A Review of Harvesting and Post-Harvesting Procedures of Marine Fish in Cage Culture with Specific Reference to Cobia Compared with Atlantic Salmon

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Abstract

A review of literature on harvesting and post-harvesting practices and potential effects on the quality of farmed fish is presented. In particular, live fish transport, stunning, killing, bleeding and chilling are targeted. Common harvesting and post-harvesting practices of Atlantic salmon are described and compared with a case study of similar processing operations during harvesting of cobia in Vietnam. The similarities between cobia and Atlantic salmon (large size, active species, high fat content) may warrant technology transfer regarding harvesting and post-harvesting practices. However, since salmon is a cold water species while cobia is a warm water species, care must be exercised regarding flesh quality issues. For large-scale production of cobia on a daily basis, the current harvesting practices may have to be reconsidered. The present individually-based handling approach may have to be replaced by a more bulk handling-oriented approach. In particular, devising a proper transport system for transferring harvested fish from the sea cage to the processing plant seems important. Concerning flesh quality, some relevant research topics are (a) effects of feed and feeding regimes, (b) comparison between wild (low fat) and farmed (high fat) cobia fillets, and, (c) cold stiffness during chilling and potential effects on fillet quality.

Introduction

The present review summarizes selected literature related to common unit operations from the time fish are harvested at sea cages until they are processed, ready for transport to market. The following topics are highlighted: live fish transport, anaesthesia and killing, bleeding and chilling. The potential effects of harvesting and post-harvesting routines on post-rigor fillet quality are discussed. The current commercial harvesting and post-harvesting routines for Atlantic salmon (*Salmo salar*) (and rainbow trout) are likewise summarized.

Due to its rapid growth, good adaptability to large-scale cage cultures in coastal waters, appreciated meat and high economic value, cobia (*Rachycentron canadum*) is a promising candidate for farming at an industrial level. In China and Chinese Taipei, cobia is already a key species in aquaculture. Other countries have similar plans for large-scale aquaculture of this species. As the cage culture of cobia is expected to increase substantially in the coming years, harvesting routines probably must change from an artisanal to a large-scale industrial approach. This is considered necessary to be able to cope with the production of large biomass. A question that might be asked is up to what extent can large-scale production of cobia (or other farmed species) benefit from the experiences gained by the salmon industry? By using a cobia case study from Vietnam as an example, cobia harvesting and post-harvesting practices are compared with those of the Atlantic salmon.

A review: Harvesting and post-harvesting routines - Preserving the quality and commercial value of the fish

Improper fish harvesting and post-harvesting practices can have adverse effects on the quality of the fish and fish products and their commercial value. Fish handling stress and gentle handling through subsequent processing steps are important factors in this context and it has been shown that by minimizing handling stress during fish harvesting, better flesh quality can be expected (Lowe et al. 1993; Sigholt et al. 1997). Proper hygienic handling practices as well as rapid post-mortem refrigeration are also well-known key factors to ensure seafood safety and good quality. In recent years, fish welfare aspects have gradually become more important for the aquaculture industry. Legislation such as the Animal Welfare Act § 30 in Norway along with increasing consumer demands in some countries are two important driving forces to improve welfare conditions in aquaculture. Adequate live fish welfare concerns all phases of fish farming (FSBI 2002).

Pre-harvesting factors

By the time farmed fish are ready for harvesting, the intrinsic quality of the fish may have been affected by genetic properties (Gjedrem 1997) and feeding regimes in the grow-out period (Johansen and Jobling 1998; Einen et al. 1999; Morris 2001). Furthermore, good environmental conditions (e.g. water quality) are important for both the fish as well as for the local environment to support sustainable aquaculture (see Feng et al. 2004).

Prior to harvesting, the fish should not be fed to reduce metabolic rates (reduce fish activity) before handling and transport, and to clean digestive tracts to minimize risks of flesh contamination during processing. Depending on seawater temperature, Atlantic salmon is commonly not fed for 1-2 weeks. Short-term fasting does not significantly reduce flesh quality. To clear digestive tracts and reduce fish metabolism, 2-3 days fasting is considered adequate (see review by Erikson 2001a).

Live fish transport

In aquaculture, transport of live fish can be employed in the following contexts: (1) transport of juveniles/smolts to sea cages for grow-out; (2) transport of market-sized fish to a processing plant for post-harvest processing; or, (3) to the live fish market. In addition, wild-caught fish are transported live to sea cages in capture-based aquaculture for farming purposes.

At harvest, Atlantic salmon are transported live by well-boats from sea cages to the processing plant. Since an open system for water exchange is used, the fish are exposed to seawater of good quality. Consequently, no adverse effects on flesh quality have been observed (Erikson et al. 1997a; Erikson 2001a) and such transports are thought to promote good fish welfare (Farrell 2006). The loading (and unloading) procedures can be more stressful than the transport itself

(Iversen et al. 1998; see Erikson 2001a). In fact, during well-boat transports the fish may actually recover (see Erikson 2001a; Iversen et al. 2005). However, during transport of salmon smolts in high seas, it has been suggested that such transports may have caused delayed mortalities after transfer to sea cages (Iversen et al. 2005). Live transportation of market-sized rainbow trout (*Oncorhynchus mykiss*) by trucks has also been shown to have limited effect on flesh quality (Ostenfeld et al. 1995).

Considering potential stress effects and live fish transport, it is important to distinguish between the cases where the fish are to be transferred to the new sites for on-growth (juveniles, smolts or capture-based aquaculture) and the harvesting of fish where they are to be slaughtered and processed immediately after transport. In the first case, handling stress and water quality issues will require more attention since the potential effects of handling and transport may be observed at a later stage. This can be delayed mortalities (Iversen et al. 2005) or reduced on-growth. The effect of stress can be monitored from blood samples such as changes in blood chemistry (e.g. stress hormones, pH, plasma ions, glucose, lactate).

On the other hand, when harvested fish are slaughtered, possible delayed effects stress will not show up even if the fish might have been exposed to several stressors. For instance, Atlantic salmon have been in a closed system at 1 ºC for about 2-3 h before slaughtering. Although water quality quickly deteriorated, no mortalities were observed. In fact, at quay the pre-chilled fish (body temperature 1 ºC) were calm. Due to inferior water quality such as low pH and elevated levels of carbon dioxide, it was likely that the fish were severely stressed. However, from a flesh quality point of view, reducing handling stress basically means minimizing excessive struggling (escape behaviour). In such cases, handling stress can be evaluated as changes in muscle biochemistry (phosphocreatine, ATP and its related products, white muscle-pH, time to rigor mortis onset) (Erikson 2001a).

During transport in a closed system with little or no seawater exchange, the water quality gradually deteriorates due to accumulation of fish metabolites, loss of mucus and scales etc. Excreted carbon dioxide reduces water pH. The low pH stabilizes total ammonium (TA-N) as NH_4^+ meaning the toxic NH_3 fraction (abundant at higher pH–values) is very low. Care must therefore be exerted concerning stripping off carbon dioxide as this would raise the pH and increase the NH_3 fraction. Moreover, mucus may cause reduced respiration due to clogging of gills. Altogether, the main effect on the fish is probably hypercapnia and a gradual loss of consciousness. If the acidosis is severe, it may be assumed that fish actually are moribund and they may not have recovered if they are transferred to fresh seawater. On the other hand, if the fish are torpid and do not show vigorous swimming behaviour, no adverse effects on fillet quality may be expected (Erikson 2001a; Erikson et al. 2006). Other than avoiding excessive struggling, only heavy oxygen supersaturation is tentatively known to have a direct effect on flesh quality. Oxygen supersaturation (>200 % at 15 ºC for 5 h) during simulated live transport of Atlantic salmon resulted in soft fillet texture (Erikson 2001a).

Regarding live transport of other species, Jittinandana et al. 2005 studied simulated chilled transport (0.3 ºC, 5.5 h) of Artic char (*Salvelinus alpinus*). The transport produced less stressed fish (higher muscle pH) than when AQUI-S^{TM} or CO_2 were added to the transport water (14 ºC). By combining carbon dioxide narcosis with hypothermia, Yokoyama et al. (1989) showed that the concept could be used successfully before live transport of carp (*Cyprinus carpio*) in a closed system. The use of the concept did not have adverse effects on flesh quality (Yokoyama et al. 1993). When Asian seabass (*Lates calcarifer*) were transported and exposed to high levels of carbon dioxide and ammonia, plasma pH was reduced to near lethal levels demonstrating the need to control water quality closely during live transport (Paterson et al. 2003). Wild-caught coral trout (*Plectropomus leopardus*) quickly responded to capture, handling and transport with elevated values of several blood parameters. However, the fish recovered after transfer to aquarium conditions, an indication that the species are appropriate for future culture use (Frisch and Anderson 2000). Capture-based aquaculture relies on transfer of fish from the catching area to the site for on-growth without major mortalities. For example, transfer of bluefin tuna (Thunnus maccoyii) caught by purse seiners may take from a few days up to several weeks. Large floating cages are required and the speed should not exceed 1-1.5 knots to avoid mortalities, accumulation of lactic acid and reduced flesh quality ('burned tuna') (Ottolenghi et al. 2004).

For further information on the different aspects of live fish transport, refer to Wedemeyer (1996) and Berka (1986) for a review of some transport designs.

Anaesthesia and killing

In aquaculture, it is possible to control harvesting and slaughtering methods to minimize the unwanted effects of rough handling on the external surfaces of the fish as well as the possible effects of handling stress on fillet quality. In both cases downgrading could reduce the commercial value of the fish. From a fish welfare point of view, an optimal slaughter method should render the fish unconscious until death without avoidable excitement (Van de Vis et al. 2003).

In Norway, salmonids are anaesthetized in a carbon dioxide tank before they are bled by cutting the gill arches. The fish are immediately transferred to an RSW tank where the fish eventually die due to loss of blood. However, the carbon dioxide anaesthesia was very stressful and the method is considered controversial from a fish welfare point of view. Eventually, RSW live chilling replaced the traditional carbon dioxide stunning. The RSW live chilling method was introduced to both pre-chilled (0 -1 ºC) and anaesthetized fish before killing. Compared with the traditional carbon dioxide stunning, lower levels of gas is used. Oxygen gas is also added to ensure sufficient amount is present for adequate respiration. Under optimized conditions (e.g. low biomasses per unit time) the method can supply unstressed fish with long pre-rigor time (Erikson et al. 2006). Nevertheless, at 2-4 min, the stunning time is similar to traditional carbon dioxide stunning and the method will eventually be banned in Norway and EU countries. Therefore, the salmon industry is currently looking at alternative methods for automated stunning and killing of farmed fish. Percussion stunning, spiking (*iki jime*), and electrical stunning in seawater or air are considered as possible alternatives. The methods are thought to promote good welfare since the fish are anaesthetized or killed instantly. A major challenge for applying these methods at a large-scale may be to establish robust solutions to fish logistics, i.e. how to transfer large biomasses to the stunning machines without exposing the fish to major stress incidents.

For other species, various slaughter methods have also been used to anaesthetize or kill farmed fish. For some species the fish are simply left to die in ice until the fish eventually die due to asphyxia. Decapitation, immersion of fish in iso-eugenol (AQUI-S™) or in an ice slurry are some of the other alternatives. Various aspects of fish welfare, slaughter methods and flesh quality have been reviewed by Robb (2001), Wall (2001), Robb and Kestin (2002), Van de Vis et al. (2003) and Poli et al. (2005). Minimizing peri-mortem handling stress is important since stress has been shown to accelerate degradation of ATP-related products (such as IMP, related to taste) (Erikson et al. 1997b), shorten time to rigor onset (Erikson 2001b), produce lighter (less red) salmon fillets (Robb et al. 2000), higher drip loss, gaping score and softer texture (Roth et al., in press) than in rested fish.

Bleeding

Bleeding is considered necessary for large fish to maintain good product quality. Residual blood in fillets may lead to reduced visual acceptance of the product (Kelly 1969). For instance, uniform white fillets are desirable for whitefish. The effects of inadequate bleeding are particularly pronounced in salted and smoked products like smoked salmon fillets (Robb et al. 2003). Residual blood, i.e. haem iron may catalyse lipid oxidation during storage of fatty fish (Richards and Hultin 2002). Moreover, bleeding of three pelagic species delayed muscle softening during chilled storage. On the other hand, bleeding had no effect on muscle firmness of three species of demersal fish (Ando et al. 1999).

The total blood volume in fish ranges from 1.5 - 3.0 % of the body weight. Only 20 % are located in muscular tissues. Since the white muscle is rather poorly vascularized, blood distribution may not be much affected during exercise (Huss 1995). On the other hand, it is known that in rainbow trout blood coagulates more quickly after a stress incident due to higher concentrations of thrombocytes, an important component involved in blood clotting mechanism in fish (Cassilas and Smith1977). This suggests that ante-mortem stress may lead to poorer blood drainage.

The fish can be bled in different ways, by cutting the gill arches, throat or caudal peduncle. Salmon is typically bled for 15-20 min in chilled water. Alternatively, the fish can be bled in air, preferably with the head down. The blood may then be collected as a by-product. Another method is simply by killing the fish and removing the viscera prior to washing. Roth et al. (2005) concluded that using this method, sufficient removal of blood is obtained. Although there are some disagreements as to which is the best bleeding method (Huss 1995), it seems clear that immediate bleeding of live fish is more important than the actual bleeding method (Kelly 1969; Valdimarsson et al. 1984; Botta et al. 1986).

Other processing operations

After bleeding, the fish are usually gutted, washed, graded and iced (or frozen) before transport to market. Gutting is necessary to maintain quality and to prolong shelf life. Otherwise, autolysis caused by (digestive) enzymes can produce off-flavours or even ‘belly-burst’ (Huss 1995).

If the fish are processed further, other typical unit operations are removal of heads, filleting, bone and skin removal, slicing, portion cutting, packing (e.g. modified atmosphere, vacuum) and quality control before the products are transported to the market. The fish should not be filleted and processed while in rigor as the fillets may be damaged and they become more susceptible to gaping (Lavety 1984). Pre-rigor filleting has several advantages over the traditional post-rigor filleting as fresher products can be available in the market (rigor may last up to 2-3 days if the fish are stored in ice). A prerequisite for consistent pre-rigor filleting is that harvesting and slaughtering is carried out without exposing the fish to excessive handling stress. Ante-mortem muscle activity is directly linked to rigor onset time. For instance, when comparing anaesthetized (unstressed) and exhausted (stressed) salmon, the post-mortem time to rigor onset is about 24 h and 2-4 h, respectively (Erikson 2001b). Thus, for the unstressed fish, we have ample time for pre-rigor processing. Stressed fish develop stronger rigor contractions (Nakayama et al. 1992; Jerrett and Holland 1998) which may have adverse effects on fillet texture (Jerrett et al. 1996; Roth et al., In press). Pre-rigor filleting have been shown to reduce incidence of fillet gaping. The fillets are thicker and the shape is different compared with post-rigor fillets. For most quality parameters, pre-rigor fillets are considered superior to post-rigor fillets (Skjervold et al. 2001a).

Chilling

Since seafoods are among the most perishable foods, rapid post-harvesting is necessary to slow down autolytic breakdown and microbial degradation. In fish farming the question is where in the production chain is the chilling going to take place? Several possibilities are conceivable. The fish may be chilled in one or several steps. This can be during live transport to the processing plant (using the vessel's RSW-system, i.e. closed system must be used) (Erikson 2001a), at the processing plant in an RSW live chilling tank (Skjervold et al. 2001b; Erikson et al. 2006) or post mortem in buffer tanks. The fish can also be chilled during the bleeding operation. Good pre-chilling before packing to a core temperature of about 0 ºC would require less ice in the boxes and lower transport costs, i.e. the purpose of the ice is then solely to maintain low temperature, not to reduce the temperature in the fish. Traditionally, flake ice, CSW or RSW have been used for chilling fish. Ice slurries are currently receiving increasing attention (Piñeiro et al. 2004). Ice slurries and superchilling (Aleman et al. 1982; Chang et al. 1998; Olafsdottir et al. 2006) have enabled subzero chilling and prolonged shelf life. To improve post-harvest quality of farmed turbot (Psetta maxima) using an ice slurry (-1.5 ºC) rather than storage in flake ice has been suggested (Rodriguez et al. 2006). However, choosing the optimal sub-zero temperature is important. For instance, when farmed gilthead seabream (Sparus aurata) were immersed in ice slurry at -2.2 ºC during slaughter, appearance of cloudy eyes reduced the commercial value of the fish (Huidobro et al. 2001). For a number of species, using ice slurries as opposed to traditional flake ice extends shelf life significantly (see Piñeiro et al. 2004).

Transport and product quality at the market

Fresh fish should be transported as quickly as possible to the market since the product storage time x temperature ultimately will determine flesh quality. If measures are taken to produce and market very fresh products (fully exploiting the quality benefits of the 'rested harvesting', 'pre-rigor filleting' concepts) a proper cold chain and quick transport are prerequisites. Otherwise, the initial beneficial effects of gentle peri-mortem handling will be offset by storage time. For instance, the clear difference in initial levels of high-energy phosphates between unstressed and stressed salmon were still discernable (IMP and inosine) up to seven days of ice storage. After this, the slower degradation of ATP-related compounds of unstressed fish was offset by storage time (Erikson et al. 1997b).

Atlantic salmon harvesting and post-harvesting procedures

During the past two decades, the salmonid fish farming industry in Norway has experienced a tremendous growth in biomass production. In 2005, a total of 588,000 and 60,000 metric tons of Atlantic salmon and rainbow trout, respectively, were produced in Norway. Atlantic salmon is hatched in closed land-based freshwater systems. After 8 -12 months saltwater-adapted smolts (large 'fingerlings') are collected by well-boats (or trucks) and transported to sea cages for on-growth. After about 1-2 years, the fish are ready for harvesting. The mean weight at harvesting is then about 4-5 kg (range 2 - 9 kg). For transport, the trend has been to develop increasingly larger and technologically more advanced well-boats transporting live fish from the sea cages to centralized fish processing plants. The hold sizes have steadily increased and today the well-boat holds can be up to 1,200 m^3. During transport, open valves are routinely used to circulate fresh seawater front to back. At harvesting, fasted fish are transferred to the well-boat hold often using the siphon principle to enable gentle transfer of fish. Fish densities typically range from 80 - 200 $kg•m^{-3}$ or more, and typical transport times range from 0.5 - 12 h. At high seawater temperatures or high fish densities, addition of oxygen gas is necessary.

For transport through areas with polluted water or through zones with risks of infections (fish diseases), it is possible to employ the closed system (water recirculation using the vessel's pumps). The well-boats are often equipped with RSW-equipment for chilling transport water which also requires the closed system. This may be used to reduce fish metabolism and to pre-chill the fish before transport. However, in such a system the water quality rapidly deteriorates and eventually this can be lethal for the fish (see above). Nowadays, closed-system transports are no longer used in Norway.

Upon arrival at the processing plant, the water level of the hold is reduced as the fish are pumped either directly to the processing line, or transferred to sea cages near the plant quayside. During unloading, water renewal is facilitated using the vessel's pumps. When the fish are transferred to cages, slaughtering will take place within the next few days (no feeding of fish). To accomplish more gentle unloading procedures, two new methods have been introduced. The fish are unloaded from the vessel either by pressurizing the hold, or by using moveable bulkheads. In both cases, the hold water level is not reduced. Consequently, excessive crowding stress can be avoided.

In some salmon producing countries, towing of sea cages, stunning, killing and bleeding operations are carried out on-site, near the sea cages. The dead fish are then transported (often by truck) in ice to the processing plant.

Even though the salmonid production volume has increased considerably over the last two decades, the number of processing plants have decreased. This is compensated for with a large increase in production capacity of each plant. Today several plants are capable of producing in excess of 100 metric tons per shift (7 h). Up to the mid 1990s, a typical slaughter line used to comprise the following unit operations: pumping or netting into the processing line, carbon dioxide anaesthesia, gill cutting with subsequent bleeding in a refrigerated seawater tank, gutting, washing, sorting and grading and packing in ice. According to a legislation in Norway (Animal Welfare Act §30), the fish must first be anaesthetized before bleeding, where the fish eventually die due to loss of blood. For the time being, carbon dioxide is the only anaesthetic allowed when the fish are slaughtered for human consumption. As the production volume steadily increased at a given plant, this resulted in excessive crowding stress in the carbon dioxide tanks. This, and other factors, often resulted in totally exhausted fish at the time of death. Sometimes fish were in rigor mortis while still in the processing line, about 2 h post mortem. Moreover, merely by visual observation of fish behaviour (violent struggling) in the carbon dioxide tank, there was also a growing awareness that this method of stunning was in conflict with the concepts of humane slaughter. The carbon dioxide tank was then gradually replaced by larger RSW live chilling tanks (water temperature 0 – 1 ºC for 20 - 60 min) where carbon dioxide is added at lower levels. In addition, the fish body temperature is reduced before killing. However, carbon dioxide still acts as the anaesthetic agent whereas hypothermia does not play a significant role in this system. Therefore, alternative stunning and killing methods (percussion stunning, spiking, electrical stunning in water or air, and eugenol) are being considered as replacements (see above). New legislation as well as consumer demands in certain markets are the main driving forces behind these changes.

After stunning, the fish are bled after cutting the gill arches. Bleeding takes place in an RSW tank at 0-4 ºC for about 30 min. An alternative bleeding method has recently been introduced. Single fish is automatically killed in a machine where percussion stunning and gill cutting occur simultaneously. The fish is placed head down in a box containing compartments for single fish. Bleeding takes place in air for about 6 min. The box is attached to a conveyor belt which moves the fish to the next unit operation, being gutting. While being transported, the fish passes through nozzles spraying water to clean the fish.

The fish is then gutted using machines, washed, sorted/graded according to quality (superior, ordinary or processing grades) and size, automatically weighed and finally packed in ice in styrofoam boxes before transport to market as whole, gutted fish. Some plants also have filleting lines where fish are beheaded and filleted using machines. Pin bones, and sometimes skin, are removed from fillets using machines. Trimming and removal of residual bones are carried out manually. Various fresh or frozen products such as whole fillets and fillet portions are among the array of products that are produced.

Up until now, it has been necessary to store the slaughtered fish for 2 - 4 days before automated removal of pin bones is possible. A new machine for automated removal of pin bones immediately after killing is currently undergoing tests at a commercial plant. If successful, this will enable production of pre-rigor boneless fillets. This may represent a major breakthrough for the fish processing industry.

Cobia farming, processing and quality compared with Atlantic salmon

The fish

Cobia is found in waters between 17 - 32 ºC (Shaffer and Nakamura 1989). Atlantic salmon on the other hand is a cold water species being farmed at seawater temperatures ranging from 3 - 18 ºC. The average growth rate of salmon is about 2-4 kg per year, compared with 5-6 kg per year for cobia. Both cobia and salmon are popular game fish because they are active species (high metabolic rates) and they exhibit very strong fighting characteristics. In the farming context, this means that the fish can easily get excited during handling processes. If less than adequate handling practices are used, these species will easily get exhausted due to excessive struggling. This in turn may affect post-mortem time to rigor onset and ultimately, fillet quality (see above).

During capture or live fish transport, pressure changes might occur. Different species may have different tolerance depending on whether they have duct from the swim bladder (like salmon) or not. For instance, if salmon are exposed to pressure changes, the swim bladder may be emptied and the fish lose their buoyancy. Cobia, on the other hand, lack the swim bladder and rely on constant swimming. This is a factor to be considered for adequate live fish transport designs. High mortality rates due to stress during live transports of juvenile and large size cobia have been reported (Liao et al. 2004).

Cobia farming

Cobia is considered as a good potential for aquaculture due to their fast growth, ease of handling and tolerance to variable environmental conditions (see Shaffer and Nakamura 1989). Under optimal conditions, growth from the fingerling stage (30 g) to 6-10 kg has been reported to occur in 280-390 days (Su et al. 2000). Cobia raised in captivity are typically fed trash fish but Chinese Taipei has developed a pellet feed especially formulated for the species.

After egg incubation, larval rearing and nursery in outdoor ponds for about 76 days all together, cobia weighing about 30 g are then transferred to nursery ponds or near shore cages where the fish grow to 600 -1000 g in 75 -105 days. Then, grow-out takes place in open ocean cages for another 6-8 months before harvesting the fish weighing 6-10 kg (Liao et al. 2004). Cobia farming is not without problems. During early life stages, high mortalities especially due to bacterial diseases have been reported (Tung et al. 2000; Liu et al. 2004). In farming areas with low winter temperatures cobia stops eating below 20 ºC and chill mortality occurs at temperatures lower than 16 ºC (Liao et al. 2004).

Composition and fillet quality

Both salmon and cobia are regarded as very good eating fish. The meat of different parts of the fish shows very distinctive features regarding fat and water contents. Both species have a versatile use, as very fresh fish can be used in sushi and sashimi markets, or they can be prepared as steamed, boiled or fried fish. A large portion of fresh salmon fillets is salted and smoked and marketed as a vacuum-packed product.

Wild cobia is usually sold gutted, head and tail on. The raw flesh has a rosy pink appearance when fresh. Fresh products of cobia (and salmon) have a maximum shelf life of about 14 days. The cobia fillet yield is 35 - 40 %. The flesh is white and flaky with an assertive, but not strong taste. In particular, the species is considered excellent smoked. Cobia is also traded as a frozen product, originating mainly from Pakistan and the Philippines (Anon. 1987).

According to body weight, cobia consists of the following characteristics: dorsal meat 26.2 %, ventral meat 23.8 %, dark (red) meat 3.3 %, viscera 12.4 % and others 34.3 %. In Asia practically all of the cobia – apart from gills and possibly livers - is used for human consumption. In most cases, only the flesh portion of Atlantic salmon is used for human consumption. The trimmed fillet ('C-trim') yield is typically 50-52 % of total body weight. The head and gut contents comprising about 11 and 12 %, respectively, are predominantly discarded. Research is currently conducted regarding the utilization of salmon by-products, cut-offs and blood. However, the market for these products has to be developed.

The gross composition of wild cobia is reported as: 74.8 % water; 2.3 % fat; 20.9 % protein; 1.9 % minerals with an energy content of 104.8 kcal per 100 g (http:// www.seafarm.com.tw), and as 5.4 % fat, 19.9 % protein and energy content 124 kcal per 100 g (Anon. 1987). Regarding farmed cobia, the similar data in dorsal and ventral flesh, respectively, are: 60.4 and 53.4 % water; 19.4 and 31.9 % fat; 16.5 and 14.1 % protein; 0.9 % minerals with energy contents of 250 and 343 kcal per 100 g (http://www.seafarm.com. tw). Furthermore, in farmed cobia weighing 3.4-4.8 kg, the fat contents of the dorsal and ventral parts ranged from 12-29 and 12-44 % fat, respectively (Liu et al. 2006). This suggests that farmed cobia are considerably fatter than their wild counterparts. If this is the case generally, the higher fat content may have a major influence on a number of flesh quality-related parameters such as fillet texture, storage time and stability, nutritional value, taste and market acceptance.

By comparison, the average total fat content of market-sized Atlantic salmon is between 8 – 18 % fat. The fat content varies considerably according to fish weight and distribution on single fish, e.g. 4 - 18 % in white muscle, 33 % in red muscle and 46 % in belly flap (see Morris 2001).

Compared with their wild counterparts, farmed Atlantic salmon generally have higher total fat contents due to intensive feeding.

To be able to deliver the highest possible quality of cultured cobia products to the market, more research is needed. Aimed at producing high-quality products and high-value fish, the following research topics (among others) have been suggested: establishing adequate husbandry techniques, identify effects of environmental conditions, identify nutritional and other factors that produce quality differences between wild and cultured cobia and how these factors are related to market demands, price etc (Rickards 2000). Another major issue that needs to be addressed is the transport of live cobia (Liao et al. 2004). Once these factors are established and large-scale production is attained, it is important to maintain the intrinsic quality by establishing adequate harvesting and post-harvesting routines.

A cobia case study: comparison with Atlantic salmon harvesting and post-harvesting practices

The cobia farm

The appraisal of cobia harvesting and post-harvesting procedures was conducted in 2005 at a commercial farm in Vietnam. Cobia were slaughtered at the fish farm, then transported to the processing plant where they were subsequently processed (fillets and by-products). Throughout the production line, the workers were told to process the fish according to standard routines. The fish farm is located at Bai Lach in the sheltered Van Phong bay area north of Nha Trang. The water quality at the cage site was good with high transparency, full salinity and temperatures in the optimal range of 25-30oC. The farm consisted of modern facilities and had about 20 people employed at the site. At present, the farming capacity for cobia is about 800 tons held in some 20 circular plastic/PEH cages of 30-60 m circumference, some with volumes up to 6,000 m3. Cobia fingerlings (about 12 cm in length) are imported from Chinese Taipei and raised in small nursery cages before eventually transferred to the larger grow-out cages. The cobia is fed specially developed pellets imported from Chinese Taipei. The feed is stored at ambient temperatures sheltered from rain and direct sunlight. The fish are fed manually 1-2 times per day. Presently, batches of 400 kg cobia (weight classes 5 -13 kg) are harvested according to customer demand.

Cobia harvesting and post-harvesting practices

Five cobia weighing 6.4-7.9 kg which were not fed for 1-2 days were harvested for the present study. About 1.5 h prior to harvesting they were transferred by individual netting from the grow-out cage to a small, shallow rectangular cage situated at a work platform. At harvesting, individual fish was netted and the throat (main artery) cut within 10-15 sec. During this operation, the fish were all relatively calm. The fish were then immediately exsanguinated by letting the fish swim in another cage adjacent to the platform until they eventually died due to blood loss. After about 1-2 min, the fish rolled over, belly up. After 5-10 min, the fish were netted into a plastic container containing ice slurry. At this point, some individuals merely exhibited some sluggish activity. The container was covered by a lid and first transported by boat (about 30 min) and then by car (about 2 h) to the subcontracted Nha Trang Seafood F17 processing plant located in the city of Nha Trang.

Upon arrival at the processing plant, the blood-containing water in the container was drained off and the fish were dipped for a few seconds in water containing 200 ppm chlorine and then placed in ice. The fish core temperature was about 7oC, corresponding to a 15-20 ºC reduction in 4 h. Notably, all fish were extremely stiff, a state that allegedly was quite normal after such transports. All processing apart from de-skinning was carried out manually. On the filleting table, the head was first cut off. Some residual blood was observed in the neck region. Then the fish were gutted and washed with running fresh water. The viscera were easily removable from the belly cavity as the various organs were kept together well. The skin surfaces were scrubbed with a brush causing some foaming probably due to the presence of mucus (glycoproteins). Before subsequent filleting, the fish were again placed in water containing chlorine (20 ppm, < 5 min). Apart from the two fillets, the middle section (backbone) contained some residual flesh which was scraped off. This, as well as the other flesh cut-offs, were collected, minced and frozen in blocks to be used as ingredients in soups. The bones located in the body cavity area were cut out by a knife. Apparently, there were 10 pin bones about 1.2 mm in diameter and 5 - 7 cm in length in fish of this size. These bones were located just beneath the surface of the body cavity. Consequently, the bones seemed relatively easy to remove. The body cavity skin (peritoneum) located in the lower body cavity was scraped off. Finally after machine de-skinning (Nock, Baden, Germany) the loins were cut. Unlike many other fish species, the anatomical distribution of red muscle was not distributed solely along the lateral line (as in salmon). Some of the red meat was scraped off to give the loin/fillet an appearance appreciated by the consumers. Little residual blood, practically no gaping and an apparently high yield were observed during the processing of the fish. Notably, no fish stiffness was observed during processing, i.e. the stiffness seemed to disappear during fish handling. The muscle fibres were apparently comparatively short and the flesh seemed to have a somewhat soft texture. However, this might be quite normal for this species. During processing, the various by-products were collected in baskets to be subsequently marketed - apart from the gill arches and liver. Thus, the overall utilization of the fish was very high. The hygienic conditions at the plant seemed adequate.

Subsequent processing of loins and fillets were as follows: First they were packed in plastic bags. Then pure CO was added to the bags to maintain the fresh red appearance of red muscles. After chilled storage at 0 - 4 ºC for a few hours, each piece of meat was packed separately in a plastic bag, which was vacuumed, sealed and subsequently frozen using contact freezers (- 40 ºC). The bags were placed in cartons and stored at -18 to -25 ºC until the products were exported. The company is currently marketing their products in Chinese Taipei, Japan (largely as sashimi product) and USA. Maximum storage time as a frozen product is about 18 months.

The company's cobia products are: (1) Fresh whole (ungutted) fish transported (gel ice) directly from the fish farm to Ho Chi Minh City where the fish are subsequently transported by air to Chinese Taipei; (2) Fresh processed products such as beheaded and gutted whole fish, cutlets etc.; (3) Frozen loins ('marbled' with red muscle); (4) Frozen whole fillets; (5) Scraped off flesh and other flesh cut-offs; (6) Flesh-containing part around pectoral fins; (7) Heads, gills removed; (8) Tongues; and (9) Skin to be used for production of leather.

Comments to cobia case study

Harvesting

The cobia handling procedures in the present study are generally considered good in terms of fish handling stress. The fish were quickly netted and bled without excessive struggling. The bleeding procedure seemed to be effective as the fish were bled immediately after the throat was cut. During transport in ice slurry, more blood was drained off. Since the loss of blood quickly reduced swimming activity, the bleeding procedure was carried out without stressing the fish severely. The cobia were bled without prior anaesthesia, whereas salmon, according to legislation, must be anaesthetized before bleeding. Since cobia handling stress was modest, a prolonged pre-rigor period could be expected. However, to be able to cope with the expected increase in slaughtered biomass, the current harvesting and the transport routines may have to be reconsidered, i.e. replacing the present individually-based handling approach with a more bulk handling-based approach. Fish pumps and a new transport system would be the factors to consider here. On the other hand, bulk handling may introduce new challenges such as less control of crowding and handling stress.

Post harvest

The post-harvest practices of cobia are generally considered adequate. Compared with the processing of Atlantic salmon, the processing of cobia was much less automated. However, the workers were skilled and they effectively produced cobia fillets. The total yield and utilization of the raw material for human consumption were considerably higher than that of the salmon industry where the by-products, with a few exceptions, currently are ensilaged to be used as ingredients in feed for terrestrial animals.

When the chilled fish arrived at the processing plant they were very stiff. Since the fish were not subjected to excessive stress during harvesting, it seemed unlikely that the stiffness could be attributed to true rigor. Cold shock reactions have been described in a number of different fish species and the phenomenon can occur during chilling of both live and dead fish. The mechanisms may however be different. A post mortem cold-shock stiffening phenomenon (a rigor-like condition) has been described in the tropical freshwater species tilapia (*Oreochromis mossambicus*) (Curran et al. 1986a). The implications of the cold-shock reaction were reduced filleting and processing yields, as well as a high drip loss and occurrence of gaping (Curran et al. 1986b). In Atlantic salmon, almost immediate rigor-like stiffening has been observed when the fish (acclimated to 5 ºC) was chilled in seawater at -1.5 ºC immediately after slaughter. The stiffness seemed to be related to the outer layer of the body (including skin). The phenomenon could not be explained by either early rigor, or tissue freezing. It was speculated that the phenomenon was related to fat hardening in lipid depots and red muscle. Notably, the stiffness disappeared when the fish were placed in ambient air (Johansen et al. 1996). When the filleting of cobia started at the processing plant, the fish were exposed to ambient air. As with the salmon, the stiffness then disappeared quite fast. If the phenomenon was related to fat hardening, the fat composition of the feed could be a factor to consider, i.e. the ratio between unsaturated and saturated fat (unsaturated fats are fluid at lower temperatures). However, at this stage it cannot be concluded whether the stiffness was related to fat hardening or if the phenomenon was related to some kind of cold-shock stiffening. Consequently, potential effects on fillet quality (see Curran et al. 1986b) cannot presently be ruled out. However, no immediate indications of reduced fillet quality were observed. Apparently, the cobia fillets seemed less fragile than salmon fillets, i.e. cobia may be more robust during processing.

The cobia fillets seemed to exhibit a somewhat soft texture. Since the potential variation in cobia fillet texture has not been investigated, this texture could be a quite normal feature for cobia. On the other hand, factors such as growth rate and the apparently higher fat contents of farmed cobia, would make comparisons with the flesh texture of wild-caught cobia interesting. In the salmon industry, issues related to flesh quality has at times been a controversial issue causing disputes between feed producers, fish farmers, processing plants, transporters or buyers of the end products. Inferior fillet quality has been associated with soft flesh, gaping and an 'oily' appearance. Soft-fleshed fish cannot simply be explained by high fat contents. The issue is not fully understood yet, but it seems that several factors such as fat contents, feeding regimes, exercise, water temperature and growth rate are factors to consider.

Conclusions

The similarities (active species, large-size at harvesting, high fat contents) between cobia and Atlantic salmon may warrant technology transfer regarding harvesting and post-harvesting technology and practices. However, the fact that salmon is a cold water species and cobia is a warm water species, should be kept in mind when flesh quality issues are considered. For large-scale production of cobia on a daily basis, the current harvesting practices may have to be reconsidered. The present individually-based handling approach may have to be replaced by a more bulk handling-oriented approach. In particular, devising a proper transport system for transferring harvested fish from the sea cage to the processing plant seems important.

Concerning flesh quality, some relevant research topics are (a) effects of feed and feeding regimes; (b) comparison between wild (low fat) and farmed (high fat) cobia fillets; and, (c) cold stiffness and potential effects on fillet quality.

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References

Aleman, M.P., Kakuda, K., Uchiyama, H. (1982) Partial freezing as a means of keeping freshness of fish. Bull. Tokai Reg. Fish. Res. Lab. No. 106, 11-25.

Ando, M., Nishiyabu, A., Tsukamasa, Y., Makinodan, Y. (1999) Post-mortem softening of fish muscle during chilled storage as affected by bleeding. J. Food Sci. 64, 423-428.

Anonymous. (1987) Cobia (Rachycentron canadum). Seafood Leader Buyer's Guide 7 (1), 60, 62.

Berka, R. (1986) The transport of live fish. A review. EIFAC Technical paper No. 48, FAO, Rome. 52 p.

Botta, J.R., Squires, B.E., Johnson, J. (1986) Effect of bleeding/gutting procedures on the sensory quality of fresh raw Atlantic cod (Gadus morhua). Can. Inst. Food Sci. Technol. J. 19, 186-190.

Cassilas, E., Smith, L.S. (1977) Effects of stress on blood coagulation and haematology in rainbow trout (Salmo gairdneri). J. Fish Biol. 10, 481-491.

Chang, K.L.B., Chang, J., Shiau, C.-Y., Pan, B.S. (1998) Biochemical, microbiological, and sensory changes of sea bass (Lateolabrax japonicus) under partial freezing and refrigerated storage. J. Agric. Food Chem. 46, 682-686.

Curran, C.A., Poulter, R.G., Brueton, A. and Jones, N.S.D. (1986a) Cold shock reactions in iced tropical fish. J. Food Technol. 21, 289-299.

Curran, C.A., Poulter, R.G., Brueton, A., Jones, N.R. and Jones, N.S.D. (1986b) Effect of handling treatment on fillet yields and quality of tropical fish. J. Food Technol. 21, 301-310.

Einen, O., Mørkøre, T., Rørå, A.M.B., Thomassen, M.S. (1999) Feed ration prior to slaughter - a potential tool for managing product quality of Atlantic salmon (Salmo salar). Aquaculture 178, 149-169.

Erikson, U. (2001a) Potential effects of preslaughter fasting, handling and transport. In: Farmed Fish Quality (S. Kestin and P. Wariss, Eds.), pp 202-219, Blackwell Science, Oxford

Erikson, U. (2001b) Rigor measurements. In: Farmed Fish Quality. S. Kestin and P. Wariss (Eds.), Blackwell Science, Oxford, pp.283-297.

Erikson, U., Beyer, A.R., Sigholt, T. (1997b) Initial distribution of white muscle high-energy phosphates related to handling stress affects K-values during ice storage of Atlantic salmon (Salmo salar). J. Food Sci. 62, 43-47.

Erikson, U., Hultmann, L., Steen, J.E. (2006) Live chilling of Atlantic salmon (Salmo salar) combined with mild carbon dioxide anaesthesia. I. - Establishing a method for large-scale processing of farmed fish. Aquaculture 252, 183-198.

Erikson, U., Sigholt, T., Seland, A. (1997a) Handling stress and water quality during live transportation and slaughter of Atlantic salmon (Salmo salar). Aquaculture 149, 243-252.

Farrell, A.P. (2006) Bulk oxygen uptake measured with over 60 000 kg of adult salmon during live haul transport at sea. Aquaculture 254, 646-652.

Feng, Y.Y., Hou, L.C., Ping, N.X., Ling, T.D., Kyo, C.I. (2004) Development of mariculture and its impacts in Chinese costal waters. Rev. Fish Biol. Fish., 14, 1-10.

Frisch, A.J., Anderson, T.A. (2000) The response of coral trout (Plectropomus leopardus) to capture, handling and transport and shallow water stress. Fish Physiol. Biochem., 23, 23-34.

FSBI. (2002) Fish welfare. Briefing paper 2. Fisheries Society of the British Isles, Cambridge.

Gjedrem, T. (1997) Flesh quality improvement in fish through breeding. Aquaculture Int. 5, 197-206.

Huidobro, A., Mendes, R.; Nunes, M.L. (2001) Slaughtering of gilthead seabream (Sparus aurata) in liquid ice: influence on fish quality. Eur Food Res Technol 213, 267-272.

Huss, H.H. (Ed.) (1995) Quality and quality changes in fresh fish. FAO Fisheries Technical Paper No. 348, Rome, FAO.195 p.

Iversen, M., Finstad, B., McKinley, R.S., Eliassen, R.A., Carlsen, K.T., Evjen, T. (2005) Stress responses in Atlantic salmon (Salmo salar L.) smolts during commercial well boat transports, and effects on survival after transfer to sea. Aquaculture 243, 373-382.

Iversen, M., Finstad, B., Nilssen, K.J. (1998) Recovery from loading and transport stress in Atlantic salmon (Salmo salar L.) smolts. Aquaculture 168, 387-394.

Jerrett, A.R., Holland, J. (1998) Rigor tension development in excised rested, partially rested and exhausted Chinook salmon white muscle. J. Food Sci. 63, 48-52

Jerrett, A.R., Stevens, A. J., Holland, A.J. (1996) Tensile properties of white muscle in rested and exhausted Chinook salmon (Oncorhynchus tshawytscha). J. Food Sci. 61, 527-532.

Johansen, S.J.S., Jobling M. (1998) The influence of feeding regime on growth and slaughter traits of cage-reared Atlantic salmon. Aquaculture Int. 6, 1-17.

Johansen, S., Rustad, T., Erikson, U. and Nordtvedt, T.S. (1996) Effect of pre-packing temperatures on Atlantic salmon quality. Proceedings from Refrigeration and Aquaculture, 20-22 March, Bordeaux, France, pp.189 -197.

Jittinandana, S., Kenney, P.B., Mazik, P.M., Danley, M., Nelson, C.D., Kiser, R.A., Hankins, J.A. (2003) Transport and stunning affect quality of Arctic char fillets. J. Muscle Foods 16, 274-288.

Kelly, T.R. (1969) Discolouration in sea-frozen fish fillets. In: Freezing and irradiation of fish. R. Kreuzer (Ed.), Fishing News (Books) Ltd., London, pp. 64-67.

Lavety, J. (1984) Gaping in farmed salmon and trout. Torry Advisory Note no 90, Torry Research Station, Aberdeen.

Liao, I.C., Huang, T.-S., Tsai, W.-S., Hsueh, C.-M., Chang, S.-L., Leaño, E.M. (2004) Cobia culture in Taiwan: current status and problems. Aquaculture 237, 155-165.

Liu, P.-C., Lin, J-Y, Chuang, W.-H., and Lee, K.-K. (2004) Isolation and characterization of pathogenic Vibrio harveyi (V. carchariae) from farmed marine cobia fish Rachycentron canadum L. with gastroenteritis syndrome. World J. Microbiol. Biotechnol. 20, 495-499.

Liu, S.-M., Hsia, M.-P., Huang, C.-M. (2006) Accumulation of butyltin compounds in cobia Rachycentron canadum raised in offshore aquaculture sites. Sci. Total Environ. 355, 167-175.

Lowe, T.E., Ryder, J.M., Carragher, J.F., Wells, R.M.G. (1993) Flesh quality in snapper, Pagrus auratus, affected by capture stress. J. Food Sci. 58, 770-773,796.

Morris, P.C. (2001) The effects of nutrition on the composition of farmed fish. In: Farmed Fish Quality, S. Kestin and P. Wariss (Eds.), Blackwell Science, Oxford, pp.161-179.

Nakayama, T., Liu, D-J., Ooi, A. (1992) Tension change of stressed and unstressed carp muscles in isometric rigor contraction and resolution. Nippon Suisan Gakkaishi 58, 1517-1522.

Olafsdottir, G., Lauzon, H.L., Martinsdóttir, E., Oehlenschläger, J., Kristbergsson, K. (2006) Evaluation of shelf life of super chilled cod (Gadus morhua) fillets and the influence of temperature fluctuations during storage on microbial and chemical quality indicators. J. Food Sci. 71, 97-109.

Ostenfeld, T., Thomsen, S., Ingólfdóttir, B., Rønsholdt, B., McLean, E. (1995) Evaluation of the effect of live haulage on metabolites and fillet texture of rainbow trout (Oncorhynchus mykiss). Wat. Sci. Tech. 31, 233-237.

Ottolenghi, F., Silvestri, C., Giordano, P., Lovatelli, A. New, M.B. (2004) Capture-based aquaculture. The fattening of eels, groupers, tunas and yellowtails. FAO, Rome, 308 p.

Paterson, B.D., Rimmer, M.A., Meikle, G.M., Semmens, G.L. (2003) Physiological responses of the Asian sea bass, Lates calcarifer to water quality deterioration during simulated live transport: acidosis, red-cell swelling, and levels of ions and ammonia in the plasma. Aquaculture 218, 717-728.

Piñeiro, C., Barros-Velázquez, J., Aubourg, S.P. (2004) Effects of newer slurry ice systems on the quality of aquatic food products: a comparative review versus flake-ice chilling methods. Trends Food Sci. Technol. 15, 575-582.

Poli, B.M., Parisi, G., Scappini, F., Zampacavallo, G. (2005) Fish welfare and quality as affected by pre-slaughter and slaughter management. Aquaculture Int. 13, 29-49.

Richards, M.P., Hultin, H.O. (2002) Contributions of blood and blood components to lipid oxidation in fish muscle. J. Agric. Food Chem. 50, 555-564.

Rickards, W.L. (2000) Sustainable cobia culture and fisheries. Cobia culture research and development planning workshop, Biloxi, Mississippi, 19-20 September 2000.

Robb, D.H.F. (2001) The relationship between killing methods and quality. In: S. Kestin and P. Wariss (Eds.), Farmed Fish Quality, Blackwell Science, Oxford, pp. 220-233.

Robb, D.H.F., Kestin, S.C. (2002) Methods used to kill fish: field observations and literature reviewed. Anim. Welf. 11, 269-282.

Robb, D.H.F., Kestin, S.C., Wariss, P.D. (2000) Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. Aquaculture 182, 261-269.

Robb, D.H.F., Phillips, A.J Kestin, S.C. (2003) Evaluation of methods for determining the prevalence of blood spots in smoked Atlantic salmon and the effect of exsanguinations method on prevalence of blood spots. Aquaculture 217, 125-138.

Rodriguez, Ó, Barros-Velázquez, Piñeiro, C., Gallardo, J.M., Aubourg, S.P. (2006) Effects of storage in slurry ice on the microbial, chemical and sensory quality and on the shelf life of farmed turbot (Psetta maxima). Food Chem. 95, 270-278.

Roth, B., Torrissen, O.J., Slinde, E. (2005) The effect of slaughtering procedures on blood spotting in rainbow trout (Oncorhynchus mykiss) and Atlantic salmon (Salmo salar). Aquaculture 250, 796-803.

Roth, B., Slinde, E., Arildsen, J. (In press) Pre or post mortem muscle activity in Atlantic salmon (Salmo salar). The effect on rigor mortis and the physical properties of flesh. Aquaculture (2006).

Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T.S. and Seland, A. (1997) Handling stress and storage temperature affect meat quality of farmed-raised Atlantic salmon (Salmo salar). J. Food Sci. 62, 898-905.

Shaffer, R.V. and Nakamura, E.L. (1989) Synopsis of biological data on the cobia Rachycentron canadum (Pisces: Rachycentridae). FAO Fisheries Synopsis 153 (NMFS/S 153). U.S. Dep. Commerce, NOAA Tech. Rep. NMFS 82, Washington D.C., USA. 21 pp.

Skjervold, P.O., Fjæra, S.O., Østby, P.B., Isaksson, T., Einen, O., Taylor, R. (2001a) Properties of salmon flesh from different locations on pre- and post-rigor fillets. Aquaculture 201, 91-106.

Skjervold, P.O., Rørå, A.M.B., Fjæra, S.O., Vegusdal, A., Vorre, A., Einen, O. (2001b) Effects of pre-, in-, or post-rigor filleting of live chilled Atlantic salmon. Aquaculture 194, 315-326.

Su, M.S., Chien, Y. H., Liao, I.C. (2000) Potential of marine cage aquaculture in Taiwan : Cobia culture. In : Cage culture in Asia. Liao, I.C., Lin, C.K. (Eds.). Asian Fisheries Society, Manila, and World Aquaculture Society-Southeast Asian Chapter, Bangkok. pp. 97-106.

Tung, M.C., Chang, L.T., Tsai, S.S., Wang, D.H. (2000) Mass mortality associated with Photobacterium Damsela subsp. piscicida in sea-cage cultured cobia Rachycentron canadum (Linnaeus, 1776) in southern Taiwan. FA COA Aquaculture Series No. 1, Reports of Fish Disease Research, vol. XX. Fisheries Administration, Council of Agriculture, Taipei, Taiwan, pp. 133 - 145.

Valdimarsson, G., Matthiasson, A., Stefansson, G. (1984) The effect of on board bleeding and gutting on the quality of fresh, quick frozen and salted products. In: Fifty years of fisheries research in Iceland. A Moller (Ed.). Icelandic Fisheries Laboratory, Reykjavik, pp. 61-72.

Van de Vis, H., Kestin, S., Robb, D., Oehlenschläger, J., Lambooij, B., Münkner, W., Kuhlmann, H., Kloosterboer, K., Tejada, M., Huidobro, A., Otterå, H., Roth, B., Sørensen, N.K., Akse, L., Byrne, H., Nesvadba, P. (2003) Is humane slaughter of fish possible for industry? Aquaculture Res. 34, 211-220.

Wall, A.J. (2001) Ethical considerations in the handling and slaughter of farmed fish. In: S. Kestin and P. Wariss (Eds.), Farmed Fish Quality, Blackwell Science, Oxford, pp.108-115

Wedemeyer, G.A. (1996) Transportation and handling In: Principles of Salmonid Culture (eds W. Pennell & B.A. Barton). Elsevier, Amsterdam, pp. 727-758.

Yokoyama, Y., Kawai, F., Kanamori, M. (1993) Effect of cold-CO_2 anesthesia on postmortem levels of ATP-related compounds, pH, and glycogen in carp muscle. Nippon Suisan Gakkaishi 59, 2047 -2052.

Yokoyama, Y., Yoshikawa, H., Ueno, S., Mitsuda, H. (1989) Application of CO_2 –anesthesia combined with low temperature for long-term anesthesia in carp. Nippon Suisan Gakkaishi 55, 1203-1209.

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Health Management Practices for Asian Aquaculture - a Key Component for Sustainability

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Abstract

The intensification of aquaculture and globalization of the seafood trade have led to remarkable developments in the aquaculture industry. Nevertheless, the industry, particularly Asian aquaculture (> 90% of world production), is paying a price for this unprecedented growth in terms of deterioration in environmental and health conditions. The industry has been plagued with disease problems caused by viral, bacterial, fungal and parasitic pathogens. In recent years, disease outbreaks are becoming more frequent in the region and the associated mortality and morbidity have caused substantial economic losses. Asian aquaculture is characterized by an enormous diversity of species e.g. several dozen marine species being farmed. Consequently, more resources are needed to understand the basic epidemiology of diseases in the various species. Epidemiology data are scarce, as are basic data on the immune systems of Asian fish species. This hampers development of effective strategies for disease control. Also, most farm operations are on a small scale, and technical support, including disease diagnosis and training, is often lacking at farm level. Increased trade of live aquatic animals and the introduction of new species for farming, without proper quarantine and risk analysis in place, result in the further spread of diseases. In Asia, most individual fish farms produce several species of fish. Trash fish are widely used as feed. Fry are often caught from the wild or derived from wild caught broodstock. Furthermore, legislation for and implementation of farming licenses and zoning policies are not in place in most Asian countries. Coupled with a 'gold rush' mentality, this often results in too many fish and too many farms in a concentrated area, which in turn promotes disease transmission. The combination of all these factors, together with the diversity of organisms in tropical waters, leads to a truly challenging disease situation. At present, many farmers still focus more on treatment than prevention. Irresponsible use of antibiotics and chemicals in aquaculture can lead to residue problems, an increasing consumer concern, and the development of drug resistance among bacterial pathogens. Good health management is the "silver bullet" for disease control. Collectively, this includes the use of healthy fry, quarantine measures, optimized feeding, good husbandry techniques, disease monitoring (surveillance and reporting), sanitation and vaccination. In Asia, with the exception of Japan, few fish vaccines are yet commercially available. The major advantages of prophylactic vaccination over therapeutic treatment are that vaccines provide long-lasting protection and leave no problematic residues in the product or environment. Overall, the emphasis of health management must be on prevention rather than treatment.

Introduction

Today, aquaculture is the fastest growing food-producing sector in the world compared with terrestrial animals and 90% of world aquaculture production is in Asia. However, from the time man started to culture fish, fish diseases have changed from being an interesting phenomenon to an important socio-economic problem. Infectious disease is considered to be the industry's single most important cause of mass mortalities and economic losses. Health problems have two fiscal consequences on the industry: loss of productivity due to animal mortality and morbidity, and loss of trade due to food safety issues. Nowadays seafood safety is under spotlight associated with the presence of food-borne pathogens or chemical residues in products.

Estimates from various organisations have indicated that approximately a third to a half of all fish and shrimp cultured in cages or ponds are lost due to diseases before they reach marketable size. The actual economic losses in the aquaculture industry worldwide are estimated to be in excess of US$ 9 billion per year, which is roughly 15% of the value of world farmed fish and shellfish production. Despite being long established, diseases and associated economic losses in aquaculture are a huge problem in the Asia (Bonadad Reantaso et al. 2005). According to Wei (2002), outbreaks of bacterial diseases caused losses of over US$ 120 million to the fish aquaculture industry in China between 1990 and 1992. In 1994, marine fish diseases caused industry losses of US$ 114.4 million in Japan alone (Arthur & Ogawa 1996). In addition, within a 3-month period, Koi herpes virus (KHV) infection of common carp led to losses of approximately US$ 5.5 million for Indonesian farmers in one area alone (Bondad Reantaso 2004). IntraFish Media reported in 2004 that, "the FAO recently sent out an alert in a press release about the dangers some of these diseases can pose not only for human health but they can also paralyze regional food producing sectors and leave thousands of farmers and producers out of work and with no income. Asia has particularly been mentioned where millions of people live off fishing or aquaculture or both". Thus, disease is undoubtedly one of the major constraints to production, profitability and sustainability of the aquaculture industry.

The aquaculture industry in Asia is characterized by an enormous diversity of fish species and most Asian farms operate on a small scale where technical support, including disease diagnosis and training, is lacking. Consequently, treatment is generally decided without proper disease diagnosis and antibiotics are often improperly used. This has led to residue problems and the development of bacterial drug resistance. Moreover, poor husbandry methods are still in practice in many places e.g. the use of fry sourced from the wild or derived from wild-caught broodstock and, in our opinion, the use of trash fish as feed. These practices open a door for pathogen infections. In addition, the increased trade of live aquatic animals and the introduction of new species for farming, without proper quarantine and risk analysis in place, have resulted in the spread of diseases within and between countries. The combination of all these factors has led to a truly challenging disease situation in Asian aquaculture where disease prevention is difficult (Tan & Grisez 2004).

Norwegian salmon farming is often taken as an example of how things should or could progress in aquaculture. However, the production of fish in tropical and subtropical areas is quite different. Differences involve not only the species cultured, but also (and mainly) the scientific knowledge that is available on reproduction, husbandry, feed requirements, diseases and immunology specific to the farmed species. Taking these differences into account, the knowledge that has been gathered in salmon health management can be used to more efficiently advance the relevant science in this region.

As Asian aquaculture continues to grow at a fast pace due to both area expansion and production intensification, the prevalence and spread of infectious diseases will unavoidably increase as a result of the intensification and therefore higher infection pressure like what we have seen in the poultry, swine and cattle industries over the last several decades. In order to become sustainable, the industry must undergo changes and pay more attention to health management strategies.

In this paper, an overview is given about the current situation regarding health management practices in Asia. Recommendations for improvement are also discussed.

Current Status of Asian Aquaculture and Challenges

Characteristics of Asian aquaculture – enormous diversity of cultured species

Aquaculture in Asia has a rich history of more than 2,500 years and is recognized as the leading aquaculture region in the world, contributing to 90% of total world aquaculture production. FAO statistics show that there are over a hundred species of finfish cultured in the region (FAO Fishstat Plus). With such species diversity, a significant amount of resources is needed to understand basic disease epidemiology, genetics/breeding and nutritional requirements for all these species. However, a more realistic approach could be to focus on a lesser number of species, as is the case in the coldwater finfish aquaculture industry. The origin of species diversification in Asia can be attributed to historical, environmental and social factors (Liao 1996). Importantly, because of the high number of species, when one is severely affected by an unknown and therefore uncontrollable disease, most farmers will opt for the most (apparently) economical way-out i.e. stop farming the problematic species and start farming a new one. For instance, KHV has severely affected the carp farming industry in several Asian countries during the last few years. In Indonesia, where the disease has wiped out entire fish populations in certain areas of Sumatra, former carp farmers are now looking at farming alternative species, such as tilapia. Another example can be seen in Thailand where between 2003 and 2006, the majority of shrimp farmers have switched from farming *Penaeus monodon* to *Litopenaeus vannamei*, considered stronger and more resistant to diseases such as WSSV. However, after several years of culture, disease and other health issues have also appeared in the latter species.

Running away from problems by switching species is only a temporary solution to an on going problem – disease. This is a consequence of intensive farming conditions and poor health management practices. Even a species such as tilapia, which was initially considered as "hardy", can be threatened by economically devastating diseases when farmed under intensive conditions. The huge diversity of farmed species in Asia, with sometimes more than one dozen species farmed in the same location, is a huge challenge in terms of disease management.

Diversity of culture system and the environment

Different species might require different culture systems. This is another challenge for Asian fish farmers. Currently the two major culture systems used to raise fish are cages and ponds. In both environments, water quality is a critical factor. In a pond, water quality management is crucial in order to avoid problems such as nitrite toxicity, plankton crash and bloom of blue green algae (causing off-flavour of the meat). In a cage environment, water quality is much less controllable. Due to crowded conditions, fish raised in cages are therefore more vulnerable to a rapid change in temperature or drop in oxygen. In addition, because of a lack of natural food sources in cage culture, fish are more dependent on a nutritionally complete diet. When farming in open water, fish are more exposed to wild species therefore there is a greater risk for disease transmission and outbreak (vice versa, concern on transmission of disease from farmed species to wild stocks also exists).

Cage farming is practiced in both freshwater and marine environments, but disease problems differ. Simple parameters such as salinity and temperature can dictate the epidemiology of disease outbreaks. For example, columnaris disease due to *Flavobacterium columnare* is a common skin disease of freshwater fish. This disease is not present in seawater or even brackish water as the bacteria involved could not grow in the presence of salt. In contrast, *Tenacibaculum maritimum*, a common bacterium causing skin lesions in marine fish (Labrie et al. 2005b), is not a problem in freshwater as it is incapable of growing without salinity. Therefore, fish reared in environments where salinity fluctuates because of seasonal variations or water availability may encounter different disease problems depending on the salinity of the water. Another example is tilapia. When reared in brackishwater, they are susceptible to the parasite ciliate *Amyloodinium* spp. (Leong et al. 2006). However, this susceptibility disappears when salinity decreases as the parasite is not adapted to freshwater.

Temperature is an additional parameter that influences the complexity of disease epidemiology. In order to infect a fish species, it is necessary that the pathogen must be able to multiply optimally within the temperature range that the fish species is farmed. For example, Lactococcus garvieae is a pathogen in fish raised in temperate waters. Therefore, it is commonly found in yellowtail and amberjack farmed in Japan but not in warm water fish raised in South East Asia, such as grouper, Asian seabass and tilapia. Another example can be found in Thailand where the tilapia industry is affected every summer with outbreaks of streptococcosis when water temperature exceeds 30°C. This temperature window coincides with the preferential temperature window of *Streptococcus agalactiae*, a pathogen involved in the disease. When water temperature is under 30°C, the mortality associated with this pathogen is low.

Comparison with salmon farming

Salmon has been considered as the model species for modern aquaculture, especially in cage farming. In the last 20 years, this industry has developed dramatically and now produces nearly 1.5 million tons annually (FAO Fishstat Plus). Produced largely by two countries (Norway and Chile), salmon products could be seen in virtually every supermarket in the world. In marine cage culture in cold water countries (Canada, Chile, Northern Europe), the focus is on only one family of cultured fish (Salmonids). Therefore, most resources available are used for optimizing (including disease control) the culture of this one family of fish. This is in stark contrast with the above-mentioned situation in Asia. A lower than 95% survival rate in salmon is a sign of a disease outbreak whereas a survival rate of 50% in groupers is often considered acceptable in Asia. It is therefore useful to highlight the main characteristics of these two very different aquaculture regional situations. Table 1 illustrates the differences.

Table 1: Differences between salmonid and Asian marine fish species farming.

	Salmonids	Asian species
Farming system and practice	mainly salmonid family integrated industry single species per farm all-in, all-out approach, with fallowing hatchery fry optimal stocking density zoning policy established market	over 50 species “backyard farming” mixed species mixed age groups no fallowing many wild-caught fry high stocking density no zoning and licensing fluctuating market
Feed technology	knowledge on nutrition optimized dry feed good FCR	little knowledge on feed largely using trash fish generally poor FCR
Health management	knowledge on diseases acceptable survival 95% focus on prevention biosecurity and sanitation documentation vaccination	lack of proper diagnosis “normal” survival < 50% focus on treatment lack of biosecurity poor record keeping & analyses few vaccines

The intensification of salmon production has led to the separation of fry production (hatcheries) and on growing sites, optimized feed and feeding strategies, good quality fingerlings (that are virtually disease-free) and good farm management. In Asia, most farms produce different species of fish at the same site. No segregation in year classes is made, something that is obligatory for salmon in Europe. Trash fish is widely used as feed, fry are often wild caught or derived from wild caught broodstock, and the culture techniques per species are not yet established. Furthermore, legislation and implementation regarding farm licenses and zoning policy are not in place in most Asian countries. With the so-called “gold rush” mentality, this often results in too many fish and too many farms in a concentrated area that promotes the spread of diseases. The combination of all these factors, together with the diversity of organisms in tropical waters, leads to a truly challenging disease situation with a variety of entry points for pathogens.

Disease status in Asian aquaculture

Disease is undoubtedly recognized as one of the biggest constraints to the production, development and sustainable expansion of aquaculture in the Asian region. As most farms operate on a small scale basis and with limited technical support, disease diagnosis and training are usually lacking at the farm level. Even if fish suffer from disease and overall survival is low, epidemiology data are rarely collected, reported and analyzed.

In the past few years, more and more attention has been given to the identification of etiological agents involved in fish disease epidemics. Pathogens can be classified into bacterial, viral, parasitic and fungal groups. Table 2 shows major pathogens affecting the fish farming industry in Asia (Tan et al. 2003; Bondad Reantaso et al. 2005; Komar et al. 2005; Labrie et al. 2005a; Leong et al. 2005; Leong et al. 2006).

Temperature zone/species	Pathogens			
	Bacteria	Virus	Parasites	Fungi
Temperate marine species (yellowtail, amberjack, red sea bream, etc.)	*Aeromonas salmonicida* *Edwardsiella tarda* *Lactococcus garvieae* *Listonella (Vibrio) anguillarum* *Mycobacterium spp.* *Nocardia seriolae* *Photobacterium* damsela ssp.piscicida *Rickettsia spp.* *Tenacibaculum maritimum*	Iridovirus Lymphocystis virus (LCDV) Nodavirus (Nervous necrosis virus) Rhabdovirus (viral haemorrhagic septicaemia) Yellowtail ascites virus (YAV)	*Benedenia* spp. *Caligus*spp. *Cryptocaryon irritans* *Heteraxine* spp. *Kudoa* spp. *Microsporidium* spp. *Myxobolus* spp. *Neobenedenia* spp. *Paradeontacylix* spp. *Philasterides dicentrachi* *Trichodina* spp.	
Warmwater marine species (Asian seabass, groupers, snappers, etc.)	*Nocardia* spp. *Vibrio* sp. (big belly disease) *Streptococcus agalactiae* *S. iniae* *T. maritimum*	Iridovirus Nodavirus	*Amyloodinium* spp. *Brookynella* spp. *Benedenia* spp. *Caligus* spp. *Cryptocaryon irritans* *Dactylogyrus* spp. *Glugea* spp. *Neobenedenia* spp. *Sphaerospora* spp. *Trichodina* spp.	*Ichthyophonus* sp.
Freshwater species (tilapia, catfish, carp, etc.)	*A. hydrophila* *E. ictaluri* *E. tarda* *Flavobacterium columnare* *Francisella-like organism* *Nocardia spp.* *S. agalactiae* *S. iniae*	Aquareovirus (grass carp hemorrhage virus: CHV) Iridovirus Koi herpes virus (KHV) Spring viraemia of carp virus (SVCV)	*Argulus spp.* *Dactylogyrus spp.* *Diplostomum spp.* *Eimeria spp.* *Ichthyophthirius spp.* *Lernea spp.* *Myxobolus spp.* *Piscicola geometrica* *Sanguinicola spp.* *Sphaerospora spp.* *Trichodina spp.*	Aphanomyces invadans Branchiomyces spp. Saprolegnia spp.

Because of the scale of resource expertise and infrastructure required for disease diagnostics of such a variety of pathogens, FAO/NACA (Bondad-Reantaso et al. 2001) recommended the use of three levels of diagnostics:

Level I. field observation of the animal and the environment, clinical examination;

Level II. laboratory observations using parasitology, bacteriology, mycology and histopathology;

Level III. laboratory observations using virology, electron microscopy, molecular biology and immunology.

In fish, clinical signs of disease are rarely obvious and it is difficult to base a diagnosis solely on field observations. Unfortunately, this is very often the only way Asian farmers "guess" the cause of the disease as they do not have access to a laboratory. The consequence is that a treatment is generally decided upon without proper disease diagnosis. Accurate disease prevention is therefore difficult. A general improvement in disease management should come from a general improvement of husbandry practices and knowledge on disease health management.

Irresponsible use of chemicals/antibiotics

Due to lack of diagnosis, farmers often apply antibiotic treatments when mortality occurs, without knowing the cause of the disease and assuming that it is caused by a bacterial pathogen. Some farmers even use antibiotics as a form of “preventative measure”, where antibiotics are administered in anticipation of an expected disease outbreak. This has resulted in the heavy use of chemicals and drugs (Choo 2000). While under certain circumstances antibiotics could provide a useful means of reducing the adverse effects of bacterial diseases, there are many problems associated with their use. An important side effect of the use of antibacterial drugs in aquaculture is the development of drug resistance among fish and shellfish bacterial pathogens (Smith et al. 1994; Huovinen 1999; MacMillan 2001; Tendencia & de la Pena 2001).

Many bacterial species multiply rapidly enough to quickly adapt to changes in the environment and survive in unfavourable conditions. The heavy use of drugs could result in the development of mutations in some bacteria. These mutations could lead to antibiotic resistance where an antibiotic is no longer capable of either killing (bactericidal effect) or preventing growth (bacteriostatic effect) of the bacteria. Emerging antimicrobial resistance, due to overuse and incorrect use of antimicrobials, is a human as well as an animal health concern worldwide.

For example, in 2004, an Asian fish farm suffered from several bacterial disease outbreaks. The primary pathogen was Edwardsiella tarda. The farm began to use a series of consecutive antibiotic treatments in the hope of stopping the on-going mortality as indicated in Figure 1 (personal communication). Antibiotic sensitivity tests were done on E. tarda isolated before and after the treatments. As indicated, the bacterium became resistant to two (trimethoprim sulfamethoxazole and florfenicol, the latter belonging to the same class of chloramphenicol) out of the three antibiotics used. This demonstrates the dangers of excessive usage of antibiotics in aquaculture.

Undoubtedly, trade restrictions imposed on some Asian aquaculture products, increasing public awareness and concern for residues in fish and crustacean products, and the development of multiple antibiotic resistant bacterial strains will lead to a shift from disease treatment through antibiotics to disease prevention by other means, such as vaccination and biosecurity.

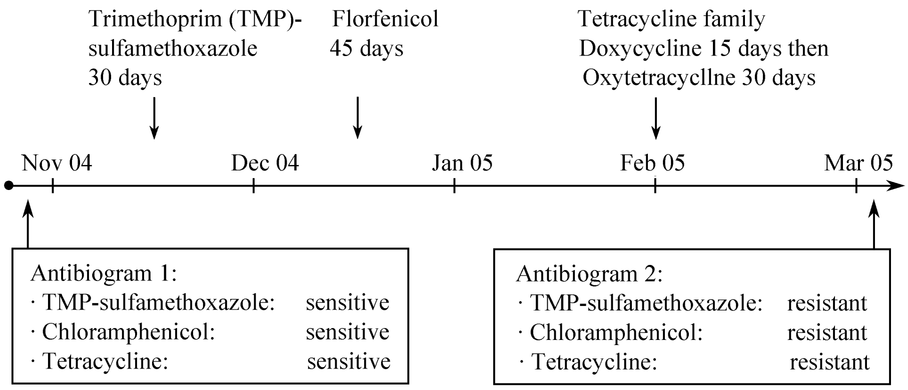

Figure 1. A real case on induction of antibiotic resistance in a fish farm.

Inadequate health management practice

In Asia, good farming and health management practices are still to be implemented. For example, the use of trash fish as feed is a common practice in small scale marine fish farming. From a health management perspective, the use of trash fish opens the door to a variety of potential pathogens and infections and it is one of the major causes of fish disease in Asia.

Fry are often sourced from the wild or derived from wild-caught broodstock. Under these conditions, the quality is inconsistent. Weak or diseased juveniles are one of the failures in Asian aquaculture.

Due to the development of the aquaculture industry and the increased globalization of commercial trade, there is more and more movement of broodstock, fry and fingerlings between countries or regions. KHV is a recent example of disease dissemination due to translocation of fish. The disease has spread to many countries within a few years (Crane et al. 2004).

Challenge to sustainability

The challenge we are facing is enormous. In tropical areas, water temperature is relatively high which facilitates the multiplication of micro-organisms and some of these can be very harmful to aquatic animals. The combination of this with all other factors mentioned above has led to a truly challenging disease situation with a variety of entry points for pathogens in Asian aquaculture.

Figure 2 illustrates how diseases are threatening the sustainability of the industry in the region. A disease causes mortality and morbidity. When antibiotics or chemicals are not used properly for treatment, there are negative consequences. One of the problems is residues in aquatic products, which in turn give food safety concerns and trigger trade barriers. In the last several years, residue problems have created a negative image for the whole aquaculture industry in Asia. Farmers in Asia tend to stock more fish or put in more cages to compensate for mortality. The low production efficiency not only increases production costs, it also wastes our natural resources and creates unnecessary pollution. This has caused huge concern from consumer activists or environmental groups (New 2003). Clearly, something must be done to keep the industry sustainable.

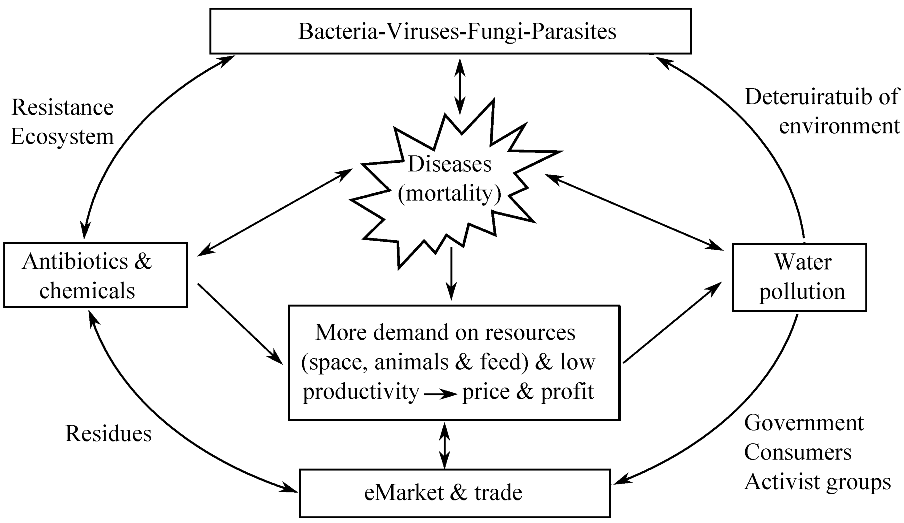

Figure 2. The negative impacts of infectious diseases on sustainability of the aquaculture industry.

Required Health Management Practices

The objective of health management is to maintain good health status, assuring optimum productivity and avoidance of diseases. In aquaculture, the economic risk associated with diseases is high. It represents a potential loss in production through mortality and morbidity, and might decrease investor confidence. Moreover, the cost to treat diseases when they are already well established is high and treatments are often initiated too late and, are therefore rarely effective. Thus, aquatic animal health management must be a global strategy that should aim to prevent diseases before they occur.

Proper disease diagnosis – a prerequisite for effective health management

As aquatic animal health management is about implementation of control measures to prevent the incidence of diseases, it is a prerequisite to have a good understanding of diseases that might occur in a particular fish species. Therefore, adequate attention should be given to disease diagnosis and epidemiology studies.

As an example, a disease investigation and epidemiology study over the last past 5 years in Asian seabass had allowed us to identify the most critical pathogens in this species (Grisez et al. 2005; Komar et al. 2005; Labrie et al. 2005a). The presence of different pathogens during the production cycle is illustrated in Figure 3.

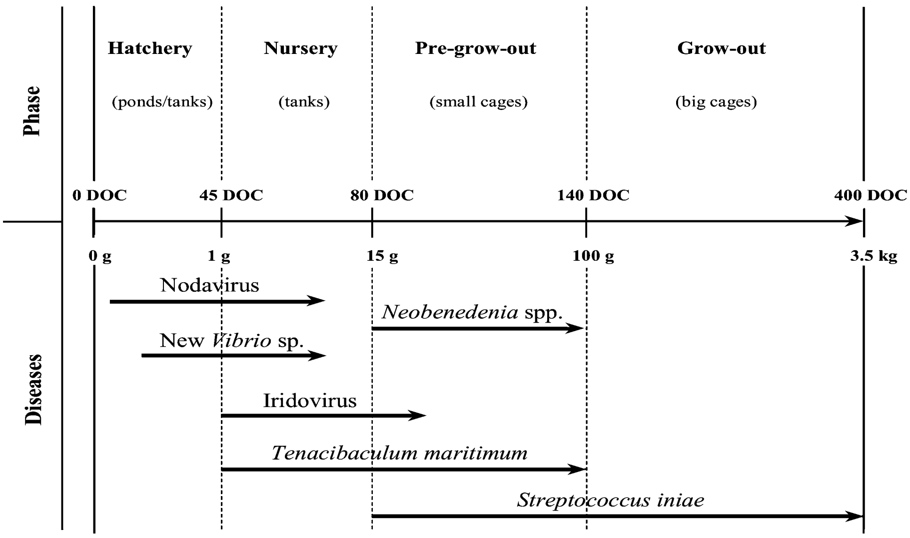

Figure 3. Major diseases affecting Asian seabass during the production cycle.

During the hatchery and nursery phases, two major viral diseases were identified. Viral nervous necrosis (VNN) was encountered in fry as young as 10 days old, causing mortality up to 100%. From 25 days of age onwards, a new Vibrio species responsible for acute mortality associated with severe clumping of internal organs, abdominal distension and muscular atrophy, was diagnosed. Subsequently, an iridovirus infection (previously never described in this species) responsible for acute hemorrhagic syndrome was identified in fingerlings as small as 1 g. Associated mortality could reach up to 90%. In addition, T. maritimum was able to induce severe skin lesions in fish after handling and/or stocking. Mortality could reach up to 30% in fish from 1 g to 100 g. During the first month of cage farming, Asian sea bass were most susceptible to monogenean parasites such as Neobenedenia spp. S. iniae was a major cause of fish mortality during the grow-out phase, right up to market size. Associated cumulative mortality could vary from 30 to 80% and the suddenness of the onset of the outbreak made antibiotic treatment ineffective.

Once a good understanding of the disease epidemiology is available, adequate treatment, control measures and prophylactic actions can be effectively formulated (Komar et al. 2005). An example of appropriate health management measures for Asian seabass farming is presented in Table 3.

Table 3: Some specific control measures for microbial diseases in Asian seabass farming.

Pathogens		Treatment	Prevention
Parasites	*Neobenedenia* spp.	Freshwater bath Formalin bath Oral anti-monogenean drugs	Regular prophylactic bath treatment
Viruses	Nodavirus	None	Egg ozone treatment Breeder selection Future broodstock vaccination
	Iridovirus	None	Future vaccination, dry-out
Bacteria	*Vibrio* sp. (big belly disease)	None	Strict hygiene Improved weaning diet Regular dry-out of tanks
	T. maritimum	Formalin bath with benzalkonium chloride if applied very early	Reduce stress Reduce fish handling Future vaccination
	S. iniae	Antibiotics in early stage	Vaccination

General approaches to health management are described below.

Aspects of health management practices – to improve fish health and survival

Responsible movement of live aquatic animals. Increased trade of live aquatic animals and the introduction of new species for farming, without proper quarantine and risk analysis in place, result in the further spread of diseases. A scientific process should be undertaken to assist decision making regarding the risks versus the benefits for the species intended to be imported. Such an import risk analysis includes hazard identification, risk assessment, risk management and risk communication (Mohan 2003; Bonadad Reantaso et al. 2005). The risk management should specify measures for proper control and emergency biosecurity when diseases do occur.

Hygiene, disinfection and biosecurity. Hygiene and biosecurity aims at preventing the introduction of any disease agent into the farm and should limit the spread of disease. Good sanitation practices in cage-farming systems are difficult to implement as there are no filters or barrier between the cage environment and its surroundings (where pathogens could be found). However, it is necessary to reduce the risk of contamination by simple management practices aimed at reducing the pathogen pressure in the environment. Such practices include proper system maintenance by removing excess suspended particles and uneaten food which is a potential substrate for pathogens. Moreover, their presence reduces water flow resulting in reduced availability of dissolved oxygen. The frequency of net cleaning depends on the severity of fouling. The removal of dead or moribund fish on a daily basis is an important sanitary measure, as well as for record keeping. Dead fish, especially in temperate and warm waters, decay quickly and can be a critical source of horizontal disease transmission as the remaining live fish have the tendency to eat the dead fish.

To minimize disease transmission, different species should not be mixed in the same farm or even in the same water area. An all-in, all-out approach, ideally with a period of fallowing in between, should be considered as a way to break the cycle of infectious disease. Zoning policy should be developed and implemented for disease control. While the above have been practiced in the livestock sector and salmon industry, it is far from the reality in Asian aquaculture.

Selection of hatchery-raised fingerlings. The overall health status of fry and fingerlings is a critical factor for a successful production cycle. When choosing a species to be farmed, preference should be given to species that are already available from hatcheries. The attention given to fish in the hatchery, and the availability of specific larval diets required to obtain strong juveniles, will allow for a constant supply of good quality fingerlings. Presently, the availability of hatchery-raised fingerlings is still limited. However, some government-owned high-tech hatcheries have been built in order to provide better quality SPF fry for stocking. The availability of hatchery-raised fingerlings should be increased in the near future.

Record keeping and disease monitoring. Often, in small scale operations, recording of farming parameters such as daily mortality, feed consumption, growth rate and water quality parameters is not standard. Record keeping is crucial in understanding the epidemiology of diseases and allows for identification of critical management points in the production cycle. The collection of this historical data helps us to take early action in case of disease outbreaks.

Good husbandry practices. Choosing the optimal fish density is important. Depending on the fish species and water quality conditions (especially oxygen saturation of the water), there is a certain fish density that should not be exceeded. A common mistake is to increase the stocking density to compensate for a decrease in survival rate. This is a source of stress for the fish which leads to skin injuries, low performance and a higher susceptibility to disease. In contrast, stocking fish optimally allows fish to grow to their best potential, thereby decreasing the risk of disease outbreaks.

Good feed management. Fish should be fed with a balanced diet as nutritional deficiency has an adverse impact on immunity and disease resistance. Dry pelleted feed adapted to each farmed species is preferred over trash fish as the former gives a consistent supply of nutrients free from pathogens. Some international feed companies have invested a considerable amount of resources in the development and supply of nutritionally-balanced pelleted feed for marine and freshwater fish. A wider usage of pelleted diet contributes to a better overall health status of the fish, thereby reducing nutrition deficiencies and the risk of disease. Dry feed should be appropriately stored in a cool and ventilated environment to avoid moulding which could lead to mycotoxicity problems.

To minimize stress. Stress is defined as any stimulus (physical, chemical or environmental) which tends to disrupt homeostasis in an animal. Under stressful conditions, fish must expend more energy to maintain homeostasis and less to combat disease. Aquatic organisms are fundamentally different from terrestrial animals: they are immersed in their environment and could not go elsewhere. Some disease agents are almost always present in the water (ubiquitous). These opportunistic pathogens will invade fish when they become stressed.

Some good practices to reduce stress include:

a) Starvation before handling of fish: handling is a source of stress as it puts fish under extreme conditions (overcrowding, manipulation outside the water). Starving the fish for 24 - 48 hours prior to handling reduces stress and avoids the deterioration of water quality when fish are overcrowded.

b) Sedation during handling and transportation: in situations such as handling or transportation, fish are overcrowded. Therefore, there is a higher risk of skin injuries. To avoid such damage, sedation using approved fish anaesthetics/sedatives is recommended as it decreases the level of stress and possible skin injuries.

c) Grading of fish to give a homogeneous population: when size variation increases in a cage, it often creates competition between larger and smaller fish. This results in stress, especially for the smaller fish. In addition, during feeding, the bigger and stronger fish get more feed. As a consequence, the smaller fish get weaker and become more susceptible to disease. When they get sick, they would also become a source of infection for the bigger fish as size variation is also a cause of cannibalism (leading to horizontal disease transmission).

d) To maintain good water quality when feasible: water quality should be monitored on a regular basis. This will be beneficial for disease diagnosis when problem arises, and for future site selection.

e) To avoid over-feeding: over-feeding could induce stress. Uneaten feed also causes water pollution.

The pitfalls of using chemicals/antibiotics. While under certain circumstances antibiotics could help to control some bacterial diseases, there are many problems associated with their use. Also, as sick fish do not eat, the efficiency of delivering antibiotics orally is often questionable.

Most countries have strict regulations on the use of antibiotics and chemicals. For example, chloramphenicol, furazolidone and malachite green are actually banned in most countries (including the major fish-importing countries) and severe measures are taken against exporters of fish and shellfish which contain residues of these chemicals. Regulations on acceptable withdrawal periods must be adhered to.

Between species, differences exist in drug disposition and metabolite formation. Moreover, temperature and composition of the water (fresh/salt water, pH value, hardness, organic material content) may affect the absorption, distribution, metabolism and excretion of drugs. Per species, relevant pharmacokinetic data are often lacking. Therefore, extrapolation of data from one species to another is difficult (Intervet 2003).

Changes in the taste of feed caused by the addition of antibiotics could negatively affect feeding. In addition, chemotherapeutics could negatively influence the immune system of fish (e.g. tetracyclines). When added to the water in recirculation systems (e.g. for catfish, tilapia and turbot), antibiotics may disturb the biological clearing systems and (bio)filters. Antibiotics in the water could rapidly lead to induction of resistant bacterial strains. The following attention should be paid regarding the use of chemicals/antibiotics:

- Antibiotics should be used only as a last resort.
- Definite disease diagnosis, including antibiotic sensitivity, should be made before administering antibiotics.
- Observe the regulations on banned chemotherapeutants. Maximum residue limits and withdrawal periods should be considered before harvesting the fish.
- The tolerance of the species should be known. For safety reasons, always first try the chemical/antibiotic at a given dose and treatment time with a small number of fish. Fish of different species and sizes under different water conditions (salinity, alkalinity and temperature) could react differently. In general, lower water temperature requires longer treatment duration and vice versa.
- Follow the correct dose and treatment time. Pay close attention to concentration of the active ingredient and adjust the dose accordingly if the chemical is not pure (<100% active).
- If using an immersion approach, add the chemical/antibiotic to a small portion of the water in a small container and make sure it is dissolved completely before use. Then pour this 'concentrate' into a tank/container to reach the desired final concentration and mix well before placing the fish into it.
- Withhold feed for 8-24 hours depending on the fish size.
- Treat during the coolest part of the day.
- Monitor water oxygen levels before, during and after treatment; if necessary, aerate as required.
- Keep a close watch on the fish during treatment and be prepared to stop treatment immediately if adverse reactions (e.g. gasping for air, strange swimming behaviour) are observed.
- In some cases, such as the occurrence of a serious disease problem, eradication should be considered. Eradication includes removal of all susceptible species followed by thorough cleaning and disinfection of the cages/nets or ponds.

Vaccination, a powerful tool that complements other health management practices. As mentioned above, there are many problems associated with the use of antibiotics. In addition to developing antibiotic resistance, sick fish often do not eat and the efficiency of delivering antibiotics orally is often questionable. Two key technical comments should be made regarding antibiotics: 1) by nature they are active mainly against bacterial pathogens and have no direct effect against viral and other pathogens; and, 2) antibiotics work only as long as they are present in the appropriate concentration in the target organ.

Whereas the use of antibiotics is a curative measure to treat an existing infection, in contrast, vaccination is a preventative measure, dependent on the immune system of the animal. Vaccines could act against bacterial, viral and, at least experimentally, parasitic infections; and they will usually act only against the targeted pathogens. The duration of protection obtained with vaccines normally largely exceeds that of antibiotics. Figure 4 clearly indicates that the introduction of vaccines has greatly reduced the use of antibiotics in Norwegian salmon production.

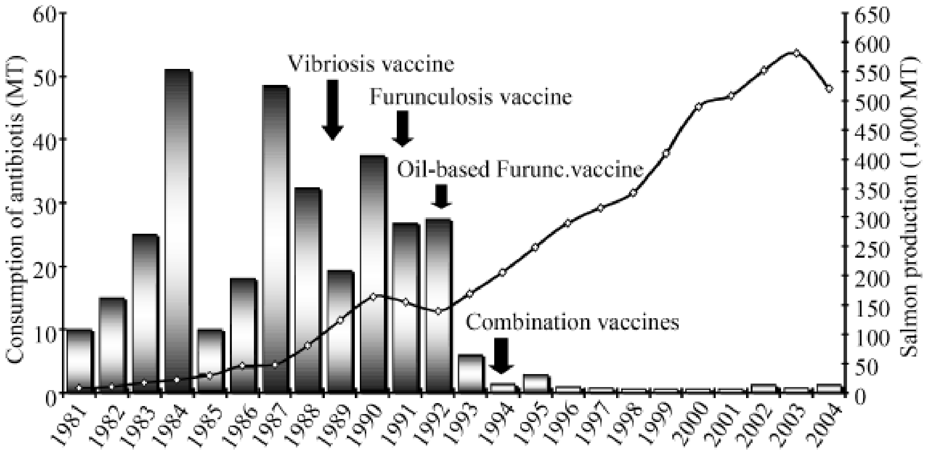

Figure 4. Norwegian salmon production, consumption of pure antibiotics and the effect of vaccines.

Vaccines are various preparations of antigens derived from specific pathogenic organisms that are rendered non-pathogenic. They stimulate the immune system and increase the resistance to disease from subsequent infection by the specific pathogen(s).

Vaccination can be compared with an insurance policy - it is worth paying a basic fee for a policy that would later cover the costs of a more expensive disease that may occur. Similarly, vaccination is a preventive measure that protects fish against future disease and the associated costs due to morbidity, mortality and therapeutic treatment. However, just as an insurance policy will cover the costs of an accident only if this fits the clauses of the insurance contract, a vaccine (generally) only protects against specific diseases. For example, a vaccine against S. iniae infection will protect the vaccinated fish against this specific species of Streptococcus but not against another streptococcal species such as S. agalactiae.

In the past, fish vaccines were only available for salmonid species. But the situation is changing with new vaccines being registered in Asia for Asian species (Grisez & Tan 2005). However, it must be remembered that vaccination is only one of the tools for good health management and it is not sufficient on its own to guarantee high survival and profitability. All the measures mentioned previously are needed to sustain a successful aquaculture industry in Asia.

In summary, some of the practices recommended for disease control in the fish farming industry are given in Table 4.

Table 4. Some practical recommendations to fish farmers in Asia.

Dos	Don’ts
1. Use healthy (not necessarily cheap) fry	1. Place your farm too close to others
2. Quarantine incoming animals	2. Stock several species in one farm
3. Use formulated pelleted feed	3. Use fingerlings from unknown sources
4. Grade fish periodically	4. Overstock (to overcome low survival)
5. Monitor water quality	5. Use trash fish
6. Record diseases and feeding	6. Overfeed
7. Observe withdrawal period of drugs	7. Use drugs without diagnosis
8. Remove dead fish at least once a day	8. Leave or throw dead fish in the water
9. Clean and disinfect equipment	9. Restock fish without cleaning the cages
10. Vaccinate if available	10. Ignore diseases until heavy mortality occurs

Conclusions and the Way Forward

Aquaculture production in Asia greatly exceeds that of the rest of the world. However, many examples show that rapid expansion of the industry has been at the cost of deterioration in fish health and environmental conditions. In general, production efficiency is low with high mortality due to disease, good health management practices are lacking, and few specific disease preventative measures or products are available. Several factors underline the present problems. The wide variety of species cultured in Asia results in the thin spread of resources across the species, resulting in sporadic and fragmented knowledge on each individual species and limiting the optimization of culture of any given species. In Northern Europe, salmon farming has been the only focus for decades and the production process is therefore fully optimized. In Asia, proper disease diagnosis and systematic collection of pathogen strains are limited. Farmers often use antibiotics without knowing the disease agent because of the lack of diagnostic support and alternatives for disease control. Use of wild fingerlings, over-stocking, mixing species, generations over-lapping and the ubiquitous use of trash fish as the principal source of feed further complicate the issue.

In recent years, an increased focus on diagnostic techniques is apparent. Furthermore, several government-owned high-tech hatcheries are being established in order to provide better quality fry for stocking. Some international feed companies are investing a considerable amount of resources in the development and supply of nutritionally-balanced eco-friendly pelleted feed for marine fish and shrimp. Significant progress has been made in the field of vaccine research and development (Grisez & Tan 2005). Besides yellowtail in Japan and grass carp in China, a commercial vaccine has recently been launched for use in Asian seabass, tilapia and other species in some Southeast Asian countries (Komar et al. 2005).

Sustainability is a shared responsibility. It rests with all stakeholders concerned directly and indirectly with aquaculture (Figure 5). Collaborative efforts from governments, non-governmental agencies, academia and the private sector are on-going in order to standardize aquaculture practices (codes of practice) and to promote good health management for disease control.

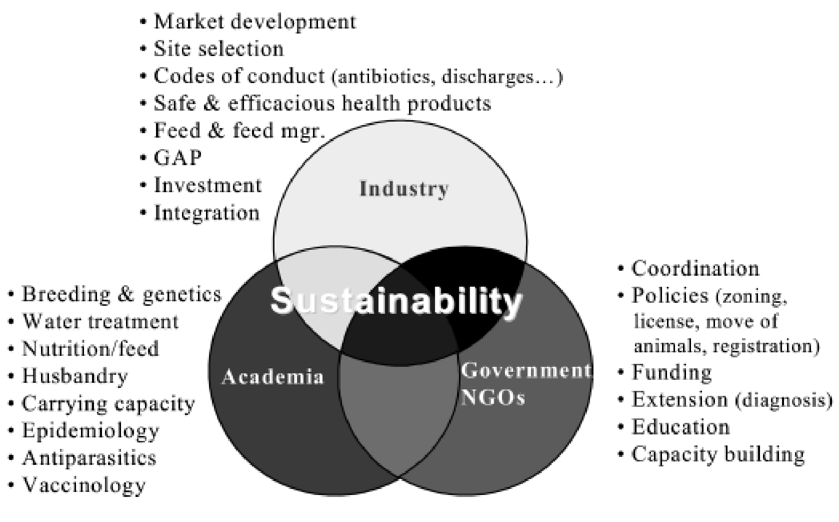

Figure 5. Sustainability is the shared responsibility of all stakeholders, including the private sector, governments and academia.

As Asian aquaculture continues to grow, disease problems will inevitably become worse unless key steps are taken. Under the threat of disease epidemic and the vigilance of governments and consumers regarding food safety, the industry must undergo changes. Therefore, disease research and the implementation of new disease control concepts are inevitable. Collectively, these include the use of healthy fry, quarantine measures, optimized feeding, good husbandry techniques, disease monitoring (surveillance and reporting), sanitation, vaccination, and the responsible use of chemicals and antibiotics when diseases occur. Overall, the emphasis must be on prevention rather than cure (treatment). This is the only way to sustain a responsible yet profitable Asian aquaculture industry.

References

Arthur, J.R. & Ogawa, K. (1996) A brief overview of disease problems in the culture of marine finfishes in east and Southeast Asia. In: Main, K.L. & Rosenfeld, C. (eds.): Aquaculture Health Management Strategies for Marine Fishes.. The Oceanic Institute, Hawaii, pp. 9–31.

Bondad-Reantaso, M.G. (2004) Transboundary Aquatic Animal Diseases: Focus on koi herpes virus, vol. IX, No. 2. Aquaculture Asia Magazine April–June, NACA, Bangkok, Thailand, pp. 24–28.

Bondad-Reantaso, M.G., McGladdery, S.E., East, I., Subasinghe, R.P. (2001). Asia Diagnostic Guide to Aquatic Animal Diseases. FAO Fish. Tech. Pap. No. 402, Supplement 2. FAO, Rome.

Bondad-Reantaso, M., Subasinghe, R. P., Arthur, J.R., Ogawa, K., Chinabut, S., Adlard, R., Tan Z., Shariff M. (2005) Disease and health management in Asian aquaculture. Vet Parasitology 132:249–272.

Choo, P.S. (2000) Antibiotic use in aquaculture: the Malaysian perspective. INFOFISH International 2:24-29.

Crane, M., Sano, M., Komar, C. (2004) Infection with Koi Herpes Virus – Disease card. Network of Aquaculture Centre in Asia-Pacific (NACA) online library. www.enaca.org

FAO Fishstat Plus, www.fao.org/fi/statist/statist.asp

Huovinen, P. (1999) Bacterial resistance; an emerging health problem. Acta Vet Scandinavia Supp. 92 :7-13.

Grisez, L., Ng, J., Bolland, A., Michel, A., Wahjudi, B., Segers, R. (2005) Demonstration and confirmation of etiology of a new facultative intracellular bacterium causing mass mortality in Asian Sea bass Lates calcarifer. World Aquaculture Society. Bali, Indonesia. May 2005.

Grisez, L. & Tan, Z. (2005) Vaccine development for Asian aquaculture. In: Diseases in Asian Aquaculture Walker, V. P.J. et al. (eds.) Fish Health Section, Asian Fisheries Society, Gold Coast, Australia, 2002, pp. 483-493.

Intervet International B.V. (2003) Guide to veterinary antimicrobial therapy. 4th ed.

IntraFish Media, http://www.intrafish.no/global/, Oct. 4, 2004.

Komar, C., Grisez, L., Michel, A., Labrie, L., Ho, E., Wahjudi, B., Tan, Z. (2005). Diseases and vaccination strategies in Asian sea bass (Lates calcarifer). World Aquaculture Society. Bali, Indonesia. May 2005.

Liao, I.C. (2000) The state of finfish diversification in Asian aquaculture. Recent Advances in Mediterranean Aquaculture Finfish Species Diversification. Proceedings of the Seminar of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM), jointly organized by CIHEAM and FAO, Zaragoza (Spain), 24-28 May 1999.

Labrie, L., Ng, J., Tan, Z., Komar, C., Ho, E., Grisez, L. (2005a). Nocardial infections in fish: an emerging problem in both freshwater and marine aquaculture systems in Asia. In: Diseases in Asian Aquaculture VI. Bondad Reantaso, M. et al. (eds.). Fish Health Section, Asian Fisheries Society, Colombo, Sri Lanka.

Labrie, L., Grisez, L., Komar, C., Tan, Z. (2005b). Tenacibaculum maritimum, an underestimated fish pathogen in Asian marine fish culture. World Aquaculture Society. Bali, Indonesia. May 2005.

Leong, T.S., Tan, Z. & Enright, W.J. (2005) Monogeneans infecting cultured green grouper, Epinephelus coioides, in the Asia-pacific region. The 5th Inter. Monogenean Symp. Guangzhou, China. August, 2005.

Leong, T.S., Tan, Z. & Enright, W.J. (2006) Important parasitic diseases in cultured marine fish in the Asia-Pacific region.

Aqua Culture AsiaPacific. Jan/Feb Issue, pp.15-16 (Part 1) and Mar/April Issue, pp.25-27 (Part 2).

MacMillan, J.R. (2001) Aquaculture and antibiotic resistance: A negligible public health risk? World Aquaculture. June issue, pp. 49-68.

Mohan, C.V. (2005) Exercising responsibilities to tackle aquatic animal diseases. Aquaculture Asia (NACA) 4:9-12.

New, M.B. (2003) Responsible aquaculture: is this a special challenge for developing countries? World Aquaculture. Sep. issue. 26-30, 60-72.

Smith, P., Hiney, M.P. & Samuelsen, O.B. (1994) Bacterial resistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning. Annual Rev. Fish Dis. 4,273-313.

Tan, Z & Grisez, L. (2004) Health management practices in Asian mariculture – current status and challenges. The 7th Asian Fisheries Forum. Asian Fisheries Society. Penang, Malaysia. November 2004.

Tan, Z., Grisez, L., Yu, X.L., Hoang, N.V., Bolland, A. (2003) Edwardsiella ictaluri isolated from catfish in China and Vietnam. Asian-Pacific Aquaculture 2003, World Aquaculture Society. Bangkok, Thailand. September 2003.

Tendencia, E.A. & de la Pena, L.D. (2001) Antibiotic resistance of bacteria from shrimp ponds. Aquaculture 195:193-204.

Wei, Q. (2002) Social and economic impacts of aquatic animal health problems in aquaculture in China. In: Arthur, J.R., Phillips, M.J., Subasinghe, et al. (eds.). Primary Aquatic Animal Health Care in Rural, Small-Scale, Aquaculture Development. FAO Fish. Tech. Pap. no. 406: 55–61.

Improvement on Aquaculture Cage Net Volume Deformation

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Abstract

The mortality of fish caused by shrinkage and deformation of the cage net during typhoons is a main concern of the marine cage aquaculture industry. To address this problem, the effect of weights at the bottom of the cage net has been investigated. These include describing the features of a cage net system, the types of external forces on a cage structure, and the estimation of net-volume deformation. The results reveal that the system with the heavier bottom weight has a higher relative net-volume coefficient while those with more pieces of bottom weights have better consequences. Thus we suggest that farmers employ the bottom weights as heavy and as many as possible, but not to exceed the diver's lifting ability which is approximately equivalent to 20 kg for each piece and the total bottom weight for each cage should not exceed 180 kg in this particular case.

Introduction

For many centuries now, fishery is one of the methods employed by humans for exploiting marine resources. The fishery resources are usually abundant, but due to over fishing in the nearshore during the last decades, reclamation and exploitation along the coastal zone, and dumping pollutants into the water body have made fish hunting harder than before. This declining trend of the fishery resources nearshore and the subsidence of coastal zones caused by land-based manual fishery have given marine cage aquaculture a new direction for the future development of fishery industries. Since Taiwan is located in the subtropical zone, every year typhoons may cause damage on the integrity of cage aquaculture structures. Moreover, the high mortality of fish is caused by the shrinkage and deformation in the volume of net cage during storms. Therefore, advancement in the engineering analysis of a flexible cage net system is needed in order to evaluate the dynamic performance. Hydrodynamic effects on net-cage systems have long attracted the interest of researchers in the marine aquacultural community. Recently, Lader et al. (2003) studied the relationship between the deformation of a flexible net and hydrodynamic forces, and later, Lader and Enerhaug (2005) developed a super-element approach to predict the global forces acting on a flexible net sheet. Tsukrov et al. (2003) employed the finite-element method with a consistent net element to model the hydrodynamic response of net panels, afterwards they evaluated the performance of a tension leg fish cage. Fredriksson et al. (2003) adopted a stochastic approach to analyze the motion response characteristics of a central spar fish cage and the tension response in an anchor line to wave forcing. Their works have obtained valuable information about the dynamic processes of a fish cage in a rigorous open sea. Suhey et al. (2005) analyzed an inflated system with structures of sufficient stiffness to provide support within a fish cage. DeCew et al. (2005) conducted an extensive experiment in a wave tank and investigated the dynamics of a modified gravity cage under the excitation of regular and random waves. Tsukrov et al. (2005) applied a finite-element model to deal with buoy mooring systems containing nonlinear elastic components such as feeding hoses and provided predictions of overall dynamics and the maximum values of tension in some critical components.

In this paper, a lumped-mass method developed by Huang et al. (2006; 2007) is adopted to investigate the net-volume deformation problem of a traditional net-cage system used in Penghu Bay, Taiwan. Section 2 describes the net cage material and the design wave conditions. Sections 3 and 4 briefly mention the external forces on the cage system and a velocity potential function applied to the wave-current flow field. Section 5 describes the methodology of estimating the relative net-volume coefficients. Finally, in the last section, conclusions and suggestions are given to the fish farmers.

Net cage material and design wave conditions

To simplify the study, a single cage is chosen for numerical simulation. The purpose is to analyze the relation between the different bottom weights and the relative net-volume coefficient under environmental loadings. Figure1 shows a cage with bottom sinkers in its four corners. However, some farmers may change the number of sinkers from 4 to 12 (Fig. 2) which means that the owner may add other sinkers at the middle points of cage bottom sides. The sinker's weight varies between 5 to 20 kg depending on farmers' preference in the operational process. Other net cage material data are shown in table 1. In order to investigate the effect of sinker's weight on the net-volume deformation, a typical design for the typhoon's wave/current conditions are used as the input data for the numerical model. The design conditions are as follows: wave height 2.8 m, wave period 7 sec, current speed 0.5 m•s^{-1}, and water depth is 10 m.

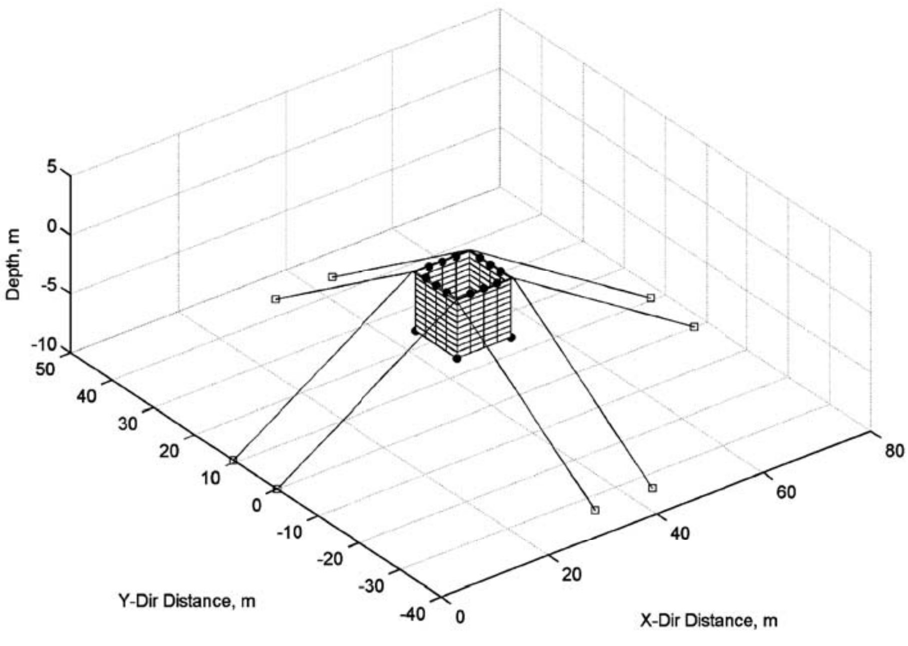

Fig.1. An aquaculture net cage system with bottom weights

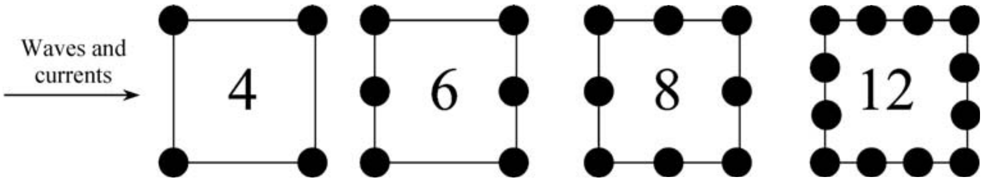

Fig.2. Four kinds of bottom weights

Cage net components	Properties
Floats	Size: 90x60x45cm Material: EPS Polyfoam Unit: 12 pieces
Mooring rope	Material: Nylon twist Diameter: 5.5cm Length: 20 m per mooring line
Net	Material: Nylon Mesh size: 5cm Twine diameter : 2.5 mm Area of net panel: 10×5 m @ 4 sides 10×10 m at bottom
Bottom weights	Sinker weights: 5, 10, 15, 20 kg per piece

Table 1. The data of materials.

External forces on the net cage

The diameter of the components of a cage net system such as mooring lines, floats, sinkers, and net twines, is small compared to the wave length; thus the net cage system is often regarded as a small body which hardly affects the wave fluid flow field. Therefore for the preliminary planning and design, the Morison equation is appropriate to be used in computing the wave forces on the structure. Aside from these environmental wave forces on the structure, other forces such as body force, buoyant force and mooring line's tension forces are also important forces which can not be ignored.

Environmental loadings

Due to the interaction of cage structures and wave fluid flow field, the modified Morison equation is applied in this study, and its expression is written as equation (1) according to Brebbia and Walker (1979).

$$\left(m+\rho\forall K_m\right)\ddot{\boldsymbol{R}}=\frac{1}{2}\rho C_D A \boldsymbol{V_R}\left|\boldsymbol{V_R}\right|+\rho\forall C_M\frac{\partial \boldsymbol{V}}{\partial t} \tag{1}$$

Where m is the mass of structure component, ρ is the sea water density, C_D is the drag coefficient, C_M is the inertia coefficient, A is the projected area, V_R is the water particle velocity (V) is relative to structure velocity ($\dot{R}$), and is the volume of the components of the net cage. For additional detailed description about the forces on the net, reader may refer to Loland (1991) while for forces on the floats, refer to Blevins (1984).

Gravitational force

The gravitational force is simply the weight of cage structures whose direction points to the earth.

$$W=mg \tag{2}$$

where g=-gk, and the upward direction of z-axis is positive.

Buoyant force

Whenever an object like cage structure components is submerged in water, the difference in pressure on the upper and lower surface will create an upward resultant force. This upward force is a buoyant force which is also equivalent to the expelled fluid weight.

$$\boldsymbol{F_B} = -\rho\forall\boldsymbol{g} \tag{3}$$

Tension force

Any long and slender elastic substance such as the twines of a flexible net or mooring lines, if subjected to external forces, may elongate a certain amount of length with respect to its original length. This elongation creates a tension force in the line and tends to pull the stretched line ends back to its original position.

$$T=A\sigma_T=AC_1\varepsilon^{C_2} \tag{4}$$

$$\varepsilon=\frac{l\text{-}l_0}{l} \tag{5}$$

Where l is the original length, l_0 is the length after elongation, ε is the strain, σ_T is the stress, A is a cross-section area subjected to force, T is the tension force, C_1、C_2 are the elastic parameters. The value of C_2 is usually set equal to 1 for practical application cases and the choice of C_1 depends on the elastic modulus of the mooring line.

Velocity potential function for wave-current flow field

Following Dean and Dalrymple (1984), we have a 3 dimensional velocity potential function for a wave field with uniform flow. The associated formulas are written as follows.

$$\eta=\frac{H}{2}\sin(k_X x+k_y y-\sigma t) \tag{6}$$

$$\phi=-(v_X x+v_y y)+\frac{Hg}{2\sigma_e}\frac{\cosh K(h+z)}{\cosh Kh}\cos(k_X x+k_y y-\sigma t) \tag{7}$$

$$\sigma_e=\sigma-\boldsymbol{V}_c\cdot\boldsymbol{K} \tag{8}$$

Take the derivative of Eq. (7) with respect to x, y and z, and we can obtain three components of velocity field.

$$\begin{cases} u = v_x + \dfrac{Hgk_x \cosh K\left(h+z\right)}{2\sigma_e \cosh Kh}\sin\left(k_x x + k_y y - \sigma t\right) \\ v = v_y + \dfrac{Hgk_y \cosh K\left(h+z\right)}{2\sigma_e \cosh Kh}\cos\left(k_x x + k_y y - \sigma t\right) \\ w = \dfrac{HgK \sinh K\left(h+z\right)}{2\sigma_e \cosh Kh}\cos\left(k_x x + k_y y - \sigma t\right) \end{cases} \tag{9}$$

Take the derivative once more with respect to t, we can obtain their correspondent local accelerations.

$$\begin{cases} \dfrac{\partial u}{\partial t} = -\dfrac{Hgk_x \sigma \cosh K\left(h+z\right)}{2\sigma_e \cosh Kh}\cos\left(k_x x + k_y y - \sigma t\right) \\ \dfrac{\partial v}{\partial t} = -\dfrac{Hgk_y \sigma \cosh K\left(h+z\right)}{2\sigma_e \cosh Kh}\cos\left(k_x x + k_y y - \sigma t\right) \\ \dfrac{\partial w}{\partial t} = -\dfrac{HgK \sigma \sinh K\left(h+z\right)}{2\sigma_e \cosh Kh}\sin\left(k_x x + k_y y - \sigma t\right) \end{cases} \tag{10}$$

Where is water surface elevation, $K=|K|$ is a wave number, $k_x=K\cos\theta$, $k_y=\sin\theta$, $v_x=|V_c|\cos\theta$, $v_y=|V_c|\sin\theta$ and θ is the incident angle between waves and currents.

Simulation and results

Prediction of relative net-volume coefficients

Using the 'lumped-mass method and plain element concepts,' (Huang et al. 2006; 2007), the entire structure can be divided into nodes and elements. According to Newton's 2nd law, the nodes on the flexible net and mooring lines may move under the environmental forces. Summing up the external forces on each node, we may form a motion equation, written as

$$\left(m_i + \sum_{j=1}^{M} k_m \rho_w \forall_j\right)\ddot{\boldsymbol{R}}_i = \sum_{j=1}^{M}\left(\boldsymbol{F_T} + \boldsymbol{F_D} + \boldsymbol{F_I} + \boldsymbol{F_W} + \boldsymbol{F_B}\right)_j \tag{11}$$

where the subscript i represents node's number, the subscript j represents the associated element's number, and M represents the total number of neighboring elements. After solving a set of motion equations for all the nodes, we can predict the new position for each node. Based on these new positions, the net-volume deformation rate is estimated as shown in the following section.

Firstly, we have to consider the net pen as a cylindrical cake. This cylindrical cake has M layers and N slices from top to bottom, as shown in figure 3.

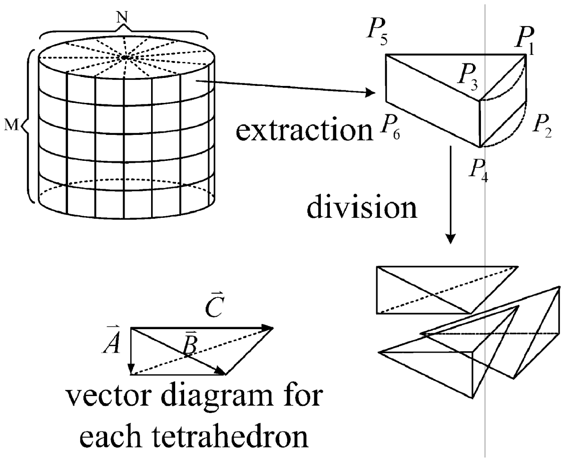

Fig. 3. Cage net volume divisions and its method of computation

There is a total of $M \times N$ pieces of small cake. A piece of cake is extracted and divided into three tetrahedrons. Choose a point as origin and assign three vectors, such as *A,B* and *C* along the sides of the tetrahedron. Using the principle of scalar triple product, $A \bullet (B \times C)$, we can compute the approximate volume of the small cake.

$$Volume = \frac{1}{6}\left\| \boldsymbol{A} \cdot \left(\boldsymbol{B} \times \boldsymbol{C} \right) \right\| = \frac{1}{6}\left[a_1 \left(b_2 c_3 - c_2 b_3 \right) + a_2 \left(b_3 c_1 - c_3 b_1 \right) + a_3 \left(b_1 c_2 - c_1 b_2 \right) \right] \quad (12)$$

Summing up all of the tetrahedrons, we may have an approximate volume of the net pen. The accuracy depends on how small is the piece of cake. At very beginning the net shape is really like as a cylindrical cake, but after several waves have passed through the cage, the net will deform and tilt along with the waves and currents. Select the smallest volume ($V_{\min}$) of net during the process, and then divide it by the total volume (V_{total}), we will have the relative net-volume coefficient (V_{ratio})for this particular wave condition.

$$V_{ratio} = \frac{V_{\min}}{V_{total}} \quad (13)$$

Results of simulation

Typical designs for typhoon's environmental conditions in Penghu Bay, Taiwan, are chosen for numerical simulation. The design wave conditions are as follows: wave height: 2.8 m, wave period: 7 sec, current speed: 0.5 $m \bullet s^{-1}$, and water depth 10 m. The results are shown in figure 4. It is clearly shown that the heavier bottom weights, the higher relative net-volume coefficients and the 12-sinkers case is the best among the tested cases. However, the maximum weight for a sinker should not exceed the lifting strength of divers or workers on the boat, which approximates to 20 kg for local farmers.

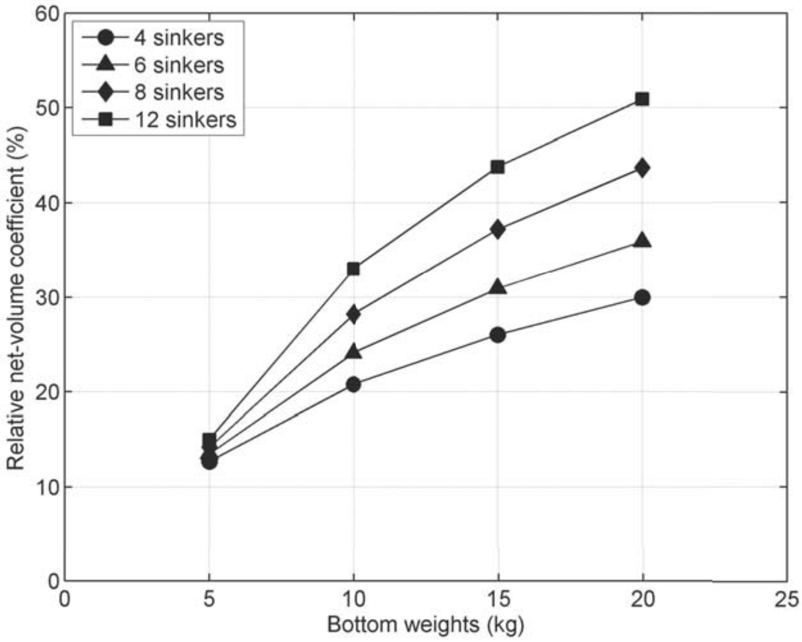

Fig. 4. Bottom weights and the corresponding relative net-volume coefficient

Figure 5 shows that when the total weights are the same, the system with more pieces of sinkers has better relative net-volume coefficients. However, it seems that 4- and 6-sinkers form a group while 8- and 12-sinkers form another group. Within the same group the volume deformation are almost the same. Therefore, the fish farmers may consider the convenience or the lifting capacity of workers to decide whether to use 8 or 12 sinkers in their farms. After long periods of field investigations, we have found out that workers including divers are capable of handling the maximum weight of about 20 kg. Another interesting fact has been found is that within the same group, as long as their total weight is the same, the difference of relative net-volume coefficients is about 2 % only.

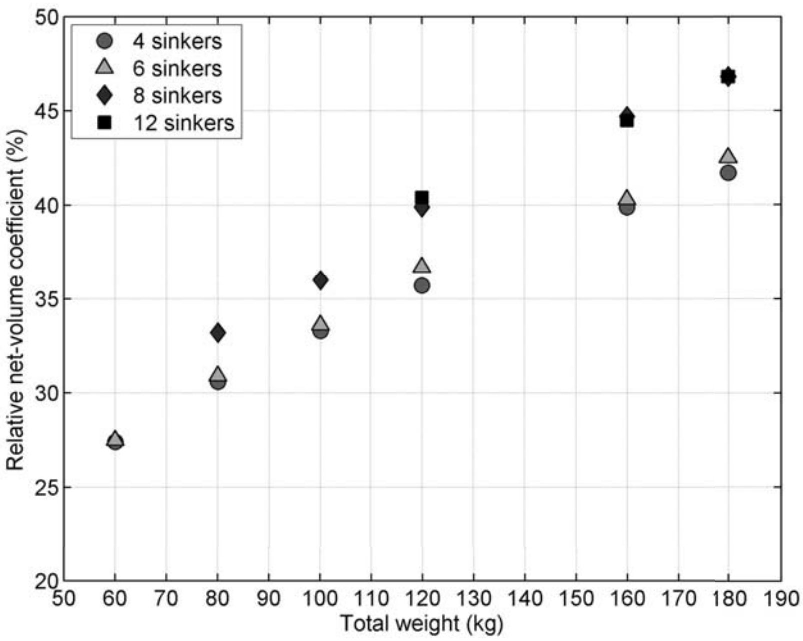

Fig. 5. Bottom weight and its corresponding deformation of cage net-volume

Conclusions and suggestions

Fish mortality caused by the shrinkage and deformation of cage net volume during typhoons are the main concerns of marine cage aquaculture farmers. In order to improve this deformation problem, the effect of sinker weights at the bottom of cage net has been studied under typical wave conditions.

A numerical simulation is presented in this article, and the results reveal that the heavier and more pieces of bottom sinkers have more advantages during environmental loading impact, since the higher relative net-volume coefficient means that the fish inside the net cage has more chances of survival during typhoons.

After long periods of field investigations, we conclude that the maximum weight for each sinker should not exceed the lifting strength of divers or workers on the boat, which approximates to 20 kg.

The result of numerical simulation also showed that the systems with 4- or 6-sinkers formed a group while the systems with 8- or 12-sinkers formed the other group. Within the group, if the total weight is the same, then the net-volume deformation is quite similar and their difference is negligible.

Finally, we strongly suggest that the fish farmers should check their cage facilities including the mooring lines and anchors at least once a year before the onset of the typhoon season.

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References

Brebbia, C.A. & Walker, S. (1979) Dynamic Analysis of Offshore Structures. Newnes-Butterworths, London.

Blevins, R.D. (1984) Applied Fluid Dynamics Handbook. Van Nostrand Reinhold Company, pp. 334.Chen, Y.Y. & Juang, W.J. (1990) Primary analysis on wave-current interaction. In: Proceedings of the 12th Ocean Engineering Conference in Taiwan. pp.248-265. (in Chinese)

DeCew, J., Fredriksson, D.W., Bougrov, L., Swift, M.R., Eroshkin, O. & Celikkol B. (2005) Numerical and physical modeling of a modified gravity type cage and mooring system. IEEE Journal of Oceanic Engineering 30(1), 47–58.

Fredriksson, D.W., Swift, M.R., Irish, J.D., Tsukrov, I. & Celikkol, B. (2003) Fish cage and mooring system dynamics using physical and numerical models with field measurements. Aquacultural Engineering 27, 117-146.

Huang, C.C., Tang, H.J. & Liu, J.Y. (2006) Dynamical analysis of net cage structures for marine aquaculture: Numerical simulation and model testing. Aquacultural Engineering 35, 258-270.

Huang, C.C., Tang, H.J. & Liu, J.Y., (2007) Modeling volume deformation in gravity-type cages with distributed bottom weights or a rigid tube-sinker. Aquacultural Engineering 37, 144-157.

Lader, P.F. & Enerhaug, B. (2005) Experimental investigation of forces and geometry of a net cage in uniform flow. IEEE Journal of Oceanic Engineering 30(1), 79–84.

Lader, P., Enerhaug, B., Fredheim, A. & Krokstad, J. (2003) Modeling of 3D Net Structures Exposed to Waves and Current. SINTEF Fisheries and Aquaculture, Trondheim, Norway.

Loland, G. (1991) Current force on and flow through fish farms. Division of Marine Hydrodynamics, The Norwegian Institute of Technology, Trondheim, Norway.

Suhey, J.D., Kim, N. H. & Niezrecki C. (2005) Numerical modeling and design of inflatable structures - application to open-ocean-aquaculture cages. Aquaculture Engineering 33, 285–303.

Tsukrov, I., Eroshkin, O., Fredriksson, D., Swift, M.R. & Celikkol, B. (2003) Finiteelement modeling of net panels using a consistent net element. Ocean Engineering 30, 251-270.

Tsukrov, I., Eroshkin, O., Paul, W. & Celikkol, B. (2005) Numerical modeling of nonlinear elastic components of mooring systems. IEEE Journal of Oceanic Engineering 30(1), 37–46.

Growth Performance of Cobia, Rachycentron canadum, in Sea Cages Using Extruded Fish Feed or Trash Fish

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Abstract

Cobia, *Rachycentron canadum,* is an attractive species for cage culture in Vietnam both for small-scale family and large-scale company farmers. However, the culture largely relies on feeding with trash fish which usually fluctuates in quantity, quality and price. Therefore the use of formulated feed is a prerequisite for the development of cobia farming. The present experiment compared the use of extruded fish feed with the present standard use of trash fish in the production of cobia up to marketable size. The experiment was carried out in duplicates with two cages for each feed type. Each 40 m^3 cage was stocked with 400 cobia fingerlings (160 ± 38.5 g) on 24 September 2004 and the grow-out trial was continued until 6 November 2005 (402 days). Both technical and economic feasibilities of the two feeding strategies were evaluated. The results indicate the advantage of using extruded feed. Biomass gained using the extruded diet was 2,213 kg compared to 1,293 kg using trash fish. Final individual weight of similar aged fish showed that the weight of fish fed with extruded diet was nearly double (6.8 ± 0.46 kg) compared to those fed with trash fish (3.5 ± 0.03 kg). Furthermore, the feed cost to produce cobia using the extruded diet was 15.8% less than using trash fish based on prevailing market prices.

Introduction

Cobia, *Rachycentron canadum,* is a migratory pelagic fish swimming alone or in small groups. It has a wide distribution in tropical, subtropical and warm temperate waters apart from the eastern Pacific region (Shaffer & Nakamura 1989). Due to its dispersed occurrence little targeted fisheries is performed but it is a popular fish for sport angling in many parts of the world. However due to its good meat quality (Su et al. 2000), fast growth rate, high market value and success of mass seed production, cobia has become increasingly popular for cage culture in Southeast Asia as well as in the Caribbean. In cage culture it can attain 4-6 kg in one year (Chou et al. 2001; Nguyen 2002; Chou et al. 2004; Wang et al. 2005). Taiwan has the longest history in cobia cage culture. Its culture production reached 1,500 tons in 1999 (Su et al. 2002) and projected to reach 5,000 tons in 2004, using mainly dry pellets with a feed conversion ratio of about 1.5 (Liao et al. 2005)

In Vietnam, cobia is a popular species for cage culture for small-scale family and large-scale corporative farmers. Being a sturdy species, cobia cage culture has expanded from more protected into more exposed areas in the ocean with better water exchange. The first successful intensive mass production of cobia fingerlings was in Vietnam in 1999 (Nguyen 2002; Nguyen et al. 2003). However, the current annual domestic hatchery production is only up to 200,000 fingerlings, and therefore cobia culture still relies on fingerling imports from Taiwan and Hainan. Cobia is considered one of the main species to make it possible to achieve the 2010-target of 200,000 tons of cultured marine fish set by the Vietnamese Ministry of Fishery. In 2004 the total production of Cobia in Vietnam was estimated at about 1,200 tons. One of the major constraints for the development of marine fish farming in Vietnam in general and cobia farming in particular is that feed supply still is largely dependent on trash fish. Trash fish supply is heavily fluctuating in quantity and quality with increasing prices. Moreover, feed conversion rate when using trash fish for cobia culture is quite high, ranging from 8-10 on wet weight basis (Nguyen 2002) which means that development of cobia culture based only on trash fish supply will not be sustainable. The present experiment was organized to compare the use of extruded fish feed with the present use of trash fish in the production of cobia up to marketable size. The technical and economic feasibility of the two strategies were evaluated.

Methodology

Cobia fingerlings were produced at the facility of Research Institute for Aquaculture No.1 (Ria-1) at Cua Lo, Nghe An province. The fingerlings were further nursed in sea cages using a farm-made moist pellet until the start of the experiment on 24 September 2004 when the fish had reached an average size of 160 ± 38.5 g (*n*=127).

The extruded fish feed (EF) used for this experiment was EWOS Marine Feed produced by EWOS Canada. The protein content ranged from 54 to 42% depending on pellets sizes (3-16 mm) and with a corresponding oil level, raising from 15 to 27%. The extruded feed was stored in cold storage at 12°C until used. Trash fish (TF) were supplied by off-shore fishing boat at Cua Hoi fishing harbour and delivered fresh to the experimental cages.

Initially, each of the four 40 m^3 hexagonal wooden cages was stocked with 400 fish. Two cages were randomly selected for feeding trash fish and the other two cages were used for the extruded diet.

As the fish in one treatment group reached an average weight of 1 and 3 kg, respectively, the fish were graded into three group sizes: small, average and large, and then counted. The total number of fish in a cage was then reduced to the predefined density of either 200 fish of 1 kg or 100 fish of 3 kg per cage respectively by selecting fish from the average size group. Twenty fish from each size group were sampled and individually measured for weight and body length. In addition at each grading, 20 average size fish from each cage were sampled for nutritional analysis (data not shown in this paper). Periodically 20 fish in every cage were sampled to estimate growth and feeding rate and returned to their cage after measurement. On 6 November 2005 the experiment was terminated as the fish in the fastest growing treatment group had reached an average market size of 6 kg and all fish in both treatment groups were measured for biological data. Feed conversion ratio (FCR, based on dry matter) was calculated by taking into account the weight of dead fish, both the observed dead fish and unseen dead fish. The weight of the 'unseen dead fish' was calculated as being the average weight between start weight and end weight in a grading period.

The EF fed group were fed to satiation twice a day using visual judgement of surface feeding activity. In contrast, the TF fed group were fed once a day till satiation when trash fish was available (present standard use of trash fish for feeding).

Data are presented as mean ± standard deviation (SD). Differences between two treatments and between grading times were tested for significance using one way ANOVA followed by Tukey's multiple comparison, $P < 0.05$ was considered statistically significant.

Results and Discussion

Environmental conditions during experiment

Seawater temperatures at the culture site at Ngu Island are summarized in figure 1. Cobia is found in nature within a temperature range of 16.8-32°C. They migrate to cooler areas in summer and to warmer areas in winter within their natural range of distribution (Shaffer & Nakamura 1989). Under culture conditions, cobia shows a strongly reduced feeding activity below 20°C. Cobia performs slow growth rate at low temperature and high mortality occurs when temperature drops to below 16°C (Liao et al. 2004; Liao et al. 2005).

The seawater temperature at the experimental site reflects the subtropical conditions and normal winter temperatures are just below 20°C. However during the experimental period very low water temperatures were experienced from December 2004 to March 2005 with monthly averages being between 17.6-20.4°C and with the lowest temperature of 15.5°C in January. Therefore some mortality related to chill-induced diseases was noted in both treatments during January and February but with no significant differences between the two. Mortality of TF fed group and EF fed group were 25.5±5.6 and 31.7±9.1%, respectively ($P > 0.05$).

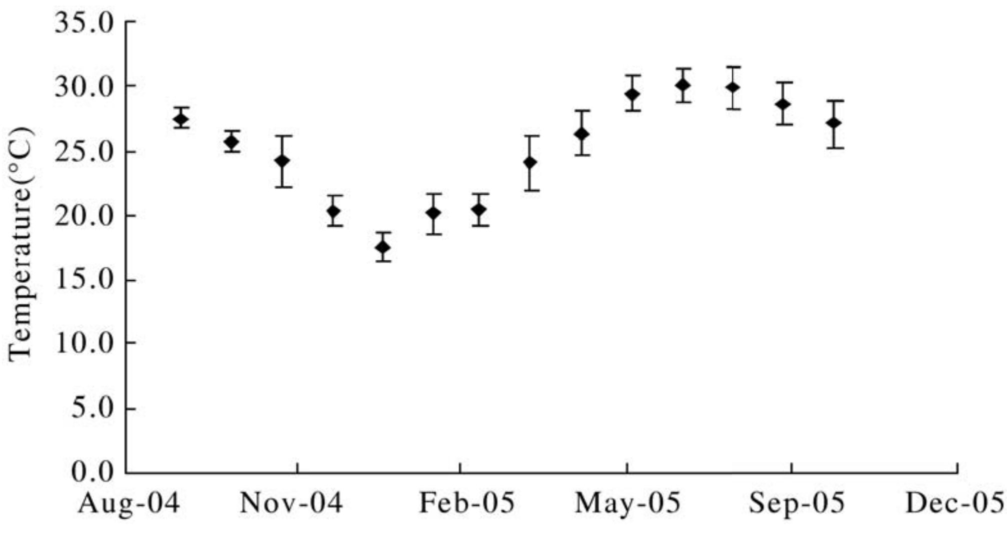

Figure 1. Sea water temperature during experimental period

Growth, utilization of feed and survival rate

EF fed group. The growth, feed utilization and survival rate of the EF group during the grow-out period are summarized in table 1. Average weights at 1st, 2nd and 3rd grading were 1,189, 3,720 and 6,844 g, respectively. Specific growth rates (SGR) of fish during the 1st period (2.4%•day^{-1}) was significantly higher compared to those during the 2nd (0.6%•day^{-1}) and 3rd periods (0.5%•day^{-1}). The FCR during the 1st period was significantly lower (1.2) compared to the 2nd (1.8) and 3rd periods (2.0). There were no significant differences between the 2nd and 3rd periods with respect to FCR or SGR. The survival rate of cobia was significantly lower during the 2nd period (64.5%) compared to the 1st (97.4%) and 3rd periods (97.5%) reflecting the abovementioned mortality during January 2005.

*T*F fed group. The growth, feed utilization and survival rate of the group fed with trash fish during the grow-out period are summarized in table 2. A significant slower SGR in the TF compared to the EF fed group gave only two grading (first and second grading) during the experimental period. As a result, when the EF fish reached the size decided for terminating the experiment (6 kg) the TF group had only attained the size qualifying for the second grading (3 kg).

Table 1. Growth, feed utilization and survival rate of extruded feed fed group(Mean ± SD, *n*=2)

	1st period (24.09.04 - 18.12.04)	2nd period (19.12. 04 – 06.07.05)	3rd period/final (07.07. 05 - 06.11.05)
Average initial weight (g)	160 ± 38.5	1,188 ± 8.3	3,720 ± 28.3
Average final weight (g)	1,189 ± 7.8	3,720 ± 28.3	6,844 ± 460
Initial no. of fish per cage	400	200	97
SGR (%•day^{-1})	2.4 ± 0.01 a	0.6 ± 0.0 b	0.5 ± 0.05 b
FCR	1.2 ± 0.07 a	1.8 ±0.04 b	2.0 ± 0.24 b
Survival rate (%)	97.6 ± 0.4 a	64.5 ± 13.4 b	97.4 ± 0.5 a

Data in the same row with different superscripts are significantly different (P<0.05)

Table 2. Growth, feed utilization and survival rate of trash fish fed group (Mean ± SD, *n*=2)

	1st period (24.09.04 - 03.05.05)	2nd period (04.05.05 - 06.11.05)
Average initial weight (g)	160 ± 38.5	1,323 ± 22.6
Average final weight (g)	1,323 ± 22.6	3,505 ± 24.7
Initial no. of fish per cage	400	170
SGR (%•day^{-1})	1.0 ±0.01 a	0.5 ± 0.01 b
FCR (based on dm)	1.4 ± 0.06 c	2.4 ± 0.01 d
Survival rate (%)	54.7 ± 5.4 e	85.6 ± 7.1 f

Dry matter (DM) of trash fish is approximately 25% of wet weight. Data in the same row with different superscripts are significantly different (P<0.05).

Table 3. Growth rate and feed conversion at 1st and 2nd grading of the two treatments(Mean ± SD, *n*=2)

	1st grading		2nd grading	
	E F group	*T* F group	*E* F group	*T* F group
SGR (%wt•day^{-1})	2.4 ± 0.01 a	1.0 ±0.01 b	0.6 ± 0.00 a	0.5 ± 0.01 b
FCR	1.2 ± 0.07 a	1.4 ± 0.06 a	1.8 ±0.03 a	2.4 ± 0.01 b

Data in the same row within each period with different superscripts are significantly different (P<0.05).

Growth rate of TF group during the 1st period was significantly lower than the EF group, while the FCRs based on dry matter were not significantly different (*P*>0.05) between the two treatments. During the 2nd period (i.e. growth up to 3 kg) the EF group had a significantly lower FCR and higher SGR compared to the TF group (Table 3). In addition, it should be appreciated that the 2nd growth period for the EF group took place during winter time while for the TF group it was during the (following) summer.

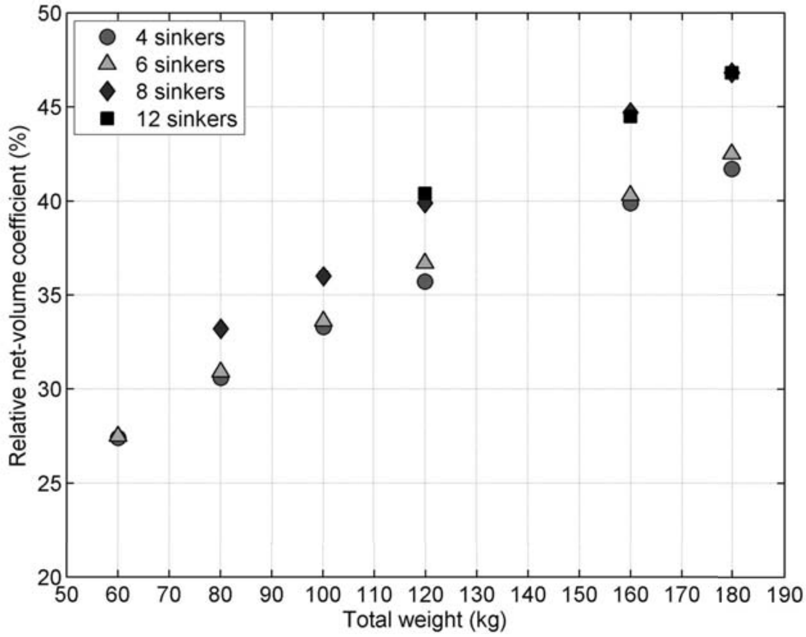

Figure 2. Weight increment of groups fed extruded feed and trash fish

The TF group grew slower than the EF group (Fig. 2). The experiment was terminated on 6 November 2005, when the fish were 16 ½ months old at which time the average weight of the EF group was 6,844 g and the TF group was 3,505 g. The growth rate in the EF group was in agreement with what has been reported in the literature (Liao et al. 2004; Liao et al. 2005) while the growth of the TF group was not. This is most likely caused by the unstable and limited supply of trash fish during bad weather periods together with some quality issues with the trash fish. In a total of 402 experimental days, there were 94 unfed days in the TF group (23.4%) while there were only 56 unfed days in the EF group (13.9%). Most of the periods of unfed days were caused by bad weather during the winter season. The average FCR in the TF group (1.9, computed from the two grading periods) when converted to wet weight is equal 7.8, close to 8-10 as reported by Nguyen (2002). However, the TF group in this experiment did not get the average size of 4-5 kg after 1 year culture as the normal cage culture condition in Northern Vietnam (Nguyen 2002). It would take at least 3 months more for the TF group to reach the average 6 kg based on growth rate in table 2.

Economic calculation

For the calculation of total biomass produced within each group, the fish removed during grading and reducing stocking density was added to the harvested volume at the termination of the experiment as well as the biomass of dead fish. Furthermore, the biomass of the initial fingerlings stocked had been deducted. Thus the total biomass produced in each experimental group and the associated feed consumption were summed from the two replicates (Table 4). The cost of the extruded diet including transportation to Vietnam was set at 1.21 USD•kg^{-1} (EWOS Canada Ltd., 2004). The cost of trash fish was computed based on the total feed purchase and its total cost (transportation cost is not included).

Table 4 shows that when using extruded fish feed one can nearly double the biomass production during the same culture period compared to when using trash fish, and at 15.8% less feed cost per kg fish produced. In addition, there may also be differences in end product quality caused by the two different feed sources.

Table 4. Feed cost of cobia production

Economic calculation	EF group	TF group
Total biomass produced (kg)	2213	1293
Feed consumed (kg)	3600.6	10559.5
Relative feed cost per kg fish produced (%)	84.2	100

The use of trash fish may be suitable for small-scale farmers having access to enough trash for their production as well as its relatively cheap price. But in reality, the price of trash fish has increased dramatically during the last five years caused by a competition between other users such as the chicken broiler industry and at the same time, the natural resources have been reduced. Another aspect of using trash fish as feed for farm fish is the negative influence on the environment as well as the risks in transmitting diseases and parasites to the farmed fish.

Acknowledgement

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References

Chou, R.L., Su, M.S. & Chen, H.Y. (2001) Optimum dietary protein and lipid levels for juvenile cobia (Rachycentron canadum). Aquaculture 193, 81-89.

Chou, R.L., Her, B.Y., Su, M.S., Hwang, G., Wu, Y.H. & Chen, H.Y. (2004) Substituting fish meal with soybean meal in diets of juvenile cobia Rachycentron canadum. Aquaculture 229, 325-333.

Liao, I.C. & Leanõ, E.M. (2005) Cobia culture in Taiwan. World Aquaculture 36(1), 31-32, 64-65.

Liao, I.C., Huang, T.S., Tsai, W.S., Hsueh, C.M., Chang, S.L. & Leanõ, E.M. (2004) Cobia culture in Taiwan: current status and problem. Aquaculture 237, 155-165.

Nguyen, Q.H. (2002) Current status of cobia (Rachycentron canadum) culture and seed production in Vietnam. Vietnamese Fisheries Journal 7, 14-16. In Vietnamese

Nguyen, Q.H., Nhu, V.C., Do, V.M., Lauesen, P., Pham, L.H., Nguyen, T.L.T., Bui, V.H. & Tran, M.T. (2003) Development of cobia (Rachycentron canadum) seed production technique. 2nd Proceedings of National Conference on Aquaculture. Research Institute for Aquaculture No.1, pp. 269-274. In Vietnamese

Shaffer, R.V. & Nakamura, E.L. (1989) Synopsis of biological data on the cobia, Rachycentron canadum, (Pisces: Rachycentridae). U.S. Dep. Commer., NOAA Tech Rep NMFS 82 [FAO Fisheries Synopsis 153], 21 p.

Su, M.S., Chen,Y.H. & Liao, I.C. (2000) Potential of marine cage aquaculture in Taiwan: cobia culture. In: Liao, I.C., Lin, C.K. (Eds), Cage Aquaculture in Asia. Asian Fisheries Society, Manila, and World Aquaculture Society-Southeast Asian Chapter, Bangkok, pp. 79-106.

Wang, J.T., Liu, Y.J., Tian, L.X., Mai, K.S., Du, Z.Y., Wang, Y. & Yang, H.J. (2005) Effect of dietary lipid level on growth performance, lipid deposition, hepatic lipogenesis in juvenile cobia (Rachycentron canadum). Aquaculture 249, 439-447.

Design, Construction and Preliminary Experiments on the Grading Device with Rigid Grates Enclosing a Volume of Frustum of Pyramid

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Zheng, G..F., Y.L. Tang, Q. Shao, L. Ding, J.K. Zhu, G.Y. Wei and G.F. Huang. 2008. Design, Construction and Preliminary Experiments on the Grading Device with Rigid Grates Enclosing a Volume of Frustum of Pyramid. In Cage Aquaculture in Asia: Proceedings of the Second International Symposium On Cage Aquaculture in Asia (ed. Y. Yang, X.Z. Wu and Y.Q. Zhou), pp ???-???. Asian Fisheries Society, Manila, Philippines, and Zhejiang University, Hangzhou, China.

Abstract

Fish aquaculture in anti-stormy wave cage has developed rapidly in China recently. Developing a suitable grading device which can shorten the operation time for fish grading and reduce fish mortality or injury can be beneficial to anti-stormy wave cage aquaculture. Based on escaping behaviors observed in laboratory and biological measurement of red seabream (*Pagrosomus major*), a grading device with rigid grid panel enclosing a volume of frustum of pyramid has been developed. The design and construction considerations of the device and its grading efficiency in preliminary experiments for grading red seabream in anti-stormy wave cages are reported in this paper. The suitable bar space of the grate is 34 mm for 500 g grading goal and 31 mm for 350 g grading goal of red seabream. The best incident angel of the grate is 45°. Average grading efficiency is 88.45 %, while the average escaping rate heavier than the grading goal is 2.58 % in the experiments in anti-stormy wave cages with 500 g grading goal of red seabream. The experiments also showed that the device has advantages such as high and stable grading efficiency with low escaping rate, ensuring all fish that needed grading to enter the device , and easier operation. Other aquaculture fish can also be graded using the device if the bar space of the grid panel is changed appropriately to correspond to the parameters of the graded fish and goal.

Introduction

Recently, fish aquaculture in anti-stormy wave cages has developed rapidly in China. With the volume enlargement of cages, the number of fish cultured in a single cage reach 1.5×10^4-4.0×10^4 and the annual output is more than 20 t. Variation of fish size in a cage is tremendously big after a certain culture period due to the large space for fish to move around, non-uniformity of feed casting and the characteristic differences of individual fish. Taking the aquaculture of red seabream (*Pagrosomus major*) in Weitou Bay of Fujian province as an example, when the number of fingerlings with individual weight of 250-350 g was 1.5×10^4 in a cage with a perimeter of 40 m, about one third of the fish with individual weights bigger than 650 g and about another third of fish with individual weights of 300 g after a 55-day culture period. In order to improve aquaculture production and economic performance, the cage-cultured fish should be graded after a certain culture period to continue culturing fish with similar size. On the other hand, the fish must be graded according to the requirements of clients when ready to sell. Manual grading lasts a long time, while the graded fish are under high stress causing injury or even death at high density for such a long time. Manual grading is also labor intensive. Therefore, developing a device, which can automatically grade fish in terms of predetermined goal of fish size, is of great importance to improve economic performance of anti-stormy wave cage aquaculture because it can shorten the grading time, thus reducing rates of fish injury and death and improve working efficiency.

The separator device or grading grid has been widely used in trawl and purse seine fisheries and has evolved into different structures. However, there is not much literature available on grading devices for aquaculture fish at present. Ivor (2002) developed a grading device of seine with Flexi-Panel made of Dyneema twine and PVC rods. Lu et al. (2004) reported the development and tests of grading systems similar to that developed by Ivor (2002) while the Flexi—Panel was made by PE and PVC fixed to PA netting. The design, construction and results of the preliminary experiments on the grading device with rigid grates enclosing a frustum of pyramid for aquaculture fish are reported in this article.

Materials and Methods

Basis for design of the grading device with rigid grate

Behavior of fish escaping through the grate. Based on laboratory observations, the behavior of red seabream escaping through the grate are as follows: a) Most fish escaped through the grate from the lower part. Some fish moved along the rods to the water surface. The small fish escaped there while the big fish moved back to deep water when the grate was set up in rear deflect state; b) Fish usually probed the rod space with its head before escaping. Fish could escape through the grate if the head or rear part of its gill cover could escape through the grate; c) Fish exerted more effort to pass through the grate under other stimulations such as disturbing the enclosed water or setting some lamps out of the enclosed water. Some fish would try to pass through the grate again and again under the stimulation, under which fish were more active than those without stimulation; d) The process of escaping could be made faster by the movement of the grading device. The number of fish escaping from the grading device increased rapidly with the decrease of enclosed volume of water, but the increase rate slowed down when the enclosed volume was reduced to a certain extent until the fish cease to escape when the enclosed volume reached its limit.

Growth equation of red seabream cultured in anti-stormy wave cages

Fish size is expressed by fish weight, while the parameters of grid can not be determined by fish weight directly. Selecting a suitable biological parameter which is closely correlated to fish weight and simultaneously accord with the selectivity of grid is the basis to determine the space between bars of grid. Red seabream was chosen as an example, and fish samples were taken randomly in anti-stormy wave cages to measure their biological parameters of length, height, width and weight. The analysis result showed that the width of fish body (L) had a positive correlation with fish weight (W). The regression curve between weight and width of 100 strips of red seabream is shown in Figure 1.

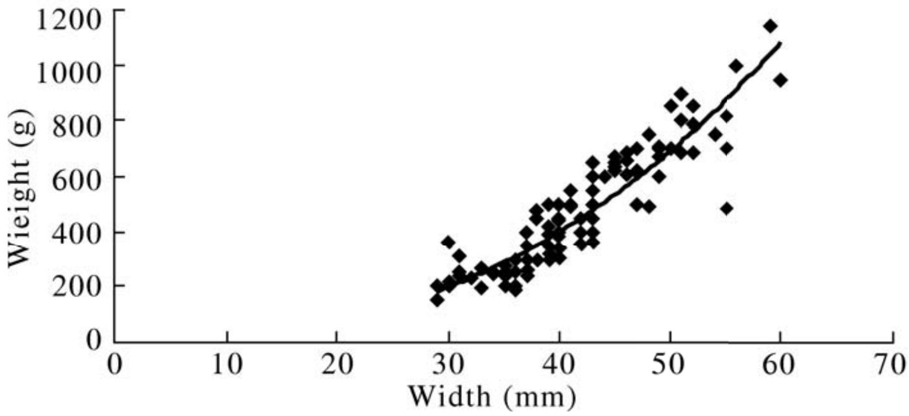

Figure 1. The correlation of weight and length of red seabream.

The regression equation was determined as below: $W = 0.047\ L^{2.4543}$, R^2=0.8222

where: W (g) = the weight of fish; L (mm) = the width of fish body.

Effect of incident angle of grate on the escaping rate

According to laboratory experiments and observations on the escaping behavior of red seabream, the highest grading efficiency which was 93.3 % occurred at the incident angel of the grid panel of 45°, while average grading efficiency was 69.2 % when the angle was 0°. Thus the side panels of the device were designed with an angle of 45° to horizontal.

Design and construction of the grading device

Design goals. In order to apply the device to anti-stormy wave cage, the design goals are: a) high and stable grading efficiency and low escaping rate; b) all fish are subject to be graded; c) convenient to operate; and, d) low rate of fish injury and mortality.

Structure design of the grading device. The structure of the grading device is shown in Figure 2 in which the black and thick lines represent the frame (38 mm × 25 mm × 0.7 mm) made of stainless steel while the fine line is made of PVC pipe of 20 mm × 2 mm.

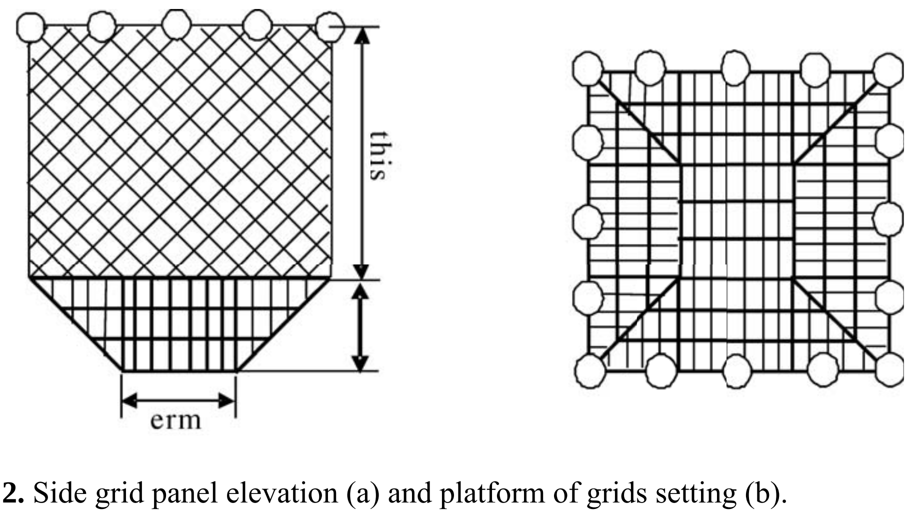

Figure 2. Side grid panel elevation (a) and platform of grids setting (b).

The device was made of five rigid grid panels joined to each other to form a shape of frustum of pyramid and netting aisle sewn up on the top brim of the frustum of pyramid. The rigid grid could secure a steady space between rods and therefore ensured the device to have a stable grading efficiency and low escaping rate. The shape of frustum of pyramid kept four side grid panels in a stable incident angle of 45° to increase the grading efficiency. The netting aisle could make all the fish enter into the device without much tension be graded and could also be an adjustment measure to control fish density during grading.

Design and construction of the grid panel. The grates consisted of stainless steel frames, strengthening bars and PVC rods. Four side grates were trapeziform (Figure 3) and the bottom was square with a total area of 8.6 m^2. The stainless steel frames and strengthening bars were welded into a whole structure and drilled with some holes to fix to the other grate or to hold the rods. The PVC rods were held in the holes of frame and strengthening bars, and could whirl in the holes. The space between rods was 34 mm for 500 g and 31 mm for 350 g of grading red seabream.

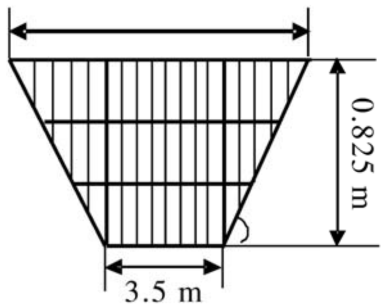

Figure 3. Side view of grid panel elevation.

Design and construction of the netting aisle. The functions of the netting aisle were as an aisle through which fish could swim from cages into the grading device without much tension and as an adjustment measure to control the fish density during grading. The netting of aisle was PE210D/15 ×3-50 and its straight height is 3.5 m. The perimeter of the lower end of the aisle was sewn up with the top brim of the grading device and its hanging ratio is 0.680. The perimeter of the top end was made into a loose structure which was convenient to mount to the perimeter of cages and equipped with 10 floats (150 mm in diameter).

Other accessories. In order to maintain the best incident angle of the grate and facility to operate the device, 8 floats (150 mm in diameter) were fixed at the middle of the four side grates. Four PE ropes were attached to the four lower corners of the frame and connected to the ring of a cage in order to lift or lower the device to maintain a suitable fish density for achieving highest grading efficiency.

Preliminary experiments

Experimental site and environment. To verify if the grading goals could be achieved by the designed grading device, preliminary experiments were carried out in the open sea of Aoqian of Pingtan County, Fujian Province. The perimeter of the anti-stormy wave cage was 30 m and the depth was 6 m. The experimental site had 5-6 levels of wind, less than 20 cm•s^{-1} of water current, and 13°C of water temperature during the preliminary experiments.

Experimental methods

Manipulation of the grading. The steps in changing net and grading in the anti-stormy wave cage are: a) cover a new net bag outside of the net bag of cage and fasten the new net bag to the frame of cage; b) unfasten a part of the attachment of the old net bag to cage floatation and let the old net bag only take about half of the cage area; c) put the grading device into the empty area of the new net bag; d) join the part of mouth of the old net bag to the upper mouth of the aisle of the grading device with loose joining and make them sink into the water; e) wring the old net bag to the ship and drive all the fish to the grading device simultaneously; f) lift or lower the grading device gradually after a while and maintain a suitable fish density to let fish smaller than the goal escape; g) harvest the fish left in the device into another cage to continue aquaculture or to sell; and, h) take back the device.

Calculation of grading efficiency. Before and after grading, the total number of fish and the number of fish bigger than the goal in the cage and grading device were counted, respectively. Then the grading efficiency can be calculated as below:

$$\alpha=(A_1-B_1)/A_1\times100\% \qquad \beta=(A_2-B_2)/A_2\times100\%$$

where: α = the grading efficiency; A_1 = the number of fish smaller than the goal in the grading device before grading; B_1 = the number of fish smaller than the goal that remained in the grading device after grading; β = the rate of escaping of fish bigger than the goal; A_2 = the total number of fish bigger than the goal before grading; B_2 = the number of fish bigger than the goal that remained in the device after grading.

Results

In the first grading experiment, the total number of red seabream was 750 before grading while the number of fish bigger than 500 g was 200. After grading, 263 fish remained in the device while the number of fish smaller than 500 g was 66, giving a grading efficiency of 88.00 % and an escaping rate of 1.50 %. In the second grading experiment, the total number of fish was 741 before grading while the number of fish bigger than 500 g was 192. After grading, 246 fish remained in the grading device while the number of fish bigger than 500 g was 185, giving a grading efficiency of 88.89 % and an escaping rate of 3.65 %. The average grading efficiency and rate of escaping from the two experiments were 88.45 and 2.58 %, respectively.

Discussion and Conclusions

The grading device developed can be used to grade farmed fish in the anti-stormy wave cages after some modifications.

The results of the preliminary experiments showed that the developed grading device performed well with high and stable grading efficiency of more than 85 % for fish smaller than 500 g and low escaping rate of less than 5 % for fish bigger than 500 g. The grading time was shortened to two hours, and all fish entering into the device did not have much tension and were easy to handle. Five grates joined to each other to form a shape of frustum of pyramid and an enclosed volume for fish not only enlarged the area of grate allowing the fish to escape from five different directions but also maintained the four side grates at the best incident angle of 45˚. The fish density in the grading device could easily be adjusted by lifting and lowering the device that increased grading efficiency and decreased fish injury.

The device is portable and can be easily moved. The whole grading process can be performed by 5-6 workers. Maintaining fish in high density can increase the grading efficiency, but it should be pointed out that high fish density may make fish gather in front of the grid and clag small fish to escape after a certain period. Thus in the course of grading operation the device should repeatedly be lowered and lifted for 3-5 times to make the fish in sparse density and in high density alternately. The lowering and lifting of the device can be easily done by four workers. In addition, the device can be used to grade other grading goal or other fish species by changing the grid panel into different spaces between rods.

The developed grading device could further be improved in the following aspects: a) the structure should be changed into a rigid frame made by stainless steel tubes and flexible grid panels assembled on the rigid frame, allowing the space between rods to have limit shift to reduce the risk of fish injury; and, b) four corners of the device should be obtused or the whole shape of the device should be changed into the frustum of a cone to avoid them hooking the netting of the cage during the operation.

The grading device developed solves the problems of soft grading systems for fish and has higher efficiency than that of vacuum fish pumps.

At present, there is no fish grading available in the aquaculture of anti-stormy wave cages in China. Lu et al.(2004) reported the experiment and study on the soft grading system for fish cultured in cages which is similar to that developed by Ivor (2002) in structure and have the advantages such as easy to fold, convenient to manipulate and deposit, and effectively reduced fish injury and mortality. However, there are also some disadvantages such as: there is no way to ensure that all farmed fish can enter the grading device because the shape of the net bag of anti-stormy wave cage is irregular due to deformation under the action of current, wave and fouling organisms. The space between bars of the grid changes with the fish rushing to pass through the grid which greatly affects the escaping rate of fish bigger than the goal weight. Huang et al. (2004) reported that vacuum fish pumps can be used to harvest and grade farmed fish in deep sea cage, but they are bulky and heavy, and need the generating set. Effects of vacuum pump on fish physiological characteristics remain to be verified further in the experiment. The grading device reported in this paper solves the existing problems in the soft grading system for fish and has higher efficiency than that of vacuum fish pumps.

The grade device can also be used to grade other cultured fish with flat ellipse shape if the bar space of the grid panel is changed appropriately to correspond to the parameters of graded fish and goal.

The space between rods of the grid must be accordance with the cultured species and the grading goal, which are different in the different periods of cage culture. At present the species cultured in anti-stormy wave cages in China are about 60, the device can be widely applied in the aquaculture of anti-stormy wave cages after their biological parameters are measured.

References

Ivor, A.J. (2002) Flexi-Panel development.www.gradingsystems.com.

Lu, W., C.T. Guan and D.F. Lin (2004) Experiment and study on the soft grading system for fishes cultured in cages. Marine Fisheries Research, 25(3):61-67.

Huang, B., C. T. Guan and D.F. Lin (2004). Technology problem of vacuum fish pump in sea cage. Fishery Modernization, 6:39-41.

Overview of Studies on Marine Finfish Reproduction and Larviculture in the United States

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Abstract

Since the 1970s, studies on marine finfish reproduction and larviculture have been carried out in the United States, and at least 20 species have been reared experimentally for stock enhancement or aquaculture purposes. Since historically, the development of larviculture technologies for marine finfish in the US has focused on the restoration of recreational and commercial fisheries rather than on the development of agribusiness opportunities to produce food for domestic consumption or export, the successful technologies of many species artificial breeding have only been limited to lab scale or in the research stage. Although there are only a few large-scale marine foodfish commercial hatcheries available in the US, hatchery production technologies have successfully been established in selected species, including striped mullet, milkfish, mahimahi, Pacific threadfin, red drum, summer flounder, greater amberjack, mutton snapper, and pompano based on successful basic studies conducted in reproduction and larviculture. In past three decades, most studies, involved in captive broodstock cultivation, induced maturation and controlled spawning, larval rearing as well as its relevance to ecology, physiology, feed and nutrition, have been carried out. The achievements and success are mainly concentrated on: controlled maturation and spawning and comprehensive larviculture technology. This paper focuses on the review of research achievements in marine finfish reproduction, especially, the methods for induced maturation and controlled spawning, and the comprehensive technologies for larviculture of some typical species typically conducted at the Oceanic Institute, Hawaii. Some factors of influence to intensive larval rearing and methods for improving larval survival rate are discussed.

Introduction

The United States is the largest exporter of edible seafood products worldwide, and second to Japan in terms of importing (FAO 1999). In 1998, the total US trade deficit in seafood products was a record US$8.2 billion (NMFS 1999a), the largest for any agricultural commodity and the second largest, after petroleum, for any natural resource product (ARS 1999). Further, the seafood trade deficit in the US has been widening over the last 10 years, largely due to weak domestic harvests from wild stocks. Although aquaculture has been rising since the 1980s, as the fastest growing sector of the US agriculture industry, aquaculture production in the US has overwhelmingly been focused on freshwater catfish, trout and the Atlantic salmon. The production of other marine finfish species presents an enormous economic opportunity for increasing the supply of high quality, safe, and wholesome US aquaculture products for domestic and global markets.

In the past, little emphasis was placed on the production of marine species for food in the United States. Marine finfish aquaculture in the US can be traced to studies on the early life histories of marine finfish, conducted in support of fisheries management objectives and, more recently, to stock enhancement efforts to replenish depleted natural populations (Lee 1997a; Lee & Ostrowski 2001). Since the 1970s, studies on marine finfish reproduction and larviculture have been carried out in the US, and more than 20 species, from near shore fish (e.g. milkfish, striped mullet) to deep-water and open ocean fish (e.g. mahimahi, greater amberjack), have been reared experimentally for stock enhancement or aquaculture purposes, because the development of larviculture technologies for marine finfish in the US historically was focused on the restoration of recreational and commercial fisheries rather than on the development of agribusiness opportunities to produce food for domestic consumption or export. The successful technologies of many species artificial breeding have only been limited in the lab scale or in the research stage. To date, mass quantities of fry can be produced for commercial purposes for only eight species in the US, including striped mullet (Mugil cephalus), Pacific threadfin (*Polydactylus sexfilis*), red drum (*Sciaenops ocellatus*), milkfish (*Chanos chanos*), summer flounder (*Paralichthys dentatus*), mahimahi (*Coryphaena hippurus*), mutton snapper (*Lutjanus analis*) and pompano (*Trachinotus carolinus*) (Lee & Ostrowski 2001).

The situation has changed in recent years in response to increasing demands for fresh seafood and natural fisheries resources constantly decline and the seafood trade deficit is steadily rising, the US government agencies recognize that marine finfish production is poised as a key growth area for aquaculture in the United States and encourage an increase support to mariculture research and industry development, especially in commercializing the production of the species studies (Sea Grant Association 1999). Furthermore, rapid growth and high-value species, such as mahimahi, greater amberjack, cobia (*Rachycentron canadum*), have been identified as candidates for the emerging offshore farming in submersible cage, associated with technologies development on reproduction, larviculture and grow-out.

Due to a relatively greater interest in marine food fish cultured in Hawaii, hatchery technology for regarded local species, such as striped mullet, milkfish, Pacific threadfin and mahimahi has been studied since the early 1970s, and a program of mass production for greater amberjack has been ongoing (Annual report of The Oceanic Institute 2001) conducted at the OI. At the Atlantic coast, techniques for spawning and larval rearing of southern flounder (*Paralichthys lethostigwa*), spotted seatrout (*Cynoscion nebulosus*) and red durm have been developed to release these species into freshwater reservoirs (Lasswell et al. 1977). Interest in aquaculture of red drum (Henderson-Arzapalo 1995) and flounder (Bengston & Nardi 1995; Smith et al. 1999a) has urged more research on hatchery technology development for these species. Commercial production of summer flounder began in 1996 (Bengtson 1999). This paper concentrates on the review of research achievements and progresses in artificial breeding and summarizes the comprehensive technology for larviculture of some typical cultured species in the US. Some specific issues in controlled maturation,spawning and hatchery operation are discussed.

Materials and Methods

Reproduction

Captive broodstock management. A successful hatchery operation is based on a healthy broodstock. The purpose of broodstock management is to supply good quality eggs and larvae in time. Captive broodstock cultivation is the first step of propagation for most selected species. The broodstock are maintained in tank, pond, or cage condition until maturation and spawning for most cultured species, such as milkfish, striped mullet, red drum, mahimahi, Pacific threadfin, greater amberjack, bluefin trevally, and summer flounder. Alternatively, fully mature broodstock for some species can be caught from the wild and stripped to obtain eggs for artificial fertilization, as common snook (*Centroppmus undecimalis*), although this method is not recommended (Lee & Ostrowski 2001).

Although different species require different ways of cultivation, the principle of good broodstock management involves providing natural and non-stressful environmental conditions, such as clean tank or pond environments, lower stocking density, proper light intensity, appropriate and stable water quality parameters, as well as a nutritious, balanced diet and regular health assessment routine. Stress factors such as handling and improper environmental conditions can easily block the normal reproductive cycle of fish (Bilload et al. 1981). The general stress response is polymorphic and subject to genetic predisposition, but different fish species respond differently to stress. Within the same species, different individuals also show different responses because of their physical and physiological conditions as well as their background experiences. So non-stressful conditioning is the most important procedure in captive broodstock management. The successful captive broodstock maintenance for Pacific threadfin is a good example, conducted at the Oceanic Institute. It will yield a year-round supply of eggs when the broodfish are maintained under the following conditions: 33-35 ppt salinity, 24-30°C temperature, > 5mg•L^{-1} dissolved oxygen (DO), 1-1.5 kg fish•m^{-3} stocking density, and 0.24 kg fish•Lpm^{-1} of seawater exchange and loading rate (Ostrowski & Molnor 1998). Sheilds et al. (2002) had also successfully produced year-round spawning of greater amberjack in natural captive management.

Although the specific nutritional requirements of broodstock are not well known for most cultured fish, the quality and quantity of n-3 HUFAs contained in fish feeds may influence the development of the gonads and quality of the egg (Navas et al. 1998; Sargent et al. 1999). Formulated feeds have been successfully used to mature broodstock of many species, e.g. striped mullet (Lee & Kelly 1991) and milkfish (Lee 1995). A combination of pelleted formulated feed and raw feed (e.g. squid, krill and smelt) are used for red drum (Colura et al. 1991) and Pacific threadfin (Ostrowski & Molnar 1998). Mahimahi broodstock require raw feed (Ostrowski 2000). The daily feeding amount recommended by the Oceanic Institute was about 3 % of broodstock body weight on pelleted diets for milkfish and striped mullet, and 3-6 and 0.6-0.9 % of broodfish body weight on raw and pelleted diets for Pacific threadfin, respectively. The requirement of protein content of pelleted diet varies in different species, for example, 40 % of crude protein is adequate for milkfish, and Pacific threadfin may require 55 % of crude protein (Liu & Kelley 1995; Ostrowski & Molnar 1998).

For some species held under captive conditions for an extended period of time, spawning takes place without any hormone treatment, such as milkfish (Lee 1995), Pacific threadfin (Ostrowski & Molnar 1998), bluefin travelly (Moriwake et al. 2001), and red drum (Colura et al. 1991). When milkfish broodstock were caught from the natural environment, hormonal induction was required to obtain mature eggs (Lee et al. 1986a; b). After nearly three years in captivity, however, the same group spawned naturally without any hormonal treatment (Liu & Kelley 1995).

Maturation and spawning. Natural spawning is suitable for obtaining fertilized eggs for hatchery production during the regular spawning season. However, when fertilized eggs were needed at a designated time, or out of a spawning cycle, environmental manipulation and/or hormone application is required (Lee& Ostrowski 2001). On the other hand, many fish exhibit reproductive dysfunctions when reared in captivity due to the fact that fish do not experience the conditions of the spawning grounds, and as a result the pituitary failed to release the maturation gonadotropin, Luteinizing Hormone (LH).

Since the 1930s, a number of researchers have made great advances in developing methods for induced breeding that can be applied closer and closer to the origins of the internal reproductive pathway (Crim 1983; Lee et al. 1986, 1987; Harmin & Crim 1992, 1993; Watanabe et al. 1995, 1998; Berlinsky et al. 1996; Tamaru et al. 1996; Zohar 1996). In summary, there are two approaches to induction of maturation and spawning in finfish: hormonal and environmental. The two approaches may be combined to achieve the best effect. After treatment, the fish in each approach may be stripped for artificial fertilization or left for natural spawning.

Environmental conditioning for maturation and spawning. Environmental control is a particularly useful technique for artificial breeding. It is effective, non-invasive and generally inexpensive. It is probably less stressful and more environmentally friendly, since it is not really adding anything to the environment. It is powerful, too, because it works at the highest level of maturation control, so all stages of maturation are controlled. Specific external stimuli that often prompt the reproductive process can include factors such as photoperiod and lunar cycles, ambient temperature or changes therein, precipitation, water flow, water depth, changes in barometric pressure, presence and behaviors of other fish, presence of suitable spawning substrate, and various changes in water quality, especially salinity, hardness, dissolved oxygen and pH (Zohar 1989; Munro et al. 1990).

The environmental conditions that lead to spawning need not always be complex or subtle. Techniques for photothermal manipulation to stimulate annual cycles have been well established for a number of species, such as Pacific threadfin (Ostrowski & Molnar 1998), greater amberjack (Shields et al. 2002), mahimahi (Szyper 1991; Ostrowski 2000), milkfish (Lee 1995), bluefin trevally (Moriwake et al. 2001). Fish designated for fingerlings production can be housed in tanks or aquaria and subjected to controlled temperature and photoperiod to mimic or even temporally compress annual cycles associated with maturation and spawning.

Depending on the temperature range encountered during a species' normal life history, equipment such as chillers and heaters may be required to acquire adequate water temperatures for maturation and spawning cycles. Photoperiod manipulation, in contrast to artificial temperature regimes, usually requires a little more than the conventional times for artificial lighting and a disciplined workforce to avoid inadvertent interruptions of a photoperiod cycle once it has been established. Although temperature or photoperiod regimes alone induce maturation or spawning in some species, in others both are essential. In general, raising the temperature and a long photoperiod can induce maturation for the species which naturally spawn in spring and summer season, and lowering the temperature and a short photoperiod for the species which naturally spawn in fall and winter.

Pacific threadfin will spawn year-round with environmental manipulation. An effective treatment is a photoperiod of 18L/6D and a temperature of 27ºC started three months prior to spawning (Ostrowski & Molnar 1998).

Red drum naturally spawns from late summer to early fall. Using an annual photothermal cycle that is condensed into four months, red drum can be induced to spawn at the desired time of the year. Once the desired temperature and photoperiod for spawning have been reached, spawning usually begins within 10 days, if spawning does not commence, it can be induced by lowering the water temperature for two days, then raising it back to the original temperature (Roberts 1990). Once spawning begins under constant photoperiod conditions, the spawning frequency can be regulated by water temperature (Arnold 1998). Red drums are capable of spawning continuously for several years under constant late summer conditions (Thomas & Arnold 1993).

Considered as the "winter spawner" striped mullet naturally undergoes maturation and spawning in winter. In Hawaii, the Oceanic Institute has successfully obtained year-round reproductive broodstock which is controlled by environmental factors. Usually a shorter photoperiod and cooler water temperatures can stimulate and induce both male and female maturation in captive. Broodstock are divided into one natural “winter” group, three phase shifted groups: “spring”, “summer”, and “fall”. Each is subjected to similar stimulatory conditions (8-12 hours daylight and 20-26ºC) and inhibitory conditions (14-18 hours daylight and 27-30ºC). The stimulatory period is 30 weeks. During the first six weeks, the fish are maintained in 35 ppt salinity. From week 7 to 30, freshwater is added which reduces the salinity to 15-25 ppt and temperatures to below 26ºC, photoperiod is shortened to eight hours. During this period, eggs will develop as if they were in normal winter conditions. The inhibitory period has a duration of 22 weeks divided into two phases. The first 12 weeks, the fish are placed under 18 hours of light and normal 35 ppt seawater. Afterwards, the 10-week final phase is under the conditions of 28ºC and 18 hours photoperiod combination (Kelley et al. 1991; Liu & Kelley 1994).

The spawning season is also controlled by environmental manipulation in other species, such as summer flounder (Watanabe et al. 1998b), and southern flounder (Smith et al. 1999b). Watanabe et al. (2000) have successfully produced naturally spawning of southern flounder when an artificial winter photoperiod of 10 L: 14 D was maintained through 3 months after 3 months in natural photothermal conditions.

Exogenous hormone-induced maturation and spawning. Reproductive hormones have been utilized since the 1930s to stimulate reproductive processes and induce ovulation/spermiation and spawning of finfish in captivity. Hormonal-induced methods began with the crude use of ground pituitary from mature fish (e.g. carp, salmon) —containing gonadotropin (GtH) which were injected into broodfish to induce spawning (Houssay 1930). Today, various synthetic hormones are available (Peter et al. 1993; Zohar 1989a, b; Crim & Bettles 1997). Now, the most common hormones used to induce maturation and spawning of marine finfish cultured in the United States are pituitary extracts, HCG (human chorionic gonadotropin), GnRHa (gonadotropin releasing hormone analogues), MT (17α-methyltesterone), LHRHa (luteinizing hormone-releasing hormone analogues).

The use of CPH to induce fish spawning has been practiced since 1930. It still is used widely now. The pituitary glands used are usually obtained from sexually maturing or mature donor fish that may be of the same or different species. They can be used fresh or stored by frozen or acetone-dried for subsequent use. Purified preparations of salmon and carp gonadotropins have been commercially available for some time (Donaldson 1973; Yaron 1995).

HCG, which is purified from the urine of pregnant females (Katzman & Dosiy 1932), has been used successfully to induce ovulation/spawning in a number of species (Lam 1982; Donaldson & Hunter 1983; Liu & Kelley 1994). Unlike LH preparation of piscine origin, HCG is often given in a single dose which ranges between 100 and 4000 international units (IU) per kg body weight. The effectiveness of HCG after a single treatment is probably due to this GtH's relatively long retention time in circulation (Ohta & Tanaka 1997).

LHRH from mammals was first used to temporarily replace GnRH in fish, triggering the pituitary release of GtH and subsequent processes. In recent years, synthetic analogs of these releasing compounds have been used with much more success. LHRH is effective in inducing gonadotropin release and ovulation in fish but its super-active analogues (LHRH-a) are more effective. Many species have been successfully induced to maturation and spawning by using LHRHa, such as milkfish (Lee et al. 1986; Liu & Kelley 1995), striped mullet (Lee et al. 1987; Liu & Kelley 1994), and rainbow (Crim & Evans 1983). There is increasing interest in the use of LHRHa as an ovulating agent in cultured fish species.

MT, a kind of super-active analog of testosterone, is usually used in male induced-maturation. In any month of the year, males of some species will produce milt in as little as three weeks after receiving a single 10 mg 17-MT capsule. An effective method of sustaining spermiation in striped mullet by steroids was reported by Lee and Weber (1986), and Lee et al. (1992). Administration of 17-MT orally (12.5mg•kg^{-1} body wt•day^{-1}) or intramuscular implantation (5μg•body wt^{-1}) can induce maturation of male mullet throughout the year.

GnRHa implants which contain100 μg of GnRHa have been successfully used to induce southern flounder ovulation and spawning (Smith et al. 1999).

Hormonal manipulation can advance maturation and ovulation by a few weeks, thus reducing losses due to pre-spawning mortality (Goren et al. 1995). Another application of hormonal manipulation is for the collection of gametes for inter-specific hybridization via artificial fertilization, since different species do not usually spawn together when placed in tanks. Finally, development of genetic selection program and hormonal manipulations can be used to enable proper maturation and timely collection of gametes. Therefore, hormonal manipulations for the induction of ovulation/spermiation and spawning will continue to play an important role in commercial broodstock management, even after various fish species become properly "domesticated".

Protocols of hormonal induction by injection. The first step of hormone injection protocols is hormone preparation. Hormones should be dissolved into saline solution or bacteriostatic water before used for injection. The dosage calculation may be various in different kinds of hormones, and depending on different species. Even within the same species, recommended dosage based on weight, volume and IUs is often necessary to conduct range-finding trails on a dosage appropriate for the particular species. In general, the recommended dosages of pituitary extracts, HCG, LHRHa are 4-8 mg•kg^{-1} body weight, 500-1000 IUs•kg^{-1} body weight, 5-10 μg•kg^{-1} body weight, respectively (Lutz 2001).

Usually an intramuscular injection into the back of the fish, either directly behind or beside the dorsal fish, generally results in less chance of injury and a more gradual and sustained uptake of the administered hormone than intraperitoneal injections. Injections should always be administered beneath the scales rather than through them. A series of two or more injections will usually produce more consistent and reliable results for females than a single injection of the total recommended dose. Males are usually injected only once, at the same time that the last, or resorting, dose is administered to the females (Lam 1982; Donaldson & Hunter 1983; Liu & Kelley 1994, 1995). Recommended intervals between injections will vary by species, and as little as 6-12 h for the tropical species. The initial gonadotrophin dose is usually 10-33 % of the total recommended dose, followed by the remaining portion of the total dose, while releasing hormones may be administered in a 20:80, 50:50, or 3- portion ratio (Lutz 2001).

Protocol of intramuscular implantation. During the last two decades, research has focused increasingly on the use of surgical implants that release various compounds in a more continuous and sustained pattern. These implants are typically used of a cholesterol-cellulose matrix. Some of the earliest works in this area were described by Crim et al. (1983) and Lee et al. (1986a). It has been successfully applied to induce milkfish, striped mullet, Pacific threadfin maturation and spawning off-season conducted at the Oceanic Institute, Hawaii.

The procedure of LHRHa pellet of implant preparation, illustrated by Lee et al. (1986), is as follows: synthetic LHRH analogue was purchased from Sigma Chemical Company, USA. Two mg of LHRHa was dissolved in 0.3 ml of 50 % ethanol. The solution was then mixed with 190 mg of cholesterol (USP grade), until a paste-like consistency was obtained. The paste was then dried in an incubator set at 35ºC. The resulting dried powder was then mixed with 10 mg of cocoa butter which serves as a binder thoroughly with a wooden stick for the uniform production of pellet using a plexiglass mold. The resulting pellet produced weighs approximately 23 mg and has an average length and diameter of 5.5 and 2.4 mm, respectively. A single pellet that is produced in this manner contains 200 μg of LHRH-a.

For the Pacific threadfin, each female is implanted with a 100-200 μg LHRH-a cholesterol pellet into the dorsal musculature. Spawning occurs approximately 36 hours after implantation. Production numbers are similar to those for natural spawning (Ostrowski & Molnar 1998).When milkfish were treated with a similar LHRH-a cholesterol pellet, a high percentage of gonadal maturation was reported in treated fish and approximately a month before its normal reproductive season (Lee et al. 1985).

In captivity, the time required for striped mullet female to reach an oocyte diameter of 600 μm is two months when it is in full maturation at the growth rate of oocytes in the vitellogentic stage averages 7 $\mu m \bullet day^{-1}$. However, the oocyte growth rate can be accelerated to 10 $\mu m \bullet day^{-1}$ with a 200 μg luteinizing-hormone-releasing hormone analogs (LHRH-a) cholesterol pellet which is implanted into the dorsal musculature. For striped mullet induced maturation and spawning, hormone treatment consist of the following steps. The female is given a “priming” injection of carp pituitary homogenate (CPH), injected at a dosage of 40 $mg \bullet kg^{-1}$ of body weight. Twenty four hours later, it is given a second injection of LHRH-a at a dosage of 100$\mu g \bullet kg^{-1}$. The female will spawn at approximately 12-14 hours after the second injection (Liu & Kelley 1994).

Smith et al. (1999) reported that GnRHa implants, which were made with a 95 % cholesterol and 5 % cellulose pellet containing 100 μg of GnRHa, were successfully used to induce southern flounder ovulation and allow strip-spawning. In addition, the female southern flounders held in photothermal conditioning for three months when the maximum diameter of oocytes had increased to 540 μm, 100 μg GnRHa implants resulted in successful tank-spawning. The GnRHa implantation method was recommended by Hodson and Sullivans (1993).

Obtaining gametes protocol

Eggs collection system for natural/spontaneous spawning. For some species held under captive conditions for an extended period of time, spawning takes place without any hormone treatment, such as milkfish (Lee 1995), Pacific threadfin (Ostrowski & Molnar 1998), bluefin trevally (Moriwake et al. 2001), mahimahi (Lee 1997), greater amberjack (Sheild et al. 2002), conducted at the Oceanic Institute, and red drum (Colura et al. 1991). Many species can spawn spontaneously after hormone treatment (Lee et al. 1988; Tamaru et al. 1989; Liu & Kelley 1995; Lee 1997b), for example, southern flounder spontaneously spawns in holding tanks almost daily for a period of 6 weeks after hormone injection or implantation (Smith et al. 1999a)

A typical egg collection system illustrated by the Oceanic Institute is as follows: Eggs are collected from the effluent with a 0.5 mm mesh net attached to a PVC frame. This is placed inside a barrel. The net is weighed down with a PVC ring. The barrel has its own outside standpipe, so eggs remain in enough water to avoid being damaged. The height of the barrel depends on the water level of the tank. The goal is to collect as many undamaged eggs as possible. A difference of no more than 6 inches in water levels between the collector system and tank is maintained (Ostrowski & Molnar 1998).

Stripped spawning protocol. The most practical approach to obtaining offspring in most finfish is clearly to allow spawning to proceed in holding ponds or tanks. When more precise control is desired or required, however, eggs and sperm must be taken directly from broodstock, such as southern flounder (Berlinsky et al. 1996; Smith et al. 1996b), summer flounder (Bengtson 1999), red snapper (*Lutianus campechanus*) (Minton et al. 1983), Nassau grouper (*Epinephelus strietus*) (Watanabe et al. 1995). While certain circumstances may require surgical removal of eggs or milt, the most common approach to collecting eggs and milt involve manual stripping of broodstock. A reliable stripped spawning protocol has been established for red snapper (Minton et al. 1983; Laidley 2001).

Hand-stripping of eggs and sperm is always associated with hormonal induction of spawning in captive condition. Once broodfish have been injected, they should be sorted by sex and held separately in tanks for monitoring and subsequent stripping. These tanks can be partially or completely covered to minimize stress and prevent fish from jumping out. Tanks for holding and monitoring should facilitate frequent netting of individual fish for examination. This is an important consideration, because one key to successful eggs has been ovulated, their quality deteriorates rapidly and fertilization must take place during a limited period of time. The common manipulation procedure of hand-stripping for most species has been summarized and described by Lutz (2001) as follow.

Female broodfish should be checked regularly for ovulation beginning several hours before the first ovulation would be expected, based on injection dosages and temperature. The appropriate interval between examinations will depend on the species, every 45min for tropical species, every 1-1.5 hour for warm-water and temperate species, and every several hours for cool/cold water species. The most common method of detecting ovulation involves physically restraining the fish in an inverted position and applying gentle pressure to the abdomen immediately behind the pectoral fins. If eggs flow freely from the vent, ovulation is at or near completion, and the eggs must be taken immediately and fertilized. Eggs must be collected without being contaminated by water or slime from the surface of the donor fish. The fish itself must be maintained under moist conditions so as to prevent the loss of the protective mucus layer from the skin if it is to be saved for recovery and future spawning. The best approach in dealing with these simultaneous requirements is the use of a damp towel to remove excess water and slime from the female fish and subsequently cover its head and abdomen during stripping. Since the fish is positioned to prevent water or slime from dripping from the vent or tail, the abdomen is stroked gently from front to rear to express eggs from the vent into a dry pan or bowl.

Milt can be stripped from males in much the same way as eggs are collected from females. Enough milt should be added to cover the eggs. The contents of the bowl are then mixed thoroughly with a plastic spoon, spatula or clean feather. Once the eggs and milt have been thoroughly mixed, water should be added to activate the sperm. Generally, only enough water to cover the eggs is required, although these proportions may vary depending on the species in question. In many species, after 5-10 min eggs should be fertilized and ready for incubation.

Larval rearing technology development

Environmental condition. In general, the environmental factors in most hatcheries are usually kept at normal ambient conditions and not manipulated, unless unfavorable ambient conditions or specific experimental needs override the costs of conducting such manipulations. If hatchery production is expected their operation is year-round for same species, however, the chiller or heater equipments and facilities are necessary for thermal manipulation to maintain optimal environmental conditions for larval growth. Besides temperature, the environmental factors include stocking density, salinity, light intensity, dissolved oxygen, pH value, ammonia, etc.

Stocking density. The stocking density depends on factors such as egg quality, percentage of fertilized eggs, facilities set-up and management strategy. In general, the stocking density ranges from 20 to 40 larvae per liter in intensive larvae rearing system initially, such as striped mullet, milkfish, Pacific threadfin conducted at the Oceanic Institute (Liu & Kelley 1994, 1995; Ostrowski & Molnar 1998). Final harvest density is not always directly related to the initial stocking density and depends on the carrying capacity of the culture system and other factors. Current hatchery operations for striped mullet, milkfish and Pacific threadfin at The Oceanic Institute usually result in a final harvest density of 5-12 fish•L^{-1}. When eggs are plentiful and survival of first-feeding larvae is low, large numbers of fertilized eggs are stocked to ensure sufficient numbers of larvae for subsequent rearing, as is common in mahimahi hatcheries (Lee & Ostrowski 2001). Daniels et al. (1996) recommended a two-step process for intensive culture of southern flounder larvae, beginning with high stocking rates (80•L^{-1}) during first feeding and lower densities (1•L^{-1}) through metamorphosis. For red drum hatchery production, larvae can be initially stocked at 10-20•L^{-1} for the first two weeks, then reduced to 1-2 fish•L^{-1} for the last few weeks (Holt et al. 1990).

Temperature. Water temperature is one of the important factors for an effective larval growth. Optimal temperature range for each species may be different depending upon habitat and geographic distribution of species. Usually, the shallow species exhibit more width in optimal temperature range than deep sea species, because the temperature of shallow water always changes sharply during a day with tidal cycle, and deep sea is more stable in environmental condition. Literatures stated the optimal temperatures of some species cultured as follows: 22-26ºC is the optimal temperature for striped mullet (Liu & Kelley1994); 24-33ºC for milkfish (Liu & Kelley 1995); 24-30ºC for Pacific threadfin (Ostrowski & Molnar 1998); 20-24ºC for southern flounder (Powell & Henley 1995). For red drum, the optimal temperature is 25-30ºC, and growth is arrested below 20ºC (Lee 1997). In general, the larvae grow faster with higher temperature at the temperature range. By increasing the water temperature about 2-4ºC, the hatchery time for striped mullet can be shortened from around 60-35 days (Temaru et al. 1993).

Salinity. The salinity requirements for larval rearing vary widely depending on different natural habitat and original early life history. Shallow species exhibit wide tolerance to salinity, especially for low salinity, such as milkfish and striped mullet, their optimal salinity range from 15 to 30 ppt (Liu & Kelly 1994, 1995). For red drum, the tolerance of salinity range should be between 10 and 45 ppt, but 20-35 ppt is preferred. Newly hatched larval can not survive at a condition under 10 ppt and fingerlings larger than 25 mm can survive in freshwater (Colura et al. 1976; McCarty et al. 1986; Lee 1997). The species living in deep sea should be kept in high salinity, such as mahimahi, greater amberjack, bluefin trevally prefer above 30 ppt (Moriwake et al. 2001; Sheilds et al. 2002). For all species, it is important to maintain salinity above the level of neutral egg buoyancy during the incubation period to allow for suspension of the eggs in water columns. This should result to better hatching than if the eggs sink to the bottom of the incubation tank (Lee & Ostrowski 2001).

Light intensity. Optimal light intensity is also very important for the success of feeding in fish larvae. Striped mullet requires a light intensity above 500 Lux on the water surface of the rearing tank (Lee & Kelley 1991). For Pacific threadfin, the light intensity should not be more than 1500 Lux (Ostrowski & Molnar 1998). Light intensity also effects larvae fish development, and required light intensities change as the fish larvae get older. Watanabe et al. (1998c) reported that higher light intensity has negative effects on yolk utilization and larval size at the time of first feeding in summer flounder. On the other hand, Deuson & Smith (1997) indicated an improvement of pigmentation in southern flounder exposed to high light intensity for 1 week post-metamorphosis. A study of the effects of photoperiod on larval survival, growth and pigmentation has been completed at the University of Rhode Island, with best results under conditions of constant light (Bengtson 1999). Photoperiod determines the duration and feeding behavior of fish larvae (Ronzani et al. 1991). Longer photoperiods are often applied in the hatchery to allow more time for the feeding of fish larvae (Lee & Ostrowski 2001).

Other factors in water quality control. Other factors affecting water quality are also important for larval rearing, such as dissolved oxygen and pH value. For the most species, the DO should be maintained above 5mg•L^{-1} and pH should range from 7.6 to 8.4 during hatchery stage. The normal acceptable level of un-ionized ammonia is less than 40 μg•L^{-1} for most warm-water marine finfish species (Tsujigado & Lee 1993). Water flow-through rearing systems are more common than closed systems, since increasing water exchange rate is necessary to maintain better water quality, for as the fish larval get older, the metabolism rate become higher. For Pacific threadfin hatchery production, daily water exchange during the incubation process is 400 %, and then reduced to 100% during the initial feeding period, and the water exchange rate is gradually increased again to 2000 % of 25-day-old larval fish before harvested to nursery (Ostrowski & Molnar 1998).

Daily routine

According to the hatchery manual series (Liu & Kelley 1994, 1995; Ostrowski & Molnar 1998) conducted at The Oceanic Institute, daily routine of finfish hatchery production involves monitoring and maintenance.

Water quality is monitored to ensure that optimum conditions exist in the larval rearing tanks. Water temperature, salinity, dissolved oxygen, and pH values should be measured and recorded twice daily in the morning and afternoon. Live feed densities should also be monitored to determine the amount of rotifers or *Artemia* to be fed according to feed regimen. Rotifers are fed 2-3 times per day and *Artemia* are fed 4-6 times per day. Dry feed should be fed hourly by hand during the early weaning stage and by automatic feeder belt during the later weaning stage. The water flow rate is determined 1-2 times daily.

In addition to everything that has mentioned, removing debris such as rotifer shell and proteinaceous oily waste from the surface of the water of rearing tanks is an important daily task. This type of cleaning, called skimming, promotes oxygen exchange between air-water interface, removes wastes that foster bacterial growth, and facilitates the ability of larval to gulp air to inflate their swim bladders. Skimming should be done at least once a day or whenever the surface appears dirty. Beakers, paper towels and styrofoam bars can be used to carefully skim to remove the waste from the water surface. An automatic surface skimming is installed when dry feed is introduced. The skimmer consists of a styrofoam square frame with one side open. A 1/2 inch diameter PVC pipe is fixed to the open side. The pipe has 1mm holes along its length. One end of the pipe is closed and the other is connected to a compressed air circuit. The air tube is adjusted so it is tangential to the surface. This will concentrate the oily film into the skimmers trap. The scum from the skimmer is removed with a beaker whenever necessary.

The tank bottom is siphoned to remove the dead larval and residues once a day. This is also an important way to prevent water deterioration to cause bacteria growth and disease. Siphoning is performed prior to the addition of algae and rotifers since it is difficult to see the bottom of the tank after algae and rotifers are added.

Feed and nutrition

Microalgae. In general, the presence of algae in the larval rearing tanks may be necessary for most species. Microalgae form the base of the larval rearing food pyramid and provide benefits that improve larval rearing survival. Algae provide nutrition for rotifers and help maintain better water quality in the rearing tanks. The nutritional value of the rotifers is degraded if algae or other feed sources are not available to rotifers in the rearing tank. *Nannochloropsis oculate,* formerly known as marine *chorella*, is most commonly used in milkfish, striped mullet, Pacific threadfin larval rearing conducted at Oceanic Institute. Although other green algae may be used, *N. oculata* is small (2-5 μm), euryhaline (contains 30 % EPA) and reproduces rapidly. It can be grown at high densities. Approximately 300,000 cell•ml^{-1} are introduced on D2 post-hatch of Pacific threadfin. The density of added algae is decreased on D3 and each day thereafter to 150,000 cells•ml^{-1} since it is based on the assumption that 100,000 cells•ml^{-1} remain in the tank from the previous day. Algae is added prior to adding rotifers in the larval rearing tank (Ostrowski & Molnar 1998).

Algae paste that is commercially available is often used in larval rearing and rotifer culture when there is insufficient supply of live algae. The paste of *N. oculata* from Algamac-2000 is used in Pacific threadfin larval rearing recommended by the Oceanic Institute.

Rotifers. Rotifers are the first food item consumed by larval of most species. Rotifers vary in size depending on strain and culture conditions. Adult size ranges from 123 to 315 μm. The mean weight of an individual is 3 μg. Rotifers are presented in rearing tanks on D2 post-hatch, one day prior to initiation of feeding. On D3 post-hatch and each day thereafter rotifers are added to the rearing tank three times per day to an optimum density of 10 rotifers/ml, the most acceptable level (Eda et al. 1990a, b; Ostrowski & Molnar 1998). Depending on the source of the rotifers and the species of fish to be cultured, different amounts of highly unsaturated fatty acids (HUFAs) are required for better larval survival, fast growth and normal development (Ostrowski & Divakaran 1991; Tamaru et al. 1993b; Baker et al. 1998; Ostrowski 2000). Craig et al. (1994) found that rotifers containing at least 0.3-0.4 mg DHA•100 mg^{-1} wt. are necessary for the maximal growth of red drum. The correlation between HUFA enrichment and pigmentation abnormalities was inconsistent among different flounder species (Devresse et al. 1994; Estevez & Kanazawa 1996; Baker et al. 1998). In general, HUFA-enriched diets increase growth and stress resistance in fish larvae and decrease pigmentation abnormalities (Lee & Ostrowski 1998). The rotifers should be enriched with live algae or algae paste for several hours before feeding to the larvae to maintain high nutritional content (Ostrowski & Molnar 1998).

Artemia. *Artemia* is usually used for feeding older, more developed larvae of most species. *Artemia* nauplii provide a high quality, high protein food item that yields high survival, rapid growth, and good pigmentation of larvae. However, research has indicated that they are insufficient in contents of unsaturated fatty acids essential for marine fish larvae. Enrichment of *Artemia* nauplii has become a standard procedure in marine fish hatcheries. The enrichment method for *Artemia* recommended by the Oceanic Institute is illustrated in the section on nutritional enrichment methods.

Copepods. Copepods are important sources of live food for the late stage of larval rearing. Copepods blooms usually occur in brackishwater ponds, but time of the blooms for larval feeding is difficult. The technology of copepod mass culture has been unstable. The nutritional value of copepods is excellent, being rich in n-3 HUFA, with an especially high DHA content (Watanabe et al. 1996). For some fish species with smaller mouth sizes, such as groupers, snappers, young nauplii of copepods can be provided as useful food source. Some research in the area of improving survival rate of red snapper larvae by feeding with copepods is ongoing, at the Oceanic Institute, Hawaii.

Food density and quality. Different fish species may also prefer different food densities. The best procedure to determine the optimal feeding density is to frequently check for satiation of the fish larvae and for available food in the rearing tank to avoid feed deprivation of the fish larvae at any time. Overfeeding of rotifers and *Artemia* nauplii will deteriorate the culture conditions, especially water quality. In addition, the unconsumed *Artemia* nauplii will grow to the adult size and compete for living space with the age of the fish larvae.

A typical feeding regimen is recommended by the Oceanic Institute for application in Pacific threadfin hatchery production as follows: N. oculata is stocked into tanks on D2 at an initial density of 300,000 cells•ml^{-1}, then reduced to 150,000 cells•ml^{-1} once larvae begin to feed. The s-type strain of rotifer (100-200 μm) is the first item from D3 to D15 at a density of 10•ml^{-1}. Enriched *Artemia* are added between D10 and D15 at increasing density from an initial density of 0.02 to 3•ml^{-1}. A commercial, salmon pellet feed is also introduced from D14 on. Increasing levels of *Artemia* and dry feed are continued through D24 or D25, when fish fry are harvested (Ostrowski & Molnar 1998).

But the rotifers for striped mullet hatchery production should be kept at a density of 20• ml^{-1} (Lee & Ostrowski 2001). Bengston (1999) reported that the larvae of summer flounder are fed rotifers for approximately 20 days after they begin feeding at D3. They can normally begin feeding on *Artemia* nauplii at D15-20. The prey consumption rates of larval sharply increase from 62 rotifers•day^{-1} at D6 to 301 rotifers•day^{-1} at D13 and from 59 *Artemia* nauplii•day^{-1} at D23 to 394 *Artemia* nauplii•day^{-1} at D47.

Nutritional enrichment methods. In the wild, marine finfish larvae obtain essential nutrients which the larvae cannot produce from natural zooplankton sources. However, many of these natural sources are difficult to mass culture. The most limited are essential nutritional components of unsaturated fatty acids (HUFAs), including EPA and DHA, key to larval development and growth. So exogenous HUFAs added to enrich *Artemia* nauplii has become a standard procedure in marine finfish hatchery production (Ostrowski & Molnar 1998).

The typical enrichment procedure was described by Ostrowski and Molnar (1998) as follows: newly hatched, separated, and rinsed nauplii are placed into enrichment tanks with seawater and aeration to maintain above 3 ppm DO. Nauplii density should not exceed 150•ml^{-1}; enrichment emulsion is measured into a 500 ml container with a fitted cap (0.15 g•L^{-1} for Super Selco, and 0.20g•L^{-1} for Aqualife DHA 30). The measured amount is placed into a kitchen blender with water and mixed for five minutes, then added directly to the enrichment tank. *Artemia* nauplii are harvested by siphon, concentrated and gently rinsed in clean seawater after 24 hours enriched, they can be either fed or cold-stored for subsequent.

Weaning methods. The success of weaning plays an important role in the final survival. Usually, the fish larvae are gradually weaned from live feed to inert feed within a 7-15-day period, after completion of the development of the digestive system. For example, southern flounder requires 20 days to change over for optimal growth and survival (Daniels & Hodson 1999). The time required to wean to dry diets may be age-related. Jenkins and Smith (1999) were able to wean young southern flounder (78 days old) to dry feed in 14 days with 80.4 % survival. For old fish, it required 160 days, with an average survival rate of 58.2 %. Bengtson et al. (1999) reported better growth, but no difference in survival, of summer flounder using a gradual weaning method versus the immediate method. They also concluded that survival improves by weaning older larvae. In contrast, greater amberjack had better growth and more uniform in size distribution as with a 3-day versus a 7-day weaning during the early nursery stage (Chambers & Ostrowski 1999). Other important factors that determine weaning success are the quality of the inert diet and the presence of attractants (Metailer et al. 1983; Lee et al. 1996; Daniels & Hodson 1999; Lee & Ostrowski 2001).

Cannibalism and deformity issues

In hatchery rearing condition, high mortality at later larval and early juvenile stages often occurs because of cannibalism during weaning to dry diets. Fish that lag in the process of weaning are always growth-retarded and, for carnivorous species, become victims of cannibalistic behavior. It is generally related to genetics and larval behavior. On other hand, the larval behavior is governed by environmental conditions, such as food availability, food type, nutritional composition of the food, population density, light intensity, refuge availability, and water clarity. In red drum, cannibalism increased fivefold as the size difference increased from 2:1 to 3:1. The cannibalism rate increased in red drum as the stocking density increased, but it did not in common snook (Dowd & Clarke 1989).

Many studies have been conducted to identify methods to reduce aggressive behavior. Shallow water and rapid current systems have proven useful in reducing mortality due to cannibalism in mahimahi (Kim et al. 1993; Ostrowski 2000) and in Pacific threadfin during transition from live to pelleted feeds (Ostrowski et al. 1996). Rapid current provides an escape mechanism for targeted fish, keeps others occupied by swimming, and animates pellets to attract fish and promotes faster weaning. Shallow water also assists in animating combination with proper diet and feeding regimen, is faster and more uniform growth of fish, with less opportunity to attack and feed on siblings. To achieve uniform growth in summer flounder, and thereby reduce cannibalism, Bengtson (1999) synchronized the time of metamorphosis. Other approaches to minimize cannibalism include satiation feeding, optimal feeding frequency, live food supplement, optimum particle size of the dry feed, determination of photic preferences, size grading of larval, removal of dominant cannibals, and determination of optimal stocking density.

Deformities and pigmentation abnormalities are common problems in many finfish hatchery productions. For example, opercular deformities have been reported in Pacific threadfin (Ostrowski & Molnar 1998) and pigmentation abnormalities in flounders (Seikai et al. 1991; Huber et al. 1999). Jaw deformities have been observed in mahimahi, striped mullet and milkfish, while spinal deformities have been observed in striped mullet. Nutritional factors and abiotic factors are all possible causes. The mass researches on mechanism of cause deformity and the methods to minimize deformity are still ongoing (Naess et al. 1995; Denson & Smith 1997; Dhert et al. 1997; Iwata & Kikuchi 1998; Burke et al. 1999; Huber et al. 1999; Jenkins & Smith 1999).

Facilities design

Facilities for broodstock. A round fiberglass tank 6 m in diameter and 1 m deep is recommended by The Oceanic Institute (Ostrowski & Molnar 1998) for broodstock cultivation. A pipe located at the center of the tank should nearly reach the surface and have holes at the bottom, collecting water from the top and bottom. The outside standpipe is secured with glue. Broodstock tanks should be covered with a double layer of 80 % shade-cloth or a roof when placed outdoor. Shading helps to control algae growth.

Hatchery system. In the US, existing marine finfish hatcheries use fiberglass tanks for larval production. Liu & Kelley (1994, 1995) and Ostrowski & Molnar (1998) summarized facilities design for hatchery production as follows: the size of the rearing tanks varies from 4000 to 8000 L. Tanks larger than 10000 L are not frequently used in the US. The shape of the tank is generally cylindrical to facilitate water circulation and waste removal. The fiberglass tanks should be painted with black epoxy inside. Interior walls of tank should be smooth, and the bottom exhibit a slight slope toward the center drain to which a center standpipe can be fitted. Water flow-through rearing systems are more common than closed systems, since water exchange is necessary for better water quality. It is standard practice to increase the rate of water-exchange as the fish larvae get older, the metabolism become higher. Each rearing tank is provided with a water inflow, air inlet, illumination and water outflow. The center standpipe should be equipped with a coupling to which a short screened pipe can be attached. The screen pipe is a section of PVC perforated with many holes and covered with 250-1000 μm nytex screen to prevent the eggs and larvae from being flushed out at different stages.

Nursery system. The nursery has been identified as the key bottleneck that limited mass production from the hatchery because fish juveniles of most species are cannibalistic and heavy losses of fish can occur if the behavior is not properly controlled. Emphasis is placed on system design and providing adequate amounts of feed continuously. Ostrowski et al. (1996) developed a shallow water system design to minimize cannibalism during Pacific threadfin nursery stage. The water level in the tank should be low and shallow because juvenile fish orient to the bottom of tanks, and spray bars are used to create current in the shallow water tank. The shallow water level and directional water current force juveniles to be preoccupied by swimming against the current. This makes them less prone to attack and assists individuals in escaping aggressors. The shallow water design also provides greater contact between fish and feed particles to more evenly distribute feed among all individuals and to minimize size disparity as they grow. Survival rates of up to 90 % were achieved with growth rates of 20 % body weight daily.

Prospect

With the increased demand for seafood in the US, it is expected that greater numbers of fish juveniles and more high-value fish species will be produced in hatcheries in the near future. Seed production must also be improved in both quantity and quality. It is expected that both producers and consumers will demand a better quality product in the future. The quality of seed or gametes, in turn, is affected by all the processes involved in seed production. Indeed, the new challenge will not be the quantity but the quality. Desired research and technology development will be focused on: (1) basic nutritional studies for fish larvae and new formulated feeds development for improving survival and growth rate; (2) the control of sexual maturation for year-round or designated production by studying the reproductive biology of a specific fish under specific conditions and environmental and hormonal manipulation based on a better understanding of the mechanism of maturation at molecular level; (3) sterility and sex differentiation, including hormonal sex control and sterilization techniques and chromosome set manipulation; (4) disease prevention and control, including development of DNA vaccines for the control of viruses in fry and juvenile fish and application of molecular diagnosis for the control of mycobacteriosis in selected species of broodstock; (5) adaptation to extreme environment, by quantitative genetic approaches to establish domestication selection population; and (5) improving efficiency of food utilization, and the enhancement of the nutritional qualities of final products.

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References

Ako, H., Tamaru, C. and P. Bass. (1994) Enhancing the resistance to physical stress in larvae of *Mugil cephalus* by the feeding of enriched *Artemia* nauplii. Aquaculture 122, 81-90.

Arnold, C.R. (1998) Controlled year-round spawning of red drum *Sciaenops ocellatus* in captivity. Contrib. Mar. Sci. 30, 65-70.

Arnold, C.R., Bailey, W.H. and T.D. Williams. (1977) Laboratory spawning and larval rearing of red drum and southern flounder. Proc. Annu. Conf. Southeast. Assoc. Fish Wildl. Agencies 31, 437-440.

ARS. (1999) Agriculture Research Service (ARS), online version at http://www.nps.ars.usda.gov/programs/106b.htm.

Baker, E.P., Alves, D. and D.A. Bengtson. (1998) Effects of rotifer and *Artemia* fatty-acid enrichment on survival, growth and pigmentation of summer flounder *Paralichthys dentatus* larvae. J. World Aquacult. Soc. 29, 494-498.

Bengtson, D. (1999) Aquaculture of summer flounder (*Paralichthys dentatus*): status of knowledge, current research and future research priorities. Aquaculture 176, 39-49.

Bengtson, D. and G. Nardi. (1995) Summer flounder culture: an update on research and the development of an industry. J. Shellfish Res. 14, 239.

Bengtson D., Lyndon, L. and J.D. Ainley. (1999) Green-water rearing and delayed weaning inproved growth and survival of summer flounder. North Am. J. Aquacult. 61, 239-242.

Berlinsy, D.L., King, W.V. and T. Smith. (1996) Induced ovulation of the southern flounder *Paralichthys Lethostigma* using gonadotropin releasing hormone analogue implants. J. World Aquacult. Soc.27, 143-152.

Berlinsy, D.L., King, W.V. and R. Hodson. (1997) Hormone induced Spawning of summer flounder *Paralichthys dentatus*. J. World Aquacult. Soc. 28, 79-86.

Bringmeyer, R.L. and G.J. Holt. (1998) Highly unsaturated fatty acids in diets for red drum (*Sciaenops ocellatus*) larvae. Aquaculture 161, 253-267.

Burke, J.S., Seikai, T. and Y. Tanaka. (1999) Experimental intensive culture of summer flounder, *Paralichthys dentatus*. Aquaculture 176, 135-144.

Chambers, M.D. and A.C. Ostrowski. (1999) Development of the bluefin trevally (*Caranx melampygus*) and greater amberjack (*Seriola dumerilli*) for offshore aquaculture. Joining Forces with Industry. Proceedings of an International Conference on Open Ocean Aquaculture, Texas Sea Grant Publication No.TAMUSG-99-103.

Colura, R.L., Henderson-Arzapalo, A. and A.F. Maciorowski. (1991) Culture of red drum. In: McVey, J.P. (Ed.), Handbook of Mariculture. Finfish Aquaculture, vol.II.CRC Press, Boca Raton, FL, USA, pp.149-166.

Crim, L.W. and S. Bettles. (1997) Use of GnRH analogues in fish culture. In: Fingerman, M., Nagabhushanam, R., Thompson, M.F. (Eds.), Recent Advances in Marine Biotechnology, Vol. 1: Endocrinology and Reproduction. Oxford and IBH Publishing, New Delhi, pp. 369-382.

Crim, L.W. and D.M. Evans. (1983) Influence of testosterone and / or LHRHa on precocious sexual development in the juvenile rainbow trout. Biol. Reprod. 29, 137-142.

Daniels, H.V. and R.G. Hodson. (1999) Weaning success of southern flounder juvenile: effects of changeover period and diet type on growth and survival. North Am. J. Aquacult. 61, 47-50.

Daniels, H.V., Berlinksky, D.L. and R.G. Hodson. (1996) Effect of stocking density, salinity, and light intensity on growth and Survival of southern flounder *Paralichthys lethostigma* larvae. J. World Aquacult. Soc. 27, 153-159.

Denson, M.R. and T. Smith. (1997) Diet and light intensity effects on survival, and pigmentation of southern flounder, *Paralichthys lethostigma.* J. World Aquacult. Soc. 28, 366-373.

Devresse, B., Leger, P. and P. Sorgeloos. (1994) Improvement of flat fish pigmentation through the use of DHA-enriched rotifers and Artemia. Aquaculture, 124, 287-288.

Donaldson, E.M. (1973) Reproductive endocrinology of fishes. Am. Zool. 13, 909-927.

Donaldson, E.M. and G.A. Hunter. (1983) Induced final maturation, ovulation and spermiation in cultured fishes. In: Hoar, W.S., Randall, D.J., Donalson, E.M. (Eds.), Fish Physiology. Reproduction. Vol. IXB, Academic Press, Orlando, FL, pp. 351-403.

Dowd, C.E. and M.E. Clarke. (1989) An experimental investigation of cannibalism in hatchery-reared juvenile redfish (*Sciaenops ocellatus*) and snook (*Centropomus undecimalis*). The Early Life History of Fish. The Third ICES Symposium, Bergen, 1988. Rapp. P.-V.Reun. Ciem., vol. 191, p.486.

Eda, H., Murashire, R. and B. Eastham. (1990a) Survival and growth of milkfish *Chanos chanos* larvae in the hatchery: Feeding. Aquaculture 89, 233- 244.

Eda, H., Murashire, R. and Y. Oozeki. (1990b) Factors affecting intensive larval rearing of striped mullet, Mugil cephalus. Aquaculture 91, 281-294.

Ellis, E.P., Watanabe, W. and O. Ellis. (1997) Effects of turbulence, salinity, and light intensity on hatching rate and survival of larval Nassau grouper, *Epinephelus striatus*. J. Appl. Aquacult. 7 (3), 33-43.

Estevez, A. and A. Kanazawa. (1996) Fatty acid composition of neural tissues of normally pigmented and unpigmented juveniles of Japan flounder using rotifer and Artemia enriched in n-3 HUFA. Fish. Sci. 62, 88-93.

FAO. (1999) The State of World Fisheries and Aquaculture 1998. Food and Agriculture organization (FAO), Rome, 112pp.

Goren, A., Gustafson, H. and D. Doering. (1995) Field trails demonstrate the efficacy and commercial benefit of a GnRHa implant to control ovulation and spermiation in salmonids. In: Goetz, F.W., Thomas, P. (Eds.) Reproductive Physiology of Fish, Fish Symposium 95, Austin, Texas, pp.99-101.

Harmin, S.A. and L.W. Crim. (1992) Gonadotropin releasing-hormone analog (GnRH-A) induced ovulation and spawning in female winter flounder, *Pseudopleuronectes americanus* (Walbaum). Aquaculture 104, 375-390.

Harmin, S.A. and L.W. Crim. (1993) Influence of gonadotropin hormone-releasing hormone analog (GnRH-A) on plasma sex steroid profiles and milt production in male winter flounder, *Pseudopleuronectes americanus* (Walbaum). Fish Physiol. Biochem. 10, 399-407.

Henderson-Arzapalo, A. (1995) Review of the nursery and growout culture techniques for red drum (*Sciaenops cellatus*). In: Culture of High-Value Marine Fishes in Asia and the United States. Proceedings of a Workshop in Honolulu, HI, 1994, Oceanic Institute, Hawaii, U.S.A, pp. 67-80.

Henny, D.C., Holt, G.J. and C.M. Riley. (1995) Recirculating-water system for the culture of marine tropical fish larvae. Prog. Fish-Cult. 57, 225-291.

Hodson, R. and C.V. Sullivan. (1993) Induced maturation and spawning of domestic and wild striped bass, *Morone saxatilis* (Walbaum), broodstock with implanted GnRH analogue and injected hCG. Aquacult. Fish. Manage. 24, 389-398.

Houssy, B.A. (1930) Accion sexual de la hipofisis en los pecesy reptiles. Rev. Soc. Arg. Biol. 106, 686-688.

Huber, M., Moore, E. and N. Marcaccio. (1999) Effects of photoperiod on survival, growth and pigmentation of summer flounder (*Paralichthys dentatus*) larvae in laboratory culture (Abstract only). J. Shellfish Res. 18, 268.

Iwata, N. and K. Kikuchi. (1998) Effects of sandy substrate and light on hypermelanosis of the blind side in cultured Japanese flounder *Paralichthys olivaceus*. Environ. Biol. Fishes, 52, 291-297.

Katzman, P.A. and E.A. Doisy. (1932) Preparation of extracts of the anterior pituitary-like substance of urine of pregnancy. J. Biol. Chem. 98, 739-754.

Jenkins, W.E. and T. Smith. (1999) Pond nursery production of southern flounder (*Paralichthys lethostigma*) and weaning to commercial diets. Aquaculture 176, 173-180.

Jenkins, W.E., Smith, T. and C.V. Sullivan. (1997) Production of southern flounder *Paralichthys lethostigma* in an outdoor nursery pond. J. World Aquacult. Soc. 28, 28, 211-214.

Kelley, C.D., Tamaru, C.S. and C. Lee. (1991) Effects of photoperiod and temperature on the annual ovarian cycle the striped mullet, *Mugil cephalus*. In: Proceedings of the Fourth International Symposium on Reproductive Physiology of fish, Norwich, UK, pp.142-144.

Kim, B.G., Ostrowski, A.C. and C. Brownell. (1993) Review of hatchery design and techniques used at The Oceanic Institute for intensive culture of the mahimahi (*Coryphaena hippurus*) on a commercial scale. In: Finfish Hatchery in Asia. Proceedings of Finfish Hatchery in Asia ’91, Tungkang, Taiwan. Tungkang Marine Laboratory, TFRI, Taiwan, pp. 179-190.

Kraul, S. (1991) Hatchery methods for the mahimahi, *Coryphaena hippurus* at the Waikiki Aquarium. In: McVey, J.P. (Ed.), Handbook of Mariculture. Finfish Aquaculture. CRC Press, Boca Raton, FL., USA, pp.241-250.

Lasswell, J.L., Garza, G. and W.H. Bailey. (1977) Status of marine fish introductions into the fresh waters of Texas. Proc. Annu. Conf. Southeast. Assoc. Fish Wildl. Agencies 31, 399-403.

Laidley, C.W. (2001) Protocol for Strip-spawning Gulf of Mexico red snapper *Lutjanus campechanus*. Personnel summarization.

Lam, T.J. (1982) Application s of endocrinology to fish culture. Can. J.. Aquat. Fish. Sci. 39, 11-137.

Lee, C.-S. (1995) The milkfish. In: Neiman-Sorensen, A., Tribe, D.E., Nash, C.E., Novotny, A.J. (Eds.), World Animal Science Series: C 8. Production of Aquatic Animals—Fishes. Elsevier, Amsterdam, pp.239-251.

Lee, C.-S. (1997a) Marine finfish hatchery technology in the USA—status and future. Hydrobiologia 358, 45-54.

Lee, C.-S. (1997b) Advanced technology in sustainable marine finfish culture: fry production. In: Sustainable Aquaculture. Proceedings of the INFOFISH-AQAUATECH 96: International Conference on Aquaculture, Kuala Lumpur, Malaysia. INFOFISH, Kuala Lumpur, pp.48-58.

Lee, C.-S. and C.D, Kelley. (1991) Artificial propagation of mullet in the United States. In: McVey, J.P. (Ed.) Handbook of Mariculture. Finfish Aquaculture. CRC Press, Boca Raton, FL, USA, pp. 193-209.

Lee, C.-S. and C.S. Tamaru. (1993) Live larval food production at the Oceanic Institute, Hawaii. In: McVey, J.P. (Ed.) Handbook of Mariculture. Crustacean Aquaculture. CRC Press, Boca Raton, FL, USA, pp. 15-28.

Lee, C,-S., Tamaru, C.S. and L.W. Crim. (1986) Preparation of a luteinizing hormone-releasing hormone cholesterol pellet and its implantation in the milkfish (*Chanos chanos Forsskal*).

Lee, C.-S. and G.M. Weber. (1986). Effects of salinity and photoperiod of 17α-methytestosterone induced spermatogenesis in the grey mullet, *Mugil cephalus* L. Aquaculture 56, 53-62.

Lee, C.-S., Tamaru, C.S. and J.E. Banno. (1986a) Influence of chronic administration of LHRH-analogue and / or 17α-methytestosterone on maturation in milkfish, *Chanos chanos*. Aquaculture 59, 147-159.

Lee, C.-S., Tamaru, C.S. and J.E. Banno. (1986b) Induced maturation and spawning of milkfish, *Chanos chanos Forsskal,* by hormone implantation. Aquaculture 52, 199-205.

Lee, C.-S., Tamaru, C.S. and C.D. Kelley. (1986c) Induced spawning of milkfish, *Chanos chanos*, by a single application of LHRH-analogue. Aquaculture 58, 87-98.

Lee, C.-S., Tamaru, C.S. and C.D. Kelley. (1988) The cost and effectiveness of CPH, HCG and LHRHa on the induced spawning of grey mullet, *Mugil cephalus*. Aquaculture 73, 341-347.

Lee, C.-S., Kelley, C.D. and C.S. Tamaru. (1991) The effects of testosterone and 17α-methytestosterone capsules on male maturation in the milkfish, *Chanos chanos*. In: Proceedings of the Fourth International Symposium on Reproductive Physiology of fish, Norwich, UK, p. 280.

Lee, C.-S., Kelley, C.D. and C.S. Tamaru. (1992) The minimum effective dosage of 17α-methytestosterone for induction of testicular maturation in the striped mullet, *Mugil cephalus*. Aquaculture 104, 183-191.

Lee, C.-S. (2000) Production Technology in the New Millennium for Desirable Finfish Seedstock. SUISANZOSHOKU 48(2), 259-260.

Lee, C.-S. and A.C. Ostrowski. (2001) Current status of marine finfish larviculture in the United States. Aquaculture 200, 89-109.

Li, J., Lee, C.-S. and J.E. Banno. (1992) Salinity tolerance of fertilized eggs and yolksac larvae in milkfish (*Chanos chanos Fosksal*). South China Sea Fish. Res. 5, 26-33.

Liu, K. and C.D. Kelley. (1994) The Oceanic Institute Hatchery Manual Series: Striped Mullet (*Mugil cephalus*). The Oceanic Institute, Waimanalo, HI, USA, 88 pp.

Liu, K. and C.D. Kelley. (1995) The Oceanic Institute Hatchery Manual Series: Milkfish (*Chanos chanos*). The Oceanic Institute, Waimanalo, HI, USA, 88 pp.

Lutz, C.G. (2001) Practical genetics for aquaculture. Published by Fishing New Books Press, pp. 235.

Lyon, D.D. and M.R. Fisher. (1998) Temperature and pH-related mortality of red drum larvae in fertilized culture ponds. Prog. Fish-Cult. 60, 227-230.

Minton, R.V., Hawke, J.P. and W.M. Tatum. (1983) Hormone induced spawning of red snapper, *Lutjanus campechanus*. Aquaculture 30, 363-368.

Moriwake, A.M., Moriwake, V.N. and A.C. Ostrowski. (2001) Natural spawning of the bluefin trevally *Caranx melampygus* in captivity. Aquaculture 203, 159-164.

Munro, A.D., Scott, A.P. and T.J. Lam. (1990) Reproductive Seasonality in Teleosts: Environmental Influences. CRC Press, Boca Raton, FL, 254 pp.

Murashige, R., Bass, P. and L. Wallace. (1991) The effect of salinity on the surbibal and growth of striped mullet M*ugil cephalus* larvae in the hatchery. Aquaculture, 96, 249-254.

Naess, T., Germain-Henry, M. and K.F. Naas. (1995) First feeding of Atlantic halibut (*Hippoglossus hippoglossus*) using different combinations of Artemia and wild zooplankton. Aquaculture 130, 235-250.

NFI. (1999) National Fisheries Institute. NFI---About our Industry. Aquaculture. http:www.nfi.org/industr2.html.

NMFS. (1999a) Fisheries Statistics and Economics. National Marine Fisheries Service (NMFS) office of Science and Technology, on-line version. http://www.st.nmfs.gov.

NMFS. (1999b) Our Living Oceans. Report on the status of U.S. living marine resources, 1999. U.S. Dep. Commer., NOAA Tech. Memo. NMFS-F/SPO-41, on-line version, http://spo.nwr.noaa.gov/olo99.htm.

Ogle, J.T., Lotz, J.M. and N. Casey. (2000) Larval culture of red snapper *Lutjanus campechanus* using copepod nauplii for first feeding. Book of Abstracts. Aquaculture America 2000, New Orleans, LA, USA, P. 249.

Ohta, H. and H. Tanaka. (1997) Relationship between serum levels of human chorionic gonadotropin (HCG) and

11-ketotestosterone after a single injection of HCG and induced maturity in the male Japanese eel, *Anguilla japonic.* Aquaculture 153, 123-134.

Ostrowski, A.C. (1997) Candidate species of the Pacific: the Hawaiian fisheries development project. In: Open Ocean Aquaculture '97. Charting the future of ocean farming. Proceedings from an international Conference, Maui, HI, USA, pp. 213-222.

Ostrowski, A.C. (2000) Dolphin (mahimahi) culture. In: Stickney, R.R. (Ed.), Encyclopedia of Aquaculture. Wiley & Sons, New York, pp.232-239.

Ostrowski, A.C. and A. Molnar. (1998) Pacific Threadfin *polydactylus sexfilis* (moi) Hatchery Manual. Center for Tropical and subtropical Aquaculture, Waimanalo, HI, USA, Publication Number 132, 96 pp.

Ostrowski, A.C., Iwai, T. and S. Monahan. (1996) Nursery production technology for Pacific threadfin (*Polydactylus sexfilis*). Aquaculture 139, 19-29.

Powell, A.B. and T. Henley. (1995) Egg and larval development of laboratory-reared gulf gulf flounder, *Paralichthys albigutta*, and southern flounder, P. lethostigma. Fish. Bull. 93, 504-515.

Roberts Jr., D.J. (1990) Photoperiod / temperature control in the commercial production of red drum (*Sciaenops ocellatus*) eggs. In: Red drum Aquaculture. Symposium on the culture of Red Drum and Oher Warm Water Fishes, TX, USA. Texas A&M Univ. Sea Grant Program No. TAMU-SG-90-603.

Ronzani C.V., Chatain, B. and P. Lavens. (1991) Photoperiodic effects on the growth and feeding rhythm of European seabass, *Dicentrarchus labrax*, larvae in intensive rearing. Spec. Publ. Eur. Aquacult. Soc. 15, 304-306.

Sea Grant Association. Marine Aquaculture: Economic Opportunities for the 21st Century [Brochure] Texas A&M University Sea Grant College Program No. TAMU-SG-99-603.

Sherwood, N.M., Eiden, L. and M. Brownstein. (1983) Characterization of a teleost gonadotropin-releasing hormone. Proc. Natl. Acad. Sci. U.S.A. 80, 2794-2798.

Shields, R.J., Laidley, C.W. and A.C. Ostrowski. (2002) Recent developments in greater amberjack *Seriola dumerili* culture at the Oceanic Institute, Hawaii. Presentation at '02 European Aquaculture Society Annual Conference.

Smith, T., Jenkins, W. and M. Denson. (1998) Tank and Pond nursery production of juvenile southern flounder (*Paralichthys lethostigma*). In: Nutrition and Techinical Development of Aquaculture. The US-Japan Cooperative Program in Natural Resources (UJNR) Techinical Peport No. 26, University of New Hampshire Sea Grant Program, pp.21-32.

Smith, T., McVey, D. and W. Jenkins. (1999a) Broodstock management and spawning of southern flounder, *Paralichthys lethostigma*. Aquaculture 176, 87-99.

Smith, T., Denson, M., and L. Heyward, Sr. (1999b) Salinity effects on early life stages of southern flounder *Paralichthys lethostigma*. J. World Aquacult. Soc. 30, 236-244.

Smith, T. and M.R. Denson. (2000) Controlled spawning of southern flounder *Paralichthys lethostigma*: issues and progress. In: Spawning and Maturation of Aquaculture Species. Proceedings of the Twenty-eighth US-Japan Natural Resources Aquaculture Panel, 1999, Kihei, HI, USA, University of Hawaii, Sea Grant Program, UJNR Tech. Rep. No. 28.

Tamaru, C., Lee, C. and C. Kelley. (1988) Characterizing the stage of maturity most receptive to an acute LHRH-analogue therapy for inducing milkfish *Chanos chanos* to spawn. Aquaculture 74, 147-163.

Tamaru, C., Kelley, C. and C. Lee. (1989) Effects of chronic LHRH-a + 17α-methytestosterone or LHRH-a + Testosterone therapy on vitellogenesis in the striped mullet (*Mugil cephalus*). Gen. Comp. Endocrinol. 76, 114-127.

Thomas, P. and C. Arnold. (1993) Environmental and hormonal contril of reproduction in sciaenid fish. Recent Adv. Aquacult. 4, 31-42.

Tujigado, A. and C. Lee. (1993) The design of a typical marine finfish hatchery in Japan using Mie Prefectural Fish farming Center as an example. In: Finfish Hatchery in Asia. Proceedings of Finfish Hatchery in Asia '91, Tungkang, Taiwan. Tungkang Marine Laboratory, TFRI, Tungkang, pp. 27-40.

Watanabe, W., Ellis, S. and E. Ellis. (1995) Progress in controlled breeding of Nassau grouper (*Epinephelus striatus*) Broodstock by hormone induction. Aquaculture 138, 205-219.

Watanabe, W.O., Carroll, P.M. and H.V. Daniels. (1998a) Recent progress in controlled reproduction of southern flounder *Paralichthys lethostigma.* In: Nutrition and Technical development of Aquaculture. Twenty-sixth US-Japan Aquaculture

Symposium, 1997, Durham, NH, U.S.A. University of New Hampshire Sea Grant Program.

Watanabe, W.O., Ellis, E.P. and S.C. Ellis. (1998b) Progress in controlled maturation and spawning of summer flounder *Paralichthys dentatus* broodstock. J. World Aquacult. Soc. 29, 393-404.

Watanabe, W.O., Freely, M.W. and S.C. Ellis. (1998c) Light intensity and salinity effects on eggs and yolksac larvae of the summer flounder. Prog. Fish-cult. 60, 9-19.

Watanabe, W.O., Ellis, S.C. and E.P Ellis. (1999) Temperature effects on eggs and yolk sac larvae of the summer flounder at different salinities. North Am. J. Aquacult. 61, 267-277.

Watanabe, W.O. and P.M. Carroll. (2000) Recent progress in controlled reproduction of southern flounder *Paralichthys lethostigma*. UJNR Technical Report No. 28, 141-148.

Weber, G.M. and C.-S. Lee. (1985) Effects of 17α-methytestosterone on spermatogenesis and spermiation in the grey mullet *Mugil cephalus* L. J. Fish Biol. 26, 77-84.

Yaron, Z. (1995) Endocrine control of gametogenesis and spawning induction in the carp. Aquaculture 129, 49-73.

Zohar, Y., Goren, A. and M. Tosky. (1989a) The bioactivity of gonadotropin-releasing hormones and its regulation in the gilthead seabream, *Sparus aurate*: in *vivo* and in *vitro* studies. Fish. Comparison with the use of HCG, Israeli J. Aquacult.—Bamidgeh 41, 105-113.

Zohar, Y., Tosky, M. and G. Pagelson. (1989b) Induced of spawning in the gilthead seabream, *Sparus aurate*, using [D-Ala^6-Pro^{9}Net]-LHRH.

Zohar, Y. and C.C. Mylonas. (2001) Endocrine manipulations of spawning in cultured fish: from hormones to genes. Aquaculture 197, 99-136.

Analysis of Genetic Diversity of *Porphyra* by RAPD

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Abstract

The genetic diversity of 11 *Porphyra* samples was confirmed by random amplified polymorphic DNA (RAPD) markers. Twenty seven primers screened from 46 arbitrary primers were available, and a total of 282 DNA bands were amplified, among which 79.4 % were polymorphic sites, including six specifically amplified bands, which could distinguish *P. haitanesis* from *P. yezoensis*. The genetic diversity was analyzed by means of Nei's genetic similarity, and the generation of dendrogran was used between groups linkage method. The results showed that a close relationship existed between two cultivars of *P. haitanesis* and *P. yezoensis*. The genetic similarity of different *P. yezoensis* ranged from 75.8 to 90.6 %, indicating the genetic diversity level in *P. haitanesis* was relatively high and could be useful in breed selection of excellent populations.

Introduction

Porphyra is one of the three most important economic algal species in China and its output value tops all other species (Shi et al. 2000). The main *Porphyra* varieties in China are *P. yezoensis* and *P. haitanesis*. For a long time, *Porphyra* culturists mainly use the mixed wild varieties as their seedling, resulting in unstable output and poor quality of *Porphyra* production. As the need to develop the industry was addressed, it was found that the variety in seed selection was one of the main limiting factors in developing and utilizing *Porphyra*. The traditional taxonomy is mainly based on the thallus morphological characteristics such as size, margin cells and the number of reproductive cells (Jia et al. 2000). It is difficult to identify the *Porphyra* variety with traditional taxonomy because *Porphyra* has a simple structure and most of its morphological traits are affected by environmental factors like temperature, salinity, tide and illumination.

The random amplified polymorphic DNA (RAPD) marker technique is an effective method in the identification of varieties based on PCR. It has been widely used in animal and plant research (Malyshev & Kartel 1997).

Recently it was used in molecular phylogenic studies of red algae (Dutcher & Kapraun 1994; Patwary et al. 1993) and in variety identification of algae (Ho et al.1995). The main objectives of this research were to detect the genetic diversity of 11 *Porphyra* species by RAPD analysis and to identify the variety of these species. This investigation will provide essential molecular marker background to facilitate breeding of *Porphyra*.

Materials and Methods

Table 1 shows the localities of the *Porphyra* samples used in this study.

Table 1. The localities of *Porphyra* samples

Porphyra lines	Origin	Characters
GL1	Pintan county of Fujian province	raised population of *P.haitanesis*
NH	Pintan county of Fujian province	wild population of *P.haitanesis*
JJS	Pintan county of Fujian province	wild population of *P.haitanesis*
BBD	Pintan county of Fujian province	wild population of *P.haitanesis*
LSD	Pintan county of Fujian province	wild population of *P.haitanesis*
LPZ	Pintan county of Fujian province	raised population of *P.haitanesis*
XSD	Fuqing county of Fujian province	wild population of *P.haitanesis*
DXY	Pintan county of Fujian province	wild population of *P.haitanesis*
LH	Longhai county of Fujian province	raised population of *P.haitanesis*
LYG	Lianyungang county of Jiangsu province	raised population of *P. yezoensis*
RD	Rudong county of Jiangsu province	raised population of *P. yezoensis*

Methods

DNA extraction of thallus. DNA was extracted according to the method by Guo et al. (2000) with some modifications. Quantification of DNA was accomplished by comparative analysis of the DNA sample in 0. 8 % agarose gel alongside diluted lambda DNA.

RAPD analysis. Amplifications were performed in 20μL of reaction mixture containing Tris-Cl 10mmol•L^{-1}, pH8.3, MgCl2 2.0mmol•L^{-1} KCl 50mmol•L^{-1}, 0.15mmol•L^{-1} of each of the four dNTP, 15 ng primer, 25 ng template and 0.3 U Taq DNA polymerase.

After an initial heat denaturation at 95°C for 3min, the reaction mixture was subjected to amplification in a Thermo-Hybaid for 35 cycles consisting of 45 s at 94°C, 1 min at 37°C, and 2 min at 72°C. Final extension was 5 min at 72°C.

The PCR products were separated in 1.2 % agarose gel, stained with ethidium bromide and visualized by illumination with ultraviolet light, then photographed and scanned with Bio-Rad Fluor-S^{TM} Multilmager scanner.

Cluster analysis. DNA fragments generated by PCR amplification were observed as the presence (represented with 1) versus absence (represented with 0). Each amplified band was named according to the primer pair and its size in base pairs (bp).

The values of similarity (GS) were obtained by using the formula $GS(i, j) = 2N(i, j) / [N(i) + N(j)]$ (Nei & Li 1979), where $N(i, j)$ was the number of RAPD fragments shared by the two clones and $N(i)$ and $N(j)$ were the number of RAPD fragments in each line. Then we obtained the genetic distance (GD) by using the formula $D = 1\text{-}S$ and made D- matrix. The SPSS computer program was used for cluster analysis and generation of a dendrogram.

Results

RAPD analysis

The 27 reproducible primers selected from 46 primers were used to amplify all 11 *Porphyra* lines and generated 282 DNA bands. Among the total 282 amplified fragments, 224 (79.4 %) were polymorphic (Table 2). As shown in figures 1 and 2, the polymorphism of the 11 thallus was very high and the result was stable after repeating many times.

Table 2. The sequence of primers and results of PCR

Primer name	Base array	Fragment number	Polymorphic fragment	Percent of polymorphic fragment (%)
S121	ACGGATCCTG	6	3	50
S122	GAGGATCCCT	5	3	60
S124	GGTGATCAGG	9	8	88.9
S125	CCGAATTCCC	7	4	57
S127	CCGATATCCC	10	10	100
S128	GGGATATCGG	10	10	100
S130	GGAAGCTTGG	10	8	80
S131	TTGGTACCCC	5	3	60
S132	ACGGTACCAG	14	13	92.9
S133	GGCTGCAGAA	7	6	85.7
S134	TGCTGCAGGT	6	3	50
S136	GGAGTACTGG	12	11	91.7
S138	TTCCCGGGTT	2	2	100
S139	CCTCTAGACC	4	3	75
S140	GGTCTAGAGG	7	5	71.4
S141	CCCAAGGTCC	7	6	85.7
S142	GGTGCGGGAA	15	9	60
S147	AGATGCAGCC	16	12	75
S148	TCACCACGGT	12	9	75
S150	CACCAGGTGA	6	4	66.7
S152	TTATCGCCCC	24	20	83.3
S155	ACGCACAACC	20	17	85
S156	GGTGACTGTG	9	9	100
S157	CTACTGCCGT	9	6	66.7
S158	GGACTGCAGA	22	17	77.3
S159	ACGGCGTATG	19	16	84.2
S160	AACGGTGACC	9	8	88.9
total		282	224	79.4

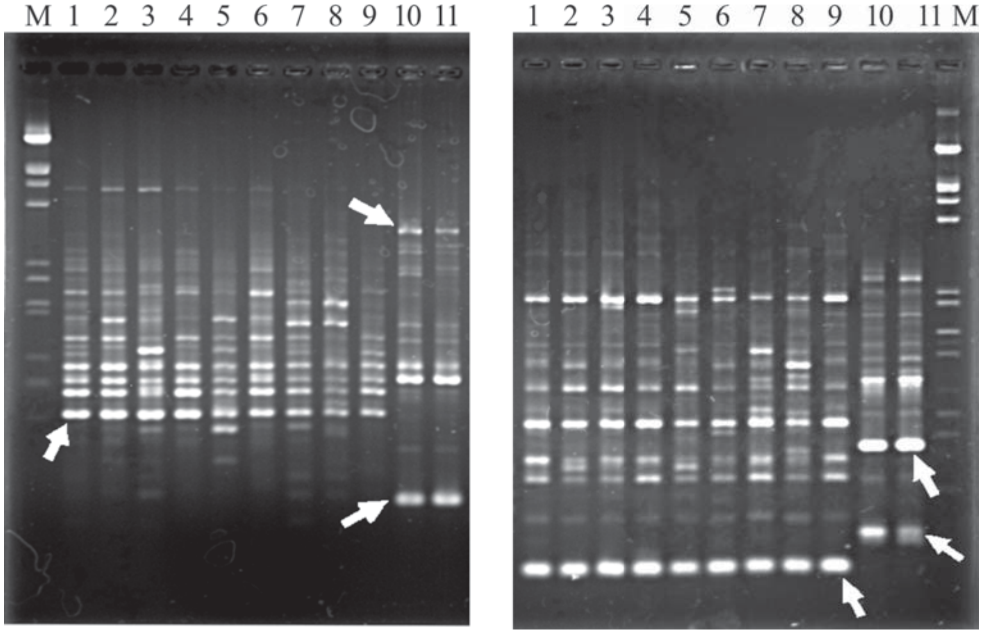

Figure 1. The RAPD electrophoresis patterns of primer of S158 and S14

M:λDNA EcoR I+Hind III; 1-11 Samples in different lanes are as follows:
GL1, NH, JJS, BBD, LSD, LPZ, XSD, DXY, LH, LYG, RD.

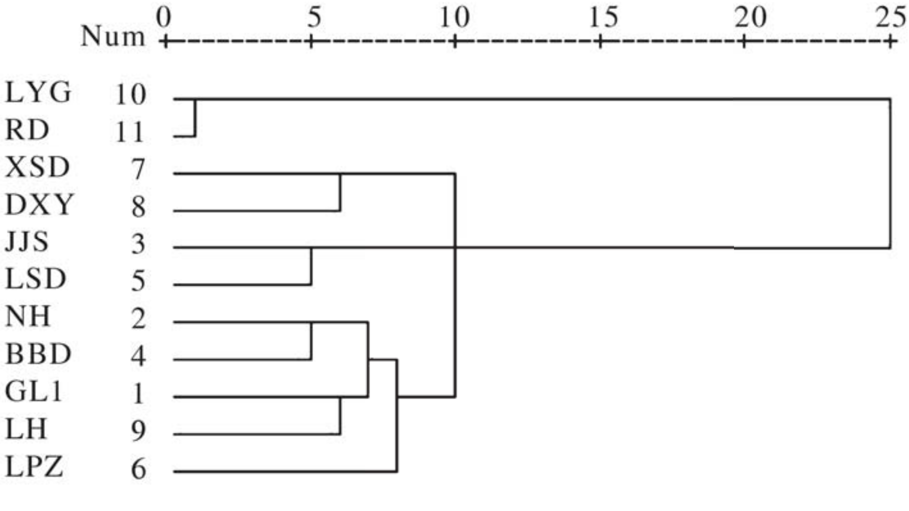

Figure 2. Dendrogram of 11 *Porphyra* samples

Group 1: RD, LYG; Group 2: GL1, NH, JJS, BBD, LSD, LPZ, XSD, DXY, LH

Genetic similarity and cluster analysis

Genetic similarity and cluster analysis was carried out using SPSS 11.0 for Windows software based on the RAPD data. The similarity (GS) of 2 *P. yezoensis* samples was 0.981.The similarity (GS) of 9 P. haitanensis samples was in the range of 0.758 - 0. 906. The similarity (GS) was about 0.5 between *P. yezoensis* and P. haitanensis. A dendrogram was developed using the between groups linkage method (Figure 2). Obviously, the 11 *Porphyra* lines were divided into 2 clusters according to *P. yezoensis* and P. haitanensis.

Discussion

Investigation and identification of the *Porphyra* resources are usually based on the traditionally morphological characteristics, such as peripheral traits, forming regions, and chromatic differences. However, all these methods have intrinsic limitations.

Compared with other molecular marker techniques, RAPD technique is superior in that it can facilitate analysis of fragment polymorphisms more rapidly and economically, and it has been widely applied in species identification, phylogenetic relationships within and among species, test of inherited mutations and hybrid advantage. The authors used data based on 27 out of 46 randomized primers, and there is significant difference in the DNA genetic makeup between and among them. The results based on the 27 primers and the amplification of 11 species samples showed high levels of polymorphisms, and the total replicons were 282, with 79.4 % as polymorphic replicons. These differences in band fragments reflected difference in genomic composition, and indicated the amplitude of resource in China. Further, our experiment provided useful information for further selection and cultivation of species. Meanwhile, both displayed inherited molecular markers (Figure 1), which indicated that primers S147 and S158 could be used to identify the PCR product of both species. We will do further analysis work on these marked bands, such as SCAR transformation and hybridization using marked-bands as probes. Judged from the genetic similarity indexes from the selected 11 resources (Table 3), there is high similarity within (98.1 %). In contrast, this similarity value is slightly lower, which is between 75.8 and 90.6 %. Lastly, the similarity between species is between 50.3 and 55.9 %, which was consistent with the results obtained by Jia et al. (2000), although the value was higher than that of Xu et al. (2001). This discrepancy may contribute to the systematic selection of species with a long history, which homogenizes the heritable background. In contrast, relatively lower selection force has been applied upon species. Different seashore regions with different production procedures could be important factors for these differences between species, and it seems promising to do the cultivation selection within species.

Table 3. Nei's similarity of coefficient matrix among 11 *Porphyra* samples

samples	GL1	NH	JJS	BBD	LSD	LPZ	XSD	DXY	LH	LYG	RD
GL1	1.000										
NH	0.860	1.000									
JJS	0.804	0.834	1.000								
BBD	0.872	0.900	0.824	1.000							
LSD	0.758	0.829	0.906	0.818	1.000						
LPZ	0.857	0.852	0.831	0.829	0.808	1.000					
XSD	0.826	0.805	0.817	0.800	0.800	0.821	1.000				
DXY	0.768	0.769	0.799	0.781	0.777	0.778	0.875	1.000			
LH	0.880	0.848	0.814	0.865	0.803	0.832	0.847	0.840	1.000		
LYG	0.514	0.548	0.523	0.532	0.528	0.540	0.559	0.537	0.506	1.000	
RD	0.510	0.539	0.526	0.522	0.531	0.537	0.557	0.546	0.503	0.981	1.000

Based on the heritable distance, we could obtain the dendrogram of 11 *Porphyra* samples (Figure 2). It is obvious that the 11 species could be categorized into two parts, with RD and LYG similar within species. Similarly, JJS and LSD are both wild types with near geographic distance. XSD and DXY showed a similar pattern. In contrast, NH and BBD live within comparatively short geographic distance. Meanwhile, high current and mixing factors in the open ocean may promote genetic exchange between species, and would display similar phonological relationship over time. LPZ, a systematically selected species, has its quality degraded recently. However, through long-term artificial selection process, genetic divergence has appeared between species.

References

Dutcher, J.A. & Kapraun, D.F. (1994) Random amplified polymorphic DNA (RAPD) identification of genetic variation in three species of *Porphyra* (Bangiales, Rhodophyta).J Appl Phycol, 6, 267-273.

Guo B.T., Bi Y.T., Shan L., Li G..C., Dai J.X. (2000) Extraction of plasmid-like DNA and high-quality total DNA from *Porphyra* yezoensis.Acta Oceanologica Sinica, 22, 87-90.

Ho C.L., Phang S.M., Pang T. (1995) Molecular characterization of *Sargassum polycystum and S.siliquosum* (Phaeophyta) by polymerase chain reaction (PCR) using random amplified polymorphic DNA (RAPD) primers.J Appl Phycol, 7, 37-41.

Shi J.F., Jia J.H., Wang P., Jin D.M., Xu P., Fei X.G., Wang B., Weng M.L. (2000) Development of Specific Molecular Markers for *Porphyra* Lines.High technology letters, 10, 1-3.

Jia J.H., Wang P., Jin M.D., Qu X.P., Wang Q., Li C.Y., Weng M.L, Wang B. (2000) The Application of RAPD Markers in Diversity Detection and Variety Identification of *Porphyra* .Acta Botaniva Sinica, 42(4), 403-40

Malyshev S.V. & Kartel N.A. (1997) Molecular markers in mapping of plant genomes. Mol Biol, 31, 163-171.

Nei M. & Li W.A . (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases [J]. Proc Natl Acad Sci USA, 76, 5269- 5273.

Patwary M.U., Mackay R.M., van der Meer, J.P. (1993) Revealing genetic markers in *Gelidium vagum* (Rhodophyta) through the random amplified polymorphic DNA (RAPD) technique. J Phycol, 29,216-222.

Xu D., Song L.S., Qin S., Alessandro P., Fei X.G., Zeng C.K. (2001) RAPD Analysis of Genetic Variation among Cultivated *Porphyra*. High Technology Letters, 12, 1-3

Cage Culture as a Source of Seed Production for Enhancement of Culture-based Fisheries in Small Reservoirs of Sri Lanka

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Abstract

Aquaculture is not a traditional practice in Sri Lanka. However, the potential of culture-based fisheries in non-perennial reservoirs (seasonal ponds, <80 ha) as an aquaculture practice is increasingly recognized. However, the main problem in popularizing this aquaculture activity is the inadequate availability of seed stock at the required time. The study was based on evaluating the potential of seed production through cage culture practices in perennial reservoirs with community participation to meet the demands for seed stock for culture-based fishery activities.

Trials were carried out with community participation in two perennial reservoirs. Fries of Chinese carps, Indian carps, common carp and cichlids (red tilapia and nile tilapia) were reared in floating cages of 20 m^3. Fries of each species of 28 days old were stocked at densities of 150 – 200 fry•m^{-3}. Ricebran (Rb), commercial feed (Cf), aqua-feed (Af) and non-fed (Nf) were the treatments in duplicate each. Feed was provided at a rate of 10 % body weight per day and was adjusted according to the bi-weekly sampling in respective cages. The rearing period lasted 50–60 days.

The cultured fish (carps and cichlids) have shown positive linear relationship (R^2=0.8929) between survival (%) and average daily weight gain (ADG) in Nf feed type and carp species only have shown positive linear relationship (R^2=0.9836) in Nf feed type in Kiri-Ibbanwewa. They have shown negative linear relationship between survival % and ADG in Af, Cf and Rb. However, the carp species have shown positive linear relationship between survival % and ADG in Cf, Af and Rb feed types and negative linear relationship in Nf feed type in Chandrikawewa. Among these feed types, Rb has shown very strong relationship (R^2=0.9059) in Chandrikawewa.

Considering SGR, ADG and survival % and linear relationship between % survival and ADG, % survival and SGR, Rb could be recommended for Labeo rohita; Cf for *Cyprinus carpio*; and Af and Nf for *Hypophthalmichthys molitrix* in Chandrikawewa. As such, Nf could be recommended for cichlids in Kiri-Ibbanwewa. Accordingly, fingerlings of carps and cichlids could be produced extensively and semi-intensively through cage culture in perennial reservoirs.

Seed production through cage culture could be used as a viable option to overcome the inadequate seed production of the Aquaculture Development Centres for the culture-based fisheries development in seasonal tanks in Sri Lanka.

Introduction

Sri Lanka, unlike most other countries in South Asia, lacks a tradition of fish culture. There are more than 12,000 small non-perennial reservoirs (seasonal tanks) (Das 2000) which for most purposes are comparable to fishponds. They are small in size, dry up for 3-4 months each year and have a potential to develop culture-based fisheries (Mendis 1977; De Silva 1988). For fishing purposes they have to be stocked at the beginning of each farming cycle which will take between 6 – 9 months, depending on the species selected and prevailing weather condition. The possibility of utilizing seasonal tanks for fish culture was recognized as early as the 1960s by fisheries officials (Mendis 1965; Indrasena 1965; Fernando & Ellepola 1969; Fernando & Indrasena 1969). The tanks are comparable to village fish ponds and could have the potential of producing much needed fish proteins for the villagers in addition to being used for irrigating paddy fields, bathing, watering cattle and domestic uses. However, programme to utilize the seasonal tanks for fish culture was first drawn up in 1980 by a planning mission from the FAO Aquaculture Development and Coordination programme (Rosenthal 1979). In the recent near past, De Silva (1983) and Chakrabathy & Samaranayake (1983) have shown the potential of seasonal tanks for extensive fish culture.

However, the large numbers of fingerlings are needed for stocking these seasonal tanks. According to Chandrasoma (1996) major constraint for the utilization of exotic carp species for development of fisheries in perennial reservoirs and seasonal tanks of Sri Lanka is the limited availability of fingerlings. Currently, 8 Aquaculture Development Centres under National Aquaculture Development Authority (NAQDA) are involved in seed production but are unable to provide the necessary requirement of seeds to be stocked the dense seasonal tanks as there are no sufficient spaces to produce the seeds. According to De Silva (1988), the competition for land between the inland fisheries sector and other sectors, primarily the agricultural sector is already increasing. Accordingly cage culture in perennial reservoirs will solve the land problem for seed production.

According to De Silva (1988), the enclosed bays and coves of the reservoirs in the vicinity of hatcheries could be utilized for rearing fry to fingerlings. Further, De Silva (2000) has emphasized recommendation that the cage culture in large reservoirs can be used effectively for rearing fry to fingerlings, particularly in nations where there is a limitation of facilities for this purpose. Chandrasoma (1996) has made comments on this difficulty that the Ministry of Fisheries and Aquatic Resources should encourage the private sector and rural farmers to get involved in the production of fingerlings. As such suggested to pay attention to introduce alternative methods for fingerling rearing using pens and cages.

Accordingly, the study based on the fry-fingerling rearing of exotic carps and cichlids in cages in perennial reservoirs and evaluate the possibility of seed production through cage culture to be used in the stocking of seasonal tanks.

Materials and Methods

Trials were carried out in two perennial reservoirs, Chandrikawewa (Ratnapura District) and Kiri-Ibbbanwewa (Moneragala District), located in the dry zone of Sri Lanka between the latitudes 6º18'- 6º24' N and longitudes 80º50'- 81º E (Fig. 1). The capacities of the two reservoirs are 312 and 256 ha in Full Spill Level, respectively.

The fish species involved are the two tilapia species (Nile tilapia, *Oreochromis niloticus* and red tilapia, *Oreochromis spp*.), two species of Indian carps (catla, Catla catla and rohu, Labeo rohita), one species of Chinese carp (silver carp, *Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*).

The net cages were imported from TAFNETS Corporation in India through Cey-Nor Foundation Ltd in Sri Lanka. Cages were fabricated using High Density Poly Ethylene (HDPE) webbing material with webbing thickness of 0.5-1.5 mm and mesh size of 4 mm knotless. The dimensions of the net cages were 2 m×4 m×2.5 m (20 m^3) and cages were fixed with a top cover. These cages were set up in the respective reservoirs using bamboo (Bambusa spinosa), empty plastic cans (130 L), coir, kuralon and nylon ropes. The cages were set up in each reservoir with the participation of the community in the respective reservoirs.

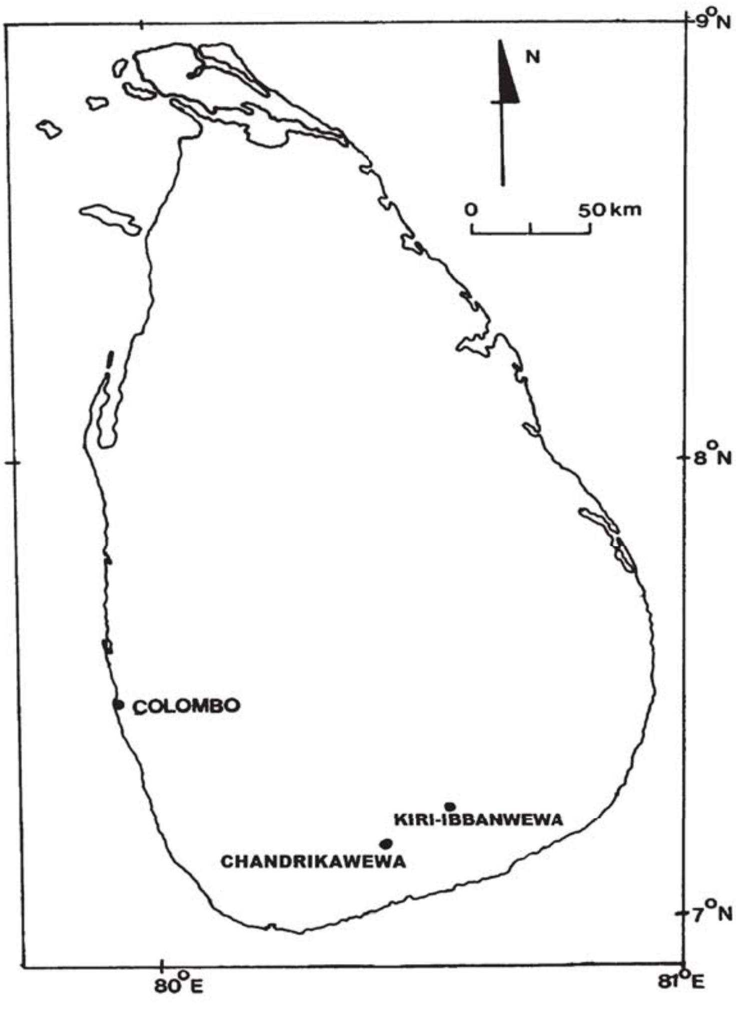

Figure 1. Map of Sri Lanka showing the experimental sites of Chandrikawewa and Kiri-Ibbanwewa

The stocking density used was 150 – 200 fry•m^{-3}. Fry of each species of 28 days old were obtained from the Aquaculture Development Centre in Udawalawa and stocked at around 0900 hrs. The top cover of the cage was fixed to the cage using nylon thread and needle, to prevent fish escapes. The top cover and side walls of cages were cleaned at an interval of 10 days to ensure water current into the cages. Before stocking fish, three fish samples (10 fish each) were collected and the total body length and initial weight were determined.

The rearing period lasted for 50–60 days. There were four treatments with duplicates each: (1) feeding rice bran; (2) feeding commercial feed; (3) feeding aqua-feed; and (4) non-feeding. Fish cages were selected for respective feed types randomly. Feed was provided at a rate of 10 % body weight per day. The amount of feed was determined according to the mean body weight during initial sampling and was adjusted according to the mean body weight of bi-weekly sampling of respective cages. Twenty fish were collected randomly from the respective cages as the sample to determine the mean body weight of the fish.

Ricebran is locally available in the research areas since agriculture is the main activity in those areas. Commercial feed was brought from Grain Elevators (Pvt) Ltd in Colombo. Aqua-feed was prepared by mixing locally prepared fishmeal, ricebran and a small amount of boiled cassava tube. Fishmeal was prepared by the fishers using minor cyprinids in respective reservoirs. Minor cyprinids were harvested through small meshed (15-52 mm stretched mesh size) gillnets without catching juvenile cichlids.

Basic physico-chemical parameters such as temperature, pH and Secchi depth were determined in situ using glass mercury thermometer, pH meter (JENWAY-3051) and 20 cm Secchi disk. These parameters were measured inside and outside of each cage bi-weekly. Water samples were collected inside and outside of the cages and fixed with magnesium carbonate ($MgCO_3$) solution for chlorophyll analysis and fixed using Winkler method to determine dissolved oxygen (DO) (APHA 1988). These samples were transported to the laboratory for analyses.

Specific growth rates (SGR) were calculated using the Ricker's equation (Ricker 1979).The average daily growth (ADG) and survival (%) of the fish were calculated at the end of the rearing period according to the following equations of (1), (2) and (3), respectively, and calculations were done in each cage.

$$(1)\ \ \text{SGR} = \frac{\text{Ln}_{\text{Final weight}} - \text{Ln}_{\text{Initial weight}}}{\text{Days of rearing}} \times 100$$

$$(2)\ \ \text{ADG}(\text{g}\bullet\text{day}^{-1}) = \frac{\text{Final weight of fish} - \text{Initial weight of fish}}{\text{Days of rearing}}$$

$$(3)\ \ \text{Survival (\%)} = \frac{\text{No. of fish harvested}}{\text{No. of fish stocked}} \times 100$$

Regression analyses were done between survival % and ADG to determine the relationships of the fish species under the treatments of rice bran, commercial feed, aqua-feed and non-feeding treatments. The software Mini-tab (version 14) was used in the statistical analysis.

The fisher community was organized with the support of Fisheries Extension Officers through the Fisheries Co-operative Societies in the respective reservoirs, during their monthly meeting. A small group of fishers was selected based on their willingness and capabilities. Both males and females were represented in the group. The team was involved in cage setting, cleaning, maintaining, harvesting, feeding and preparing feed ingredients, mainly fishmeal through minor cyprinids.

Results

The carp species showed remarkable survival %, ADG and SGR in non-fed treatment in both reservoirs (Table 1).

The community in Kiri-Ibbanwewa was new to the cage culture practices but the community in Chandrikawewa had some previous experience.The community in Kiri-Ibbanwewa mostly learned cage culture through this 1st trial (common carp rearing). Their lack of experience (mainly in the preparation of aqua-feed, feeding and maintenance of cages etc.) affected the results of common carp rearing in Kiri-Ibbanwewa).

Table 1. The specific growth rate (SGR), average daily growth (ADG) and survival of the cultured fish species in the ricebran, commercial feed, aqua-feed and non-feeding treatments in floating cages, respectively in Kiri-Ibbanwewa and Chandrikawewa.

Fish species	Feed type	Kiri-Ibbanwewa			Chandrikawewa		
		SGR	ADG($g \cdot day^{-1}$)	Survival(%)	SGR	ADG($g \cdot day^{-1}$)	Survival(%)
Common carp	Rb	3.59±0.46	0.013±0.003	38	4.11±2.12	0.012±0.011	38
	Cf	3.15±0.64	0.010±0.004	48	3.88±0.35	0.009±0.002	64
	Af	7.26±0.46	0.084±0.018	10	2.05±0.54	0.003±0.001	36
	Nf	3.67±0.20	0.014±0.002	22	2.89±0.67	0.005±0.002	34
Rohu	Rb	3.16±0.421	0.016±0.004	53	3.99±0.49	0.032±0.003	63
	Cf	4.48±0.02	0.035±0.004	42	2.10±0.21	0.331±0.002	51
	Af	3.47±0.02	0.031±0.004	39	2.57±0.42	0.014±0.005	42
	Nf	3.52±0.36	0.030±0.003	31	2.25±0.10	0.010±0.001	34
Catla	Rb	5.95±0.52	0.023±0.006	23	9.75±1.56	0.117±0.074	<1
	Cf	5.35±0.81	0.012±0.003	46	7.34±2.96	0.014±0.002	<1
	Af	4.44±0.16	0.010±0.001	25	5.19±0.59	0.014±0.004	<1
	Nf	5.42±0.30	0.017±0.003	25	6.75±0.63	0.028±0.008	<1
Silver carp	Rb	1.93±0.41	0.012±0.005	19	3.96±0.78	0.016±0.001	31
	Cf	1.76±1.11	0.012±0.008	40	2.88±0.18	0.015±0.002	28
	Af	2.28±0.43	0.014±0.007	34	3.88±0.15	0.015±0.002	21
	Nf	2.25±0.38	0.016±0.007	24	3.44±0.75	0.015±0.001	23

Rb=ricebran, Cf=commercial feed, Af=aqua-feed, Nf=Non-fed

The cichlids showed high average daily growth in non-fed feed type and high survival % in rice bran feed type (Table 2).

The regression analysis of ADG and survival % of carps showed positive relationship in the three feed types of ricebran, commercial feed and aqua-feed. Among them a stronger positive relationship was shown in Rb feed type in Chandrikawewa (Figure 2).

Table 2. The specific growth rate (SGR), average daily growth (ADG) and survival % cichlids(red tilapia and Nile tilapia) in the ricebran, commercial feed, aqua-feed and non-feeding treatments in floating cages, in Kiri-Ibbanwewa.

Fish species	Feed type	SGR	ADG(g•day^{-1})	Survival(%)
Nile tilapia	Rb	4.02 ±0.63	0.022 ±0.008	112
	Cf	5.76 ±0.78	0.066 ±0.028	40
	Af	4.76 ±0.97	0.053 ±0.027	26
	Nf	4.70 ±0.57	0.048 ±0.015	30
Red tilapia	Rb	5.27 ±0.19	0.053 ±0.027	26
	Cf	4.67 ±0.57	0.048 ±0.015	30
	Af	5.27 ±0.19	0.025 ±0.002	82
	Nf	6.69 ±0.10	0.082 ±0.006	38

Rb=ricebran, Cf=commercial feed, Af=aqua-feed, Nf=non-fed

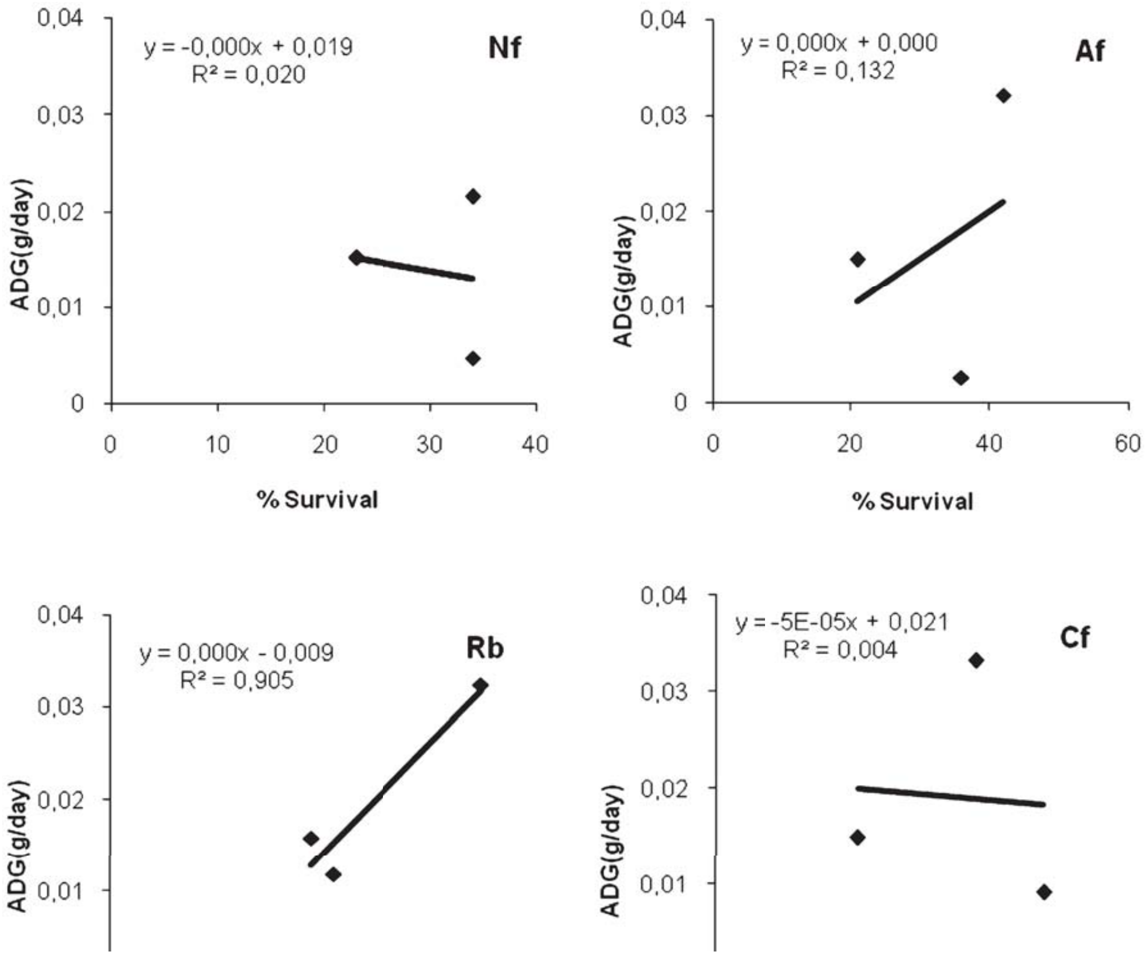

Figure 2. The relationship of survival % and average daily growth (ADG) of carps in ricebran (Rb), commercial feed (Cf), aqua-feed (Af) and non-fed (Nf) feed types in Chandrikawewa

Fish species of carps and cichlids showed positive linear relationship between the % survival and ADG in Nf feed type but showed a negative linear relationship with Rb, Cf and Af feed types in Kiri-Ibbanwewa (Fig. 3). As such, the used carp species showed a strong positive relationship in survival % in ADG in Nf feed type in Kiri-Ibbanwewa (Fig.4).

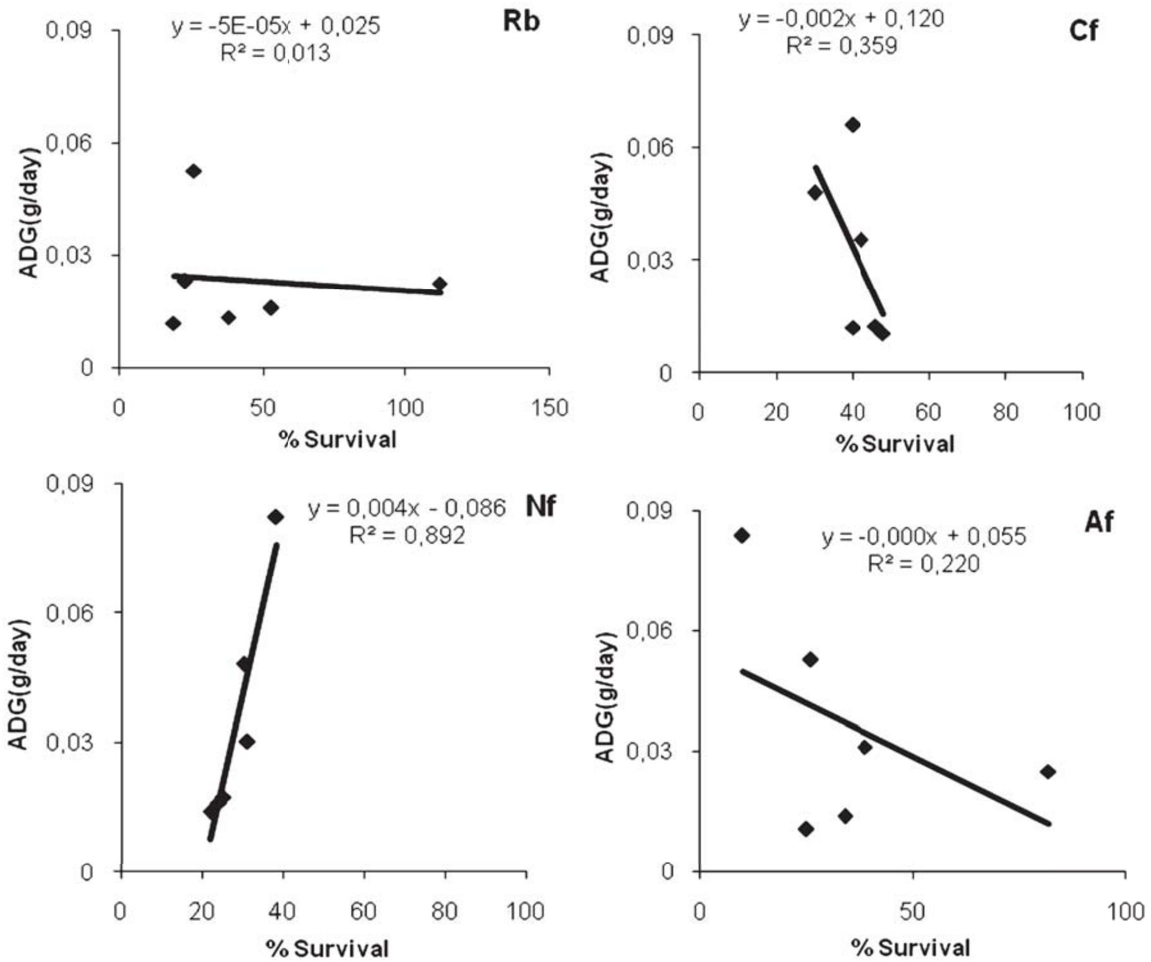

Figure 3. The relationship of % survival and average daily growth (ADG) of carps and Tilapia on rice bran (Rb), commercial feed (Cf), aqua-feed (Af) and non-fed (Nf) feed types in Kiri-Ibbanwewa

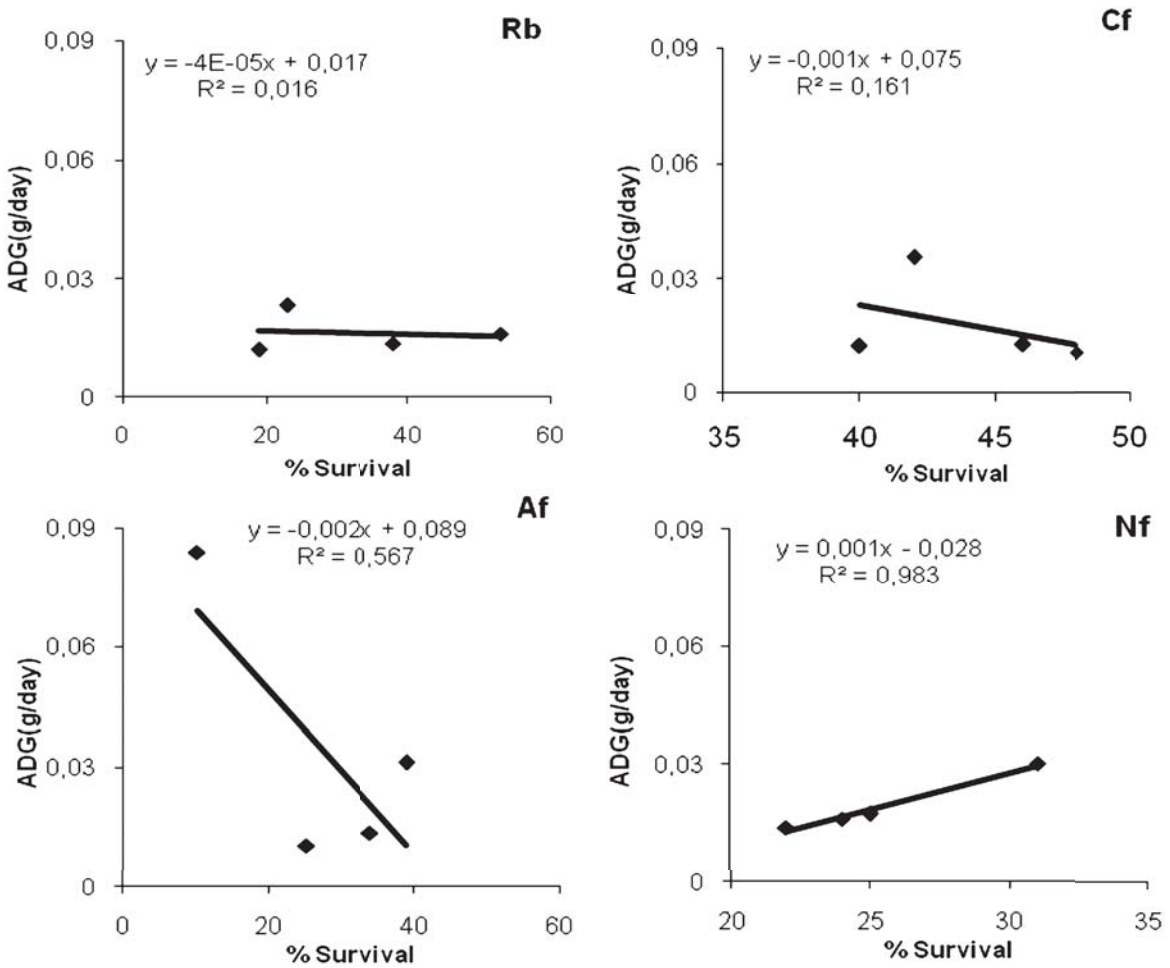

Figure 4.The relationship of survival %and Average Daily Growth (ADG) of carps in rice bran (Rb), commercial feed (Cf), aqua-feed (Af) and non-fed (Nf) feed types in Kiri-Ibbanwewa

Discussion

Seed production through cage culture could be used as a viable option to overcome the inadequate seed production of the Aquaculture Development Centres for the development of culture-based fisheries in seasonal tanks in Sri Lanka. Carps (common carp, rohu, catla and silver carp) and cichlids (red tilapia and Nile tilapia) could be reared in cages extensively in Kiri-Ibbanwewa. As such the same carp species could be reared in Chandrikawewa with supplementary feed including ricebran, commercial feed and aqua-feed.

The regression analysis showed the relationship of ADG and survival % of fish in three feed types (Cf, Rb and Af) in Kiri-Ibbanwewa as a negative linear relationship (Fig. 2). It may be due to the failure of utilizing feed due to the high wave action in Kiri-Ibbanwewa. The feed provided may be displaced and washed away from the cages in Kiri-Ibbanwewa due to heavy wave action. However, the regression analysis of ADG and survival % of fish in the same three feed types in Chandrikawewa has shown a positive relationship (Fig. 3). It may be due to the calm situation in Chandrikawewa. The absence of high wave action enabled the fish to utilize feed.

It envisaged that the supplementary feed could be used in seed production through cage culture in Chandrikawewa. Accordingly the coves and bays of perennial reservoirs where wave action is not too strong would be more suitable for semi-intensive cage culture, particularly for rearing fry up to fingerlings. As such, the time period without winds and high wave action in perennial reservoirs would be more suitable for fingerlings production through cage culture. However, future research on cage culture should be focused on the selection of suitable site and suitable time periods for fry-fingerlings rearing through cage culture.

Fish species of carps and cichlids showed strong positive linear relationship between the survival % and in ADG in Nf feed type (R2=0.8929) and (Fig. 3) carps alone showed a stronger relationship (R2=0.9836) in the same feed types in Kiri-Ibbanwewa (Fig. 4). Nevertheless both carps and cichlids and carps only showed negative linear relationships in survival % and ADG in three feed types (Af, Cf and Rb) in Kiri-Ibbanwewa (Figs. 3 & 4). Accordingly, it has proven that the use of supplementary feed for fry-fingerlings rearing in cages in the perennial reservoirs similar to Kiri-Ibbanwewa is not required. It could be done extensively. According to Pantastico et al. (1986), the diatom Navicula and blue green algae Chroococcus were the most acceptable feed for tilapia (O. niloticus) fry. Increasing the amount of these species was assimilated as the tilapia fry grew to fingerling stage. Nevertheless the highest survival rate was shown with Oscillatoria. As such, the larval rearing of silver carp showed that better growth is acheived when they feed on cyanophyte (Spirulina). However, these should be productive reservoirs. The productive reservoirs could be selected through Geographical Information Systems (GIS) or by testing the water quality. For the abundance of natural food in the cages it is necessary to clean the top and sides of the cages to enable water exchange into the cage.

The carp species showed positive linear relationship between the % survival and in ADG in three feed types(Af, Cf and Rb) and negative linear relationship in Nf feed type in Chandrikawewa (Fig. 2). It has proven that supplementary feed could be used in fry-fingerlings rearing in the reservoirs similar to Chandrikawewa, effectively. However among these feed types, Rb showed a higher and strong R2 value (R2=0.9059). Accordingly Rb feed type could be recommended for rearing carp fry up to fingerlings through cage culture in Chandrikawewa.

Feed were provided twice per day once in the morning and once in the evening. Accordingly, fish should utilize all the feed requirements during these two times. Nevertheless, since cyprinids are stomach less, they cannot store feed accordingly. This may be one of the reasons for low R2 value in carp species with supplementary feed (Rb, Cf and Af) in Kiri-Ibbanwewa. Therefore, it is necessary to feed cyprinids several times within the day for better feed utilization when carp species are reared in cages.As such, the method of providing feed (use of feeding table) should be improved to keep feed from being washed away due to wave action. It would be considered in future research on cage culture in perennial reservoirs.

Based on the results of cage culture trials in two perennial reservoirs, it could be clearly seen that the Rb and Nf feed types could be considered in seed production of carps and tilapias through cage culture. As such the community could be involved in the system productively.

De Silva et al. (2006) have pointed out the major Chinese and Indian carps and the Common carp possess most of the desired features for culturing in seasonal tanks in polyculture system. These fish species could be produced through cage culture in perennial reservoirs and could be used in the stocking of seasonal tanks in poly culture system. It is therefore necessary to encourage the community to engage in this seed production process. As such the technical know-how should be given to them through practical participation (Farmer Field School). It could be done by the Extension Officers through co-operative societies in the respective reservoirs. Currently, most of the NGOs (Non-Governmental Organizations) are involved in the culture based fisheries in seasonal tanks purchasing fingerlings from NAQDA and stocking them in seasonal tanks. Instead of this task, they could provide cages and accessories to the fishers and promote the production of fingerlings in perennial reservoirs through cage culture with community involvement. Further, NGOs could provide transport facilities from the purchase site to the cages. As such, produced fingerlings through cage culture could be transported to the seasonal tanks until fishers could carry out these activities independently, through the co-operative societies.

Based on the experimental project, funded by the UNDP carried out during 1981–1983, it was revealed that culturing fish in seasonal tanks is technically feasible and to ensure its sustainability the participation of village community should be encouraged (Thayaparan 1982; Chakrabathy & Samaranayake 1983; De Silva 1988; Chandrasoma 1986).

The fisher community could obtain much benefit in the production of fingerlings through cage culture in reservoirs such as reduced cost of fingerlings, minimized transportation fee and fish mortality that occur during long distance fish transport. As such this could provide extra income for the fishers. Since all the seasonal tanks and most of the perennial reservoirs are in the dry zone of Sri Lanka, the fingerling production through cage culture in perennial reservoirs and stocking seasonal tanks could be done easily. Accordingly it may be the solution for inadequate fingerlings in proper time to be stocked in dense seasonal tanks in Sri Lanka.

Fish species have shown different ADG, SGR and survival % in four different feed types (Rb, Cf, Af and Nf) in the same rearing period (50-60 days) in these two reservoirs (Tables 1 & 2). The fish species with high ADG, SGR and survival % should be considered for the production of seeds.Accordingly rohu, common carp and silver carp reared with Rb could be highly recommended for cage culture in Chandrikawewa. As such these three fish species could also be reared using aqua-feed . However Common carp has shown lower ADG than other fish species. Accordingly the rearing period should be increased for common carp to produce big size fingerlings as the size of fingerling is very important in the stocking of seasonal tanks.The production of cichlids (red tilapia and Nile tilapia) with non-fed and ricebran feed types could also be recomended as they have obtained high ADG and survival % in these feed types (Table 2). As cited by De Silva et al. (2002), Indian carp, Chinese carp and some tilapia species augmented by very few indigenous species if available should be considered for culture-based fisheries. Accordingly, the process is highly recommended for seed production of carps and cichlids and the enhancement of culture based fisheries in small reservoirs in Sri Lanka.

Recommendations

The use of bamboo in making cage frames has a great advantage for it is cheaper than other materials such as metals or PVC pipes. Furthermore, it is available almost everywhere and also easy to work with. However, transportation and cutting of bamboo have been prohibited by the Forest Conservation Department in Sri Lanka. Accordingly it is necessary to get approval from the Provincial Secretaries in the area and from the Forest Conservation Department of Sri Lanka. The procedure should be flexible for communities which use bamboo in this type of rural development activities. Furthermore the rearing period should be extended to get large size fingerlings.

Acknowledgements

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References

APHA. (1988) Standard Methods for Examination of Water and Waste Water, 17th Edition, Washington DC:American Public Health Association.

Chakrabathy, R.D and R.A.D.B. Samaranayake. (1983) Fish culture in seasonal tanks in Sri Lanka. Journal of Inland Fisheries 2, 125 – 140.

Chandrasoma, J. (1986) Primary productivity and fish yield in ten seasonal tanks in Sri Lanka. J. Inland fish 3, 56- 62.

Chandrasoma, J. (1996) Impact of stocking of exotic carp species on the fish production of man-made lakes in Sri Lanka-A reappraisal. Sri Lanka Journal of Aquatic Sciences 1, 71-80.

DAS. (2000) Data Book for Village Irrigation Schemes of Sri Lanka (23 volumes). Colombo: Department of Agrarian Services.

De Silva, S.S. (1983) The reservoir fishery: present status and future strategies. Journal of Inland Fisheries, Sri Lanka 2, 3-13.

De Silva, S.S. (1988) Reservoirs of Sri Lanka and Their Fisheries. FAO Fisheries Technical. Paper No.298. 128 pp.

De Silva, S.S. (2000) Asian Reservoir Fisheries: Broad Strategies for Enhancing Yields. Paper presented at the Workshop on Reservoir Fisheries Biology & Management, Bangkok, Thailand, 15th to 18th February 2000.

De Silva, S.S., Moehl, J., Satia, B., Bartley, D., and R. Subasinghe. (2002) Inland Fisheries and Aquaculture:A Synergy for Sustainable Food Fish Production.In:Interactions between inland capture fisheries and aquaculture and their contribution to food security and poverty aaleviation in sub-saharan Africa, Twelfth Session of the Committee for Inland Fisheries for Africa (CIFA), Yaounde, Cameroon, 2-5 December 2002.

De Silva, S.S., Amarasinghe, U.S. and T.T.T. Nguyen. (2006) Better-practice approaches for culture-based fisheries development in Asia, p.p 1-96, Australian Centre for International aricultural Research, Australia (ACIAR) Publication. Internet source: www.aciar.gov/publication/MN120-1

Fernando, C.H. and W.B. Ellepola. (1969) A preliminary study of two village tanks (reservoirs in Ceylon. Bulletin Fisheries Research Station., Ceylon 20, 3 - 13.

Fernando, C.H. and H.H.A. Indrasena. (1969) The Freshwater fisheries of Ceylon. Bulletin of Fisheries Research Station, Ceylon 20, 101-134.

Indrasena, H.H.A. (1965) The development of freshwater fisheries in Ceylon. Bull. Fish. Res. Stn., Ceylon. 17, 287-289.

Mendis, A.S. (1965) A preliminary survey of 21 Ceylon lakes.2. Limnology and fish production potential. Bulletin of Fisheries Research Station, Ceylon 16, 7-16.

Mendis, A.S. (1977) The role of man-made lakes in the development of fisheries in Sri Lanka. Proceedings of Indo-Pacific Fisheries Council 17, 245- 254.

Pantastico, J.B., Baldia, S.F. and J.P. Baldia. (1986) Comparative efficiency of some cyanophytes as larval feed for Silver carp (Hypophthalmichthys molitrix) and the culture of Spirulina platensis. Paper presented at the FIRST Asian Fisheries Forum. Phil. Interl.Conv. Center, Manila, Philippines, 26-31 May 1986.

Ricker, W.E. (1979) Growth rate and models. In: Fish Physiology VIII (W.S.Hoar, D.J.Randall & J.R.Brett, eds). Pp.677-743. Academic Press, New York.

Rosenthal, H. (1979) Preliminary report and recommendations on reservoir and tank stocking activities in inland fisheries in Sri Lanka. Interim Report to FAO for the TCP/SRL/8804, Development of fisheries in the man-made lakes and reservoirs. FAO, Rome.

Thayaparan, K. (1982) The role of seasonal tanks in the development of freshwater fisheries in Sri Lanka. Journal of Inland Fisheries, Sri Lanka 1, 133-167.

Assessment of Submerged Tilapia Fish Cage Farming in Lake Buhi, Philippines

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Abstract

An assessment of submerged fish cage farming in Lake Buhi; Camarines Sur, Philippines was conducted using a combination of field survey interview, key informant's interview and actual farm trial. Related biological aspects such as plankton, fouling organism, gut content, and bottom sediment were also collected.

Results obtained reveal that submerged fish caging has significant contribution to the socio-economic well being of the people around the lake. Fish caging is centered on the monoculture of Nile tilapia (*Oreochromis niloticus*) and their hybrids with or without supplemental feeding at stocking densities ranging from 5,000 to 10,000 fingerlings per crop, reared from 6 months to one year depending on the season. The average production ranges from 7.2 kg per square meter (harvest size 2 to 6 pieces per kg) with the lowest recorded production of 1.10 kg per square meter. Mortality ranges from 31% to more than 60%, mostly attributed to poor quality of fingerlings and seasonal occurrence of sulfur upwelling.

As regards the factors affecting technology application and performance, it revolves around the environment, the technology, the resources and capacity of the beneficiaries and the prevailing government policies. It should be noted that aquaculture is always subject to climatic risk and since it has always been treated as a family business, experience is always equated with technological success. It is therefore recommended that better management and regulation of the lake's carrying capacity be initiated to ascertain the limits at which primary production could support.

Introduction

Fish cage farming of tilapias in Lake Buhi is of paramount importance because of its contribution to the economy and society as it provides livelihood, income and employment (Escover and Claveria 1985; Luceña 2000) to fishers along the lake. However, despite its long existence, submerged cages have not been properly assessed. Studies on tilapia cage farming in the region are concentrated on fixed cages as practiced in neighboring Lake Bato, Camarines Sur, and therefore deserve to be investigated.

Being submerged a few meters below water surface, there is a presumption that food availability may be a problem due to light penetration difficulty and placing cages some meters above the lake bottom may correspondingly affect fish growth as a result of organic load decay and oxidation.

It is also worth noting that while fish caging is becoming an increasingly important source of cheap protein, it also creates adverse impacts on lake ecosystem, ranging from localized nutrient enrichment to fish kill at its worst. It should be noted that Lake Buhi is the home of the world's smallest commercial fish sinarapan (*Mistichthys luzonensis, Smith*) and therefore should be protected due to its national significance.

This paper highlights the assessment of submerged cage farming of tilapia in Lake Buhi, with emphasis on the status and performance based on on-farm trial conducted, and the problems and constraints affecting the industry.

Materials and Methods

A total of 150 fish cage farmers/practitioners were interviewed using a Rapid Resource Assessment (RRA) technique to assess the status of submerged fish caging and the factors affecting technology application and performance in Lake Buhi.

On-farm trial was also conducted for dry (April to September) and wet (October to March) seasons using three (3) units of 10 m×16 m×5 m submerged cages with all sides enclosed by a double C-net (10 mm mesh size). Each cage was provided with 8 plastic buoys on edges and counter-weighed by 12 boulders as sinkers. This was installed at a depth of 15 meters immersing the topside of the cages at one-meter distance from the surface level to facilitate navigation, feeding, monitoring, sampling and retrieval of stocks.

The farmer-cooperator's management practice was adopted to elicit the true picture of submerged fish caging. Data on growth, survival, feed conversion ratio and efficiency and recovery were taken during the entire culture trials. Economic performance was analyzed using simple cost and analysis.

Associated biological factors of relevance to fish cage farming such as plankton, fouling organisms, gut content and soil samples were also monitored. Kemmerer water sampler was used to collect samples from the surface, middle and bottom zones of the lake. Plankton was collected within and outside the cages using 28-micron plankton net for phytoplankton and 50-micron for zooplankton. A 250-ml plankton bottle was used for storing and concentrating samples for laboratory examination. All samples were examined microscopically for counting and taxonomic identification purposes using resource book of taxonomic classification made by Umaly and Cuvin (1988) for Phytoplankton and Guide to the Philippine Flora and Fauna (Mamaril 1986) for zooplankton. Fouling organisms were collected from the cage walls and samples were stored in plastic bottles containing 5% formalin.

Live tilapia samples weighing about 98 g were collected both inside and outside the cage for gut content analysis. The samples were dissected and the gut was taken and divided into three portions (fore-gut, mid-gut and hind-gut) for subsequent laboratory analysis. Soil samples from the cage bottom were taken by diving using a compressor. The samples were then air-dried, pulverized using a mortar and pestle and analyzed for soil texture.

Results and Discussion

Status of submerged tilapia fish cage farming in Lake Buhi

Lake Buhi is a 1,800-hectare tropical mountain lake located 105 meters above sea level with an average water depth of 15 meters. It has an outlet where a hydraulic control system is constructed and 11 tributaries that contribute to the large volume of water in the lake. It is also the home of the world's smallest commercial fish, *Mistichthys luzonensis*, locally known as "sinarapan" (Lake Buhi Profile 2003).

At least 70% of the lakeside communities depend on fishing and lake utilization is primarily focused on tilapia caging (182.27 ha), fish corral (21.04 ha) fish pen culture (1.58 ha), and fish coral (21.04 ha) (Lake Buhi Profile 2003). There are some 705 submerged fish cage operators mostly using 16m×10m×5m submerged cages. The average number of cages is 14 units with a total area of 2,240 m^2. The cage size ranges from 112 to 22,600 m^2, averaging at 1,519 m^2 (Bersabal 2004).

Cage farming is centered on the monoculture of Nile tilapia (Oreochromis niloticus) and their hybrids with or without supplemental feeding. Stocking densities range from 5,000 to 10,000 fingerlings per crop, cultured 6 to 12 months depending on the season and the feed used. The average production ranges from 7.2 (2 to 6 pieces per kg) to 1.10 $kg \bullet m^{-2}$. Mortality ranges from 31 to 60% or more, mostly attributed to poor quality of fingerlings and seasonal occurrence of sulfur upwelling (Bersabal 2004). Fish caging has always been treated as a family business (Luceña 2000), thus operation and management are important tasks of family members.

On the overall, submerged fish cage farming of tilapia is a major economic activity that contributes to the livelihood, employment and income of the people. Excluding them from the industry would create serious economic problems on those who depend on it.

Performance of submerged fish cage farming (On-farm trial result)

On-farm trial results showed that wet season farm trial (October to March) had better growth compared to dry season (April to September) (Table 1). This was verified when the farm cooperator deliberately increased the stocking density during the second trial. Similar observation was noted in most fish cage farmers in the area. It should also be noted that farmers' practice include stocking 5,000 fingerlings in three separate cages for six months with feeding given at 5% of the biomass. After which, the stock in each cage are recounted and restocked in one cage for another three months till harvest.

Table 1. Summary of performance of tilapia grown in 10m×16m×5m submerged fish cage over a 9 month rearing period fed with commercial pellets in Lake Buhi, Camarines Sur, Philippines.

Stocking Data	Cropping Trials	
	1	2
Stocking Density (Pieces)	15,000	18,000
Stocking Weight (g)	7.0	15
Total Harvest (kg)	458	2,250
% Survival	9.12	25
ABW (g)	300 – 400	500 –350

Findings showed an overall low survival rate in all trials, which could be attributed to stress during transport, stocking and transfer. In addition, localized dissolved oxygen (DO) deficiency also contributes to mortalities especially when growth of fouling organisms in the cage walls is extensive that it restricted water circulation in the cages. Similarly, as water flow is hindered, removal of waste and organic metabolites was slowed down creating oxygen deficiency due to decay of uneaten and excess feeds.

The cost and return analysis of the on-farm trial is shown in **Table 2.** Results obtained showed negative net benefits of PhP 5,248.50 in the first trial but the same was recovered in the second trial with a gross benefit of PhP 99,168.00. It appears from this result that farmer's experience and proper timing of stocking are important factors in the success or failure of fish cage farming.

Table 2. Cost and return analysis of 10m×16m×5m submerged fish cage for the farming of tilapia (O. niloticus) fed with commercial pellets in Lake Buhi, Camarines Sur, Philippines.

Particulars	Cropping	
	1	2
Revenue:		
Sales from marketable tilapia	25,064.00	146,250.00
Total Sales	25,064.00	146,250.00
Less Expense		
Fingerlings	5,250.50	11,700.00
Feeds	19,462.50	29,632.00
Labor	800.00	800.00
Depreciation	2,500.00	2,500.00
Permit/License	1,500.00	1,500.00
Marketing cost	700.00	700.00
Miscellaneous Expenses	100.00	100.00
Total Expenses	30,262.50	47,082.00
Gross Income Before Tax	(5,198.50)	99,168.00
Assumptions:		
Fingerlings cost	*@ Php 0.35 t0 0.65*	
Feed cost (Tateh)	*@ Php 206.50/bag (fry mash)*	
	@ Php 463.00/bag (grower	
	@ Php 453.00/bag (finisher)	
Tilapia market price	*@ Php 39.00 /kilo (Medium size)*	
	@ Php 65.00/kilo (Large)	
Cost of Cage	*@ Php 12,800.00/unit*	
Cage Life Span	*@ 3 years*	
Depreciation Cost	*@ Php 4,200/unit/year*	

Results also confirm the findings of Lovell (1989) and Shang (1981) that feed constitutes 50-70% of the operational cost. The study obtained 64.21% feed cost. Findings also point to the fact that excess feed contributes to the organic load of the lake bottom as evidenced by the bottom sediments characteristics. This may in turn induce algal bloom, bacterial growth and pollution at the extreme end.

Associated biological factors affecting fish caging

Plankton

Phytoplankton plays a vital role as a primary producer in the aquatic ecosystem contributing over 90% of the total productivity of the sea and large lakes (Bell 1983) and virtually forms the only major food chains in these bodies of the nutritive condition of the water (Welch 1935)

Plankton monitoring showed that majority of the phytoplankton identified in the water within and outside the cage belongs to the division *Chlorophyta* (green algae) followed by Bacillariophyta (diatoms), *Cyanophyta* (blue-green algae), *Pyrrophyta, Chrysophyta and Euglenophyta.* The most common was *Hormidium subtile and Ulothrix sonata.* The most dominant zooplankton identified belongs to the family *Corycaeidae* and the most common species was *Thermocyclops crassus.* A similar finding was reported by Cano (2003) in the study on zooplankton distribution and biomass in the Sinarapan sanctuary in Lake Buhi.

According to Bell (1983) and Thompson (1995) plankton occupies the shallow layer of water called photic zone which is considered as the most productive layer of water for plankton growth but they are also found in the lower layers. It could be inferred therefore that stocked fish in submerged cage have benefited from the abundance of phytoplankton and zooplankton available within the cage perimeter.

Fouling organisms

Findings show that fouling organisms which normally grow in the cage walls are both beneficial and detrimental. On the positive side, tilapia feeds on algae growing in the cage walls, may serve as an extra food source for the stock. On the other hand, luxuriant growth may also cause damage to the stock by blocking the pathway of water circulation. As a matter of fact, dead fish were observed floating every now and then during the farm trials, which could be attributed to dissolved oxygen (DO) deficiency. It should be noted that increased algal growth increases oxygen in the daytime due to photosynthesis. At night, they use it up, thus the dense algal bloom produced during daytime cause the suffocation of fish and other animals. In addition, waste from uneaten and excess feeds used to increase production have a significant environmental effect in the lakes.

Fouling organisms collected include green filamentous algae, *Chlorophyta* (Charra sp.), cladocerans (*Daphnia*) and mollusks (*Pila luzonica*), also referred to as *Ampullaria lagunaensis*. Their growth might have been stimulated through feeding via excess feed and metabolic waste that represents addition of phosphorus, nitrogen and carbon in the aquatic environment. Daphnia was observed free-swimming within and outside the cage. In the study of Ramos (2004) on aquatic productivity in the benthic zone in Lake Buhi, a similar finding was noted.

Gut content

Gut content analysis confirms that tilapia feeds on algae that grow in the cage walls. A similar finding confirmed that tilapia does not only feed on macrophytes, but also ingest the attached algae, bacteria and detritus as reported by Bowen (1982) Fish samples from test cages had a greater percentage of ingested food belonging to filamentous green algae *Chlorophyta,* followed by phytoplankton. This finding strongly supports the fact that fouling organisms are also important food sources of fish in submerged cages. However, no documentation was made on the biomass of eaten fouling organism. A small percentage of zooplankton, *Daphnia* in particular, was noted with rotifers dominating the animal composition of food they eat.

Bottom sediments characteristics

Samples of bottom sediments taken from the culture sites exhibit the general soil characteristics as being silty-loam. It contains a moderate amount of finer grades of sand and clay. Its appearance is cloudy and can readily be broken and pulverized to powder. In dry state, it forms shapes that can be freely handled. In wet state, soil runs together and puddles. It feels smooth and is slightly plastic. It is this textural feature of sediment that may pose a catastrophic chain reaction effect on fish stock because they can easily be carried up by upwelling process together with the organic load accumulated via supplemental feed. In terms of soil texture, the composition of bottom sediments reveals the following features: coarse (26.01%), clay (35.85%) and silt (38.14%). Freshly taken sediment samples from a depth of 10.7 meters indicated sulfur odor indicative of the organic material decomposition. This clearly confirms the idea that uneaten and excess feeds used to increase production have a significant environmental effect if not properly planned and managed.

Problems and constraints

Fish cage farming of tilapias in Lake Buhi has a long history. As a matter of fact, those who have been in this business before have earned from the bounty of the lake's resources. Today, fish caging is beset with problems and constraints associated to the industry's effect on the environment and the environmental effect on the industry (Table 4). It appears that fish cage intensification in the lake to meet the demand for food fish have posed eminent danger to the sustainability of the industry. While it significantly contributed to the socio-economic well being of the people, it also created important problems of alarming concerns. Worth mentioning are sulfur upwelling and typhoons. These are natural phenomena with limited defensive options except proper timing. Poaching appears to be alarming but can be managed if the cage farmers will organize themselves into a cohesive force.

Siltation, fish kill, pollution, and slow fish growth, although not presented according to order of priority, can be considered as interrelated impacts of the environment to fish caging due to its expansion over the years. It should be noted that the increasing the number of fish cages coupled by intensive feeding without considering the limits of what nature can provide is too dangerous. This practice often results to interrelated conditions of organic loading, aquatic pollution and siltation, which at its severe state will result to slow fish growth and fish kill at its worst (de los Trinos 1993).

Table 3. Summary of the problems and constraints encountered by tilapia fish cage farmers in Lake Buhi

Problems and constraints in fish cage farming	F	%
1. Sulfur upwelling	26	17.33
2. Typhoon	23	15.33
3. Poaching	22	14.67
4. Siltation	20	13.33
5. Fish kill	12	8.00
6. Pollution	12	8.00
7. Low fish price	11	7.33
8. Lack of capital	10	6.67
9. Availability of fingerlings	4	2.67
10. Slow fish growth	4	2.67
11. Lack of technical assistance from government	4	2.67
12. Fish cage congestion	1	0.67
13. High cost of feeds	1	0.67
Total	150	100.00

These threats are considered deeply rooted on the mix of economic activities within the lake that placed considerable stress and immense pressure on the lake's resource system, typical of a resource with open access nature

On the other hand, low price, lack of capital, availability of fingerlings and lack of technical assistance from the government are problems that can be easily addressed by concerned government agencies. These can be resolved if the cage farmers will organize themselves into cooperatives, which would in turn handle production up to post-harvest and marketing activities.

It is also worth noting that submerged fish caging has inherent advantages. Worthy to mention are the minimum damage during typhoons, freedom from obstruction to navigation, they are undisturbed by motorized watercraft and do not interfere much on the visual quality of the lake.

In summary, the factors affecting technology application and performance revolve around the environment, the technology, the resources, the capacity of the resource-users and the prevailing government policies and priorities.

Conclusion

On the basis of the findings of the study, the following conclusions are arrived at:

1. While submerged tilapia fish cage farming contributed much to the socio-economic well being of the people in terms of employment and livelihood, it also created negative impacts on the lake's ecosystem.

2. The factors affecting technology application and performance revolve around the integrity of the present environment, the technology, the resources, the capacity of the resource users and the prevailing government policies and priorities.

It is therefore recommended that the carrying capacity of the lakes be studied, managed and protected. A moratorium for the construction of new cages should also be enacted by the municipality and finally, fish cage farmers should organize themselves into a cooperative and work for the sustained growth of the industry and its environment.

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References

Bell, W. (1983) Plankton Grolier International. U.S.A. 331p.

Bersabal, R.B. (2004) Assessment of Fish Cage and Fishpen Production in Lake Buhl, Camarines Sur. Unpublished Undergraduate Thesis. Bicol University Tabaco Campus, Tabaco City, the Philippines.

Bowen, S.H. (1982) Feeding, digestion and growth - qualitative considerations. In: R.S.V. Pullin and R.H. Lowe-McConnell (Eds), The Biology and Cultured of Tilapias. ICLARM Conference Proceedings 7. International Center of Living Aquatic Resources Management, Manila, the Philippines, pp.141-156.

Cano, C.A.C. (2003) Zooplankton Distribution and Biomass in Sinarapan (*Mistichys luzonensis*) Sanctuary in Lake Buhi, Camarines Sur. Unpublished Undergraduate Thesis. Bicol University Tabaco Campus, Tabaco City, the Philippines.

Escover, E.M. and R.L. Claveria. (1985) The Economics of Cage Culture in Bicol Freshwater Lakes, Philippines. Makati, M.M. Philippines, *In*: I.R. Smith, E.B. Torres and E.O. Tan (*Eds*), p50-56, Philippine Tilapia Economics, ICLARM Conference Proceedings 12. 126p. Philippine council for Agriculture and Resources Research and Development, Los Baños, Laguna and International Center of Living Aquatic Resources Management, Manila, the Philippines.

Lovell, T. (1989) Nutrition and Feeding of Fish. Van Rostrand Reinhold.p167.

Luceña, D.V. (2000) Comparative Analysis of Production and Economic Performance of Tilapia Fish Cage Farming in Freshwater Lakes in the Bicol Region. Unpublished Special Problem for the Degree of Master of Economics, Bicol University Graduate School, Legazpi City. March 2000.

Ramos, R.R. (2004) Aquatic Productivity in Benthic Zone in Lake Buhi, Camarines Sur. Unpublished Undergraduate Thesis. Bicol University Tabaco Campus, Tabaco City. March 2004.

Shang, Y. C. (1981) Aquaculture Economics: Basic Concepts and Methods of Analysis. Westview Press, USA. 153pp.

Thompson. E.F. (1995) Plankton. Grolier International, Inc. USA 178 p.

Umaly, R.C. and M.L.A. Cuvin. (1988) Limnology, Laboratory and Field Guide. Physico-chemical Parameters. Biological Factors. MG Reprographics, Manila 257-p.

Welch, D.S. (1979) Limnology. McGraw Hill, Inc. New York, 211 p.

Comparisons of Growth and Yield of Carps Cultured in Boat-Frame and Floating Cages at the Pengxi Stream of the Three Gorges Reservoir Area in China

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Abstract

A study was carried out at the Pengxi stream, a branch of the Yangtze River, in Yunyang county at the Three Gorges Dam Reservoir Area from 1998–2000, to compare the performance of boat-frame cages and floating cages. The total area of boat-frame cages of 6.0×2.5×1.5 m^3 size was 150 m^2, while the total area of floating cages of 6.0×6.0×2.0 m^3 and 4.0×4.0×2.0 m^3 sizes was 288 m^2. During the three-year experimental period, the Jian strain of common carp (*Cyprinus carpio var. Jian*), grass carp (*Ctenopharyngodon idellus*) and crucian carp (*Carassius auratu*s) were the major species raised in these cages. Fish were fed with granulated formulated feed (31.5% crude protein) throughout the experimental period.

The annual fish yields in boat-frame cages were 101.0, 115.8 and 131.2 kg•m^{-3} in 1998, 1999 and 2000, respectively, while those in floating cages were 56.1, 67.6 and 72.0 kg•m^{-3} in 1998, 1999 and 2000, respectively. The average fish yield from the boat-frame cages was 116.0 kg•m^{-3}, which was 77.9% higher than that (65.2 kg•m^{-3}) from the floating cages. Feed conversion ratios were 1.8 and 2.0 in boat-frame and floating cages, respectively. Economic analysis showed that the net revenue in boat-frame cages was US$ 10•m^{-3} higher than that in floating cages. The experimental results suggest that the boat-frame cage is more appropriate than the floating cage in rivers.

Introduction

The Three Gorges Dam Project is one of the biggest hydroelectric projects in the world, which is located in the midstream of the Yangtze River in Sandouping township of Yichang city in Hubei province of China. The completion of the dam construction will result in a huge reservoir. Due to the large variation of water volume and depth in the upstream of Yangtze and its branches (Shi 1990) the traditional aquaculture is not appropriate in the Three Gorges Reservoir Area. Capture fisheries depend only on natural fish reproduction, and production is very low in the rivers and streams. Intensive cage aquaculture using both boat-frame cages and floating cages in those streams or rivers may give large socio-economic impacts on the local poor communities (Guo et al. 1996; Wang and Sun 1997; Zhang et al. 2000).

At present, an important issue is how to develop fisheries/aquaculture in the Three Gorges Reservoir Area. "Demonstration Project of Outstanding Aquaculture Techniques in the Three Gorges Reservoir Area", a key research project granted by the Chinese Ministry of Agriculture, was carried out from 1998-2000, to efficiently utilize the water resources in the Three Gorges Reservoir Area, develop technological packages of intensive cage culture suitable to the ecological condition in the Three Gorges Reservoir area, promote healthy aquaculture development, and help in the poverty alleviation of the poor immigrants. A component project was conducted to compare the growth and yield of fish cultured in boat-frame and floating cages at the Pengxi stream, a branch of Yangtze River, in Yunyang county, located at the Three Gorges Reservoir Area.

Materials and Methods

The experimental site and water condition

The experimental site was located at a backflow arm of the Pengxi stream, a branch of Yangtze River, in Gaoyang township of Yunyang county at the Three Gorges Reservoir Area. Water was clear without pollution, while water depth was 4.0-7.0 m and the current velocity 0.2-0.9 $m•s^{-1}$. The annual variation of silt content was 0.0-7.6 $kg•m^{-3}$, while the transparency was 30-100 cm from October to April, and 5-50 cm from May to September. The ranges of dissolved oxygen and pH were 5.8-13.2 $mg•L^{-1}$ and 6.7-8.0, respectively. Annual average water temperature was 18.7ºC, ranging from 6-32ºC, and water temperature was higher than 15ºC during late March to November, and ranged from 22-31 ºC during May to September.

Cage structure and setting

Cages were made of polyethylene net. One boat had 10 cabins for cage culture, and the outer appearance of the boat was similar to the inland river un-powered barges (Wan and Sun 1997). The boat-frame cages were not covered at the top and had a single layer net with a mesh size of 3-4 cm and a dimension of 6.0×2.5×1.5 m^3. The total area of the boat-frame cages was 150 m^2. The floating cages were covered on all sides by a double layer net with a dimension of 6.0×6.0× 2.0m^3 and 4.0×4.0×2.0 m^3, with mesh size of 3 cm for the inner net and 4 cm for the outer net. The total area of floating cages was 288 m^2.The boat-frame cages were arranged as double rows, while the floating cages were arranged either as single or double rows fixed by bamboos. Both floating cages and boats were fixed by anchors or cables.

Fish stocking

Fingerlings of Jian strain of common carp (*Cyprinus carpio var. Jian*), grass carp (*Ctenopharyngodon idellus*), and crucian carp (*Carassius auratus*) were stocked in the cages at a ratio of 7:2:1 before the end of February every year when water temperature was 12-15 ºC . The average stocking size and rate in these cages were 122 $g•fish^{-1}$ (99-140 $g•fish^{-1}$) and 25 $kg•m^{-2}$, 136 $g•fish^{-1}$ (110^{-1}58 $g•fish^{-1}$) and 30 $kg•m^{-2}$, and 152 $g•fish^{-1}$ (125-180 $g•fish^{-1}$) and 35 $kg•m^{-2}$ in 1998, 1999 and 2000, respectively.

Feed and feeding

During the experimental period, fish were fed with granulated formulated feed (31.5% crude protein) at daily feeding rates as shown in **Table 1.** Fish were fed once to twice daily for 20-25 minutes per feeding time at a water temperature below 15ºC, two to three times at a water temperature of 15-20ºC, three times at a water temperature of 20-25ºC, and four times at a water temperature of 25-30ºC.

Table 1. Daily feeding rates (%) at different temperatures for different sizes of fish cultured in cages.

Water temperature (ºC)	Fish body weight (g•fish^{-1})			
	100-200	200-300	300-500	>500
15-20	1.8-2.5	1.5-2.0	1.0-2.0	1.0-1.5
20-25	2.5-3.5	2.0-3.0	2.0-2.5	1.5-2.0
25-30	3.5-5.0	3.0-4.0	2.5-3.5	2.0-3.0

Routine management

A record for every cage was maintained. According to the water level changes in the stream, the berths for floating cages and boats should be adjusted timely to ensure that the cages were always kept in safety places with slow water current. The cages must be checked and cleaned regularly, to prevent damages from floods, escape and diseases.

During the experimental period, water temperature, transparency and velocity of water current were measured daily, while dissolved oxygen, silt content and fish growth rate were determined regularly. Three cages were randomly sampled from each type of cage, and at least 60 fish were randomly selected from each of the three cages for counting and weighing. The average body weights were used to estimate the average weights in all boat-frame cages and the floating cages, respectively.

Data Analyses

Economic analysis was conducted using a full enterprise budget. Farm gate prices were used for harvested fish, while market prices were used for all other items. The exchange rate used in this study was US$ 1 to 7.97 Chinese Yuan during the early 2006. Data were analyzed by descriptive statistics using Excel software.

Results

Growth

Carps in boat-frame cages grew better than those in floating cages, especially during the flooding season, probably due to higher resistance against floods and high current velocity of the boat-frame cages than the floating cages (Table 2).

Table 2. Comparison of growth performance of carps cultured in boat-frame and floating cages in 1999.

Month	Boat-frame cages	Floating cages		
	Mean weight ($g•fish^{-1}$)	Daily weight gain ($g•fish^{-1}•day^{-1}$)	Mean weight ($g•fish^{-1}$)	Daily weight gain ($g•fish^{-1}•day^{-1}$)
April	197.6	1.42	174.4	0.98
May	274.5	2.48	237.6	2.04
June	393.0	3.95	336.3	3.29
July	478.9	2.77	398.9	2.02
August	572.5	3.02	466.5	2.18
September	708.7	4.54	579.0	3.75
October	794.0	2.75	642.9	2.06
November	827.1	1.10	662.7	0.66

The daily weight gains in both types of cages fluctuated with the changes in water temperature (Figure 1). Two growth peaks appeared in June and September, while carps showed slower growth during July and August when water temperature was higher and more suitable for carp growth. The possible reasons were large fluctuation of water temperature caused by floods and high current velocity to which carps swam against thus exerting large amount of energy and lowering fish growth. It can also be seen from figure 2 that the monthly average daily weight gains tended to decrease with increasing current velocity.

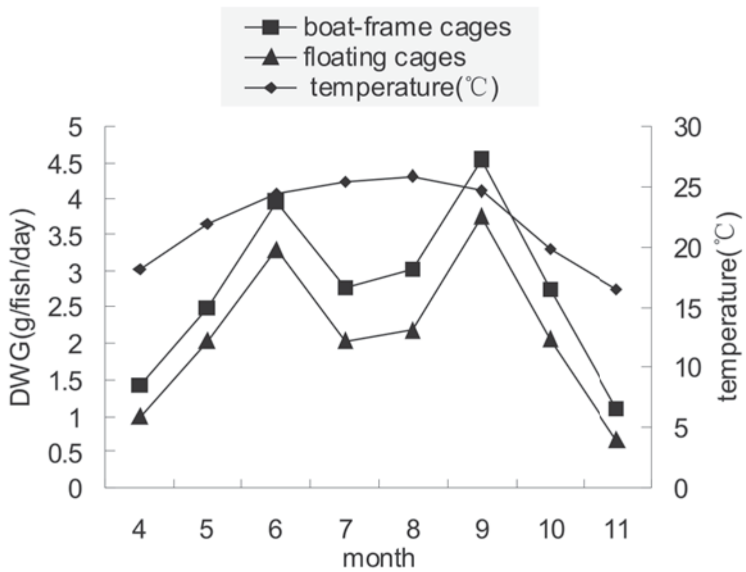

Figure 1. Changes in monthly average DWG ($g•fish^{-1}•day^{-1}$) reared in boat-frame cages and floating cages with water temperature (ºC)

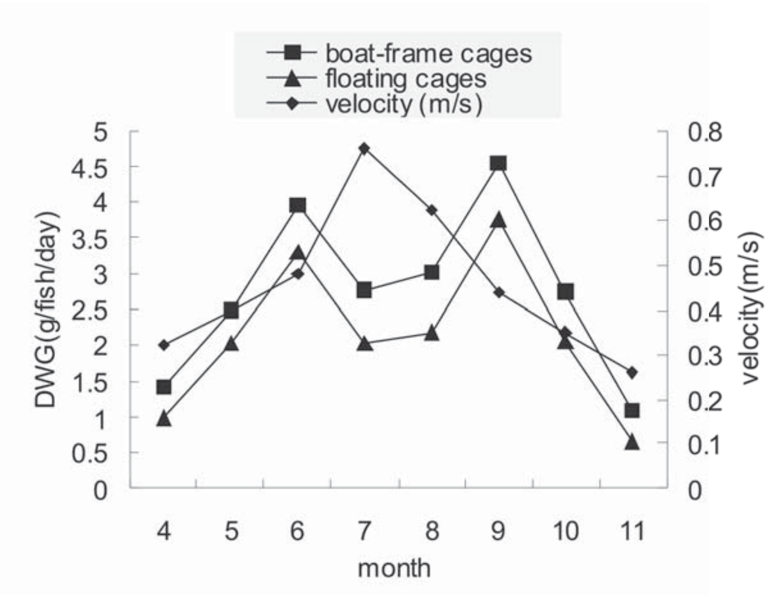

Figure 2. Changes in monthly average DWG (g•fish^{-1}•day^{-1}) reared in boat-frame cages and floating cages with current velocity (m•s^{-1}).

Fish yields

Stocking sizes and yields in boat-frame and floating cages during the three-year experimental period are shown in **Table 3.** The annual fish yields in boat-frame cages were 101.0, 115.8, and 131.2 kg•m^{-3} in 1998, 1999 and 2000, respectively, while those in floating cages were 56.1, 67.6, and 72.0 kg•m^{-3} in 1998, 1999 and 2000, respectively. The average fish yields from boat-frame cages were 116.0 kg•m^{-3}, which was 77.9% higher than that (65.2 kg•m^{-3}) in floating cages. Feed conversion ratios were 1.8 and 2.0 in boat-frame and floating cages, respectively.

Table 3. The stocking sizes and yields in both boat-frame and floating cages during 1999-2000.

Year		Stocking size (g•fish^{-1})	Harvest size (g•fish^{-1})	Total fish production (kg)	Average fish yields (kg•m^{-3})		FCR
Boat cage	1998	122.0	776.9	22725.0	101.0	116.0	1.8
	1999	136.0	827.1	26055.0	115.8		
	2000	152.0	894.6	29520.0	131.2		
Floating cage	1998	122.0	590.5	32313.6	56.1	65.2	2.0
	1999	136.0	662.7	38937.6	67.6		
	2000	152.0	669.8	41472.0	72.0		

Economic analysis

Economic analysis for boat-frame and floating cage culture during 1999 to 2000 is shown in **Table 4.** Average gross revenue from fish sale for boat-frame and floating cages were 195.75 and 281.181 thousand Yuan, respectively, while the total costs in the two types of cages were 154.77

and 222.84 thousand Yuan, respectively, giving the same cost/revenue ratio of 1:1.27 (Table 4). Average net revenue in unit area was 182 Yuan•m^{-3} in boat-frame cages, which is higher than that (102 Yuan•m^{-3}) in floating cages (Table 4), indicating that boat-frame cage culture is more profitable than floating cage culture.

Table 4. Economic analysis for boat-frame and floating cage culture during 1999-2000.

Culture System		Gross revenue (‘000 Yuan)	Gross revenue (Yuan•m^{-3})	Total cost ('000 yuan)	Net revenue ('000 yuan)	Net revenue (Yuan•m^{-3})	Cost/revenue ratio
Boat-frame cage culture	1998	170.44	758	138.87	31.57	140	1:1.23
	1999	195.41	868	154.57	40.84	182	1:1.26
	2000	221.40	984	170.87	50.53	225	1:1.30
	Total	587.25	2,610	464.31	122.94	546	1:1.27
	Average	195.75	870	154.77	40.98	182	
Floating cage culture	1998	242.35	421	199.44	42.91	74	1:1.22
	1999	292.03	507	226.41	65.62	114	1:1.29
	2000	311.04	540	242.66	68.38	119	1:1.28
	Total	845.42	1,468	668.51	176.91	307	1:1.27
	Average	281.81	489	222.84	58.97	102	

Discussion

Production and socio-economic benefits

Boat-frame and floating cage culture require high capital inputs and have high risk, thus economic return is the first thing to be considered by cage operators. It can be seen from the economic analysis in the present study (Table 4) that floating and boat-frame cage culture at the Pengxi stream in the Three Gorges Reservoir Area produced average fish yields of 65.2 and 116.0 kg•m^{-3}, and net revenues of 102 and 182 Yuan•m^{-3}, respectively. Therefore, cage culture in rivers and streams in the Three Gorges Reservoir Area may be a good way of poverty alleviation for the immigrants, however, the cage culture development should be environmentally sustainable (Wu and Yi 2000; Zou et al. 2002; Li et al. 2005).

The results of the present study show that the boat-frame cage culture is better than the floating cage culture in terms of fish yields and economic returns, probably due to the small volume of the boat-frame cages, the high strength, good buoyancy and strong resistance against floods of the boats. Due to the large variations in water volume and level in the Three Gorges Reservoir Area boat-frame cage culture should be the first choice.

Low-cost feeds should be developed to lower the production cost, thus further increase the economic returns. On the other hand, culturing high-value species especially indigenous species of the upstream Yangtze River can enhance the economic returns of cage culture.

Influence of environmental factors

In July-August which is the period for good fish growth, water temperature at the Pengxi stream is relatively lower than the other places at the reservoir area, although water temperature change is very fast and may decrease by 3-5ºC within several hours during the flood season. The relatively low and fast fluctuating temperature may reduce fish growth.

It has been reported that low current velocity (0.04-0.20 m•s^{-1}) was in favor of fish growth while higher velocity may cause excessive movement of fish thus lower fish growth rate (Wu 1987; Xu 1989). The practice of boat-frame cage culture has shown that the most appropriate current velocity at the berth location of boat-frame cages then the maximum current velocity within boat-frame cages is lower than 0.20 m•s^{-1} (Chen 1994; Chen and Tang 1995). It was observed in the present study that fish swam against the water current when the velocity was greater than 0.60 m•s^{-1} at the berth location then the velocity within boat-frame cages was greater than 0.23 m•s^{-1} causing stronger swimming against the water current with the increasing velocity thus slow fish growth (Figure 2). In addition, a current velocity lower than 0.10 m•s^{-1} and higher than 1.20 m•s^{-1} may negatively affect fish growth and even cage safety (Chen and Tang 1995). Both boat-frame and floating cages should be placed in the backflow arms or in places with low water velocity.

Transparency in streams and rivers is mainly dependent on silt content (Zhang 1991). Silt content in water lowers transparency, and may reduce fish's feed intake, growth and resistance to diseases, thus increase production costs. The observations in the present study indicate that caged fish did not show any unusual response when the silt content was below 5 kg•m^{-3} or transparency was about 5 cm; caged fish showed low response to feeding and swam towards the surface but did not die when the silt content reached 10 kg•m^{-3} or when transparency was below 3 cm. In addition, large amounts of drifts during flood seasons may cause serious damages to cage culture.

Fingerlings and stocking

Fingerlings were nursed in cages, and this practice guaranteed the quality and quantity of fingerlings for stocking the grow-out cages by eliminating fish seining in large water bodies and transportation of fingerlings. This resulted in savings in production costs, reduced disease risk and increased survival rate. Compared to the fingerlings purchased from local farms, the fingerlings nursed in cages had already adapted to the water environment for grow-out cage culture, thus had better growth performance. Therefore, it can be said that farmers could benefit both technically and economically from fingerling nursery and grow-out in cages in the same water body.

Fingerlings should be stocked in cages as early as possible in the spring season, in order to make them adapt to the water environment in cages. Once water temperature rises to 15ºC, feeding should start. Stocking size has direct effects on market size, yields and marking time. In order to achieve better economic benefits, multiple-size fingerling stocking and multiple-harvests should be practiced. The results of the present study show that the proper stocking size was 100-200 g• fish^{-1} for Jian strain of common carp, grass carp and crucian carp.

Stocking density is an important factor affecting fish growth and yields. At a certain range, higher stocking density results in higher yields, lower feed conversion ratio and greater economic returns (Dai 1987; Yang and Pan 1992; Guo et al. 1996; Wan and Sun 1997). Stocking rate is dependent on the growth characteristics of cultured fish species, marketable size, management, and the number of days with water temperature above 18ºC as well as other water quality parameters such as dissolved oxygen. The stocking rate ranges from 10 to 25 kg•m^{-3} in cages in China and the stocking density is usually higher in boat-frame cages than that in floating cages. Further research should be conducted to optimize the stocking density in boat-frame and floating cages in the Three Gorges Reservoir Area.

Resistance to floods and escape

Site selection is very crucial in preventing losses caused by floods, while it is also important to check cages regularly, fix cages well, block and remove drifts during flood seasons. Using double-layer cages and metal-frame cages is an alternative option. Farmers' practice showed that boat-frame cages have good buoyancy, high strength, good resistance to floods, water current as well as escape. All of these indicate that boat-frame cages are better than other cages.

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References

Cao, H. T. and Wu, D. H. (1996) Study on high-yield techniques of cage culture special species in Dongting Lake. Inland Fisheries 5, 4-5 (in Chinese).

Chen, C. Q. (1994) Preliminary study on boat-frame cage culture in rivers. Fisheries Science & Technology Information 21(1), 3-7 (in Chinese).

Chen, C. Q. and Tang, Y. (1995) Characteristics of fisheries environment in rivers in Chongqing and its effects on boat-frame cage culture. Resources and Environment in the Yangtze River Watershed 4(2), 163-169 (in Chinese).

Dai, C. L. (1987) High-Yield Techniques of Carp Cage Culture. Science Press, Beijing, China (in Chinese).

Guo, D. M., Lu, Z. Y., Yu, S. B. and Ji, R. L. (1996) Preliminary Report on high-yield techniques of carp cage culture. ACTA Hydrobiologica Sinica 20(2), 190-191 (in Chinese).

Li, C. M., Huang, Z. L., Chang, J.B. (2005) Analysis of the pros and cons of cage culture in the Three Gorges Reservoir. China Three Gorges Construction 4, 49-52 (in Chinese).

Shi, B. N. (1990) Fisheries Resources and Divisions in rivers in Sichuan Province. Southwest Normal University Press, Chongqing, China (in Chinese).

Wan, Q. and Sun, J. (1997) Study on high-yield techniques of cage culture of Wuchang fish. Journal of Anhui Agriculture University 24(2), 181-185 (in Chinese).

Wu, Z. D. (1987) Flow-Through Fish Farming. Jiangsu Science and Technology Press, Nanjing, China (in Chinese).

Wu, Z. W. and Yi, Y. (2000) Feasibility of sustainable development of cage culture in reservoirs. Scientific Fish Farming 1, 5-6 (in Chinese).

Xu, P. C. (1989) Study on the relationship among dissolved oxygen, water current and fish grow. In: Freshwater Fisheries Research Collection of China Society of Fisheries. Scientific Press, Beijing, China, pp. 21-27 (in Chinese).

Yang, Q. X. and Pan, D. M. (1992) Trial of cage culture in a plateau reservoir. Reservoir Fisheries 5, 9-12 (in Chinese).

Zhang, J. M. (1991) Handbook for Survey of Inland Fishery Resources. Agricultural Press, Beijing, China (in Chinese).

Zhang, Z. X., Guan, P., Huang, G. X. (2000) Study on high-yield techniques of carp cage culture in Xingyun Lake. Reservoir Fisheries 20(4), 33-34 (in Chinese).

Zou, Q., Wu, S. G., Gao, S. B. (2002) Study on the relationship between reservoir cage culture and environmental protection. Reservoir Fisheries 22(1), 42-43 (in Chinese).

Technical and Economical Evaluation of Small-Scale Fish Cage Culture for Youth in the River Nile of Egypt: 1. Effect of Stocking Density of Monosex Nile Tilapia (Oreochromis niloticus) Fingerlings

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Abstract

Monosex Nile tilapia (Oreochromis niloticus) fingerlings (mean weight = 64.78±5.57 g) were stocked at three densities (40, 80, and 120 fish•m^{-3}) in six rectangular cages (submerged dimensions of each cage measuring 12m×12m×5m and 720 m^3 volume) at the Damietta branch of the River Nile, Egypt and reared for 194 days, in order to investigate their growth performances, survival rates (%), feed conversion ratios (FCR), yields and probability. Fish in each treatment (twice cages) were fed ad libitum with commercial extruded fish feed (30% crude protein) through a set of demand feeders for six days each week. The results indicated the following: (1) average daily gain (ADG g•fish^{-1}•day^{-1}); specific growth rate (SGR%•day^{-1}) and survival rates significantly ($P< 0.05$) increased with decreasing fish stocking density. However, the highest stocking density resulted in the highest fish yield (24.48 kg•m^{-3}) with a condition factor of 2.93; (2) the value of FCR in the highest stocking density group (120 fish•m^{-3}) was 1.73 which was poorer than the other tested groups (1.57 in 80 fish•m^{-3} and 1.30 in 40 fish•m^{-3}) with low stocking densities; and, (3) the highest net income•m^{-3} (92.35 LE, about $15.92) was obtained with the highest stocking density (120 fish•m^{-3}) groups. In conclusion, although fish growth rate has been affected by increasing stocking density, this can be partly compensated by the market values of the excess of production. In addition, the production value of the highest stocking density (120 fish•m^{-3}) was 81% higher than that of lower stocking density (40 fish•m^{-3}). However, the lower stocking density (40 fish•m^{-3}) was more profitable than the other tested groups with higher stocking densities (80 or 120 fish•m^{-3}). Therefore, the use of 40 fish•m^{-3} as the optimum level for the small-scale cage culture of monosex Nile tilapia fingerlings in the northern area of River Nile of Egypt could be recommended.

Introduction

Cage culture is commonly practiced worldwide in both freshwater and marine environments, including the open ocean, estuaries, lakes, reservoirs, ponds and rivers (Beveridge 1983). The production of fish in cages has been practiced for many years in various countries worldwide. The earliest record of cage culture practices dates back to the late 1800s in Southeast Asia. Similar culture practices have been reported in both freshwater and marine environments, including open ocean, estuaries, lakes, reservoirs, ponds and river (Eng and Tech 2002). Cage culture practices have numerous advantages over other culture systems. By integrating the cage culture system into the aquatic ecosystem, the carrying capacity per unit area is optimized because the free flow of current brings in water and removes metabolic wastes, excess feed and faecal matter (Beveridge 1983). Tilapia was later transplanted and became established as a potential farmed species in the late 1940s in the Far East and a decade later, it spread in the Americas. The growing popularity of tilapia among consumers and the ever increasing need to improve food production, impose the need to seek production alternatives to culture tilapia. The popularity is due to its market acceptability and relative tolerance to a wide range of water temperature, dissolved oxygen (DO), salinity, pH, light intensity and photoperiods. However, the determination of stocking density for cultured tilapia is essential for the maximization of its production, profitability and sustainability.

Stocking density as a management tool strongly influences the maximum feed intake of the fish. It is an important parameter when establishing optimal husbandry condition of fish. The use of high stocking density as a technique to maximize water usage and thus increase stock production in fish culture has been shown to exert severe adverse effect on fish growth (Schreck et al. 1985).

It is assumed that if environmental factors do not become limiting, the optimal stocking density of certain species for obtaining the highest possible yield depends on the amount and the quality of food available, however the highest possible yield does not have to coincide with the highest economic production because the latter is also determined by the preferred market size and price. Intensive cage culture of tilapia is becoming more common. Recently, several large commercial projects have started in Africa and the Caribbean. Fish are stocked at higher rates and fed complete feeds. Stock rates at harvest are typically around 25 kg• m^{-3}. The maximum density is limited by the availability of oxygen. Therefore, the aim of the present study was to evaluate the effect of stocking density of monosex Nile tilapia (Oreochromis niloticus) on growth performance, survival, feed utilization production as well as profitability of the project.

Materials and Methods

The experiment described in the present study was conducted in the River Nile branch at El–Bostan region, Damietta Governorate, Egypt for 194 days from June 10 to December 21, 2004.

Experimental net cages

Six floating rectangular net cages each with a submerged volume of 720 m^3 (12m×12m×5m per cage) were used.

Stocking

Tilapia fingerlings with an average initial weight of 64.78 ±5.57 g were cultured at three stocking densities (40, 80 and 120 fish•m^{-3}).

Water quality

Dissolved oxygen, pH and water temperature were determined daily at 7:00 am, whereas ammonia, nitrite, nitrate and phosphate concentrations in water of the cage area were monitored once a week according to Golterman et al. (1978).

Feeding regime

Fish were fed *ad libitum* using commercial extruded fish feed pellets with 30% crude protein. Demand feeders were used to feed the fish during day light only (07:30 to 16:00 hrs).

Feed ingredients and chemical analysis

The feed ingredients and chemical analysis of the tested commercial diet used in the present study are presented in **Table 1.** The composition of vitamin and mineral mixture are likewise listed in **Table 1.** Chemical analysis of the dietary dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash contents were conducted according to AOAC (1993). Kjeldahl method was used to determine total nitrogen content in the feed sample. The factor 6.25 was used to estimate crude protein according to ISO (1979).

Growth parameters and fish survival (%)

Random fish samples from each cage were caught bi-weekly to determine total fish weights. Survival rate was determined for each cage by counting all the live fish remaining at the end of the experiment.

Total weight gain, average daily gain, specific growth rate and feed conversion ratio were determined according to Ricker (1975) and Castell & Tiewes (1980) based on the following formulae:

Total weight gain (g•fish^{-1}) = $W_T – W_I$

Average Daily Gain (g•fish^{-1}•day^{-1}) = $W_T – W_I/n$

Specific Growth Rate (%•fish^{-1}•day^{-1}) = 100 × ($Ln\ W_T – L_n\ W_I$)/n

Survival (%) =)No. of fish at the end of the experiment/No. of fish at start of the experiment) ×100

Feed Conversion Ratio = Dry matter intake (g)/Weight gain (g)

Table 1. Percentages of feed ingredients and chemical analysis of the commercial diet used during the study.

Feed ingredient	%
Soybean meal	21.50
Fish meal	15.00
Yellow corn	15.00
Wheat milling by products	27.20
Corn gluten (60%CP)	10.00
Rice bran	10.00
Mineral mixture*	1.00
Vitamin mixture**	0.30
Chemical analysis	
Dry matter	90.20
Crude protein	29.85
Crude fat	4.61
Crude fiber	4.20
Ash	10.03
Nitrogen free extract (NFE)	51.31

*Mineral mixture: Magnesium (300 mg); Iron (50 mg); Copper (5 mg); Cobalt (0.20 mg); Zinc (80 mg); Manganese (40 mg); Iodine (0.25 mg); Selenium (0.30 mg); Anti- oxidant (150 mg).
**Vitamin mixture: Vit. A (20000 IU), Vit. D_3 (25000 IU); Vit. C (300 mg); Vit. E (10 mg); Vit. K_3 (8 mg); Vit. B_1 (15 mg); Vit. B_2 (13 mg); Vit. B_3 (30 mg); Vit. B_6 (15 mg); Niacin (100 mg); Folic acid (2.5 mg); Biotic acid (1 mg); Choline (1000 mg).

Statistical analysis

To analyze the differences between the two treatment means (feeding methods), data collected from the experiment was analyzed using t-test (Steel & Torrie 1980). Analysis of Variance (ANOVA) ($P \leq 0.05$) and Duncan's Multiple Range Test were used on feeding forms statistical analysis to compare the means between individual treatments (Duncan 1955; Snedecor & Cochran 1967).

Economical evaluation

Economical evaluation was conducted according to Loverich (1996). Profitability was measured as:

Feed cost (LE•kg^{-1}) •kg^{-1} body weight gain = FCR× cost per kg diet.

Total Production cost (LE•kg^{-1}) = Sum of variable costs•kg^{-1}/sum of fixed cost•kg^{-1}) / No. of kg(s)

Results and Discussion

Water quality parameters in cages area

Results in table 2 show the values of water quality parameters in the cages. The average values of water temperature were 26.80 ± 2.08ºC. The pH average value was 7.71 ± 0.15 and dissolved oxygen was 6.95 + 0.18. The values of total alkalinity averaged 310 ± 3.46 ppm while ammonia concentrations never exceeded 0.01 mg•L^{-1}. The concentrations of nitrite (NO_2) nitrate (NO_3) and phosphate (PO_4) in the cages were moderate indicating that nutrients influx in the area was still in balance with the biological activity of different microorganisms.

Table 2. Water quality parameters in the floating cages from June to December, 2004.

Parameters	Average values
Water temperature°C	26.80 ± 2.08
Dissolved oxygen (ppm)	6.95 + 0.18
Total alkalinity, $CaCO_3$ (ppm)	310 + 3.46
pH	7.71 + 0.15
Ammonia, NH_3 (ppm)	< 0.01 + 0.00
Nitrite, NO_2 (ppm)	0.28 + 0.02
Nitrate, NO_3 (ppm)	3.61 + 0.82
Phosphate, PO_4 (ppm)	1.04 + 0.31

The water quality results indicated the suitability of water for fish culture. Hepher & Pruginin (1981) reported that water pH values ranging from 6.5 to 9.0 are reasonable for fish farming. Swingle (1969), Neill & Bryan (1991) as well as Daniel et al. (2005) considered that dissolved oxygen concentrations below 3.5 mg•L^{-1} and ammonia concentrations of more than 0.2 mg•L^{-1} are undesirable in fish culture. Maar et al. (1974) showed that total water alkalinity over 50 mg•L^{-1} CaCo3 is beneficial for fish culture.

Water, being the medium of all aquatic organisms, including fish, provides support, oxygen and enhances the availability of nutrients for the fish to utilize. Maintaining good water quality means maintaining a suitable environment for healthy fish growth.

Tilapia is one of the few fish species that exhibits a wide range of tolerance to environmental conditions and has low susceptibility to disease. In intensive rearing systems, however, the high density of fish and fish wastes may lead to deterioration in water quality and induce problems such as out-gassing and disease from parasitic, bacterial and fungal infections (Tonguthai 1995). Lethal temperature limits for Nile tilapia are 11°C and 42°C (Philippart & Ruwet 1982) while temperatures up to 30°C have been found to promote growth rate.

Growth performance

Table 3 shows the growth performance of monosex fingerlings of Nile tilapia reared in floating net cages for 194 days grow-out period, at different stocking densities (40, 80 and 120 fish•m^{-3}). Average daily gain was significantly higher ($P \leq 0.05$) in fish stocked at 40 fish•m^{-3} (1.51 g•$fish^{-1}$•day^{-1}) than fish stocked at 80 fish•m^{-3} (1.15 g•$fish^{-1}$•day^{-1}) while fish stocked at 120 fish•m^{-3} obtained the lowest value (0.98 g•$fish^{-1}$•day^{-1}). Specific growth rate (%•day^{-1}) followed the same trend; 0.88, 0.77 and 0.71 in 40, 80 and 120 fish•m^{-3}, respectively. It is clear that values of growth performance parameters increased with decreasing stocking density per cubic meter. In agreement with the present study, Guerrero & Guerrero (1985), McGinty (1985), Hargreaves et al. (1991), Tidwell & Webster (1993), Watanabe et al. (1993) and El-Sagheer (2001) found that increasing stocking density per cubic meter led to decreased values of growth performance parameters. Survival (%) and condition factors were similarly decreased with increasing stocking density of monosex fingerlings of Nile tilapia.

Table 3. Effect of stocking density on growth performance, survival and condition factors (K) of monosex fingerlings of Nile tilapia reared at different stocking densities in cages for 194 days.

Items	Stocking density (fish•m^{-3})*		
	40	80	120
Initial weight(g•$fish^{-1}$)	64.78	64.7	64.78
Final weight (g•$fish^{-1}$)	356.87a	288.09b	255.26c
Weight gain (g•$fish^{-1}$)	292.09a	223.31b	190.48c
Average daily gain (g•fish-1•day^{-1})	1.51a	1.15b	0.98c
Specific growth rate (%•$fish^{-1}$•day^{-1})	0.88a	0.77b	0.71c
Initial number of fish per cage	28800	57600	86400
Final number of fish per cage	27328	288.09	255.26
Survival (%)	94.89a	90.06b	79.93c
Condition factor (K)	3.02a	2.98b	2.93c

*Means in the same row having different superscript letter are significantly different ($P< 0.05$).

Feed conversion ratio

Table 4 indicates that fish stocking density significantly ($P\leq0.05$) affected the feed conversion ratio (FCR). The FCR was improved significantly ($P\leq0.05$) with decreasing stocking density throughout the experimental period. The best FCR was observed at 40 fish•m^{-3}, followed by 80 and 120 fish•m^{-3}. These findings are in agreement with the results obtained by Jorgensen et al. (1993), Tidwell & Webster (1993) and El-Sagheer (2001).

Table 4. Effect of stocking density on feed conversion ratio and production performance of monosex fingerlings of Nile tilapia reared in cages for 194 days.

Items*	Stocking density (fish•m^{-3})		
	40	80	120
Feed conversion ratio	1.57a	1.68ab	1.73b
Total fish production (kg•m^{-3})	13.55a	20.76b	24.48c
Total fish production (t•$cage^{-1}$)	9.75a	14.94b	17.63c

*In the same row, means having different superscripts are significantly different ($P< 0.05$).

Fish yield

Increasing stocking density up to 120 fish•m^{-3} led to increasing total yield•m^{-3} as shown in **Table 4.** These results are in agreement with Eid & El-Gamal (1997), Al-Azab (2001) and El-Sagheer (2001).

Economical evaluation

Table 5 shows that increasing stocking density from 40 to 80 and 120 fish•m^{-3} led to increasing total operation cost from 35,250 to 58,850 and 74,550 LE, respectively. Net return•

m^{-3} and per cage increased with increasing stocking density. This is in agreement with the findings of Guda (1996), Eid & El-Gamal (1997), El-Shandawiely (1999), Al-Azab (2001) and El-Sagheer (2001) in terms of economical value and returns.

Table 5. Cost and return (LE) of cage cultured monosex fingerlings of Nile tilapia at different stocking densities for 194 days.

Items	Stocking density (fish•m^{-3})		
	40	80	120
Cage construction depreciation•$year^{-1}$	1700	1700	1700
Fingerlings	5800	11400	17200
Feed	27000	45000	54900
Labor	750	750	750
Total operation cost (LE•$cage^{-1}$) *	35250	58850	74550
Return (LE•$cage^{-1}$)	87750	119520	141040
Net return (LE•m^{-3})	72.92	84.26	92.35
Net return (LE•$cage^{-1}$)	52500	60670	66490
Net return (%)	149	103	89

* US$ 1 = 5.80 LE

However, the profitability (%) of the operation was higher with the lower stocking density (40 fish•m^{-3}) followed by 80 and 120 fish•m^{-3}, respectively. From the results, it could be concluded that stocking density of cages with 40 fish•m^{-3} of monosex tilapia fingerlings was the optimum density for higher growth performance, feed utilization, survival and net return profitability under the experimental conditions.

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References

Al-Azab, D.S.M. (2001) Studies on Feeding of Nile Tilapia under Intensive Culture Condition. Ph.D. Thesis, Faculty of Agriculture, Al-Azhar University.

AOAC (Association of Official Analytical). (1993) Official Methods of Analysis, 15th edn. AOAC. Arlington, VA, USA.

Beveridge, M. (1983) Current Status and Potential of Aquaculture in Bolivia. ODA Report, Institute of Aquaculture, University of Stirling, Stirling, UK, 65 pp.

Castell, J.D. & Tiewes, K. (Eds). (1980) Report of the EIFAC, IUNS and ICES Working Group on the Standardization of Methodology in Fish Research, Hamburg, FRG, 212-23 March, 1979. IFAC Tech. Paper 3, 24.

Daniel, S., Larry, W.D. & Joseph, H.S. (2005) Comparative oxygen consumption and metabolism of striped bass (Morone saxaatilis) and its hybrid. Journal of World Aquaculture Society 36 (4), 521 – 529.

Duncan, D.B. (1955) Multiple range and multiple F tests. Biometrics 11, 1-42.

Eid, A.E. & El- Gamal, A.A. (1997) Effect of Stocking Density on Growth Performance of Nile Tilapia in Three Culture Systems. Conference of Animal Production. Faculty of Agriculture. Monofiya Univ., Shibin El-Kom.

El-Sagheer, F.H. (2001) Effect of Stocking Densities, Protein Levels and Feeding Frequencies on Growth and Production of Tilapia Monosex in Earthen Ponds. Ph.D. Thesis, Faculty of Agriculture, Alexandria University.

El-Shandawiely, M.Z.Y. (1999) Effect of fertilization and feeding system on performance of Nile tilapia (Oreochromis niloticus) and grey mullet (Mugil cephalus) in polyculture earthen ponds. Ph.D. Thesis, Faculty of Agriculture, El-Fayoum University.

Eng, C.T. & Tech, E. (2002) Introduction and history of cage culture. p. 1-39. In: P.T.K Woo, D.W. Bruno, and L.H.S. Lim (eds.). Diseases and Disorders of Finfish in Cage Culture. CAB International.

Golterman, H.L., Clymo, R.S. & Ohnstad, M.A.M. (1978) Methods for Physical and Chemical Analysis of Fresh Waters. Intern. Biol. Prog. Hand Books 8, 213 pp.

Guda, A.M.A. (1996) Types of Nutrition for Fresh Water Fish. Ph.D. Thesis, Faculty of Agriculture, Cairo University.

Guerrero, R.D. & Guerrero, L.A. (1985) Feasibility of commercial production of sex reversal Nile tilapia fingerlings in Philippines. Aquaculture 137, 312-322.

Hargreaves, J.A., Rakocy, J.E. & Bailey, D.S. (1991) Effect of diffused aeration and stocking density on growth, feed conversion ratio and production of Florida red tilapia in cages. Journal of the World Aquaculture Society 22(1), 24-29.

Hepher, B. & Pruginin, Y. (1981) Commercial Fish Farming. A Wiley Interscience Publication, New York.

ISO. (1979) International Standard Organization. Animal Feeding Stuffs Determination of Nitrogen Content and Calculation of Crude Protein Content. Switzerland Method No. 5983.

Jorgensen, E.H., Christiansen, J.S. & Jobling, M. (1993) Effect of stocking density on food intake, growth performance and oxygen consumption in Arctic charr (Salvelinus alpinus). Aquaculture 110, 191-204.

Loverich, G.F. (1996) Offshore Aquaculture Harvest and Transport Concept Feasibility and Development. A Report of Ocean Spar Technologies. LLC Pursuant to National Oceanic and Atmosph. Admin., A. Word No. NA56 FD 0071, Bainbridge, Washington.

McGinty, A.S. (1985) Effects of predation by largemouth bass in fish production ponds stocked with tilapia (Oreochromis niloticus). Aquaculture 46, 269-274.

Neill, W.H. & J.D. Bryan. (1991) Responses of fish to temperature and oxygen, and response integration through metabolic scope. p. 30-57. In: D.E. Brune and J.R. Tomasso, editors. Aquaculture and Water Auality (Advances in World Aquaculture, Volume 3), The World Aquaculture Society, Baton Rouge, Louisiana.

Philippart, J.C.L. & Ruwet, J. (1982) Ecology and distribution of tilapias. p. 15-59. In: R.S. V. Pullin and R.H. Lowe-McConnell (Eds.). The Biology and Culture of Tilapias, ICLARM Conference Proceeding 7. Manila Philippines. 432 pp.

Ricker, W.E. (1975) Computation and Interpretation of Biological Statistics of Fish Populations. Fish. Res. Board Can. Bull., 191.

Schreck, C.B., Patino, R., Pring, C.K., Winto, J.R. & Holway, J.E. (1985) Effect of rearing density on indices of smoltification and performance of coho salmon, Oncorhynchus kisutch. Aquaculture 45, 345-358.

Snedecor, G.W. & Cochran, W.G. (1967) Statistical Methods. 6th edn. Iowa State Univ. Press, Ames, Iowa, USA, 341 pp.

Steel, R.G.O. & J.H.Torrie, (1980) Principles and Procedures of Statistics: A Biometrical Approach, 2nd edn. McGraw Hill, New York, USA. 633 pp.

Swingle, H.S. (1969) Methods of Analysis for Water, Organic Matter and Pond Bottom Soils in Fisheries and Research. Auburn Univ., Auburn, Alabama, USA. 119 pp.

Tidwell, J.H. & Webster, C.D. (1993) Effect of stocking density and dietary protein on green sunfish (Lepomis cyanellus)× bluegill (L. macrochirus) hybrids overwintered in ponds. Aquaculture 113, 83-89.

Tonguthai, K. (1995). Fish Disease. Asian Institute of Technology, Bangkok, Thailand, 87 pp.

Watanabe, W.O., Ernst, D.H., Chazar, M.P., Wicklund, R.I. & Olla, B.L. (1993) The effect of temperature and salinity on growth and feed utilization of juveniles, sex-reversed male Florida red tilapia cultured in a recirculating system. Aquaculture 112, 309-320.

Use of Artificial Seawater in Larviculture of the Giant Freshwater Prawn (Macrobrachium rosenbergii) in Fine Mesh Cage in a Closed Water Recirculating System

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Abstract

This study aimed to decrease the use of saline water when nursing the giant freshwater prawn during development stage 1 to post-larval stage. This experiment used a completely randomized design which consisted of two treatments with three replications. The control treatment used an open water recirculating system while the other treatment used a closed water recirculating system with a trickling filter unit packed with fiberglass and bioballs. The artificial seawater used with a salinity of 15 ppt was prepared from commercial sea salt powder (Marinium trade mark). Healthy first larval stage of *Macrobrachium rosenbergii* was randomly selected and transferred to a 50 L aquarium with a stocking density of 80 larvae·L^{-1}. Survival rates of post-larvae were not significantly different (P>0.05) at 13.34+7.42 and 11.26+7.78% from the open and closed systems, respectively. The metamorphosis period from the first larval to post larval stages was not significantly different (P>0.05) between the two larviculture systems. Water volume used in the open system was significantly higher (P<0.05) than in the closed system. It was seen that artificial seawater can be efficiently used as a saline water source. The closed water recirculating system with a trickling filter unit packed with fiberglass and bioballs has a high potential. However it might be necessary to supplement the culture medium with some elements especially magnesium, potassium and sodium to maintain a suitable concentration.

Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii* (de Man), is highly esteemed as food by the Thai people. Previously, the giant freshwater prawn was highly abundant in rivers, lagoons and all freshwater reservoirs. Nowadays there is an extremely significant reduction of natural stocks because of water pollution and over fishing. The above situation could no longer meet the increasing demand for freshwater prawn to supply the market, thus the number of giant freshwater prawn farms has increased.

Since the major breakthrough by Ling (1962) when he discovered that salinity was an important basic requirement for prawn larvae to survive and develop during their early life stages, various hatchery techniques have been developed to mass produce post-larvae for commercial purposes.

In 2000, the world production of giant freshwater prawn was 118,501 tons (US$ 410,001 thousand million) (FAO 2000). The production in Thailand reached approximately 15,000 MT in 2002 (DOF 2004) which was about 12.6% of the total world production. Giant freshwater prawn became an important economic aquatic species which can be cultured in all freshwater areas throughout Thailand, with the central region the most popular area for seed and grow-out production. However, since production was still not enough, the culture area expanded to the north and northeast regions which are of a considerable distance from the sea and the source of seed. In the past when the cost of transportation was affordable, it was possible to transport seed from the source to distant farms. However, the longer transport distance resulted in a lower survival rate of larvae which led to the establishment of local hatcheries. Concentrated seawater from salt farms was transported to these local hatcheries, which was, at that time, considered as the best way to operate a hatchery. But this is not possible now due to the increase in fuel price, resulting in a relatively higher cost of transport. Thus these local hatcheries could not continue larval production. Therefore, it is necessary to find a suitable source of saline water for lower cost of operation. Artificial seawater has the potential to replace concentrated seawater transported from long distances. However there is a lack of information on its use in prawn hatcheries. To solve these problems, this study investigated the use of commercial artificial seawater under a closed recirculating saline water system with a trickling filter unit packed with fiberglass and bioballs.

Materials and methods

Preparation of the experimental larvae

Immature berried *M. rosenbergii* females from a commercial farm were used in this experiment. They were kept in freshwater until they matured. Then they were introduced to 15 ppt artificial seawater overnight. The hatched larvae were separated and transferred to 50 L aquaria at a density of 80 larvae·L^{-1} the following day.

Larviculture system

The experiment had two treatments, namely, the open recirculating water system in glass aquaria without a filter unit (as the control) and the closed recirculating water system in glass aquaria with a trickling filter unit packed with fiberglass and bioballs (Figure 1). The rectangular aquaria had a dimension of 30.5×61×38.0 cm. Each aquarium had three chambers. The first chamber (29.3cm×49.0cm×38.0cm) with fine mesh net cages inside was provided for larval rearing area. The fine mesh net cage with a dimension of 15cm×15cm ×13cm was used for 1-10 day old larvae while the 20cm×20cm×20cm cage was used for larvae older than 10 days (Figure 2). The second chamber (10.0cm×18.7cm×27.8cm) was provided for the trickling filter unit. It contained two layers of fiberglass at the top and 720 bioballs at the bottom. The third chamber (10.0cm×10.0cm×27.8cm) contained the water pump (600 L•hr^{-1}) for water circulation.

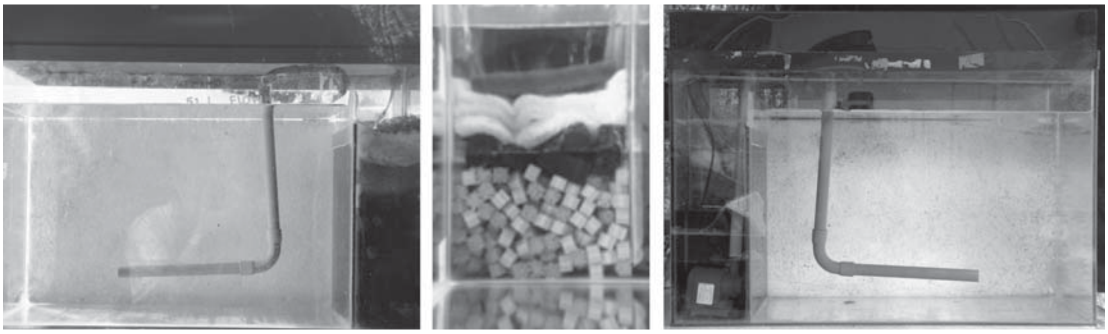

Figure 1. Closed recirculating water system glass aquaria for *M. rosenbergii* larviculture.

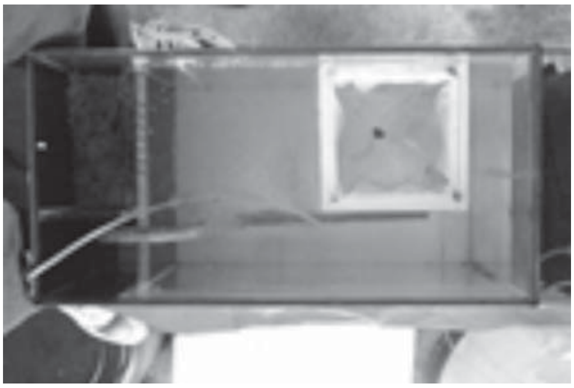

Figure 2. Floating fine mesh cage in closed recirculating saline water system.Saline water.

The saline water used in this experiment was prepared from commercial artificial sea salt powder (Marinium trade mark). The Jenway conductivity meter (model 4200) was used to ensure that the salinity level has reached the desired 15 ppt.

Rearing of larvae

The 4,000 larvae (80 ind•L^{-1}) were randomly stocked into each aquarium. The larvae had been fed with newly hatched Artemia nauplii since hatching. They continued to be fed with Artemia nauplii at a feeding rate of 5 ind•ml^{-1} twice a day throughout the experiment until they metamorphosed into post-larvae. For the open system (Control), debris was siphoned out daily. Water was also exchanged daily at 50%, while there was none for the closed recirculating water system. However, the debris was also removed in the same way. Larval development was checked daily at 8.00 am. Nitrite and ammonia concentrations in the water were monitored using a commercial test kit.

Rearing water sampling and analysis

Water sample from each aquarium was collected at the beginning of the experiment and three times a week until the experiment ended. The water samples were kept in a refrigerator. The presence of calcium, chloride, magnesium, potassium and sodium in the water were determined through high performance energy dispersive X-ray fluorescence spectrophotometry (Oxford ED^{2000}). An automatic pipette was used to draw 1 ml from the water samples for analysis (Pratoomchat et al. 2002a; 2003).

Data analysis

All data were analyzed by regression analysis, ANOVA and Tukey's using SPSS program ver. 9.0.

Results

The survival rate of post-larvae in the open system was 13.34+7.42% which was not significantly different from that of the closed system (11.26+7.78%) (Table 1). There was also no significant difference in the metamorphosis period between the two systems (P>0.05). After stocking, it took the larvae 26 days to develop from stage 1 to 12 (post larvae) in both systems. It was obvious that water use in the open recirculating system was significantly higher (6 times more) in terms of cost and volume (Table 1).

Table 1. Larval density and survival, water use and ammonia and nitrite levels during the experiment.

Treatment	Initial density (larvae•L^{-1})	Survival rate (%)	Volume of saline water used (L)	Cost of saline water used (USD)	Average NH_3^+ (ppm)	Average NO_2^- (ppm)
Open recirculating system	80	13.34+7.42[a]	300	34.29	ND	ND
Closed recirculating system	80	11.26+7.78[a]	50	5.71	0.08+0.08	0.03+0.03

Means with same letters in the rows are not significantly different at P<0.05; *ND*=not determined.

In the closed recirculating system, the magnesium concentration (Figure 3a) in the rearing water continuously declined (P<0.01, R^2 =0.7733), while sodium and potassium (Figures 3b and 3c) gradually declined (P<0.05, R^2 = 0.1687 and R^2= 0.1788). Calcium concentration (Figure 3d) was rather stable (P>0.05, R^2 = 0.0063). In contrast, chlorine concentration gradually increased (Figure 3e). Very low concentrations of ammonia and nitrite in the closed recirculating systems were observed.

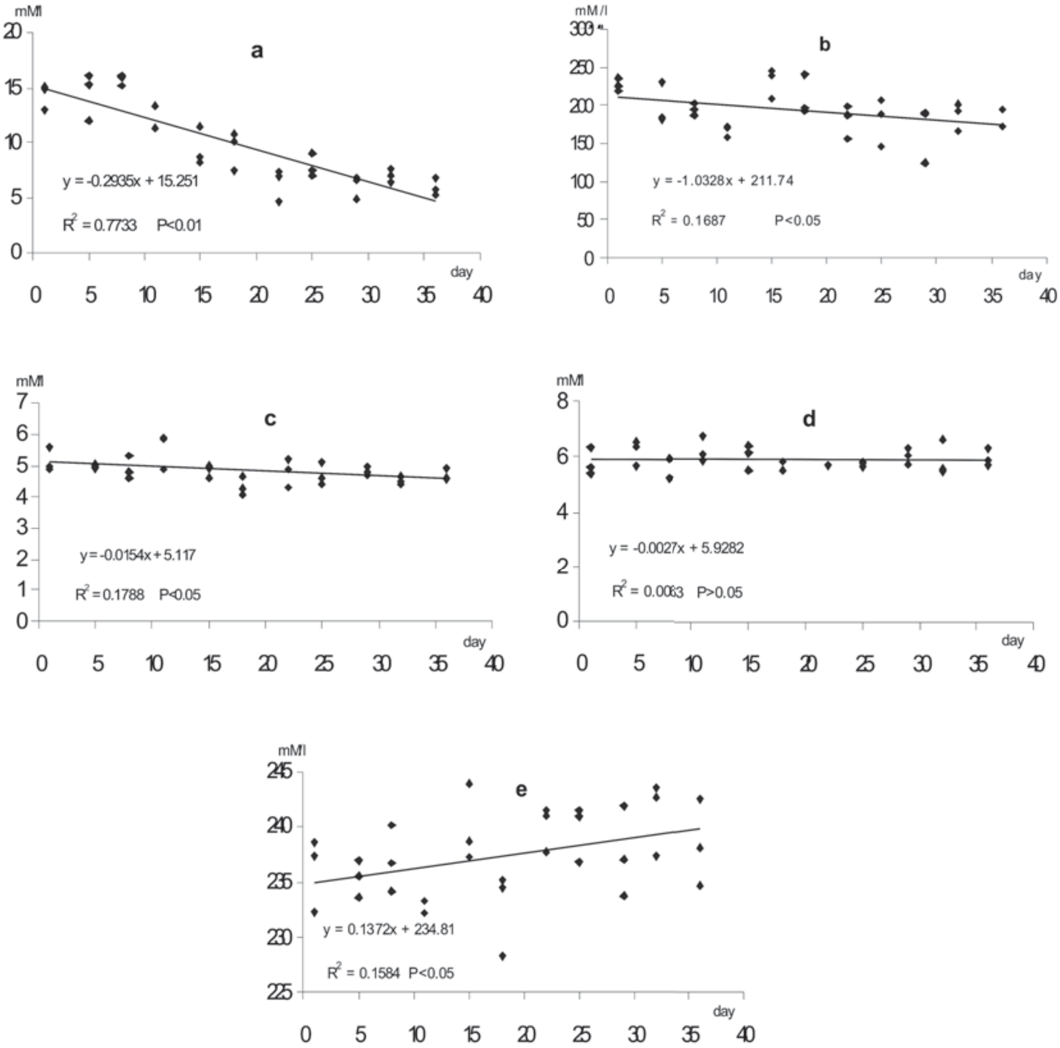

Figure 3. Concentrations of (a) magnesium, (b) sodium, (c) potassium, (d) calcium and (e) chloride for 36 days in the closed recirculating system.

Discussion

In the past, several trials have attempted to reduce the use of concentrated seawater in *M. rosenbergii* larviculture. Artificial seawater was used instead such as that reported by Tansakul (1983) which resulted in a 15% survival rate. The stocking density used by Tansakul (1983) was 10 larvae•L^{-1}, stocked in 12 ppt artificial seawater in an 8 L static water system with 1 L exchange of seawater every five days. Qureshi et al. (1993) also reported that M. malcolmsomii larvae could survive and develop to post-larval stage in artificial seawater at salinities of 12-14 ppt, in a study area that was a considerable distance from the coast.

The survival rate obtained in this experiment is comparable to the results obtained by Tansakul (1983) where he obtained a 15% survival rate from nursing larvae in a static water system with 12 ppt artificial seawater. Likewise, the results obtained by Menasveta and Piyatiratitivorakul (1980) were similar, which ranged from 15.9-18.7% at a density of 20 larvae•L^{-1}. They used a closed recirculating system with separate sub-sand filter unit and with sub-sand filter inside the rearing tank. However, it has to be noted that the initial stocking densities of larvae in the experiments by

Menasveta and Piyatiratitivorakul (1980) and Tansakul (1983) were significantly lower than the densities used in this present study. This indicates that the system used in the present study is more efficient for *M. rosenbergii* larviculture.

The survival rate in the closed system was a bit lower than that in the open system although not significantly different. It might be due to the negative effect of decreasing amounts of magnesium, potassium and sodium concentrations in the water during the 36 day culture period. Magnesium concentration especially decreased to more than two times from the initial concentration. According to Pratoomchat et al. (2002) and Visudtibhan (1993), magnesium is essential for cuticle formation and neurosystem function while potassium is necessary for osmoregulatory system and membrane potential. Visudtibhan (1993) reported that the optimum levels of magnesium and potassium for *M. rosenbergii* larvae were 16.45 mM•L^{-1} and 7.67 mM•L^{-1}, respectively. According to Damrongphol et al. (2001), calcium, magnesium and sodium are also necessary for supporting normal embryonic development, survival, hatching or survival of newly hatched larvae *M. rosenbergii*. Thus magnesium, potassium and sodium should be added into the rearing water to maintain suitable concentrations during culture. This also includes calcium although it has a minor effect on survival rate.

The average concentrations of ammonia and nitrite during the experiment in the closed system were 0.08 and 0.03 ppm, respectively. They are within the recommended levels of less than 1.0 ppm for total ammonia and 0.25 ppm for nitrite (Jayachandran 2001). Therefore, mortality was not caused by ammonia and nitrite from larvae and live feed.

One serious problem observed during this experiment was the abnormal appearance of the bodies of the brooders. Their abdominal muscles were opaque and the hepatopancreas and hemolymph became pale orange in color. In addition, the mortality rate of larvae was quite high. However, the reasons for this occurrence were not known because there had been no reports of an epidemic or virus disease outbreak for a long time. After the experiment, it was learned that *M. rosenbergii* hatcheries and farms in Thailand were facing an extremely serious problem of high mortalities. Both brooders and larvae were infected with white tail disease (WTD) caused by *M. rosenbergii* nodavirus (*Mr*NV) and extra small virus (XSV). The infected prawns showed symptoms of lethargy with opaque abdominal muscles. Other signs of this disease included orange colored hepatopancrease and haemolymph. The presence of *Mr*NV and XSV in brooders suggested that they were likely to transmit the viruses to their larvae and post-larvae (Yoganandhan et al. 2006). The organisms in this experiment showed all the pathological signs which meant that they were infected by this disease, resulting in a rather low survival rate.

The closed water recirculating system in this experiment had no impact on the metamorphosis of the larvae. The larvae developed into post larvae after 26 days, similar to what is happening in commercial production nowadays (Pitipornchai pers. comm).

In conclusion, this experiment has shown that artificial seawater prepared from commercial sea salt powder could be used as a source of saline water instead of concentrated seawater from salt farms. The closed saline water recirculating system with trickling filter unit packed with fiberglass and bioballs could be efficiently used to reduce saline water usage. It is highly possible to apply this system to commercial production of *M. rosenbergii* larvae in the future.

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References

DOF. (2004) Fisheries Statistics of Thailand 2002. Fishery Information Tecnology Center, Department of Fisheries, Ministry of Agriculture and Cooperatives.

Damrongphol, P., Jaroensastraraks, P. and Poolsanguan. B. (2001) Effect of Various Medium Compositions on Survival and Hatching Rates of Embryos of the Giant Freshwater Prawn (*Macrobrachium rosenbergii*) Cultured *in vitro*. Fisheries Science 67:64-70.

FAO. (2000) FAO Year Book Fisheries Statistic. Aquaculture production.

Jayachandran, K.V. (2001) Palaemonid Prawns : Biodiversity, Taxonomy, Biology and Management. Enfield Science Publishers, Inc.

Ling, S.W. (1962) Studies on the Rearing of Larvae and Culturing of Adults of *M.rosenbergii* (de Man). FAO Indo-Pac. Fish. Counc. Curr.Aff.

Menasveta, P. and Piyatiratitivorakul, S. (1980) A Comparative Study on Larviculture Techniques for the Giant Freshwater Prawn, *Macrobrachium rosenbergii* (de Man) Aquaculture 20:139-249.

Pratoomchat, B., Sawangwong, P. and Machado, J. (2002a) Organic and Inorganic Compound Variations in Haemolymph, Epidermal Tissue and Cuticle over the Molt Cycle in *Scylla serrata* (Decapoda). Comp.Biochem.Physiol. 131A:243-255.

Pratoomchat. B., Sawangwong. P., Guedes. R., Reis. M. L., and Machado. J. (2002b) Cuticle Ultrasructyre Changes in the *Crab Scylla serrata* over the Molt Cycle. Jour Exper Zoology 293:414-426.

Pratoomchat, B., Sawangwong, P. and Machado, J. (2003) Effects of Controlled pH on

Organic and Inorganic Composition in Haemolymph,Epidermal Tissue and Cuticle of Mud *Crab Scylla serrata*. Jour Exper Zool. 295:47-56.

Tansakul, R. (1983) Progress in Thailand Rearing Larvae of The Giant Prawn *Macrobrachium rosenbergii*. Aquaculture 31:95-98.

Visudtibhan, C. (1993) Effect of Different Levels of Magnesium Ions and Potassium Ion on the Survival of *Macrobrachium rosenbergii* (de Man) Post Larvae in the Rock Salt Water. Master of Science Thesis, Kasetsart University, Thailand.

Yoganandhan, K., Lertvibhas, M., Sriwongpuk and Limsuwan, C. (2006) White Tail Disease of The Giant Freshwater Prawn *Macrobrachium rosenbergii* in Thailand. Dis Aquat Org 69:255-258.

Cage Culture of *Pangasius* Catfish in the Mekong Delta of Vietnam: A Case Study in Dong Thap Province

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Abstract

A survey was conducted in Hongngu district, Dongthap province of Vietnam during November 2001 to November 2002, to assess the status of the cage culture of *Pangasius* catfish and the environmental awareness. Ninety-five cage farmers were randomly selected for interviews, using a survey to investigate socio-economic characteristics of farmers, cage culture practices, investment cost and return, problems and other information. These farmers managed 156 cages out of total 204 cages in the study area. The survey consisted of a structured checklist and open-ended questions. The survey showed that the major species cultured in cages are *Pangasius hypophthalmus* and *P. bocourti* in either monoculture or polyculture with *P. conchophilus* or red tail barb (*Barbodes altus*). Almost all farmers used homemade feed prepared with locally available ingredients, and the homemade feed accounted for about 98% of the total feed input. More than 50% of farmers produced negative net economic returns, and most cage farmers listed lack of capital, unstable costs and unstable fish prices as the top three constraints for cage culture.

Introduction

Cage culture is commonly practiced worldwide in both freshwater and marine environments, including open ocean, estuaries, lakes, reservoirs, ponds and rivers (Beveridge, 1987). In Southeast Asia, cage culture plays an increasingly important role for fish production, which involves many small-scale farmers in Vietnam, Cambodia, Indonesia and Thailand (Liao and Lin, 2000). However, the environmental impact of cage culture is often ignored and rarely subjected to study.

Cage culture of *Pangasius* catfish originated many years ago in Cambodia and has spread widely to other Indochinese countries. The best known areas for intensive catfish production from cage culture are in An Giang and Dong Thap provinces of the Mekong delta, Vietnam (Andriesz, 2000), where annual production in 2000 was 42,000 and 20,000 tons by 3,000 and 2,000 cages, respectively (Anon., 2000). Total fish production has increased 5 fold between 1995 and 2000. Most catfish cages are concentrated along the banks of Mekong River near Chau Doc district (An Giang province) and Hongngu district (Dong Thap province) , which are also the major suppliers of fingerlings for cage stocking. The main species being cultured are *Pangasius* bocourti and P. hypothalamus along with minor species like Chana micropelpte and Puntius gonionotus.

Cage cultured fish are entirely dependent on formulated diet (Phuong, 1998), and the waste produced from this consumption is released directly to the river. The cage culture contributing nutrients, organic matter and turbidity may cause deterioration of water quality and biota downstream (Pillay, 1992). The quantity of wastes discharged from a fish cage depends on quantity and quality of feed inputs (Cho et al., 1991). With relatively low protein and high carbohydrate diet, nutrient loading from culture of *Pangasius* is likely to be much lower than for salmonid culture in cages. However, waste in the form of organic matter, particulate matter and suspended solids may result in sediment accumulation and BOD near the site of cage culture systems. As a result of rapid expansion of cage culture in Chau Doc and Hongngu districts, water quality is reported to have deteriorated so much that fish disease outbreaks have occurred when the river is low and water flow sluggish during the dry season. While research has been done on seed propagation and diet formulation, little effort has been expended on environmental issues of cage culture. Therefore, the purpose of this study was to assess the status of the cage culture of *Pangasius* catfish and the environmental awareness.

Materials and Methods

A survey was conducted in villages from Hong Ngu district, Dong Thap province of Vietnam during November 2001 to November 2002. The cage culture areas are located in the confluence of So Thuong Canal and one branch of the Mekong River. Cages distributed on both sides of So Thuong Canal and the southern side of Mekong River. The cage culture areas cover 5,252 m in the So Thuong Canal and 2,530 m the Mekong River.

The survey was conducted using a structured checklist and open-ended questions in three villages along So Thuong Canal and five villages along Mekong River. The questionnaire examined socioeconomic characteristics of farmers, cage culture practices, investment cost and return, problems and other information. Ninety-five cage farmers including 38 along So Thuong Canal and 57 along Mekong River were randomly selected for interviews in the survey, which covered 156 out of 204 cages in the study area. Among the 156 cages, 49 were in Sothuong Canal and 107 in Mekong River. They included 26 nursery cages and 130 grow-out cages, which was proportional to the ratio of nursery to grow-out cages (Table 1).

MS Excel was used to store all survey data, and to generate tabular and graphical outputs for different types of data. A simple cost-benefit analysis was conducted to compare economic returns of different cage culture practices (Shang, 1990).

Table 1. Study areas and distribution of interviewed farmers and cages in Hongngu district of Dongthap province in Vietnam.

River/canal	Villages	Number of interviewed farmers	Types of cages					Total number of cages	
			Nursery		Grow-out				
			Small (<200 m^3)	Medium (200-300 m^3)	Small (<200 m^3)	Medium (200-300 m^3)	Large (>300 m^3)		
So Thuong canal	Tan Hoi	8	0	1	6	2	4	13	49
	Thuong Lac	12	1	0	6	2	7	16	
	Hong Ngu	18	0	0	8	4	8	20	
Mekong River	Thuong Thoi Tien	5	0	0	4	1	2	7	107
	Longkhanh A	1	0	0	3	0	0	3	
	Longkhanh B	7	1	0	8	1	4	14	
	Anbinh	2	0	0	2	0	4	6	
	Longthuan	42	20	3	29	9	16	77	
Total	8	95	22	4	66	19	45		156
			26		130				

Results

Farmers' background

All cages were operated and managed by farmers (owners). The age and gender of farm owners had a bearing on the decision-making process. Men dominated in the cage culture industry, comprising 90.5% compared to 9.47% for women (Table 2). Among the interviewed farmers, ages between 40-49 years old were most common (Table 2). Educational background of the owners was poor, with only 3.45% of owners trained in colleges and vocational schools (Table 2). Among the interviewed farmers, about 38% of nursery farmers had run farms for 1-5 years, 38% for 6-10 years, 12% for 11-15 years, and 12% for more than 16 years. In comparison, about 36% of grow-out cage farmers had run farms for 1-5 years, 36% for 6-10 years, 22% for 11-15 years, and only 6% for more than 16 years.

Cage culture was the main occupation of cage owners, and all respondents were working part-time in this field, so they also had a subsidiary occupation. About half (55%) were also working in paddy fields, and 18% involved in business (Table 3). Before starting cage culture, about 31% cage owners were rice farmers, followed by pond aquaculture farmers (25%) and businessmen (23%).

About 35% of the interviewed nursery cage farmers hired permanent workers with an average of 1.4 workers per household, and none of them hired casual workers. Grow-out cage farmers hired both permanent workers (61%) and causal workers (13%). The average number of workers hired by the grow-out farmers was 2.3 permanent workers and 5.7 casual workers per household (Table 4).

Table 2. Percent of cage farmers of different gender, age and educational backgrounds.

Items	Types of farmer		Total
	Nursery	Grow-out	
Gender and age (*N* = 95)			
Man			
20-29 years old	2.11	0	2.11
30-39 years old	4.21	18.9	23.2
40-49 years old	9.47	25.3	34.7
50-59 years old	8.42	15.8	24.2
>60 year old	1.05	5.26	6.31
Subtotal	25.3	65.3	90.5
Woman			
20-29 years old	0	0	0
30-39 years old	1.05	3.16	4.21
40-49 years old	1.05	1.05	2.11
50-59 years old	0	2.11	2.11
>60 years old	0	1.05	1.05
Subtotal	2.1	7.37	9.47
Total	27.4	72.6	100
Education background (*N* = 87)			
Primary school	11.5	21.8	33.3
Secondary school	8.05	24.1	32.2
High school	10.3	20.7	31.0
College/vocational school	0	3.45	3.45
Total	29.9	70.1	100

Cage construction and dimensions

Most cages were topped with wooden houses, thus the cages were also home for the cage farmers. Most cages were constructed using hardwoods with high water resistance, making them strong enough to withstand the constant pressure of water current. Cages could last from 20-30 to even 50 years. All cages were rectangular in shape except very small squared cages, which also had no homes on them.

Cages were categorized as small, medium and large (Table 5). Small cages had volumes less than 200 m^3, medium cages 200-300 m^3, and large cages more than 300 m^3. The average dimensions of the small, medium and large cages were 8.4×4.2×3.5 m, 11.1×5.2×4.5 m, and 14.4 ×7.0×5.1 m, respectively. Fingerlings were mostly nursed in small, and sometimes medium cages, but not in large cages. Grow-out was practiced in all three sizes of cages (Table 5). The mean number of cages was 1.6 small and 1.8 medium cages per household for nursery farmers, and 1.4, 1.6 and 1.8 cages of small, medium and large size, respectively, for grow-out farmers (Table 5).

Production systems

Among the interviewed farmers, most farmers (57.1%) cultured P. hypothalamus in cages, followed by red tail barb (*Barbodes altus*) and *P. conchophilus* (18.2% each), and *P.*

bocourti (6.5%) (Table 6). All seed of *P. bocourti* came from hatcheries, while most seed for P. hypothalamus and B. altus also came from hatcheries, but only half of *P. conchophilus* seed was from hatcheries (Table 6).

Table 3. Percent of cage farmers in current and former employment categories.

Items	Type of farmer		Total
	Nursery	Grow-out	
Current subsidiary occupation (*N* = 60)			
Rice farmer	11.7	43.3	55.0
Business	5.00	13.3	18.3
Hired labor	3.33	3.33	6.66
Rice processing	0	5.00	5.00
Tailor	3.33	0	3.33
Worker	0	3.33	3.33
Government officer	0	1.67	1.67
Livestock breeding	1.67	0	1.67
Sawing factory	0	1.67	1.67
Pull-dozer driver	0	1.67	1.67
Wood selling	0	1.67	1.67
Total	25.0	75.0	100
Former main occupation (*N* = 91)			
Rice farmer	9.89	20.9	30.8
Pond aquaculture	8.79	16.5	25.3
Business	4.40	18.7	23.1
Government officer	1.10	4.39	5.49
Sawing factory	1.10	3.30	4.40
Rice processing	0	3.30	3.30
Goods transportation	0	3.30	3.30
Hired labor	2.19	1.10	3.29
Fish sauce processing	0	1.10	1.10
Total	27.5	72.5	100

Table 4. Employment provided by cage farmers (*N* = 95).

Items	Type of farmer	
	Nursery	Grow-out
Percentage of farmer hiring workers (%)		
Permanent workers	34.6	60.9
Casual workers	0	13.4
Total	34.6	74.3
Mean number of workers hired by cage culture farmers (workers/household)		
Permanent workers	1.4±0.2	2.3±0.3
Casual workers	0	5.7±1.6

Table 5. Information on cage sizes (*N* = 95). Mean (+SE) and ranges (in parentheses).

Items	Type of farmer		Total
	Nursery	Grow-out	
Percentages by size of cage (%)			
Small	14.10	42.31	56.41
Medium	2.56	12.18	14.74
Large	0	28.85	28.85
Total	16.66	83.34	100
Mean size of cage (m^3/cage)			
Small	109±7.4	137±9.0	----
	(45-180)	(38-200)	----
Medium	252±14.3	255±8.1	----
	(225-292)	(210-300)	----
Large	----	523±38.3	----
	----	(302-1,077)	
Mean number of cages per household			
Small	1.6±0.2	1.4±0.1	----
Medium	1.8±0.3	1.6±0.3	----
Large	----	1.8±0.2	----

Table 6. Fish species cultured in cages and sources of seed (*N* = 95).

Species	Percent of farmers	Sources of seed	
		Wild seed (%)	Hatchery propagated seed (%)
Pangasius hypophthalamus	57.1	15.0	85.0
P. bocourti	6.5	0	100
Barbodes altus	18.2	16.0	84.0
P. conchophilus	18.2	48.3	51.7

Cage culture operated year round, especially with more artificially propagated seed from hatcheries. Although fish could be stocked any time of year, there were two main periods of fish stocking – November to December and April to June. Cage farmers sought the best time to harvest their fish to maximize economic benefits.

Before stocking into grow-out cages, fry were nursed for 1-5 months depending on fish species, while grow-out lasted for 3-14 months, varying with different culture practices (Table 7). Fish nursery was normally done in monoculture cages, while grow-out was practiced in both monoculture and polyculture. About 46% of the interviewed cage farmers cultured P. hypothalamus in monoculture, while only 7% monocultured the other three species (Table 7). The remaining 46% polycultured with species in different combinations.

Grow-out farmers stocked nursed fingerlings of *Pangasius* catfish (28-141 g in size) and B. altus (12-40 g in size) in cages at densities varying from 33 to 238 fish/m^3 for both monoculture and polyculture (Table 8). Except for the monoculture of B. altus, stocking densities in small cages were higher than those in medium and large cages. Average size of *Pangasius* catfish at harvest ranged from 700 to 1,629 g, while B. altus had smaller average size at harvest (67 – 221 g) (Table 8).

Table 7. Culture systems in grow-out cages (*N* = 69).

Cage size	Culture system	Fish species	Percent	Culture duration (mons/crop)
Small	Monoculture	*P. hypophthalamus*	20.3	8.6±1.1 (3-13)
		B. altus	2.90	
	Polyculture	*P. bocourti + P. conchophilus + B. altus*	1.45	
		P. hypophthalamus + P. conchophilus + B. altus	1.45	
		P. hypophthalamus + B. altus	2.90	
		P. hypophthalamus + P.conchophilus	4.35	
		P. conchophilus + B. altus	4.35	
Medium	Monoculture	*P. hypophthalamus*	13.0	10.7±1.4 (6-13)
		P. conchophilus	1.45	
	Polyculture	*P. bocourti+ P. conchophilus*	1.45	
		P. bocourti+ P. conchophilus+ B. altus	1.45	
		P. bocourti+ P. hypophthalamus+P.conchophilus+B.Altus	1.45	
		P. hypophthalamus + B. altus	1.45	
Large	Monoculture	*P. hypophthalamus*	13.0	10.0±0.7 (6-14)
		P. bocourti	2.90	
	Polyculture	*P. bocourti + P. conchophilus + B. altus*	10.1	
		P. bocourti + B. altus	1.45	
		P. hypophthalamus + P. conchophilus + B. altus	1.45	
		P. hypophthalamus + P. conchophilus	8.70	
		P. hypophthalamus + B. altus	1.45	
		P. conchophilus + B. altus	2.90	
Total			100	

Table 8. Fish stocking, harvest and production in grow-out cages (*N* = 67).

Culture system	Cage size	Fish species	Stocking size (g/fish)	Stocking density (fish/m^3)	Harvest size (g/fish)	Production	
						(kg•m^{-3}•crop^{-1})	(kg•m^{-3}•year^{-1})
Monoculture	Small	*P. hypophthalmus*	91±15.0	143±26.5	1,064±44.0	90±8.9	152±15.0
		B. altus	12±0.0	922±357.1	67±33.0	27±0.0	45±0.0
	Medium	*P. hypophthalmus*	133±31.0	106±7.8	1,094±50.0	118±21.2	135±24.2
		P. conchophilus	100±0.0	33±0.0	700±0.0	19±0.0	22±0.0
	Large	*P. hypophthalmus*	126±27.0	87±7.7	1,050±49.0	95±21.9	115±26.6
		P. bocourti	93±8.0	55±11.3	1,400±100	59±34.6	72±41.9
Polyculture	Small	*P. hypophthalmus*	80±17.0	238±36.9	978±96.8	89±6.9	150±11.6
		P. bocourti	166±0.0		1,200±0.0		
		B. altus	25±5.4		179±12.6		
		P. conchophilus	141±21.5		700±70.7		
	Medium	*P. hypophthalmus*	28±5.5	157±29.1	1,350±50.0	103±16.1	118±18.4
		P. bocourti	108±50.7		1,400±100		
		B. altus	35±10.1		197±31.2		
		P. conchophilus	107±51.5		900±100.0		
	Large	*P. hypophthalmus*	83±0.0	123±23.9	1,178±101	93±8.1	113±9.9
		P. bocourti	122±21.5		1,629±129		
		B. altus	40±3.4		212±14.5		
		P. conchophilus	122±16.4		780±31.3		

Feed and Feeding

Almost all farmers use home-made moist feed that they prepared for cage culture. The home-made feeds were prepared using locally available agro- and/or fishery by-products such as rice bran and trash fish (the main ingredients of home-made feeds). Feed formulation varied with different cultured species, fish size and seasonal supply of ingredients. The average percentages of rice bran and trash fish were 54% and 45% during nursery stage, 65% and 34% during grow-out stage 1 (<500 g), and 65% and 33% during grow-out stage 2 (>500 g) (Table 9). Pelleted feed only accounted for 1.5% and 2% of total feed input during nursery and grow-out stages, (Table 9). Average feed conversion ratio for home-made feed was 4.09±0.43, ranging from 3 to 6.

Table 9. Composition of feed and feeding practices (*N* = 91).

Item	Culture stages		
	Nursery	Grow-out 1 (< 500 g)	Grow-out 2 (> 500 g)
Feed ingredients for home-made feed (%)			
Trash fish	45.4	34.4	33.5
Rice bran	54.3	65.2	64.7
Vegetables	0.37	0.40	1.87
Feed types (%)			
Home-made	98.5	97.3	
Pelleted	1.50	2.38	
Feeding rate (%)	11±0	5.3±0.6	4.8±0.7
Feeding frequency (times/day)	4.4±0	2.7±0.1	2.7±0.2

Table 10. Average net returns and percent of cages with negative and positive net returns based on simple cost-benefit analysis (*N* = 82).

Farmer types	Cage sizes	Culture systems	Negative net return		Positive net return		N
			Amount (US$/cage)	Percent	Amount (US$/cage)	Percent	
Nursery	Small	Monoculture	-4,432±2,142	71	1,665±762	29	21
	Medium	Monoculture	-5,856±3,070	67	1,216±0.0	33	3
Grow-out	Small	Polyculture	-5,734±3,375	50	1,121±509	50	10
		Monoculture	-4,855±2,677	56	2,304±1,026	44	16
	Medium	Polyculture	-150±0.0	25	3,157±1,1764	75	4
		Monoculture	-7,242±2,965	30	7,469±3,606	70	10
	Large	Polyculture	-7,357±2,751	87.5	10,012±1,811	12.5	8
		Monoculture	-11,223±3,029	30	11,677±4,674	70	10

Cost-benefit analysis

The overall average net returns from cage culture varied with cage size and culture system (Table 10). Overall, about 55% of cages lost money, and 45% generated profit. Most nursery farmers lost money, and only about one third produced positive net returns, ranging from US$ 5,856±3,070 to US$1,665±762 (Table 10). About half small grow-out cages of both polyculture and monoculture produced negative net returns, while more then two-thirds of medium grow-out cages of both polyculture and monoculture produced negative net returns. For large grow-out cages, most polyculture cages (87.5%) resulted in negative net returns, while 70% of monoculture cages resulted in positive net returns.

Constraints faced by cage farmers

Most cage farmers listed lack of capital, unstable market and unstable fish price as top three constraints to cage culture (Table 11). Among constraints faced by cage farmers, capital and market issues surpassed technical issues for both nursery and grow-out farmers. The three same constraints were the main ones faced by both nursery and grow-out farmers - lack of capital, unstable market prices and unstable fish prices. Both nursery and grow-out farmers were satisfied with widely used homemade feeds. Only 2.17% of nursery farmers and 0.64% of grow-out farmers indicated that high feed prices were constraints; moreover none of the cage farmers complained about quality of the homemade feeds. Only a few farmers complained about low quality of fish seed, probably due to wide availability of artificial seed.

Environmental awareness

Local government officials at provincial and district levels have been aware of the importance of environmental regulations and enforcement, and also showed positive attitudes to environmentally related studies. However, there are no any existing regulations or documentation on environmental issues related to cage aquaculture either in rivers or in lakes/reservoirs. Generally, cage farmers have little environmental awareness.

In 2002, the Aquaculture Research Institute No.2 (RIA2), following suggestions from the Department of Science and Technology, Ministry of Fisheries of Vietnam, drafted "Standards for Aquaculture: Cage Culture of Basa Catfish (P. bocourti), Sutchi Catfish (P. hypothalamus) – Conditions for Food Safety" (Anon., 2002). This document outlined basic conditions to secure food safety on cage culture in rivers. However, this document dealt only with requirements for cage culture such as site selection, water quality, and environment, but not important issues on environmental impacts such as number of cages and waste loading.

Discussion

Cage culture has a long history in Mekong delta, and culture technologies are well known. Until 1996, most seeds of *Pangasius* catfish were collected from the wild (Phuong, 1996). However, with successful artificial breeding programs, artificially propagated seeds of P. hypothalamus and *P. bocourti* are available from local hatcheries year round (Table 6). Successful artificial breeding has provided the potential for the further development of cage culture in the area. However, cage culture in the Mekong delta has become a large-scale commercial production sector of high investment, depending upon the complicated interaction among the biotechnological, socio-economic and environmental factors. The development of cage culture for *Pangasius* catfish in southern Vietnam has fluctuated for decades with the political and economic changes in Vietnam, in the region, and in the world.

Economic analyses in the present study showed that more than 50% of grow-out cages produced negative net return (Table 10) during 2001-2002, which is similar to the situation in 1996 (Phuong, 1998). This is due largely to the reduced export of *Pangasius* catfish to USA and thus the drop in selling price. For that reason, most cage farmers listed lack of capital, unstable market and unstable fish price as top three constraints for cage culture (Table 11).

Table 11. Constraints faced by cage culture farmers.

Constraints	Nursing farmers (%, *N*=26)	Grow-out farmers(%, *N* =69)
Lack of capital	23.9	29.9
Unstable market	21.6	31.2
Unstable fish price	15.2	15.9
Disease	13.0	7.64
Lack of culture techniques	6.52	7.64
High fish mortality	6.52	0.64
Low water quality	6.52	4.46
High cage density in rivers	2.17	1.27
Low seed quality	2.17	0
High feed price	2.17	0.64
High interest rate on loans	0	0.64
Total	100	100

Pelleted feed has been promoted for cage culture for some years, but the adoption of it has been slow. Pelleted feed only accounted for about 2% of the total feed input in the present study, and the percentage was similar to the 1% reported 7 years ago by Phuong (1996). Homemade feeds, prepared by farmers themselves using locally available low-cost ingredients, have many disadvantages such as low and unbalanced nutritional values, as well as unstable quality and supply of ingredients. These may cause slow fish growth, high fat deposition in visceral areas of fish, and other problems. A comparison between homemade feed and pelleted feed showed that cost of homemade feed for producing one kg of fish may be higher than that of pelleted feeds. However, use of pelleted feed could not only increase economic return, but also reduce the pressure of catching trash fish from the nature, reduce nutrients, organic matter and solid wastes released into rivers, and reduce other wastes such as ash and fuel produced during preparation of homemade feeds. Adoption of pelleted feed will take considerable promotion and extension effort. Research should be conducted in the Mekong River to determine the appropriate number of cages and/or amount of feed input the river can sustain (the carrying capacity for cages) and investigate the environmental impacts of cage culture.

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References

Andriesz, P. (2000). Aquaculture in Vietnam. Global Aquaculture Advocate 3:36-38.

Anon. (2000). Annual report on rural development of An Giang and Dong Thap Provinces. Department of Agriculture, An

Giang and Dong Thap Provinces, Vietnam.

Anon. (2002). Standards for aquaculture: cage culture of Basa catfish, Sutchi catfish – conditions for food safety. Aquaculture Research Institute No.2 (RIA2), Vietnam.

Beveridge, M. (1987). Cage Aquaculture. Fishing News Books Ltd., England.

Cho, C. Y., J. D. Hynes, K. R. Wood and H. K. Yoshida (1991). Quantification of fish culture wastes by biological (nutritional) and chemical (limnological) methods; the development of high nutrient dense (HND) diets. Pages 37-50 in C. B. Cowey and C. Y. Cho, Editors. Nutritional Strategies and Aquaculture Waste. Proceedings of the First International Symposium on nutritional strategies in management of aquaculture waste (NSMAW). University of Guelph, Canada.

Liao, I-C. and C. K. Lin (2000). Cage Culture in Asia. Proceedings of International Symposium on Cage Aquaculture in Asia. Asian Fisheries Society and World Aquaculture Society, Manila, Philippines.

Phuong, N.T. (1996). On-farm prepared feed and feeding regimes for the *Pangasius* catfish (*Pangasius* bocourti) cultured in cages in the Mekong River in Vietnam. Paper presented at the EIFAC Workshop on Fish and Crustacean Nutrition Methodology and Research for Semi-Intensive Pond-Based Farming Systems. 3-5 April 1996, Szarvas, Hungary.

Phuong, N.T. (1998). Cage Culture of *Pangasius* Catfish in the Mekong Delta, Vietnam: Current Situation Analysis and Studies for Feed Improvement. Ph.D. Thesis, National Institute Polytechnique of Toulouse, France.

Pillay, T.V.R. (1992). Aquaculture and Environment. Blackwell Scientific Publications Inc., Cambridge, England.

Shang, Y.C. (1990). Aquaculture Economic Analysis: An Introduction. World Aquaculture Society, Baton Rouge. 211 pp.

Pen Culture Technologies in Lake Gaobao, Yangzhou, China

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Abstract

Located in the Central part of Jiangsu province, Lake Gaobaois is shared by the provinces of Jiangsu and Anhui and connects three municipalities, namely Yangzhou, Huai'an and Chuzhou. The total area covers 792 km^2 ranking Lake Gaobao as the third largest lake in this province. The lake is characterized by constant water flow and provides great advantages for aquaculture activities in the lake. Pen culture is one of the farming practices started in the early 1990s. The lake is rich in aquatic grasses. As seasons change, the grass rot and decay in the lake making the water highly fertilized. Aquaculture through pen culture practices can effectively control the rapid and excessive growth of aquatic grass in this open water body. Farmers in the lake adopt intensive pond farming technologies for the lake pens with care. Moreover, various species such as crabs (*Eriocheir sinensis*), shrimps (*Macrobrachium nipponense*), and non-traditional species apart from conventional fish species are employed for pen aquaculture as an important step for farming system renewal. The practical technologies in this lake farming have revealed the high skills of the farmers in lake fisheries management by running, for example, pen culture or cage culture in the pen enclosures.

This paper presents the aquaculture technologies, site selection for pen placement, appropriate ratio between water areas and pen dimensions, pen size, mesh size for various farmed fishes, species selection, and appropriate stocking size. Environment-friendly practices such as cage-in-pen management, harvesting technologies, managerial skills, predator control and methods to prevent fish escape are also presented.

Lake Gaobao is a constant flowing water body. The annual water replacement is about 21 times, averaging almost twice a month. This water body cannot retain great fertility, particularly during the high-flow seasons when the fish are at their peak growth rate. The approaches for making use of this unique environment are discussed.

Introduction

Lake Gaobao Shaobo is located in the central part of Jiangsu province, at the west side of the Grant Canal. The lake from north to south is respectively called Baoying Lake, Gaoyou Lake and Shaobo Lake. The total size of these three lakes is 792 km^2 (equivalent to 1,188,000 mu; a mu is equal to 1/15 ha) (Figure 1). Lake Gaoyou is the largest with an area of 650 km^2, followed by Lake Shaobo and the smallest is Lake Baoying with an area of only 44 km^2. Lake Gaobao Shaobo is characterized by unique meteorological features from each lake. Lake Baoying is located in the northern part with stable water level, rich natural food, abundant aquatic grasses which are all welcome by the farmed fish species. Lake Gaoyou, located at the southwest and along the south corner is the bypass of flood waters from the upper reaches of Lake Hongzhe. There are many changes in meteorological features that influence the production of natural food organisms. In general, the lake has clear and good water quality, heavy flow, and high water exchange at the rate of 21 times a year. Thanks to the separation of the Grant Canal, the lakes have little source of pollution from agricultural residues. Lake Baoying is also a bypass for the national project on "South-North Water Transfer".

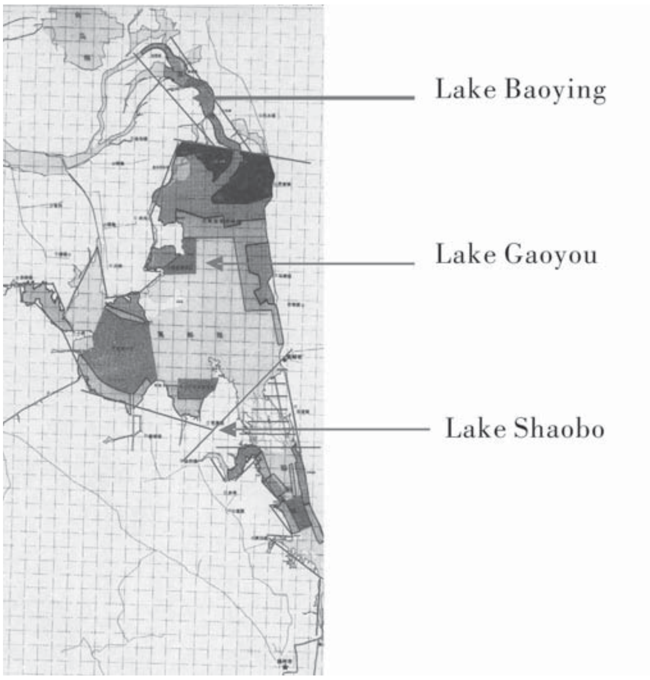

Figure 1. Map showing the location of Lakes Baoying, Gaoyou and Shaobo in Jiangsu, China.

Fishery Status in Lake Gaobao Shaobo

Lake Gaobao Shaobo is an important resource for freshwater fish production with an annual production of 12,000 tons, of which 41% is from capture fisheries and 59% is from aquaculture, mainly from pen and cage culture. The capture fisheries consist of 19.8% *Macrobrachium nipponese*, 14.8% *Palaemon modestus*, 21.7% *Coilia ectenes*, 10.2% common carp, crucian carp, and bream, and 4.1% snakehead (Figure 2). The aquaculture sector contributes 23.7% from crabs, 72.4% from grass carp and breams, and 3.9% from *Macrobrachium nipponese* (Figure 3). Crab provides 70% of income for the farmers (Yong and Zhu 2005).

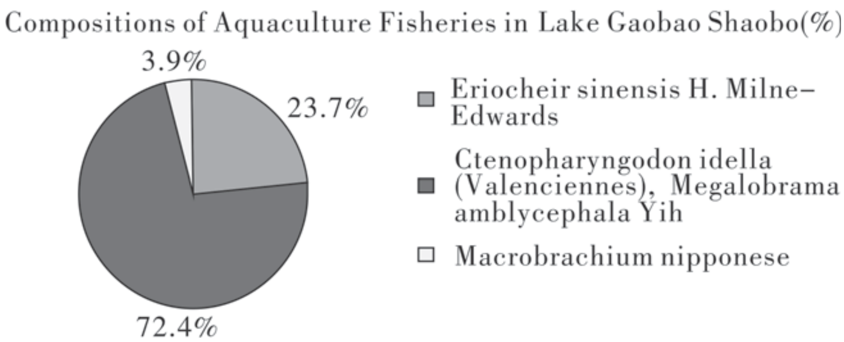

Figure 2. Compositions of the Capture Fisheries in Lake Gaobao Shaobo.

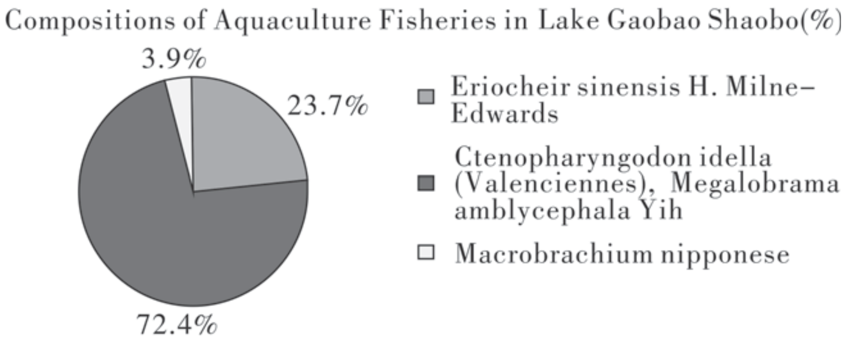

Figure 3. Compositions of the fisheries from aquaculture in Lake Gaobao Shaobo.

Pen and cage culture and other enclosures are the major approaches for aquaculture. Cage culture started in the Baoying Lake as early as the 1980s with an annual yield reaching 5 t•mu^{-1}. Till the 1990s, more and more people took interest in cage aquaculture, and the culture area expanded. At present, the total aquaculture area has reached 9,500 ha.

Pen culture

Pen culture is a major activity in the lake. Apart from the areas for transportation and flood bypass, the entire lake is available for the construction of fish pens. Each pen is 100 m×3000-4000 m in width and length with an area of 40-60 mu (Figure 4). Pens have uniform sizes for easy operation.

Figure 4. Pen culture in lakes.

The species stocked in pens should be in line with the expected yield and managerial technique. Crabs are now very popular for farming. The yield can reach 40-70 kg•mu^{-1}. Apart from crabs, other species such as mandarin fish (*Siniperca chuatzi*) and spotted steed (*Hemibarbus maculates*) are also cultured for the control of unwanted wild species that serve as food competitors. Production of these species is given in Table 1.

Table 1. Stocking, survival rate and harvest of species cultured in fish pens in Lake Gaobao Shaobo.

Species	Stocking		Survival	Harvest	
	Size	Quantity	(%)	Size	Quantity
Crab	80 pc•kg^{-1}	400 pc•mu^{-1}	80%	100-150-50(g•pc^{-1})	40%-35%-25% 40-70 kg•mu^{-1}
Mandarin fish	3-5 cm•fish^{-1}	10-15 fish•mu^{-1}	90%	500-750 g•fish^{-1}	5-10 kg•mu^{-1}
Spotted steed	3-5cm•fish^{-1}	30 fish•mu^{-1}	90%	300-550 g•fish^{-1}	8-14 kg•mu^{-1}

Cage culture in pens

Cage culture in pens is a traditional farming technique that is farmed intensively (Figure 5). On the average, fish yield can reach 5-6 tons/mu through the effective use of feeds. Residues from the cages can be further used in pens through the movement of the fish and flow of water, thus reducing the organic load in the open water environment and lowering feed cost. Cage aquaculture in pens is mainly for grass carp and Chinese bream (*Megalobrama* spp.) that some farmers mix with channel catfish. As shown in Table 2, the quantity of grass carp stocked ranges between 2,100 to 2,300 fish with a stocking size of 250 g•fish^{-1} (Table 2). In one rearing cycle the fish can reach 3,500 g with a production of 6.5 t•mu^{-1}. The stocking for the Chinese bream is 20% of grass carp. The stocking of channel catfish is 6500 fish•mu^{-1} with the stocking size of 25 fish•mu^{-1} (40 g•fish^{-1}). In one farming cycle, channel catfish could reach 750 g-900 g•fish^{-1} which is the marketable size for this species. The cage size, based on farmers' experience should not be bigger than one mu (666.7 m^2) in the 40 mu pen. If more cages are intended to be placed, several smaller cages are used. Regardless of the size of the cages in pens, these should be similar in size so that preparations for catching nets, feeding, and routine management is easy.

Table 2. Stocking density, survival and harvest of grass carp, Chinese bream and channel catfish cultured in cages within pens.

Species	Stocking		Survival	Harvest	
	Size	Quantity	(%)	Size	Quantity
Grass carp	2100 - 2300	250	98	3500	6000 - 6500
Chinese bream	420 - 460	30 - 50	95	500 - 750	200 - 327
Channel catfish	6500	40	95	750 - 900	4500 - 5500

Figure 5. Cage culture in pens.

Sustainable development with high economic returns

Pen culture is the main source of livelihood of farmers in these lakes. High economic returns are the fundamental goals of farmers (Gong 2006). With these goals in mind, adequate infrastructure and necessary inputs are essential for the farmers to support their families and continue farming.

Infrastructure capacity

As earlier mentioned, the southwest of Lake Gaoyou extending to Lake Shaobo is a water bypass during flooding season at the Hongzhe Lake. If flood occurs in the upper reaches, then the sluice gates for flood control have to be lifted to direct the water flow through Lake Shaobo to the Yangzhi River. When this happens, farmers sacrifice their means of livelihood as water level continuously rises and pens and cages are destroyed. Farmed fish escape and natural food such as aquatic grass die, settling down to the bottom and becoming decayed organic substances, which leads to over-fertile water conditions. Therefore, necessary infrastructure should be provided to prevent any untoward incidents affecting farmers' livelihood. At the same time, farmers' capabilities should be enhanced so that they will be able to have stronger resistance against risks and natural disasters.

Recommendations to establish appropriate rules and regulations for lake development

Aside from being site specific, the development of the lake should also be size specific, and with great responsibility. Through this, the development of the lake can be effectively controlled and rationalized thus avoiding over-exploitation and damage to proper function of the lake. The result of this study shows that it is appropriate to operate pen culture systems at the rate of less than 1% in open water space so as to effectively avoid an overload of the organic discharge from the farming activities (Min 2003).

Restricting operation license for enclosure aquaculture in the lake

All development processes should strictly follow the rules and regulations set by the lake authority, neither institutions nor individuals should occupy and develop a space as they wish. Furthermore, all producers should be responsible enough and follow the laws so as to ensure safety food production

Conserving fishery biodiversity and environment

More efforts should be exerted on maintaining the functions of the lake, balance between inputs and outputs. The lake should be closed for fishing activities during the breeding period. Also, the protected areas should be zoned for ecological conservation. This implementation requires joint efforts and collaborations among governmental agencies, research institutions, and legal enforcers (Yang et al. 2004).

Improving farming systems for the promotion of sound development in the lake

At present most of the pen culture apply feeds for fish to a great extent, which has a negative impact in speeding up the eutrophication process. Therefore, one way of reducing the impact is to improve farming systems by stocking well-balanced multi-species as a biological control. The research results show that lake water environment can be effectively maintained by stocking silver carp and bighead carp which can play an important role in water purification by consuming planktons, the common carp and crucian carp can make full use of the residues and detritus at the bottom of the lake.

A change in the farming systems can also be made by stocking quality and non- traditional species in pens and cages. The pen is stocked with high-valued species such as mandarin fish, Takifugu, snakehead, and large-mouth bass. Other wild species of fish such as top-mouth culter (*Eryghroculter ilishaeformis*), yellow catfish (*Pelteobagrus fulvidraco*) and spotted steed (*Hemibarbus maculates*) can also be domesticated. These fish species have a strong environmental tolerance, rich resources, good market acceptability and are more competitive against exotic fish species (Kang 2007).

Developing recreational fisheries for higher economic returns

With the development of farming systems and an improvement in the standard of living of the people, recreational fisheries should be developed in some areas. Recreation fisheries can provide relaxation, fun, participation, knowledge and experience apart from the fish catch. Recreation fisheries utilize fishing facilities, space, operation ground, fishing gears, products and human and natural resources.

Conclusion

Presently, Lake Gaobao Shaobo is recognized as a natural aquatic reserve for its richness in biodiversity, including 131 plant species in 53 families, 63 species of aquatic animals in 16 families, 200 species of planktons in 80 families, and 150 kinds of terrestrial birds. Many kinds of animals are listed nationally as top class or second class protected animals which are long inhabitants in the area. In the past, many developers have focused on economic progress, resulting in about 30% of the area that are not appropriately developed (Yangzhou Daily 2006). In order to balance economic development and wetland conservation in the area, the local government has set up a new and special agency for better and environment-friendly development program which will play a positive role for sound human living.

Acknowledgement

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References

Yong, N. and C.D. Zhu (2005). Fishes of the Taihu Lake.

Gong , J.H. (2006) Cage Culture of Wild Leiocassis longirostris in Reservoirs. Reservoir Fisheries, 2006(1): 54.

Min, K.H. (2003). Effective Approaches of Open water Fishery Development in China. China Fishery Economics. 5: 17-19.

Yang, Z.F., W.G. Shi, L.Q. Chen, Y. Chen, Z.L. Zhou and H. Liu (2004). The Effects of Pen Fish Culture on the Ecological Environments of Taihu Lake. China Agricultural Sciences, 37(6): 928-932.

Kang, C.D. (2007) Appropriate Approaches toward the Pen Culture in Lakes and Reservoirs. China Fishery News. September 2007.

Yangzhou Daily. (2006). The Center of the Wetland, Lake Gaobao Shaobo. April 10, 2006.

The Impact of Nylon Net Cages on the Development of Red Tilapia Culture in Peri-urban Areas of Ho Chi Minh City, South Vietnam

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Abstract

Red tilapia was first introduced into Vietnam in the 1990s. It took more than a decade to establish itself as part of the local diet and now it is considered an important restaurant dish for city consumers, particularly in Ho Chi Minh City. After a limited expansion at the beginning due to production constraints, red tilapia production in Ho Chi Minh City has developed exponentially with 717% annual growth between 2002 and 2005, largely due to the introduction of innovative nylon net cages in 2002.

Since the nylon net cage introduction, red tilapia culture has switched from ponds to cages with a production of 13,500 metric tons in 2005 in the peri-urban areas of Ho Chi Minh City. Various cage systems contributed to this production, such as stainless steel cages (4%), traditional wooden cages (42%) and nylon net cages (54%). Thus, nylon net cages have become the dominant structure as they cost 4.3 times less than wooden cages, thereby many poor farmers could afford them. In 2005, Ho Chi Minh City cage farmers produced US$ 1,964,000 worth of fish, of which 61% in value came from nylon net cage production.

This new approach seems to be successful as it has been spreading throughout the Mekong delta. However, the rapid expansion may lead to overproduction and environmental pollution.

Introduction

Vietnam is a country with strong economic growth, for e.g. in 2005 growth was 8.4%. This has resulted in rapid changes and new challenges at all levels. One of the challenges is the modification of consumption patterns, which are now diversified with the increase in incomes and creation of new market opportunities.

How to satisfy this new demand by using local resources is a challenge we have to face. For instance, the fast expansion of red tilapia production in the peri-urban areas of Ho Chi Minh

(HCM) City is responding to the growth of demand from urban areas. In fact, the development of red tilapia production was relatively slow during the early 2000s; it only took off after the introduction of an innovative technology, the nylon net cage which was supported by the Sustainable Development of Peri-Urban Agriculture in South-East Asia (SUSPER) Project with funding from the French Ministry of Foreign Affairs (about 991,000 euros for 4 years). SUSPER is implemented in Cambodia, Laos and Vietnam by the World Vegetable Centre and CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement). The project focuses on four cities, namely Hanoi, HCM City, Phnom Penh and Vientiane, and aims at enhancing the contribution of peri-urban agriculture for food security. One of its objectives is to strengthen peri-urban agriculture by testing technical innovations and disseminating the efficient ones. In South Vietnam, the project was carried out by the Faculty of Aquaculture at the University of Agriculture and Forestry, HCM City.

The red tilapia, a hybrid between strains of *Oreochromis niloticus x Oreochromis mossambicus* was first studied in Taiwan (Liao and Chang 1983). It was introduced into Vietnam in the 1990s. It took more than a decade to establish the local diet and now it represents an important food fish for city consumers in South Vietnam, especially in restaurants (Hung and Huy 2007). Adaptation to culturing red tilapia was slow at the beginning because the species was a new exotic food product, and it took time to cultivate consumers' eating habits. Moreover, the fish was first cultured in ponds where the growth to the marketable size was slow, limiting the output but high production costs. It was available only in high-end restaurants. Therefore, increasing red tilapia production became a priority for development projects, researchers and fish producers.

Transferring the fattening phase of red tilapia from ponds to cages is a technical and economical issue for increasing its production volume. The existing traditional wooden cage is such an expensive system that most fish farmers could not afford to use it (Hung and Huy 2007).

This article presents how a multidisciplinary approach was able to overcome the technical constraints of this production and allow the exponential development of red tilapia supply chains.

Materials and Methods

After having assessed the technical and economic problems, the SUSPER project team introduced a new cage model to farmers, i.e. the nylon net cage, which has iron water pipes as frames encircled by a nylon net (Figure 1). This costs about 4 and 7 times less than the traditional wooden cage (wooden frame covered by planks) and the stainless steel cage (a stainless pipe frame surrounded with a stainless net), respectively.

The nylon net model cage was introduced by Dr Philippe Cacot, a CIRAD aquaculture researcher together with the team from HCM City Faculty of Aquaculture. Dr Cacot drew the model's inspiration from some cages observed in Vientiane, along the Nam Ngum and Mekong rivers.

The large grid of rivers in HCM City region was divided into five main cage culture sites, namely, Long Thanh My (District 9th, HCM City), Long Binh, Tan Hanh and Tan Mai (Bien Hoa municipality, Dong Nai province), and Thien Tan (Vinh Cuu district, Dong Nai province) (Figure 2).

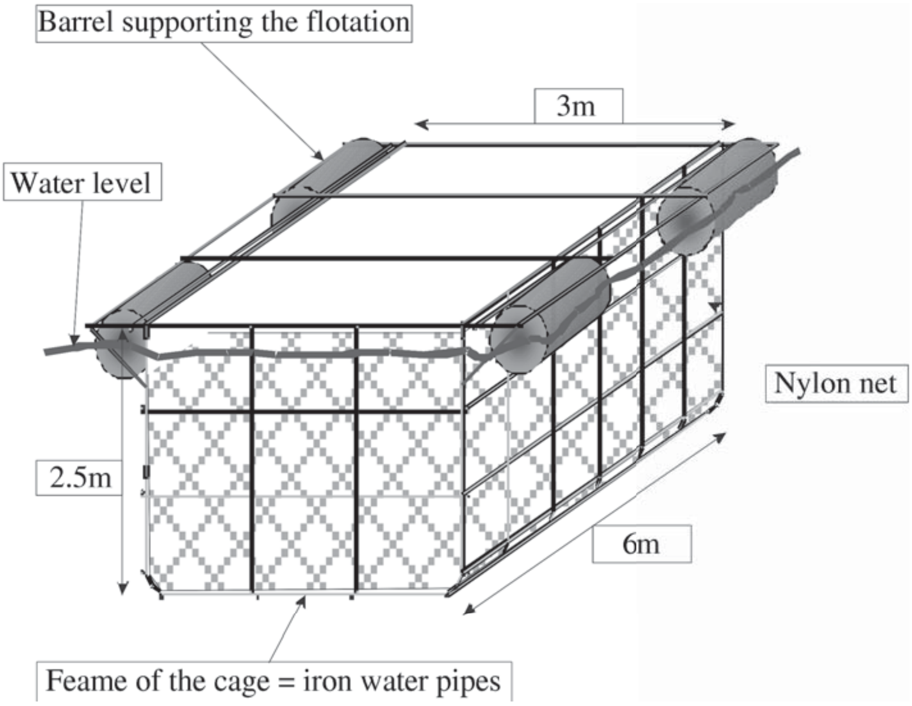

Figure 1. Diagram of the nylon net cage proposed to farmers in Ho Chi Minh City

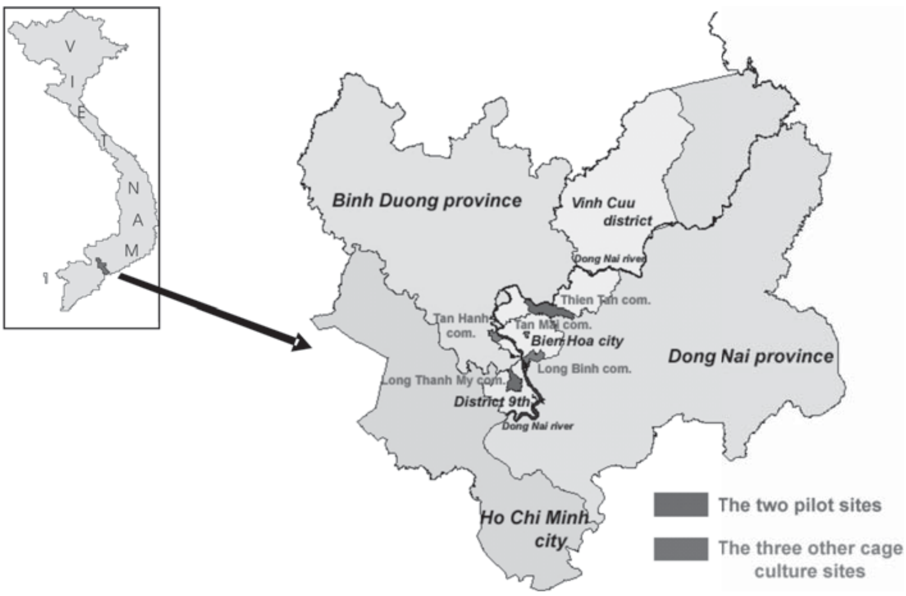

Figure 2. Location of Ho Chi Minh City and the five cage culture zones

The two pilot sites of SUSPER project, Long Than My and Thien Tan, were chosen for the following reasons: the local authorities of the two communes did not freeze nor ban cage culture; and, the proximity of Long Thanh My to the Faculty of Fisheries as it facilitated the monitoring of the four demonstration cages stocked with red tilapia. Thien Tan is located about 30 km from the Faculty and the project only provided technical advice to build the nylon net cages and to culture red tilapia to two of the three cage farmers. No pilot nylon net cage was installed there.

SUSPER project provided funding only for the demonstration cages and technical leaflets, thus no cage farmer was given any money to implement the project model with nylon net cage. All the farmers built this type of cage with their own capital.

The following tasks were performed to assess the impacts of the nylon net cage: (1) interview of the first group of cage farmers and local agriculture officers to evaluate the previous cage types, number and volume; (2) categorization of cage types, number and volume at each place in 2005, through brief interviews of 31 producers at Long Thanh My and 15 farmers at Thien Tan and via agricultural services of the three other sites; (3) conducting in-depth economics evaluation of seven chosen farmers (15% of total cage farmers covering the three types of cages, including wooden (2), stainless steel (2) and nylon (3) net cages. The data were recorded and analysed using the Excel software. Due to this small size sample, economic results should be taken as indicative rather than strictly accurate.

Results and Discussion

The growing popularity of red tilapia production after the introduction of nylon net cages

In 2002, before the introduction of the nylon net cage into the two pilot sites, there were eight cage farmers. In December 2005, the two sites had a total of 134 cage farmers (Figure 3). This represents a growth of 1,575% after only 3 years of introducing net cages. Starting with a small number of farmers, Thien Tan presents a higher development rate of 2,767% versus 860% in Long Thanh My. This result is due to two factors: better water quality in Thien Tan and the migration of cage farmers from Tan Hanh, where cage culture was banned by the local authorities in 2004.

Among the 126 new comers, 80% were new farmers who were able to practice cage culture due to the low cost of the nylon net cage, while the remaining 20% were former cage farmers who moved in from other places. All farmers used nylon net cages.

In fact, the large majority of these new farmers did not decide to practice cage culture prior to SUSPER project intervention. They first observed the few farmers who tested the innovative nylon net cage for about one year and when the operations were successful, these new farmers decided to follow and accept this cage culture production system.

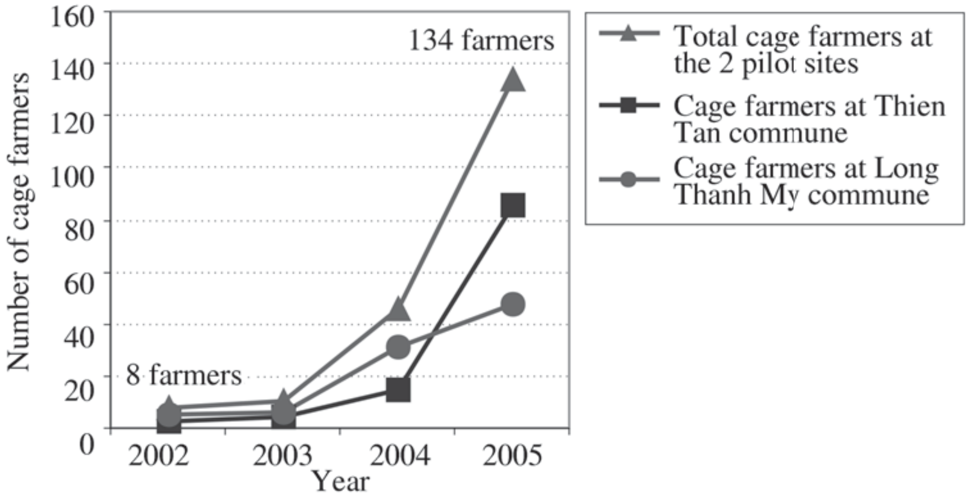

Figure 3. The phenomenal increase in the number of cage farmers at the two pilot sites

The total cage volume at the two pilot sites increased by 4,187% between 2002 and 2005, i.e. 1,396% per year (Figure 4). These figures show an indisputable explosion in the volume capacity of cages being operated at the pilot sites. This trend also extended further into HCM City peri-urban areas. In 2002, the volume capacity of cages in the two pilot sites represented only 7% of the total volume in HCM City, but in 2005, it increased to 75% (Table 1).

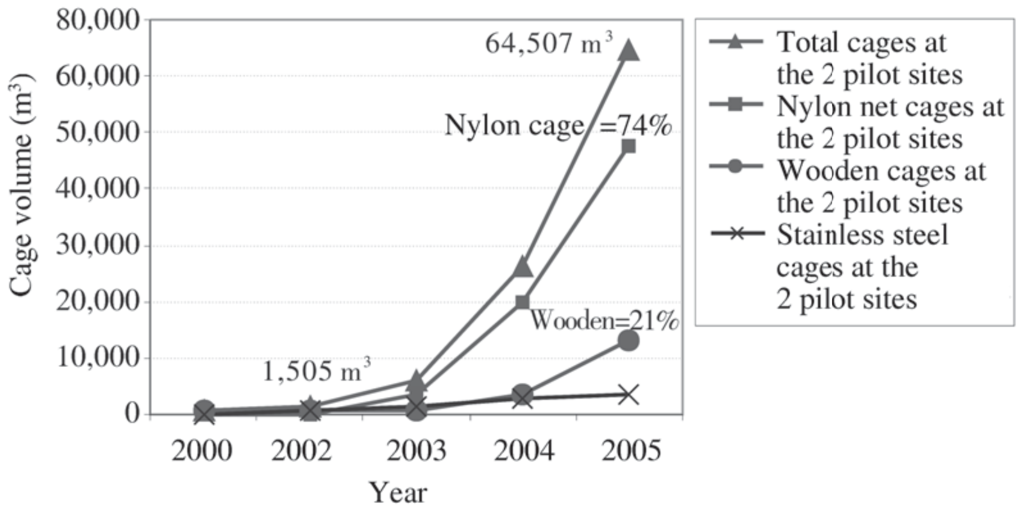

Figure 4. Evolution of cages volume at the two pilot sites

In HCM City, the total cage volume increased by 276% between 2002 and 2005. But this growth was 15 times slower than the one at the two pilot sites (Table 1). This relatively slow development was the result of banning cage culture since 1998 by the local authorities of the two original cage culture sites, Tan Mai and Long Binh. In these two sites, only traditional wooden cages were used which represented 25% of HCM City cage volume in 2005. The authorities feared for the environmental impact of too many cages and water pollution which could affect drinking water supply.

The nylon net cage volume accounted for 56% of HCM City total cage volume in 2005, indicating that the introduced cage model was well accepted by farmers, particularly at the pilot sites where they accounted for 74% of the total cage volume. Wooden cage volume represented 40% and stainless steel cages 4% (Table 1).

Table 1. Cage volume (m^3) in HCM City peri-urban areas

Type of cage	2000	2002	2003	2004	2005
Wooden cage volume at HCM City	21,869	22,061	22,195	25,056	34,435
Stainless steel cage volume at HCM City	158	554	1,426	2,772	3,564
Nylon net cage volume at HCM City	-	144	3,852	21,096	47,628
Total of cage volume at HCM City	22,027	22,759	27,473	48,924	85,627
Total of cage volume at the two pilot sites	773	1,505	5,899	26,388	64,507

Source: SUSPER project survey

The differences in the rate of cage development were due to investment cost. The nylon net cage is the cheapest thus the easiest to be developed. However, wooden and stainless steel cages continue to be accepted and developed, even though at a much slower pace than the nylon net. Indeed, a wooden or stainless steel cage is still used by wealthy farmers to house workers and store feed as its strong structure can prevent destruction by drifting logs. Once this solid base is installed, farmers would usually expand by adding nylon net cages. A cluster of three wooden cages linked to 110 nylon net cages was seen in Long Thanh My site (Figure 5). But at the same place, some less

wealthy farmers built a light house on the top of their nylon net cage (Figures 6 & 7).

Figure 5. A wooden cage with a house connected to a number of nylon net cages

Figure 6. A less wealthy farmer built a nylon net cage with a light house (April 2004)

Figure 7. The same farmer with more nylon net cages six months later (October 2004)

In 2002 at the two pilot sites, Long Thanh My and Thien Tan communes, cage farmers stocked different fish species, namely grass carp, common carp, giant gouramy and red tilapia. In 2005 in the same area, despite the strong arrival of new cage farmers, all farmers except one were stocking red tilapia exclusively, and only for fattening. The one exception was a farmer in Thien Tan commune who has been culturing grass carp for 20 years; even then, he also built a nylon net cage to raise red tilapia.

Table 2 shows the rapid specialisation on red tilapia cage culture in HCM City. Since 2002, all nylon net and stainless steel cages have been dedicated to red tilapia production. Then, this year, only 15% of HCM City cage volume was used to produce red tilapia. However, when the nylon net demonstration cages were introduced for red tilapia culture, this ratio totally reversed. Thus, in 2005, 96% of the total cage volume in HCM City was again producing red tilapia.

Table 2. Wooden cage volume share (%) dedicated to produce red tilapia in HCM City

Location	SUSPER actions from 2002 to 2006	2000	2002	2003	2004	2005
Tan Mai	None	5	10	30	60	80
Long Binh	None	5	5	20	40	60
Tan Hanh	Indirect information	0	50	100	100	-
Thien Tan	Technical Advices	75	100	100	100	100
Long Thanh My	Nylon net pilot cages	15	60	90	100	100

Source: SUSPER project survey

The large increase in red tilapia production in HCM City peri-urban areas could then be attributed to the exponential development of cage culture, particularly using nylon net cages. Between 2002 and 2005, red tilapia production and the increase in nylon net cage volume had an almost perfect correlation coefficient of 0.999. Based on the figures above, it can be concluded that SUSPER project enhanced the expansion of cage culture and red tilapia production by introducing the nylon net cage in HCM City peri-urban areas.

Until 2000, the red tilapia production in HCM City peri-urban areas fluctuated between 100 and 260 tons per year. In 2002, this output increased to 600 tons and exceeded 13,500 tons in 2005 (Figure 5). This phenomenal increase to 717% per year between 2002 and 2005 is to be credited firstly to the boom in nylon net cage investment wherein 10,992% of volume increased per year. This resulted in an exponential increase of 27,864% per year of red tilapia produced from nylon net cages, where farmers could have two to three production cycles per year. Secondly, the change of species produced in wooden cages (Table 2) into red tilapia increased production from 510 to 5,640 tons (333% per year) even though the volume of wooden cages increased only by 19% per year (Table 1). Red tilapia production from stainless steel cages was considered minor, with only an increase of 181% per year over the same period.

In 2002, red tilapia from wooden cages was 85% of total production from all types of cages in HCM City. But with the boom of nylon net cage production, this dropped to 42% in 2005, while production from nylon net cage reached 54%.

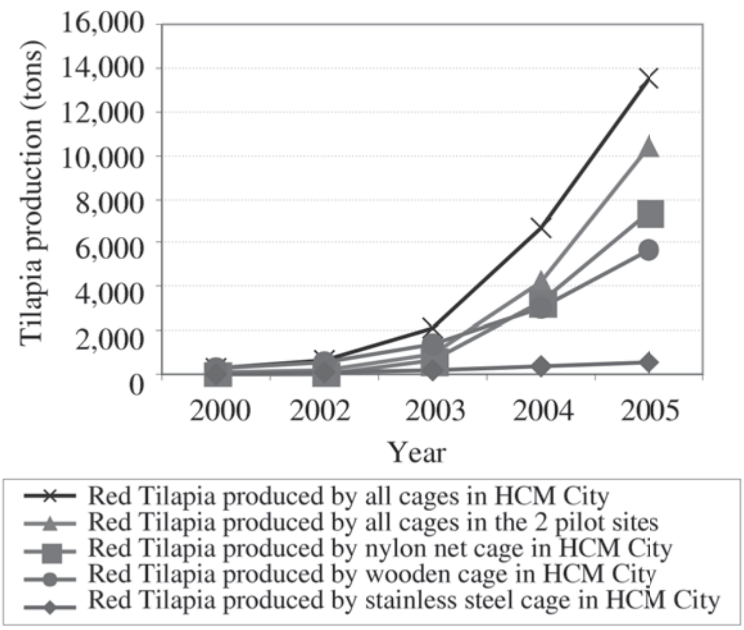

Figure 5. The development of red tilapia production in HCM City (in tons)

Through HCM City fish wholesale market surveys, the SUSPER team estimated the demand of red tilapia for HCM City to be between 12,000-13,000 tons per year in 2005. Therefore, red tilapia produced in HCM City in 2005 was able to meet this demand. Moreover, the two pilot sites contributed 77% to HCM City red tilapia production.

The introduced innovation, the nylon net cage, was adopted by all cage farmers in HCM City because of its simple technology which improved red tilapia productivity compared to pond culture and traditional wooden cage systems. Given the same water and management conditions, it would take four months for a 70 g juvenile red tilapia to reach the marketable size of 0.5 kg if grown in a wooden cage, whereas it required only two weeks at the most if grown in a nylon net cage. Thus, using nylon net cages could speed up red tilapia growth by 10%. Furthermore, it was observed that the colour of red tilapia from nylon net cages was nicer than from a wooden cage. Indeed, the weak water flow inside the wooden cage and the shade from the house built above the cages result in weaker fish and slower growth. The overall benefit from using nylon net cages including the nice form and healthier appearance of red tilapia may favour a better selling price, thus a higher income for the farmer.

Table 3. Technical and economic characteristics of the different cages (2004)

	Stainless steel	Wooden	Nylon net
Advantages	- Very solid, used upstream - No risk of fish loss - Needs little care - Can support a heavy house - Longest life time, over 20 years - Easy to maintain - Better water flow, higher productivity	- Very solid, used upstream - Low risk of fish loss - Needs little care - Can support a heavy house - Long life time, 20 years	- Relatively solid * - Relative low fish loss risk - Needs relatively more care - Can support only a light house - Easy to maintain - No roof, healthier and nicer fish - Better water flow, higher productivity - Cheap, lowest economic risk
Constraints	- Expensive, high economic risk	- Needs care under cage - Weak water flow inside - Slower growth (-10%) - Expensive, high economic risk	- Light steel, short life time, 5 years - Needs medium to slow water flow
Average volume (m^3)	40	77	36
Investment ($\$\bullet cage^{-1}$)	3,870	4,130	450
($\$\bullet m^{-3}$)	98	54	13
Cycle (months)	3.70	3.75	3.67
Net revenue per crop ($)	690	1,415	820
Return to investment	5.6 crops 21 months	2.9 crops 12 months	0.6 crop 2 months

* no farmer reported fish was lost due to torn nylon nets; Source: SUSPER project survey.

The main strength of the nylon net cage is its cheap cost making it more economically accessible and is correlated to acceptable technical constraints and risks (Table 3). Indeed, with only 13 ($\$\bullet m^{-3}$), the nylon net cage is 4.3 and 7.8 times cheaper than the wooden and stainless steel cages, respectively. This economic advantage offers established cage farmers the possibility to increase their production volume. Moreover, it allows less wealthy new farmers to operate cage fish culture systems. Between 2003 and 2004, among the 36 new nylon net cage farmers at the two pilot sites, nine did not own any wooden or stainless steel cage. Thus, the SUSPER cage model contributed to the increase of new cage farmers by 25%, especially the less wealthy ones. The remaining 75% were wealthier as they could afford a wooden cage and/or a stainless steel cage. The richest farmer in each pilot site invested three wooden cages in addition to the 100 and 110 nylon cages in Thien Tan and Long Thanh My, respectively.

Stocking red tilapia in nylon net cages represents a low economic risk. It needs less than one crop to get back its investment. The return to investment in using a nylon net cage is only 0.6 crop or about 2 months, which is 5.3 and 10.2 times faster than that of the wooden and stainless steel cages, respectively. Moreover, investing in a wooden or stainless steel cage locks up an important capital for 20 to 40 years. This fund immobilization may represent a serious risk on a fast changing market and the uncertainty of producing in the river, a public property. As what has already happened in Long Binh and Tan Mai, the two original cage culture sites, the local authorities may freeze cage culture development. But they can also totally ban cage culture, like in Tan Hanh in 2004 or recently, at Dau Tieng lake in Tay Ninh province, where about 1,200 wooden cages were dismantled in order to preserve the lake as a reservoir for drinking water. Therefore, with its moderate cost, the nylon net cage represents a good compromise for a medium term investment.

Once again, we must emphasize that the figures in Table 4 are indications only as the sample of seven farmers surveyed for economic indicators was quite small. However, the figures on nylon net cages may be considered as relatively reliable as the surveyed volume (4,841 m^3) was high. Moreover, compared to the nylon net cage figures, the ones for wooden cages seem to be quite reliable. The weakest data belong to the stainless steel cage. Its technical characteristics are closely similar to the nylon net cages, but despite the same farm gate price, the net revenue differs by up to 30%.

If we accept these figures as quite reliable indications, the net revenue from nylon net cage culture seems to be the most profitable, as it exceeds that of the wooden and stainless steel cages by 24 and 30%, respectively (Table 4). Despite the large difference in investment costs, the depreciation (cost of investment/duration of use) of the three cage types is nearly similar (0.8-0.9 $•$m^{-3}$). The higher priced cage has a longer life time.

Table 4. Economic indicators of red tilapia production from different cages in 2004 at HCM City

	Unit	Stainless	Wooden	Nylon net
Number	farmers	2	2	3
Surveyed volume	m^3	285	240	4,851
Average yield	kg•m^{-3}	46	43	48
Farm gate price	VND•kg^{-1} ($•$kg^{-1}$)	23,000 (1.5)	23,000 (1.5)	23,000 (1.5)
Total input	VND•m^{-3} ($•$m^{-3}$)	822,857 (53.1)	669,579 (43.2)	840,353 (54.2)
Output	VND•m^{-3} ($•$m^{-3}$)	1,120,352 (72.3)	987,083 (63.7)	1,227,699 (79.2)
Added value	VND•m^{-3} ($•$m^{-3}$)	297,495 (19.2)	317,504 (20.5)	387,346 (25.0)
Gross revenue	VND•m^{-3} ($•$m^{-3}$)	281,621 (18.2)	292,796 (18.9)	366,547 (23.6)
Depreciation	VND•m^{-3} ($•$m^{-3}$)	13,125 (0.8)	13,402 (0.9)	13,625 (0.9)
Net revenue	VND•m^{-3} ($•$m^{-3}$)	270,544 (17.5)	285,657 (18.4)	352,922 (22.8)

Source: SUSPER project survey

Finally, the figures in Table 4 allow us to estimate the total added value and net revenue obtained by HCM City cage farmers. In 2004, they generated $ 1,093,600 and 990,500 of added value and net revenue, respectively; with 48% of the values coming from the nylon net cages. If we consider the same economic values for 2005, HCM City cage farmers produced $ 1,964,000 and 1,781,300 of added value and net revenue, respectively; with 61% of the values from nylon net cages. Thus, in 2005, the added value generated by nylon net cages covered almost the whole SUSPER project budget. The return on capital employed to finance this project was realised within one year through a single action.

The nylon net cage spreads into the Mekong delta, but at what risks?

In October 2005, the SUSPER team observed some nylon net cages close to My Tho, in the Mekong delta, at about 100 km from the closest pilot site. Farmers adapted the cage frame by using V iron bars instead of galvanised water pipes. They also stocked red tilapia. Thus, this technology is also spreading to the Mekong delta, without any intervention by the SUSPER project (Hung et al. 2007).

Moreover in April 2005, basing on the success of introducing the innovation in HCM City peri-urban areas, the project transferred the model to Phnom Penh (Cambodia), where cage culture is also practiced. At the end of 2005, it was reported that a few neighbouring farmers of the demonstration sites started to copy the project model by adapting it. Furthermore, we may expect a larger expansion as a project funded by the Mekong River Commission copied our project nylon net cage model to implement it in their own target areas.

In HCM City, due to available technical information, input-output facilities, presence of feed traders and collectors needing easy access to producers, cage farmers like to set-up cages in the same area, forming informal clusters. This means that the area has a large concentration of cages, and with high stocking densities used, this may lead to serious disease outbreaks. This situation may also present a potential for environmental pollution. Therefore, further studies on environmental risks are needed to provide clear information to decision makers, in particular the maximum volume of cages at a particular site with specific cage management measures. These data are of the highest importance to prevent sudden intervention of local authorities to freeze cage culture development or worse to completely ban cage culture operations at a particular site, due to reasons that they would lead to environmental pollution.

Finally, rapid development of red tilapia production may lead to overproduction and oversupply, resulting in decreased farm gate prices. The drop in income may be fatal for less wealthy farmers who had to borrow money to invest in their cages in the first place.

Conclusions

This paper presented how the introduction of a technically and economically efficient innovation has resulted in the development of the red tilapia supply chain to respond to an expanding local demand, in just three years. Beyond the technique itself and the spectacular results, it seems necessary to constantly apply what has been learnt through the experimentation, in order to develop research/extension policies which may support the frenetic economic development of Vietnam.

Contrary to some common opinions, investing into Research & Development is profitable. In one year, the added value created directly through a single project action was able to pay back the whole project budget.

However, some conditions must be met to achieve this kind of success, such as:

(a) an accurate initial assessment should be based on a multidisciplinary approach, both technical and economical. The technique could not have been adopted without the increase of the urban demand of red tilapia. The supply could not have succeeded to satisfy the demand without the development of the technique. Both are linked.

(b) a serious and close monitoring of the test/demonstration, realised with some pilot farmers chosen for their representativeness and their leadership capacity. Once again, this monitoring should be both technical and economical.

(c) Researchers/extension technicians should be skilled in techniques and economics. They should be sufficiently paid in order to work fulltime on their research/extension assignments.

These very encouraging results raise some new questions, which should be answered by deeper analysis, so that the success would not become "a failure of success", particularly where environmental issues and development policies are concerned. Resolving these new issues will certainly require supporting the work with farmers' organisations for a sustainable supply chain, in addition to the technical and economic approaches. Here again, a multidisciplinary approach is obviously needed. The links between research/extension/ growth policies are to be studied in depth through new research directions. This work looks very promising and fascinating as to the present economic and technical challenges.

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References

Hung L.T. and Huy P.H.V. (2007) Analysis of feeds and fertilizers for sustainable aquaculture development in Vietnam. In: Hassan M.R., Hecht T., De Silva S.S. and Tacon A.G.J. (eds). Study and Analysis of Feeds and Fertilizers for Sustainable Aquaculture Development. FAO Fisheries Technical Paper. No. 497, 331-361. Rome, FAO.

Hung L.T., Truc L.T.T., Huy H.P.V. (2007) Case study on the use of farm made feeds and commercially formulated pellets for pangasiid catfish culture in the Mekong delta, Vietnam. In: Hassan M.R., Hecht T., De Silva S.S. and Tacon A.G.J. (eds). Study and Analysis of Feeds and Fertilizers for Sustainable Aquaculture Development. FAO Fisheries Technical Paper. No. 497, 363-377. Rome, FAO.

Liao I.C. and S.L. Chang. (1983) Studies on the feasibility of red tilapia culture in saline water. In: Fishelton L. & Yaron Z. (eds.) International Symposium on Tilapia in Aquaculture, pp 588-596. Tel Aviv, Israel.

Integrated Cage-cum-Pond Culture System with African Catfish (*Clarias gariepinus*) in Cage and Carps in Open Pond

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Abstract

Two consecutive experiments, one on-station and another in a farmer's field, were conducted in subtropical Nepal for 166 and 153 days from 16 September 2004 to 28 February 2005 and from 24 July 2005 to 4 January 2006, respectively. The objectives were to adopt the cage-cum-pond systems in local conditions in Nepal and determine the appropriate stocking density, growth and production of catfish (*Clarias gariepinus*) in cage, and carps such as silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), common carp (*Cyprinus carpio*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), and sahar (*Tor putitora*) in open pond. The on-station experiment included one 2 m^3 cage suspended in a 100 m^2 pond. There were four treatments and one control with three replicates each.These were (1) carps at 1 fish m^{-2} in open pond without cage (control); (2) catfish at 50 fish m^{-3} in cage and carps at 1 fish m^{-2} in open pond; (3) catfish at 100 fish m^{-3} in cage and carps at 1 fish m^{-2} in open pond; (4) catfish at 150 fish m^{-3} in cage and carps at 1 fish m^{-2} in open pond; and, (5) catfish at 200 fish m^{-3} in cage and carps at 1 fish m^{-2} in open pond. Carps in open pond for treatments 2-5 were the same as that in the control ponds. Caged catfish were fed with a locally made pellet feed (28% CP), while no feed or fertilizer was added into the open pond water. The control ponds were fertilized weekly with diammonium phosphate (DAP) and urea at the rate of 2 kg N and 1 kg P $ha^{-1}\cdot d^{-1}$. Based on the results of the on-station experiment, a verification trial was conducted in a farmer's field in three locations using T1 (control) and T3 (catfish at 100 fish·m^{-3} in cage and carps at 1 fish·m^{-2} in open pond).

Results of the on-station experiment showed that carp yields were significantly higher in the control pond than in cages due to limited nutrients that were available in the cages. However, combined yields were similar showing better yield in treatment 3. Survival rates of carps and catfish were similar. Relatively lower food conversion ratio (FCR) for catfish was achieved in treatment 3. Net fish yield (NFY) of carps in farmer field trials was not significantly different between the pond with cage and that without cage, but combined yields of carps and catfish were significantly higher in the pond with cage. Carp survival was similar between two treatments. In addition, FCR of catfish was lower than that in on-station

experiment. Results showed that small farmers could achieve more than double the yield from a single pond (32.4 $kg \cdot crop^{-1}$ i.e. 64.8 $kg \cdot year^{-1}$ from 100 m^2 pond) using the cage-in-pond system, with the catfish in cage and carps in pond, than from just carp polyculture in pond.

Introduction

African catfish (*Clarias gariepinus*) is generally cultured at high stocking densities with intensive feeding while carps are cultured in semi-intensive systems with fertilization and with or without supplementary feeding (Rai and Lin 1999). One of the most problematic aspects of intensive catfish culture is its effects on the environment (Muir 1982). Since African catfish utilizes only less nutrients, huge amounts of nutrients are wasted and released into the water, deteriorating water quality in the culture system (Sundar 1989). To utilize these wasted nutrients from intensive catfish culture, the cage-cum-pond integrated culture system with African catfish and carps seems ideal to minimize nutrient loading of the culture water and improve water quality through nutrient recycling, leading to increased fish production (Uddomkarn 1989; Lin 1990; Lin and Diana 1995; Yi et al. 2003).

Earlier experiments on cage-cum-pond integration systems were limited to cages set up in Nile tilapia monoculture ponds (Lin 1990; Lin and Diana 1995; Sethteethunynhan 1998; Uddomkarn 1989; Ye 1991) although this system could also be applied in carp polyculture systems. An on-station experiment was conducted in subtropical Nepal during September 2004 to February 2005 to adopt this integrated cage-cum-pond system with African catfish in cage and carps in open pond (Shrestha et al. 2005). Based on the results of this on-station experiment, a verification trial was conducted in farmers' fields in three different locations.

The purposes of this study were to adopt the integrated cage-cum-pond systems in local conditions in Nepal, to assess the appropriate stocking density, growth and production of catfish and to verify the best results of the on-station experiment in the farmers' fields.

Methods and Materials

Two consecutive experiments, one on-station and another on-farm, were conducted in subtropical Nepal for 166 (16 September 2004 to 28 February 2005) and 153 days (24 July 2005 to 4 January 2006), respectively.

Experiment 1

The on-station experiment was conducted at the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal, utilizing one 2 m^3 cage suspended in a 100 m^2 earthen pond. There were four treatments including one control, with three replicates per treatment, namely (1) carps in open pond at 1 $fish \cdot m^{-2}$ without cage (control); (2) catfish in cage at 50 $fish \cdot m^{-3}$ and carps in open pond at 1 $fish \cdot m^{-2}$; (3) catfish in cage at 100 $fish \cdot m^{-3}$ and carps in open pond at 1 $fish \cdot m^{-2}$; (4) catfish in cage at 150 $fish \cdot m^{-3}$ and carps in open pond at 1 $fish \cdot m^{-2}$, and (5) catfish in cage at 200 $fish \cdot m^{-3}$ and carps in open pond at 1 $fish \cdot m^{-2}$. African catfish fingerlings (1.8–4.8 g) were stocked in cages, while fingerlings of silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), common carp (*Cyprinus carpio*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*) and sahar (*Tor putitora*) with average weights of 10, 7, 13, 6, 12 and 17 g, respectively, were stocked in the open ponds. The stocking ratio of silver carp, bighead carp, common carp, rohu, mrigal, and sahar was 4:2:1:1:1:1 in each pond.

Fish in cages were fed twice daily with a locally made pellet feed (28% CP) at the rate of 5% body weight per day, while no feed or fertilizers were added into the pond water. Feeding rates were adjusted fortnightly to compensate for growth. The control ponds were fertilized weekly with diammonium phosphate (DAP) and urea at rates of 2 kg N and 1 kg P $ha^{-1}\cdot d^{-1}$.

Weekly and biweekly measurements of water quality parameters were conducted at 0600 – 0800 h starting from 15 September 2004. In situ water temperature, dissolved oxygen (DO), pH (0.15m depth), and Secchi disk depth were measured weekly using DO meter (YSI meter model 50B), pH meter (ATC Pocket Meter) and Secchi disk, respectively. Water samples were collected biweekly from each pond using a plastic column sampler and analyzed for total alkalinity (as CaCO3), total ammonium nitrogen (TAN), nitrite nitrogen (nitrite-N), nitrite-nitrate nitrogen (nitrite-nitrate-N), total nitrogen (TN), soluble reactive phosphorous (SRP), total phosphorous (TP) and chlorophyll-a (APHA 1985).

Fortnightly growth of African catfish was determined to adjust the feeding rate by sampling the total population from each cage. Similarly, monthly growth measurements of carps were done by randomly sampling and bulk weighing at least 20% of each species. All study ponds were harvested on 28 February 2005 by seining twice followed by complete draining to determine the final total number and weight of all fish species in each pond. Net fish yield (NFY) was calculated as $kg\cdot pond^{-1}\cdot crop^{-1}$. Based on the quantity of feed given and the NFY, food conversion ratio (FCR) for catfish, and for catfish and carps combined, were calculated by dividing the amount of total feed consumed by the NFY.

Experiment 2

Based on the results of experiment 1, a verification trial was conducted in 18 earthen ponds of varying sizes (85-130 m^2) in farmers' fields located at 3 different sites, namely Nepal, Kawasoti, Nawalparasi ($Site^{-1}$), Kushana, Kathar, Chitwan ($Site^{-2}$) and Gothauli, Kathar, Chitwan ($Site^{-3}$). The experiments used the treatments T1 (control) and T3 (Catfish at 100 $fish\cdot m^{-3}$ in cage and carps at 1 $fish\cdot m^{-2}$ in open pond) from experiment 1. Each experimental site had 3 replications of non-caged and caged treatments. One 2 m^3 (1.5 m×1.5 m×1 m) cage covered with a 1 cm mesh net was suspended in each of the three ponds at each site, and three ponds at each site served as control without cages. Water depth in each pond was maintained at 1.1±0.1 m by topping up with canal water to replace water loss, while water depth in each cage was 0.9 m to achieve a water volume of 2 m^3 in the cages.

African catfish fingerlings (12.8–13.2 g average size) were stocked in cages, while fingerlings of silver carp (4.6 g), bighead carp (2.2 g), common carp (4.2 g), rohu (0.5 g) and mrigal (0.7 g) were stocked in the ponds. The stocking ratio of silver carp, bighead carp, common carp, rohu and mrigal was 4:2:2:1:1 in each pond. Catfish fingerlings were stocked on 24 July 2005 while the carps were stocked on 5 August 2005. Fish in cages were fed twice daily at 0900-1000 h and 1500-1600 h with a locally made pellet feed (28% CP). Feed ration was calculated based only on African catfish biomass estimated from fortnightly fish sampling. Daily feeding rates of 5 and 3% body weight were used for African catfish smaller and greater than 100 g, respectively, while no feed or fertilizers were added into the ponds. Control ponds were fertilized with DAP and urea at rates of 2 kg N and 1 kg P ha^{-1}·d^{-1} throughout the experimental period. Proximate analysis of locally made pellet feed with composition of fish meal, rice bran and mustard oil cake (5:3:2, respectively) was conducted according to AOAC (1980). In situ water temperature and pH (0.15m depth) were measured fortnightly at 1000 – 1200 h by using glass thermometer and pH meter (ATC Pocket Meter), respectively.

Growth of African catfish was determined through fortnight sampling of the total population from each cage to adjust the feeding rate. All cages and ponds were harvested on 4 January 2006. Ponds were seined twice and drained completely. The final total number and weight of all fish species in each cage and pond were then determined. NFY was calculated as kg pond^{-1} crop^{-1} and extrapolated to obtain the annual value. Based on the quantity of feed given and the NFY, the FCR for catfish, and for catfish and carps combined, were calculated by dividing the amount of total feed consumed by the NFY.

A partial budget analysis was conducted based on farm-gate prices for harvested fish and market prices for all costs in Nepal (Shang 1990). From this study, the African catfish and carps had farm-gate prices of 120 and 100 NRs·kg^{-1}, respectively, based on their sizes at harvest. Prices for African catfish and carps fingerlings were 5 and 0.25 NRs per piece, respectively. Prices of feed, DAP and urea were 15, 28 and 18 NRs·kg^{-1}, respectively. The cages cost 1200 NRs per cage with an estimated life span of 3 years (6 culture cycles). The calculation for cost of working capital was based on an annual interest rate of 8% (US$ 1 = 71 Nepali Rupees, NRs).

Analysis of variance (ANOVA) was used to analyze the data obtained from both experiments using SPSS (version 11.0) statistical software package (SPSS Inc., Chicago). Differences were considered significant at the 95% confidence level ($P<0.05$). All means were given with ± standard error (S. E.).

Results

Experiment 1

Results showed that African catfish stocked at the highest density (T5) had a significantly lower survival (30.8%) than those of other treatments (54.6-67.2%; Table 1, Figure 1), while carps in the control had a significantly lower survival (54%) than those in other treatments (64-67%; Table 2). Final mean weights and daily weight gains of African catfish were 47.2 to 89.0 g and 0.26 to 0.49 g fish^{-1}day^{-1}, respectively, which were highest in treatment 2, intermediate in treatments 3 and 5, and lowest in treatment 4 (Table 1; Figure 2). There were no significant differences in net and gross yields of African catfish among all treatments ($p>0.05$; Table 1). FCR for African catfish ranged from 3.2 to 4.1 and was significantly different among treatments ($p>0.05$; Table 1). For carps, results showed that net and gross yields were significantly higher in the control pond than in treatments with cages, but were not different between caged treatments ($p>0.05$; Table 2). There were no significant differences in the net and gross yields of all carps in the control, and combined catfish + carps in caged treatments (P 0.05; Table 3). The overall FCRs in the treatments ranged from 1.41 to 1.64, and were not significantly different ($P>0.05$; Table 3).

Table 1. Performance of catfish in cage at different stocking densities in experiment 1 (Mean ± SE).

Parameter	Sto^{c}king density (fish·m^{-3})			
	50	100	150	200
Sto^{c}king				
Total weight (kg ^{c}age^{-1})	0.5±0.1[a]	0.4±0.1[a]	0.5±0.1[a]	0.8±0.2[a]
Mean weight (g fish^{-1})	4.8±0.8[a]	1.8±0.4[b]	1.8±0.3[b]	2.0±0.6[b]
Harvest				
Total weight (kg cage^{-1})	5.6±1.0[a]	8.9±1.9[a]	7.7±0.9[a]	7.9±2.0[a]
Mean weight (g fish^{-1})	89.0±8.2[a]	64.9±8.0[ab]	47.2±1.3[b]	63.6±9.3[b]
Daily weight gain(g fish^{-1}day^{-1})	0.49±0.05[a]	0.37±0.05[ab]	0.26±0.01[b]	0.35±0.05[ab]
Gross yield (kg cage-1 crop^{-1})	5.6±1.0[a]	8.9±1.9[a]	7.7±0.9[a]	7.9±2.0[a]
Net yield (kg cage^{-1} crop^{-1})	5.1±1.0[a]	8.5±1.8[a]	7.2±0.8[a]	7.1±1.7[a]
Survival (%)	62.0±9.2[a]	67.2±6.5[a]	54.6±5.9[a]	30.8±6.2[b]
FCR	4.1±0.1[a]	3.2±0.1[c]	3.6±0.0[b]	3.9±0.1[a]

Mean values with the same superscripts are not significantly different at P= 0.05.

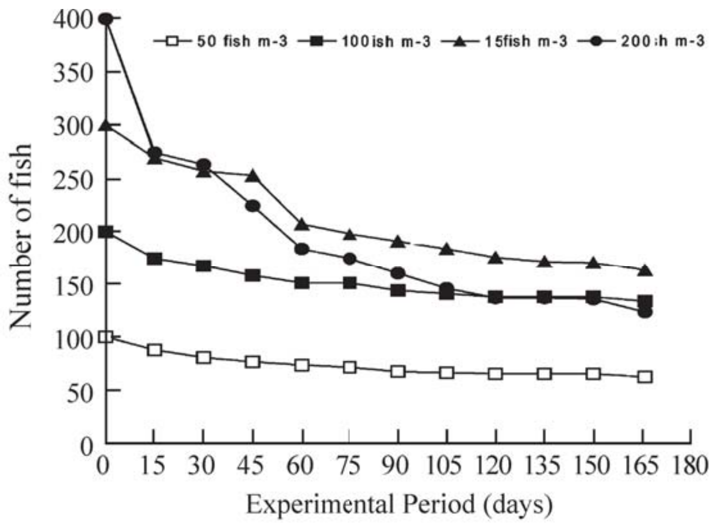

Figure 1. Fortnightly survival of catfish in cages in experiment 1 (on-station trials).

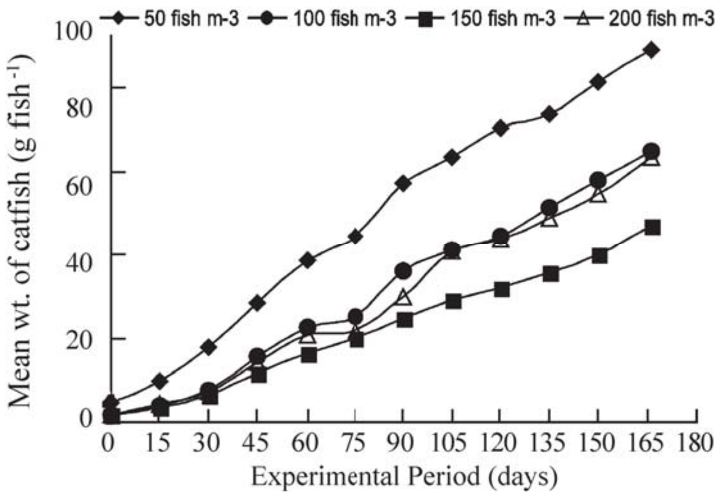

Figure 2. Mean weight of African catfish in cages at different sampling dates in experiment 1 (on-station trials).

Table 2. Performance of carps stocked in open pond at different stocking densities in experiment 1 (Mean ± SE). Mean values with the same superscripts are not significantly different at P= 0.05.

P^{a}r^{a}meter	Stocking density (fish m^{-3})				
	0	50	100	150	200
Common c^{a}rp					
Me^{a}n stock weight (g fish^{-1})	12.67±0.17^{a}	12.83±0.19^{a}	12.57±0.15^{a}	12.63±0.19^{a}	12.53±0.13^{a}
Mean harvest weight (g fish^{-1})	1145.50±200.45^{a}	358.81±59.31^{b}	393.92±42.67^{b}	343.51±35.81^{b}	381.67±28.92^{b}
Silver carp					
Mean stock weight (g fish^{-1})	9.94±0.87^{a}	10.35±0.72^{a}	10.17±0.78^{a}	9.71±0.84^{a}	9.89±0.94^{a}
Mean harvest weight (g fish^{-1})	368.68±51.34^{a}	113.90±27.65^{b}	118.26±8.32^{b}	107.22±31.18^{b}	90.25±15.89^{b}
Bighead carp					
Mean stock weight (g fish^{-1})	6.83±0.51^{a}	7.78±0.04^{a}	7.10±0.46^{a}	6.77±0.52^{a}	6.83±0.41^{a}
Mean harvest weight (g fish^{-1})	446.25±77.48^{a}	228.65±32.70^{b}	245.40±16.32^{b}	157.64±19.17^{b}	211.79±43.87^{b}
Rohu					
Mean stock weight (g fish^{-1})	5.80±0.06^{a}	5.93±0.28^{a}	5.90±0.06^{a}	5.77±0.07^{a}	5.70±0.15^{a}
Mean harvest weight (g fish^{-1})	360.37±30.77^{a}	124.83±17.26^{b}	190.42±22.08^{b}	163.39±41.90^{b}	168.97±51.53^{b}
Naini					
Mean stock weight (g fish^{-1})	12.10±0.36^{a}	12.53±0.15^{a}	12.40±0.21^{a}	12.33±0.22^{a}	12.50±0.10
Mean harvest weight (g fish^{-1})	203.72±27.15^{a}	148.27±7.69^{ab}	147.66±13.10^{ab}	112.96±26.87^{b}	131.37±30.38^{b}
Sahar					
Mean stock weight (g fish^{-1})	17.40±1.06^{a}	17.13±0.94^{a}	17.20±0.76^{a}	16.93±0.82^{a}	16.07±0.12^{a}
Mean harvest weight (g fish^{-1})	95.04±5.15^{a}	91.08±8.18^{a}	93.95±4.55^{a}	78.92±2.31^{a}	78.62±6.35^{a}
All carps					
Gross yield (t ha^{-1} crop^{-1})	2.23±0.31^{a}	1.06±0.14^{b}	1.17±0.11^{b}	1.03±0.19^{b}	1.06±0.12^{b}
Net yield (t ha^{-1} crop^{-1})	2.13±0.31^{a}	0.96±0.14^{b}	1.06±0.10^{b}	0.93±0.19^{b}	0.96±0.13^{b}
Survival (%)	54±6	65±3	64±7	67±3	66±2

ANOVA showed that mean values of chlorophyll-a, SRP, TP, TAN, nitrite-N, nitrite-nitrate-N, and TN, in the control treatment were significantly higher than those in caged treatments (P<0.05; Table 4). However, there were no significant differences in temperature, transparency and total alkalinity levels among the treatments (p>0.05). The DO concentration in the control was lower than that in the caged treatments (P<0.05) but they were not significantly different. Most of the water quality parameters were within the acceptable ranges for fish culture according to the standards set by Boyd (1990). However, mean water temperature during the experimental period was low (21°C) and remained below 20oC for 3.5 months out of the 5-month experimental period (Figure 3). Fluctuations in DO concentrations could be attributed to the varying intensity of photosynthesis caused by alternating cloudy and sunny weather conditions as well as variations in the rate of oxygen production in different treatments. Nitrite-N, although not detected in most ponds during the initial stage of the experiment, was very low during most part of the experiment.

Results show that the net return in the control was significantly higher than that of the treatments (P<0.05), although all the treatments produced positive net returns. Among these treatments, the one with catfish stocked at 100 fish·m^{-3} in cages gave the highest returns.

Table 3. Total yield of carps and catfish at different stocking densities in experiment 1 (Mean±SE).

Parameter	Sto^{c}king density (fish m^{-3})				
	0	50	100	150	200
Initial fish biomass (kg)	1.01±0.04^{a}	1.54±0.08^{bc}	1.40±0.05^{b}	1.54±0.06^{bc}	1.79±0.22^{c}
Final fish biomass (kg)	22.27±3.10^{a}	16.18±2.35^{a}	20.52±0.82^{a}	18.00±2.65^{a}	18.49±3.10^{a}
Fish biomass gain (kg $pond^{-1}$)	21.26±3.06^{a}	14.65±2.34^{a}	19.12±0.77^{a}	16.46±2.59^{a}	16.70±2.94^{a}
Gross fish yield (t ha^{-1} $crop^{-1}$)	2.23±0.31^{a}	1.62±0.24^{a}	2.05±0.08^{a}	1.80±0.27^{a}	1.85±0.31^{a}
Net fish yield (t ha^{-1} $crop^{-1}$)	2.13±0.31^{a}	1.46±0.23^{a}	1.91±0.08^{a}	1.65±0.26^{a}	1.67±0.29^{a}
Overall FCR	0.00±0.00^{a}	1.41±0.07^{b}	1.42±0.24^{b}	1.59±0.10^{b}	1.64±0.15^{b}

Mean values with same superscript are not significantly different at P= 0.05.

Table 4. Overall mean and range of water quality parameters in different treatments in experiment 1 (Mean±SE).

Parameter	Stocking density (fish m^{-3})				
	0	50	100	150	200
Temperature (°C)	20.9±0.1^{a}	21.0±0.0^{a}	21.0±0.0^{a}	21.0±0.0^{a}	21.0±0.0^{a}
DO (mg L^{-1}1)	4.1±0.2^{a}	5.6±0.5^{b}	5.9±0.3^{b}	5.7±0.3^{b}	5.8±0.2^{b}
pH	8.3	8.4	8.4	8.4	8.4
Transparency (cm)	30.0±1.8^{a}	30.9±1.8^{a}	33.2±2.1^{a}	32.9±2.1^{a}	30.7±1.3^{a}
Total alkalinity (mg L^{-1} as $CaCO_3$)	124.5±1.3^{a}	126.5±3.3^{a}	126.7±1.6^{a}	127.7±1.0^{a}	123.6±1.0^{a}
Chlorophyll-a (mg m^{-3})	139.3±23.5^{c}	104.1±9.9^{bc}	91.4±11.2^{a}	88.3±6.6^{a}	92.8±5.9^{a}
Total nitrogen (mg L^{-1})	4.36±0.13^{b}	1.98±0.29^{a}	2.47±0.09^{a}	2.42±0.32^{a}	2.21±0.11^{a}
Total ammonium nitrogen (mg L^{-1})	0.392±0.030^{b}	0.116±0.012^{a}	0.139±0.003^{a}	0.120±0.014^{a}	0.119±0.013^{a}
Nitrite nitrogen (mg L^{-1})	0.016±0.000^{b}	0.003±0.000^{a}	0.004±0.000^{a}	0.003±0.001^{a}	0.003±0.000^{a}
Nitrite+Nitrate nitrogen (mg L^{-1})	0.073±0.003^{c}	0.044±0.005^{ab}	0.055±0.002^{b}	0.041±0.007^{ab}	0.036±0.003^{a}
Total phosphorous (mg L^{-1})	1.33±0.13^{b}	0.25±0.01^{a}	0.43±0.02^{a}	0.39±0.04^{a}	0.37±0.04^{a}
Soluble reactive phosphorous (mg L^{-1})	0.491±0.022^{a}	0.077±0.007^{b}	0.132±0.000^{c}	0.115±0.019^{bc}	0.122±0.012^{bc}

Mean values with the same superscripts are not significantly different at P= 0.05.

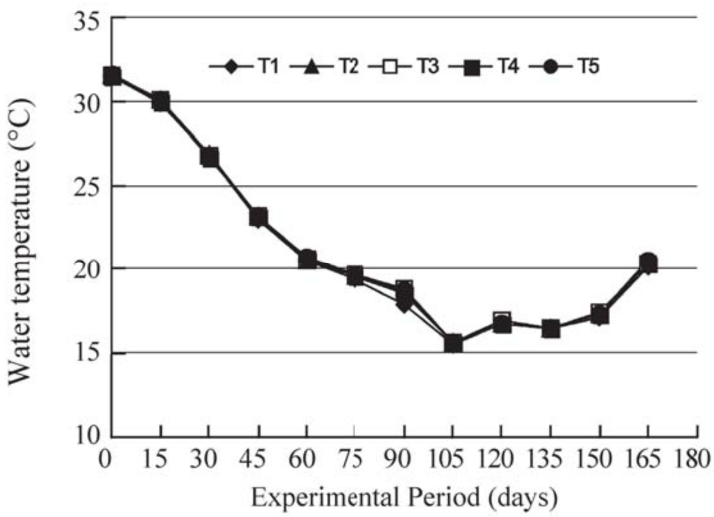

Figure 3. Fortnightly pond water temperature in experiment 1 (on-station trials).

Experiment 2

Survival of African catfish, ranging from 53.3 to 56.8%, was not significantly different among all sites (*P*>0.05; Table 5). Final mean weights (188.5 to 229.0 g), daily weight gains (1.2 to 1.4 g fish^{-1}day^{-1}), gross fish yield (GFY) (19.0 to 25.4 kg cage^{-1} crop^{-1}), NFY (16.6 to 22.9 kg cage^{-1} crop^{-1}) and feed conversion ratio (2.6 to 3.3) of African catfish were not significantly different among all sites (*P*>0.05; Table 5). African catfish grew steadily during the whole culture period (Figure 4). Survival of catfish decreased drastically within a month and remained almost stable afterwards (Figure 5). Pond water temperature remained above 20oC during most of the experimental period except in the last 15 days (Figure 6). Most of the growth and production parameters of all carps were better in the non-caged treatment than in the caged treatments (Table 7). Site-wise and overall means of net and gross yields of African catfish, all carps and catfish-carps combined in caged and non-caged treatments are shown in Tables 6 and 7. Combined yields of catfish and carps were almost double that of carps alone (Table 7). Results show that both caged and non-caged ponds produced positive net returns ranging from 1030 to 2509 NRs·pond^{-1} and 1238 to 1702 NRs·pond^{-1}, respectively (Table 8).

Table 5. Mean stocking and harvest sizes, weight gain, GFY, NFY, survival and FCR of African catfish at different sites in experiment 2 (Mean±SE).

Parameters	Experimental Sites			
	Site 1	Site 2	Site 3	Average
Stocking				
Total weight (kg cage-1)	2.6±0.0	2.6±0.0	2.6±0.0	2.6±0.0
Mean weight (g fish-1)	12.8±0.0	13.2±0.0	13.2±0.0	13.1±0.1
Harvesting				
Total weight (kg cage-1)	25.4±5.0	19.0±2.3	25.0±3.7	23.1±2.1
Mean weight (g fish-1)	229.0±29.5	188.5±24.3	220.6±5.1	212.7±12.3
Weight gain (g f-1d-1)	1.4±0.18	1.2±0.15	1.4±0.03	1.3±0.1
Gross yield (kg cage-1 crop-1)	25.4±5.0	19.0±2.3	25.0±3.7	23.1±2.1
Net yield (kg cage-1 crop-1)	22.9±5.0	16.4±2.3	22.3±3.7	20.5±2.1
Survival (%)	54.5±4.8	53.3±12.5	56.8±9.1	54.9±1.0
FCR	2.6±0.5	3.1±0.2	2.8±0.1	2.8±0.1

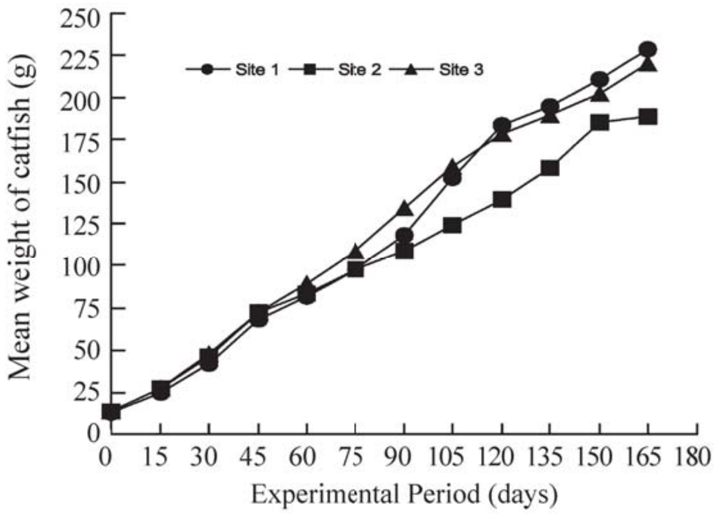

Figure 4. Mean weight of African catfish in cages at different sampling dates in experiment 2 (field trials).

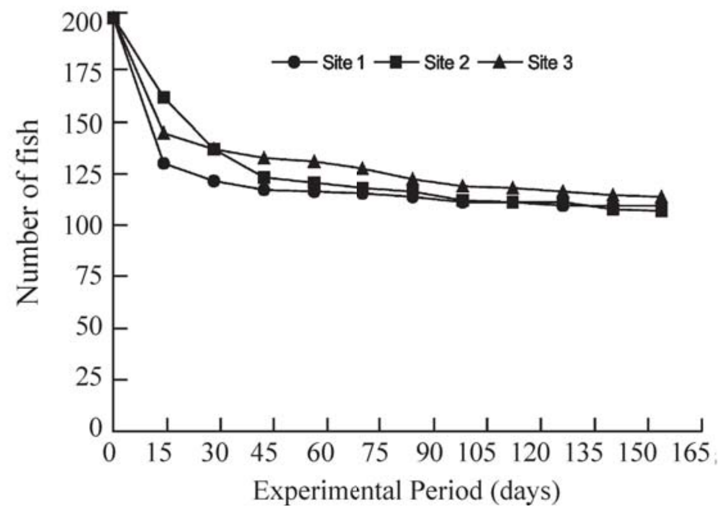

Figure 5. Fortnightly mean survival of catfish in cages in experiment 2 (field trials).

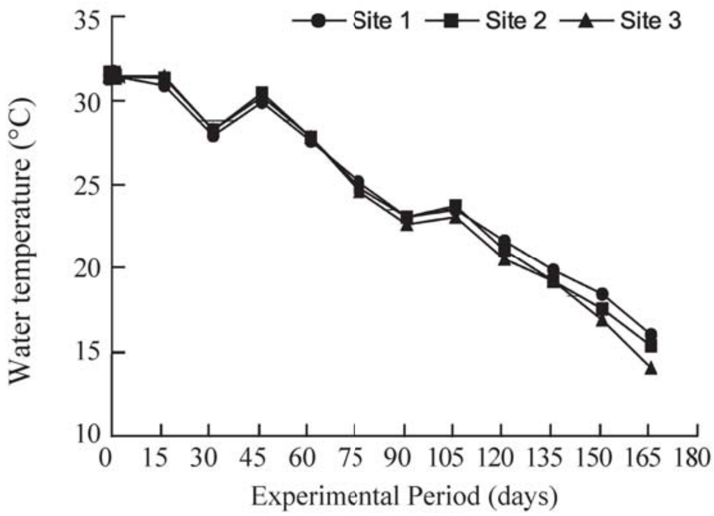

Figure 6. Fortnightly pond water temperature in experiment 2 (field trials).

Table 6. Mean stocking and harvest sizes of carps at different sites in experiment 2 (Mean±SE).

Parameter	Site 1		Site 2		Site 3	
	Cage	Non-cage	Cage	Non-cage	Cage	Non-cage
Common carp						
Mean stock wt. (g fish-1)	4.2±0.0	4.2±0.0	4.2±0.0	4.2±0.0	4.2±0.0	4.2±0.0
Mean harvest wt. (g fish-1)	247.9±11.3	351.0±15.3	433.0±92.1	337.2±36.1	412.5±52.9	455.7±48.3
Silver carp						
Mean stock wt. (g fish-1)	4.6±0.0	4.6±0.0	4.6±0.0	4.6±0.0	4.6±0.0	4.6±0.0
Mean harvest wt. (g fish-1)	158.6±20.0	225.8±18.3	128.0±7.0	220.6±30.7	226.6±25.0	286.7±21.4
Bighead carp						
Mean stock wt. (g fish-1)	2.2±0.0	2.2±0.0	2.2±0.0	2.2±0.0	2.2±0.0	2.2±0.0
Mean harvest wt. (g fish-1)	149.8±23.1	210.1±23.5	162.2±14.8	216.5±28.3	237.1±44.8	278.0±26.8
Rohu						
Mean stock wt. (g fish-1)	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0	0.5±0.0
Mean harvest wt. (g fish-1)	117.2±15.8	117.1±10.1	61.3±6.8	104.2±11.9	155.8±44.9	139.3±21.0
Mrigal						
Mean stock wt. (g fish-1)	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0
Mean harvest wt. (g fish-1)	92.8±3.3	96.1±1.6	60.4±15.1	95.2±34.1	123.8±29.2	138.1±36.0

Note: no data on weight gain, NFY, GFY and survival

Table 7. Mean GFY and NFY of catfish and carps at different sites in experiment 2 (Mean±SE).

Site	Fish species	Cage		Non-cage	
		GFY (kg)	NFY (kg)	GFY (kg)	NFY (kg)
Site 1	Catfish	25.4±5.0	22.9±5.0	-	-
	Carps	11.0±1.1	10.7±1.1	15.5±0.7	15.2±0.7
	Combined	36.4±6.0	33.5±6.0	15.5±0.7	15.2±0.7
Site 2	Catfish	19.0±2.3	16.4±2.3	-	-
	Carps	8.1±0.5	7.7±0.5	14.8±0.8	14.5±0.8
	Combined	27.1±2.8	24.1±2.8	14.8±0.8	14.5±0.8
Site 3	Catfish	25.0±3.7	22.3±3.7	-	-
	Carps	17.6±2.4	17.3±2.4	17.5±1.7	17.2±1.7
	Combined	42.6±5.7	39.6±5.6	17.5±1.7	17.2±1.7
Mean	Catfish	23.1±2.1	20.5±2.1	-	-
	Carps	12.2±2.8	11.9±2.8	15.9±0.8	15.6±0.8
	Combined	35.4±4.5	32.4±4.5	15.9±0.8	15.6±0.8

Table 8. Economic analysis (NRs pond^{-1}) of cage and pond systems at each site in experiment 2 (Mean±SE).

Parameter	Site 1		Site 2		Site 3		Mean	
	Cage	Non-cage	Cage	Non-cage	Cage	Non-cage	Cage	Non-cage
Gross Revenue								
Catfish	3051.1	-	2284.7	-	2997.8	-	2777.9	-
Carps	1096.7	1506.7	806.7	1543.3	1760.0	2026.7	1221.1	1692.2
Total	4147.8	1506.7	3091.3	1543.3	4757.8	2026.7	3999.0	1692.2
Operational								
Catfish fingerlings	1000.0	-	1000.0	-	1000.0	-	1000.0	-
Carp fingerlings	25.0	24.2	25.0	25.8	25.0	29.2	25.0	26.4
Urea	-	55.3	-	59.2	-	66.8	-	60.4
DAP	-	180.0	-	192.3	-	217.3	-	196.5
Feed	812.0	-	765.0	-	945.5	-	840.8	-
Cage depreciation	200	-	200	-	200	-	200.0	-
Working capital cost (8%)	73.2	9.3	71.5	10.0	78.0	11.3	74.2	10.2
Total	2110.2	268.8	2061.5	287.3	2248.5	324.6	2140.1	293.6
Net Return	2037.6	1237.9	1029.8	1256	2509.3	1702.1	1858.9	1398.7

Discussion

In the first experiment, growth of catfish in the low density treatments was higher than those in high density treatments. The daily weight gains of catfish in this experiment showed density-dependent growth which ranged from 0.26 to 0.49 g·fish^{-1}·day^{-1}. These were lower than those in the second experiment which ranged from 1.2 to 1.4 g·fish^{-1}·day^{-1}. The daily weight gains of catfish in both experiments were lower than that in an integrated cage-cum-pond system which was at 2.1-2.2 g·fish^{-1}·day^{-1} (Uddomkarn 1989; Lin and Diana 1995), and integrated pen-cum-pond system which ranged from 2.5 to 2.6 g·fish^{-1}·day^{-1} (Yi et al. 2003). The main reason for lower daily weight gains in this experiment was probably the lower feed input, low quality feed, small stocking size and low water temperature, compared to the experiments conducted by Lin and Diana (1995) and Yi et al. (2003). FCRs of 3.2-4.1 in the first experiment were higher compared to those in the second experiment (2.6-3.1) which was probably due to the small stocking size in the first experiment. FCRs in both experiments were higher than in the above-mentioned integrated systems as reported by Lin and Diana (1995), Yi et al. (2003) and Long and Yi (2004), which had FCRs of 1.9-2.2, 1.3 and 1.1-1-2, respectively. The main reasons for poor FCR in this study were low quality feed and high mortality of fish during the later part of the experiment. Survival of African catfish in the present experiments (30.8-67.2%) was lower than those reported by Uddomkarn (1989), Lin and Diana (1995), Yi et al. (2003), and Long and Yi (2004).

Average harvest size and production of carps in ponds without cages were significantly higher than those in ponds with cages ($p>0.05$) which denote nutrient limitations in caged treatments. No relationship was observed between the separate and combined yields of catfish and carps and stocking density of catfish in cages as commonly observed in other integrated cage-cum-pond system experiments (Lin and Diana 1995; Yi et al. 1996). The combined net yields in the first experiment ranging from 1.5 to 1.9 $t \cdot ha^{-1} \cdot crop^{-1}$ were lower than those achieved in the second experiment (3.2 $t \cdot ha^{-1} \cdot crop^{-1}$), and in other experiments with integrated cage-cum-pond systems and other species combinations (Lin 1990; Lin and Diana 1995; Yi et al. 1996; Rai and Lin 1999; Yi 1999; Yi and Lin 2000; Shrestha 2002).

All water quality parameters except water temperature in all ponds during both experiments were within the acceptable ranges for fish culture according to Boyd (1990). They were less affected by the stocking ratio of catfish in cages (Table 4). Water temperature became the limited factor for fish growth during most part of experiment 1 as it remained below 20°C for about three and half months from November 15 to February 28. Higher levels of TN, TAN, SRP and TP were found in ponds without cages, which may be due to increased nutrient input from fertilization. The high biomass of plankton in control ponds resulted in lower levels of DO and higher levels of TAN and SRP than in other treatments. Since no water quality parameters exceeded the lethal levels for carp growth, the lower yield in ponds with cages was due to poor primary production due to insufficient nutrients from catfish cages in the form of catfish wastes.

The income from these experiments was estimated by simple analysis. Fixed costs were not included as the analysis was intended to only compare relative differences in efficiency between treatments, and it was assumed that the fixed costs were the same for all treatments. The estimated costs were based on local market prices of fingerlings, fertilizer, feed, fish and cage materials. Results showed that net returns in the first experiment were higher in the non-caged than in caged treatments. Among caged treatments, the high returns were produced by the treatment with catfish at 100 $fish \cdot m^{-3}$ in cages. In the second experiment, a significant increase in net returns was observed in an integrated cage-cum-pond culture system (1860 $NRs \cdot pond^{-1}$) as compared to the semi-intensive culture system of carps (1400 $NRs \cdot pond^{-1}$) (Table 8).

The results of these experiments showed that African catfish has a great potential to be cultured in an integrated cage-cum-pond culture system, but it is necessary to fine-tune stocking ratios of catfish to carps and avoid the winter season for culture. Growth and production of African catfish can be improved by stocking larger size fingerlings and providing higher quality feed.

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References

AOAC (1980) Methods of Analysis, 13th edition. Association of Official Analytical Chemists, Benjamin Franklin Station, Washington DC, 20044. 1018 p.

APHA (1985) Standard Methods for the Examination of Water and Waste Water, 16th edition. American Public Health Association American Water Works Association 1015, Fifteenth street NW, Washington DC. 1268 p.

Boyd, C.E. (1990) Water Quality in Ponds for Aquaculture. Alabama Agriculture Experiment Station, Auburn University, Alabama, USA.

Lin, C.K. (1990) Integrated Culture of Walking Catfish (*Clarias macrocephalus*) and Tilapia (*Oreochromis niloticus*). In: Hirano, R.& Hanyu, I. (eds) The Second Asian Fisheries Forum. Asian Fish. Soc., Manila, Philippines. Pp. 209-212

Lin, C.K. & Diana, J.S. (1995) Co-culture of Catfish (*Clarias macrocephalus*×*C. gariepinus*) and Tilapia (*Oreochromis niloticus*) in Ponds. Aquatic Living Resources: 8, 449-454.

Long, N.T. & Yi, Y. (2004) Stocking Ratios of Hybrid Catfish (*Clarias macrocephalus*×*C. gariepinus*) and Nile tilapia (*Oreochromis niloticus*) in an Intensive polyculture. In: Boliver, R., Mair, G. & Fitzsimmons, K. (eds.) New Dimension in Farmed Tilapia, Vol. II, Proceeding from the Sixth International Symposium on Tilapia and Aquaculture. Sept. 12-16, 2004, Manila, Philippines. pp. 518-528.

Muir, J.F. (1982) Economic aspects of Waste Treatment in Fish Culture. In: Albaster, J.S. (ed.) Report of the EIFAC Workshop on Fish Farm Effluents. EIFAC Technical Paper 41: 123-135.

Rai, S. & Lin, C.K. (1999) Effect of Co-culture of Hybrid Catfish with Carps on Pond Water Quality and Fish Yield. J. Inst. Agric. Anim. Sci. Vol. 19-20: 83-89. (1998-1999).

Sethteethunyahan, R. (1998) Recycling Wastewater from Intensive Hybrid Catfish (*Clarias macrocephalus*×*C. gariepinus*) Culture for Semi-intensive Nile Tilapia (*Oreochromis niloticus*) Culture in Cement Tanks. Unpublished M.Sc. Thesis, Asian Institute of Technology, Thailand.

Shang, Y.C. (1990) Aquaculture Economic Analysis: An Introduction. World Aquaculture Society, Baton Rouge, Louisiana, 211 pp.

Shrestha, M.K. (2002) Mixed sex Nile tilapia Culture Model for Subtropical Nepal. Paper Presented in World Aquaculture, 23-27 April 2002, Book of Abstracts, Beijing, China, pp. 699-699.

Shrestha, M.K., Pandit, N.P. Yi, Y. Lin, C.K. & J Diana, J.S. (2005) Integrated Cage-Cum-Pond Culture System with African Catfish (*Clarias gariepinus*) in Cages and Carps in Open Ponds. Unpublished Report Submitted to the Aquaculture CRSP.

Sundar, V.S. (1989) Intensive Culture of Walking Catfish (*Clarias macrocephalus*) in an Outdoor Recirculating System Integrated with Tilapia. AIT Thesis No: AE – 89 – 37. Asian Institute of Technology, Bangkok, Thailand.

Uddomkarn, C. (1989) Integration of Catfish (*Clarias macrocephalus*) Cage Culture with Tilapia in Earthen Ponds. AIT Thesis No: AE – 89 – 39. Asian Institute of Technology, Bangkok, Thailand.

Ye, Q.F. (1991) Nutrient Budget and Water Quality in Integrated Walking Catfish – Tilapia Culture Unpublished M.Sc. Thesis, Asian Institute of Technology, Thailand.

Yi, Y. (1999) Modeling Growth of Nile Tilapia (*Oreochromis niloticus*) in a Cage-Cum-Pond Integrated Culture System. Aquacultural Engineering: 21, 113-133.

Yi, Y. & Lin, C.K. (2000) Integrated Cage Culture in Ponds: Concepts, Practices and Perspectives. In: Liao, I.C. & Lin, C.K. (eds.) The Proceedings of the First Symposium on Cage Culture in Asia. Asian Fisheries Society, Manila, Philippines, and World Aquaculture Society – Southest Asian Chapter, Bangkok, Thailand. Pp. 233-244.

Yi, Y., Lin, C.K. & Diana, J.S. (1996) Influence of Nile Tilapia (*Oreochromis niloticus*) Stocking Density in Cages on Their Growth and Yield in Cages and in Ponds Containing the Cages. Aquaculture: 146, 205-215.

Yi, Y., Lin, C.K. & Diana, J.S. (2003) Hybrid Catfish (*Clarias macrocephalus*×*C. gariepinus*) and Nile Tilapia (*Oreochromis niloticus*) Culture in an Integrated Pen- Cum-Pond System: Growth Performance and Nutrient Budgets. Aquaculture: 217, 395-408.

A Rapid Appraisal Approach to Identify Locally Available Feed Ingredients for Small-Scale Cage Aquaculture

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Abstract

This paper discusses a rapid appraisal approach to identify locally available feed ingredients for cage aquaculture. The approach includes a short questionnaire survey to identify feed ingredients and socio-economic status of the target group in addition to transect construction and resource mapping. Among the ingredients identified, snails and earthworms were relatively seasonal but available in sufficient quantities and could effectively reduce the dependency on fish meal for formulated diets. A diet of 25% crude protein (CP) content was formulated with earthworm, duck weed, mustard oil cake and snails as supplementary protein sources. Catfish (*Pangassias pangassias* and *Clarius batrachus*) and climbing perch (*Anabas testudineus*) were the choice species because of their higher farm gate prices. Three management scenarios were designed for cage aquaculture. Overall production ranged from 14.4 kg•m^{-3}•yr^{-1} to 36.0 kg•m^{-3}•yr^{-1} with 23.6 kg•m^{-3}•yr^{-1} for the realistic scenario. The five-year cash flow analysis projected a higher net present value (NPV) for both optimistic (US$ 235.80•m^{-3}) and realistic (US$ 162.25•m^{-3}) scenarios. Researchers would be able to identify locally available feed ingredients and the socio economic conditions required for low-cost low-input cage aquaculture in a brief period. This study highlights the possibility of applying this methodology to promote other aquaculture systems such as tilapia pond aquaculture to improve the livelihood status of resource poor people in Asia and other continents.

Introduction

Cage aquaculture has been widely practiced in most Southeast Asian countries including Cambodia, China, Malaysia, Philippines, Thailand and Vietnam. Production systems range from large commercial farms in lakes or estuaries to small single cage operations owned by marginal farmers. The role of cage culture in poverty alleviation for fringe populations in developing countries has been discussed in earlier studies (Hambrey et al. 2001a; 2001b). In Bangladesh, cage aquaculture is a relatively recent system introduced by some NGOs as a poverty alleviation and income generation tool for the marginal or landless farmers (Brugere et al. 2000; Hambrey et al. 2001; Huchette & Beveridge 2002) and for empowering rural women (Chowdhury & Rahman 1998; Brugere et al. 2001). Cage culture is being introduced in Bangladesh in lakes (Chowdhury and Yakupitiyage 2000; Kibria 2004), rivers (Huchette & Beveridge 2002), and other water bodies (Hambrey et al. 2001). However, to sustain and expand cage aquaculture for the resource poor, Edwards & Allan (2004) stressed the need for improved feed and feeding practices, and suggested further intensification of the production systems.

In countries like Bangladesh, the cost of commercially produced formulated feed limits the ability of marginal and landless farmers to adopt such production practices (Brugere et al. 2001; Chowdhury & Bureau 2006; Chowdhury et al. 2007). Alternatives to expensive commercial diets were investigated in periphyton based systems (Huchette & Beveridge 2002) and in the use of aquatic plants e.g. *Lemna* sp., green vegetables or rice bran as supplemental feed (Morrice 1998) in Bangladesh. Apart from feeds, most of these studies were focussed on the culture of indigenous fish species and cage construction materials (Bulcock & Brugere 2000). However, a low growth rate of fish observed in these systems with alternative feeding strategies (Azim et al. 2004) raised questions regarding the feasibility of these techniques. Another alternative to reduce the cost without sacrificing the productivity is to prepare a diet compatible to the commercial feed using locally available ingredients. A combination of low-cost diet and feed optimization techniques could provide farmers an effective tool to minimize cost and reduce dependency on external resources. Therefore, it is important to develop a method to identify locally available feed ingredients that could be used to formulate a diet for the sustainability of cage culture systems.

Rapid appraisal methodology has been widely used in techno-socio-economic studies related to aquaculture and fisheries (Pido et al. 1997; Chowdhury & Yakupitiyage 2000; Pitcher & Preikshot 2001; Jim-Saiki et al. 2004; Chowdhury et al. 2007) but hardly any has dealt with low-cost high-quality feed production except for Ofori & Prein (1996), and none has addressed the issue of identifying locally available feed ingredients for low-input cage aquaculture. The objectives of this paper are to develop a rapid appraisal tool to identify locally available feed ingredients, to formulate a low cost diet with locally available feed ingredients, and to assess the socio-economics of different management scenarios for cage aquaculture in oxbow lakes.

The rapid appraisal approach process (RAA)

The RAA used was divided into three sections: 1) household and physical resource mapping; 2) resource survey and identification of fish feed ingredients; and, 3) compilation of data, interpretation and diet formulation (Figure 1). It started with the formation of a multi-disciplinary appraisal team composed of one member specialist from aquaculture, animal or fish nutrition, and socio-economics disciplines. Pre-appraisal consultation meetings with external agencies and among the team members were organized to (a) determine the target group and targeted regions, (b) prepare and test the survey questionnaire, and (c) determine the maximum allowable cost of the formulated diet for a specific region on the basis of its socio-economic configuration for cage aquaculture. After the initial field visit, the rapid appraisal team constructed a model transect of the study area followed by resource mapping (Figure 2).

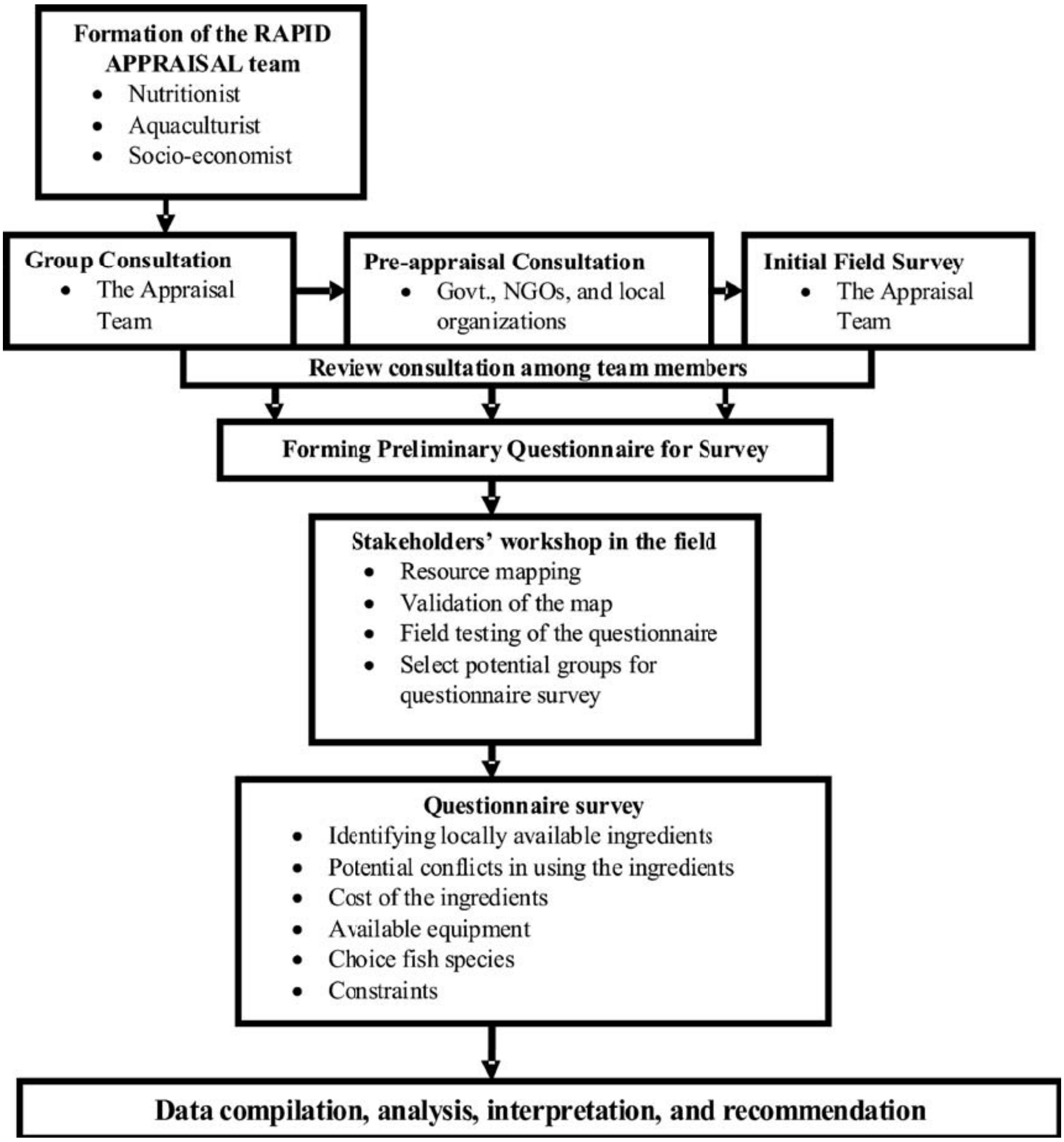

Figure 1. The rapid appraisal process.

Resource mapping is generally done by mapping the physical capital such as taking an inventory to acquire information on community resources directly from the people living in the area. The basic principle of resource mapping is to reflect and respond to the needs assessment. Key informant members of a community whose livelihoods partially or completely depend on an aquatic resource are required to actively participate in drawing the resource map. Following the above process and principles of RAA, a workshop was organized for each community where participants were briefed on the perspectives of resource mapping, its importance in locating resources and their availability. Participants were provided with necessary materials to draw the water body and the resources around it including houses, crop fields, ponds, vegetable gardens, canals and other social landmarks such as mosque, school or community centre (Figure 2).

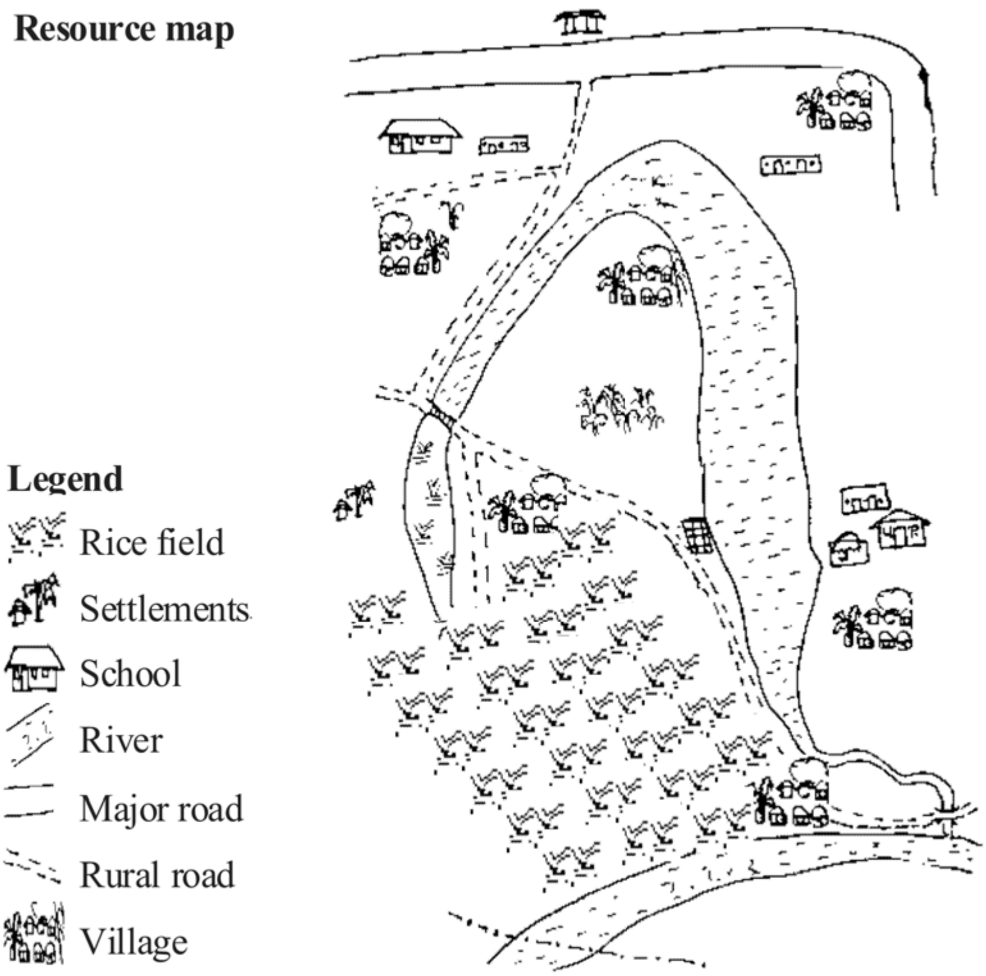

Figure 2. A resource map of the study area drawn by workshop participants.

The questionnaire was divided into three parts and contained basic questions on farm household identification including demographics. The first part included questions on types of feed used for livestock, poultry and fish and their seasonal availability as well as seasonal price fluctuation. The second part dealt with information on existing aquaculture practices and available feed processing equipment at the household level. The third part collected information on socioeconomic condition which included assets and the capital base of farmers.

An example from Bangladesh

The rapid appraisal study was conducted in three districts (*Chuadanga, Jessore and Jhenidah*) from the southwestern part of Bangladesh where aquatic resources are available. Two sets of samples were drawn for the study from each survey location i.e. 10 key informants from the fisheries community for focus group discussion (FGD) and administering the structured questionnaires, and 10 samples randomly selected from each sample site targeting particularly women living around the aquatic resource area. Before the workshops and the surveys, a list of ingredients was prepared from published and unpublished sources in each region. The information was further validated and corrected by specific questions on locally available feed ingredients collected during FGD and again from individual respondents. Information on availability and seasonality of the ingredients, market price, and opportunity cost for the ingredients was collected (Table 1).

Linear programming was applied to formulate a diet with at least 25% crude protein content. Sensitivity analysis was performed to produce three scenarios. The fixed factors were cage size and cost, labour cost, fingerlings and feed cost, transportation cost, and number of crops per year. Outputs for each scenario were the internal rate of return (IRR), net present value (NPV), cost benefit ratio, return on labour, and production. Assumptions for different scenarios were taken from the information collected during the field survey.

Table 1. A list of selected locally available feed ingredients identified by the participants and their nutrient compositions.

Source	Seasonal availability	Local market price(US$ kg^{-1})	Opportunity cost (US$ kg^{-1})	Imported/local
Plant source				
Rice bran	Year round	0.025	-	Local
Wheat bran	Year round	0.025	-	Local
Broken rice	Year round	0.125	-	Local
Mustard cake	Year round	0.100	-	Local
Dry duckweed	Jul to Oct	-	0.25	Local
Defatted bran	Year round	0.035	-	Local
Water hyacinth	Year round		0.05	Local
Animal source				
Fish meal	Year round	0.500	-	Imported
Poultry viscera	Year round	0.030	-	Local
Snails	Jul to Oct	-	0.20	Local
Shrimp meal	Year round	0.200	-	Local
Bone meal	Year round	0.050	-	Local
Other sources				
Household waste	Year round	-	0.05	Local
Egg shell	Year round	-	0.05	Local

Key findings

Differences were observed between existing management systems from the resource maps drawn by the stakeholders. Paved or brick-soiled roads around the lakes were common in lakes managed by the Directorate of Fisheries and Oxbow Lake Project, whereas most NGO-operated and privately-owned lakes did not have an established road network, making transportation of fish and raw materials for cage operations harder for the farmers. Marketing facilities were present in lakes under all the systems to a varied extent.

Ingredients such as earthworm and snails were collected from the wild and they could be used as protein supplements. A list of selected potential feed ingredients identified during the survey is provided in table 1. In formulating a low cost diet, selected input constraints were: (1) the percentage of plan protein source (15%) because of their anti-nutritional factors; and (2) fish meal (10%) because of their high cost. The output constraints were set for the crude protein content (25%) and price per kg of feed (US$ 0.22).

Feed formulation

Table 2 provides a simple example of a formulated diet for cage aquaculture using locally available ingredients. From the survey, it was found that the most common fuel used by farmers to process feed ingredients was dried cow manure followed by dry plant materials, chopped wood then kerosene. Processing equipment such as knives, rice mill, graters, grinders and extractors were available in most of the households. Although a single household did not own all the equipment, a community sharing practice made all equipment available to all the farmers in a group.

Table 2. A formulated feed for cage aquaculture using locally available ingredients

Ingredients (%)	Percentage (%)	Cost (US$)	Final nutrient composition
Mustard oil cake	15	0.025	Crude protein –25
Rice bran	35	0.027	Crude lipid – 6
Wheat flour	2	0.007	Crude fibre – 9
Brown wheat flour	18	0.03	Ash – 15
Fish meal	10	0.10	NFE – 45
Earthworm	5	0.013*	
Snails	10	0.01*	
Duckweed	5	0.008)	
Total	100	0.22	

* Opportunity cost

Farmers preferred high value species as choice species for cage aquaculture such as catfish (*Pangassias pangassias*, *Clarius batrachus*) and climbing perch (*Anabas testudineus*), and popular species such as Indian major carps and silver carp. However, farm gate prices of carps were too low to make cage culture operations sustainable. In addition, the limited availability of fingerlings of *Pangassius* sp. and *A. testudineus* made year-round culture of these two species almost impossible. The market price of catfish during the time of the survey ranged from US$ 2.25-2.75 kg^{-1} while that of *Pangassius* and climbing perch ranged from US$ 1.50-2.00 and from US$ 2.00 to 2.50 kg^{-1}, respectively. Because of the availability of its fingerlings and its high market price, catfish (*Clarius* sp.) was deemed suitable and selected for financial analysis. The variables for the financial models were stocking density (m^{-3}), transportation and labour (US$), final weight (g) and farm gate price (US$ kg^{-1}) (Table 3). Benefit-cost ratios were >1 in both optimistic and realistic scenarios but <1 in the pessimistic scenario. The payback period of 0.22 yr for the optimistic scenario appeared to be unreasonable but the 1.28 yr for the realistic scenario was reasonable. Overall production ranged from 14.4 to 36 kg yr^{-1} with 23.6 kg yr^{-1} for the realistic scenario. The cash flow analysis for five years showed a higher net present value (NPV) could be obtained for both the optimistic (US$ 235.80 cage^{-1}) and realistic scenarios (US$ 162.25 cage^{-1}).

Table 3. Considered factors and their assumed values for three different scenarios

	Factors	Optimistic	Pessimistic	Realistic
Variable factors	F.C.R	2.5	3	2.75
	Survival	100	80	90
	Stocking density·m^{-3}	80	60	70
	Harvesting weight (g)	150	100	125
	Farm gate price (US$)	4.50	3.00	3.75
Benefit : cost ratio		1.13	0.93	1.28
Payback period (yr)		0.22	-	1.28
RoL		4.45	0.00	0.83
IRR (%)		366	-16	136
Production (kg·yr-1)		36	14.4	23.6

Note: RoL - return on labour; IRR - internal rate of return

Discussion

Most rapid appraisal methods, whether participatory rural appraisal (PRA) or rapid rural appraisal (RRA), never work far beyond resource mapping (for examples see Ofori & Prien 1996; Pido et al. 1997). In this proposed method, basic processes were followed, such as existence of a multidisciplinary team, recording data by transects, and mapping resources (Chambers 1980; Pitcher & Priekshot 2000) but a brief individual questionnaire survey was added to identify feed ingredients and to accommodate individual perception on the identification of feed ingredients, feed processing and culture management. Instead of generating a snapshot of available resources as a base for the large questionnaire survey, the proposed rapid appraisal method for identifying feed ingredients dealt with the complete process from resource survey to feed formulation to socio-economics in a reasonably brief period. To facilitate this, the questionnaire for individual survey needs to be very brief i.e. not more than two pages, and should include the key questions only to address the main objectives of the study.

At present, only a commercially available formulated diet (US$ 0.35 kg^{-1}) is available for use by some of the cage farmers. Few farmers have tried with a mixture of rice bran and mustard oil cake. The rest of the farmers have been using kitchen and garden waste along with the occasional use of rice bran (Hambrey et al. 2001a). Use of snails and earthworms as protein supplement was also tried but no study was done to formulate a diet composed of conventional and non-conventional feed ingredients. Hasan (2001) stressed the need for a formulated diet for the development of cage aquaculture in oxbow lakes and elsewhere in Bangladesh. It is shown that by identifying and selecting locally available ingredients through a rapid appraisal methodology and incorporating them in the feed formulation, it is possible to formulate a diet with a similar crude protein content (25%) with that of the commercial diet at a fraction of its cost. However, caution must be taken to limit the content of plant protein sources because of their nutrient limiting factors. Although anti-nutritional factors in oilseed meals can be destroyed by heat treatment, some cannot be destroyed, such as gossypol, glucosinolates and phytic acid (Tacon 1987). We also propose the culture of high value species because financial projection suggested that growing species with a farm gate price of less than US$ 2.25 kg^{-1} was not profitable.

This study showed the applicability of the proposed rapid appraisal methodology to identify locally available feed ingredients for cage culture in oxbow lakes. Two major steps of the process are: 1) pre-field visit activities, such as team formation, consultation and formulation of the survey questionnaire; and 2) feed formulation, interpretation of and inference from the socio-economic analysis. However, further in-depth research is needed with a multi-locational approach covering different agro-ecological environments depending on locally available feed ingredients, their availability and adaptability through participatory research involving government and non-government agencies and farmers. Secondly, research on feed processing and production technologies of local ingredients need to be translated in an easiest and simplest way using the local language. Finally, a feed plant to formulate and process feeds suitable for a small-scale processing unit to encourage local entrepreneurs should be designed and developed.

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References

Azim, M.E., Rahman, M.M., Wahab, M.A., Asaeda, T., Little, D.C. & Verdegem, M.C.J. (2004). Periphyton based pond polyculture system: a bioeconomic comparison of on-farm and on-station trials. Aquaculture 242, 381-396.

Brugere, C., McAndrew, K. & Bulcock, P. (2000) Social impacts of cage aquaculture on communities in Bangladesh. Aquaculture Asia 5(3), 29- 33.

Brugere, C., McAndrew, K. & Bulcock, P. (2001) Does cage aquaculture addresses gender goals in development? Results of a case study in Bangladesh. Aquaculture Economics and Management 5, 179-190.

Bulcock, P. & Brugere, C. (2000) Identifying research methods in adoption of cage culture, Bangladesh. DFID Aquaculture News, 7-12, UK.

Chambers, R. (1980) Rural Development: Putting the Last First. Harlow, England.

Chowdhury, M.A.K. & Bureau, D.P. (2006) A rapid appraisal approach to identify feed ingredients for low-input cage aquaculture: a case from Bangladesh. International Aquafeed 9(1), 14-18.

Chowdhury, M.A.K., Bureau, D.P., Bose, M.L. & Dey, M.M. (2007) Relevance of a rapid appraisal approach to identify locally available feed Ingredients to small-scale Nile tilapia (*Oreochromis niloticus* L.) aquaculture. Aquaculture Economics and Management 11, 151-169.

Chowdhury, M.A.K. & Rahman, S. (1998) Women’s participatory role in oxbow lake fisheries management: A case from Bangladesh. Aquaculture Asia 3(2), 3-7.

Chowdhury, M.A.K. & Yakupitiyage, A. (2000) Efficiency of the existing oxbow lake management systems in Bangladesh to introduce cage culture for resource poor fishers. Fisheries Management and Ecology 7, 65-74.

Edwards, P. & Allan, G. (2004) Fish feed and feeding in the countries of the lower Mekong Basin. World Aquaculture 35(3), 30-32.

Hambrey, J., McAndrew, K. & Beveridge, M. (2001a) Aquaculture and poverty alleviation 1: cage culture in freshwater in Bangladesh. World Aquaculture, 32(1), 50-58.

Hambrey, J. Tuan, L.A. & Thuong, T.K. (2001b) Aquaculture and poverty alleviation 2: cage culture in coastal waters of Vietnam. World Aquaculture 32(2), 34-38.

Hasan, M.R. (2001) Nutrition and feeding for sustainable aquaculture development in the third millennium. In: R.P.

Subasinghe, Bueno, P., Phillips, M.J., Hough, C., McGladdery, S.E. & Arthur, J.R. (Eds.), Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000, NACA, Bangkok and FAO, Rome, pp. 193-219.

Huchette S.M.H. & Beveridge M.C.M. (2002). Technical and economical evaluation of periphyton-based cage culture of tilapia (*Oreochromis niloticus*) in tropical freshwater cages. Aquaculture 218, 219-234.

Jim-Saiki, L.O., Ogunbadeio, H.K. & Ayanda, J.O. (2004) Small scale fish production, processing and marketing in Ijebu-Lekki Local Government area of Lagos State, Nigeria. Proceedings of the 18th annual conference of the Fisheries Society of Nigeria (FISON). Nov 2004, pp 179-185.

Kibria, G. (2004) Feasibility study of cage aquaculture in oxbow lakes of Bangladesh. World Aquaculture 35(4), 23-30.

Morrice, C.P. (1998) A trial to test the suitability of a low-cost, silver barb *Puntius gonionotus*- based polyculture model for seasonal ponds in northwest Bangladesh. Asian Fisheries Science 11(2), 121-132.

Ofori J.K. & Prein, M. (1996) Rapid appraisal of low-input aquaculture systems. ICLARM Conference Proceedings. Manila.

Pido, M.D., Pomeroy, R.S., Garces, L.R. & Carlos, M.B. (1997) A rapid appraisal approach to evaluation of community-level fisheries management systems: Framework and field application at selected coastal villages in the Philippines and Indonesia. Coastal Management 25(2), 183-204.

Pitcher, T.J. & Preikshot, D. (2000) RAPFISH: a rapid appraisal technique to evaluate the sustainability status of fisheries. Fisheries Research 49, 255-270.

Tacon, A.G.J. (1987) The nutrition and feeding of farmed fish and shrimp – a training manual, Vol. 2. Nutrient Sources and Composition, GCP/RLA/075/ITA, FAO/UNDP, Rome.

Yakupitiyage A. (1984) A feasibility study of the culture of earthworms on cesspool slurry and evaluation of their nutritive value for the catfish (*Clarius macrocephalus*). AIT Thesis no. AE 84.21, 95pp.

Effects of Fish Meal Partial Replacement by Soybean Meal on Growth and Body Composition of Fingerling Black Seabream, *Acanthopagrus schlegeli*

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Abstract

An experiment was conducted to evaluate the effect of dietary levels of soybean meal on growth performance, feed utilization efficiency and body composition of black seabream, *Acanthopagrus schlegeli.* Six isonitrogenous (43 % crude protein) and isocaloric (18.70 kJ of gross energy•g^{-1}) diets containing soybean meal at levels of 0, 11.86, 23.72, 35.58, 47.44 and 59.30 % as replacements of 0, 12.5, 25.0, 37.5, 50.0 and 62.5 % of Peruvian fish meal on an equal protein basis were fed to fingerling black seabream (5.31±0.19g) for 50 days. Average final weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish fed diet 3 were not significantly different (P>0.05) from the group fed the basal diet. Fish fed diet 4 had WG and SGR similar to those of fish fed diet 3, but had significantly poorer (P<0.05) FCR and PER than those of diet 3. Survival was not affected by dietary treatments. There were no significant differences (P>0.05) in body contents of moisture, crude protein, crude ash and condition factor (CF) among fish fed various experimental diets. Lipid contents of fish fed soybean meal-containing diets were significantly higher than that of fish fed the control diet. Results of this study indicated 23.72 % soybean meal can be included in the diet of fingerling black seabream as a replacement of 25 % of Peruvian fishmeal protein without affecting their growth performance and feed utilization efficiency.

Introduction

Commercial feeds for marine fish or shrimp usually contain high levels of fish meal (FM), typically ranging from 30 to 50 % by weight (Lim & Dominy 1990). Fish meal has high nutritive value, palatability and digestibility, but is a relatively expensive feed ingredient compared with protein-rich ingredients of plant origin. The continued increase in the demand of FM coupled with the shortage of global FM supply has led to considerable increase in the price of FM (e.g. U.S. \$1,500•ton^{-1} in China). In recent years, considerable efforts have been devoted by aquaculture nutritionists to use plant ingredients as alternative protein sources to replace FM in aquaculture feeds. (Hu et al.1995; Adelizi et al.1998; Allan et al. 2000a; 2000b). Due to its availability, consistent quality and supply, soybean meal (SM) has received the most attention among alternative plant protein sources. It is known to have one of the best amino acid profiles, compared with other plant protein ingredients, for meeting the essential amino acid requirements of aquatic species. Partial or total substitution of FM with SM in diets of several aquaculture species, such as Japanese seabass (*Lateolabrax japonicus*), rainbow trout (*Oncorhynchus mykiss*), giant freshwater prawn (*Macrebrachium rosenbergii*), white shrimp (*Penaeus vannamei*), yellow tail (*Seriola quinqueradiata*), red drum (*Sciaenops ocellatus*), Australian silver perch (*Pagrus auratus*), Pacific white shrimp (*Litopenaeus vannamei*), Siberian sturgeon (*Acipenser baerii*) (Hu et al.1995; Lei et al.1998; Dong & Niu 2000; Shimeno et al. 1993; McGoogan & Gatlin 1997; Catacutan & Pagador 2004; Allan 2000a; Mendoza et al.2001; Rónyai et al. 2002) have been investigated. No studies have been conducted on the use of SBM in diets of black seabream (*Acanthopagrus schlegeli*), one of the most popular aquaculture fish species in Southeast Asia.

This study was conducted to evaluate the effects on dietary levels of SBM as partial replacements of FM on growth performance, feed utilization efficiency and body composition of fingerlings black seabream.

Materials and Methods

Experimental fish

Black seabream was obtained from the hatchery of Xiangshan Damutu in Zhejiang Province. Prior to the start of the experiments, fish were acclimated in an indoor concrete tank and fed a commercial diet for 2 weeks. The fish (mean weight ± SEM of 5.31 ± 0.19g) were then divided into 18 groups of 30 fish each and stocked in randomly assigned 0.75 m^3 concrete tanks.

Experimental system

The experiment was conducted at the wet laboratory of the Zhejiang Institute of Freshwater Fisheries (ZIFF). Stored natural seawater which had been filtrated through a sand filter and a 200-mesh nylon screen was used in this study. Each tank was continuously aerated through an air stone supplied by a central air compressor. During the experiment period, salinity, water temperature and pH were kept at 22 ppt, 27±1.5C, and 7.4-8.2, respectively. Dissolved oxygen was maintained above 5.8 $mg•L^{-1}$. Nitrite-N and ammonia-N, measured once weekly using Nitrite and Ammonia Reagent Kits were less than 0.02 and 0.03 $mg•L^{-1}$, respectively. Approximately 50 % of the water in each tank was exchanged daily, in the morning before feeding, with stored filtered seawater.

Experimental Diets

Six isonitrogenous (43 % crude protein) and isocaloric (18.70 $kJ•g^{-1}$ gross energy) diets were formulated to contain 0, 11.86, 23.72, 35.58, 47.44 and 59.30 % SBM (diets 1 to 6) as replacements of 0, 12.5, 25.0, 37.5, 50.0 and 62.5 % of Peruvian fish meal on an equal protein basis, respectively (Table 1). All ingredients were ground to pass through a 60-mesh sieve and mixed thoroughly before a small amount of water was added. The moist mixture was pelleted through a 1.5-mm diameter die using a meat grinder. The resulting moist pellets were air-dried at room temperature, broken into small pieces, then transferred into plastic bags and stored at 0°C in refrigerator until such time that it will be used.

Table 1. Ingredient composition and proximate analyses of the experiment diets (%)

No.	1	2	3	4	5	6
Peru Fishmeal(68%)[a]	60.00	52.50	45.00	37.50	30.00	22.50
Soybean meal(43%)[a]	0.00	11.86	23.72	35.58	47.44	59.30
Wheat middling	10.00	10.00	10.00	10.00	10.00	10.00
Squid meal	2.00	2.00	2.00	2.00	2.00	2.00
α-starch	24.50	19.74	15.00	10.28	5.54	0.80
Fish oil	2.00	2.40	2.80	3.14	3.52	3.90
vitamin mix[b]						
Mineral mix[c]	0.75					
0.75	0.75					
0.75	0.75					
0.75	0.75					
0.75	0.75					
0.75	0.75					
0.75						
Proximate analysis (%) [d]						
Fishmeal protein substituted (%)	0.00	12.50	25.00	37.50	50.00	62.50
Moisture	9.21	9.28	9.35	9.41	9.48	9.54
Crude protein	43.32	43.32	43.32	43.32	43.32	43.32
Crude lipid	7.77	7.79	7.81	7.77	7.77	7.77
Crude ash	9.09	8.76	8.42	8.09	7.76	7.43
Gross energy ($kJ•g^{-1}$)[3]	18.62	18.66	18.73	18.71	18.71	18.70

[a] Sources: Peru fishmeal (68 %), pesquera Hayduk S.A.,Peru; soybean meal (43 %), Local vendor.
[b] Vitamin mix (per kg feed): vitamin A, 10mg; vitamin D, 0.05mg; vitamin E_E, 400mg; vitamin K, 40mg; vitamin B_1, 50mg; vitamin B_2, 200mg; vitamin B_3, 500mg; vitamin B6, 50mg; vitamin B_7, 5mg; vitamin B_{11}, 15mg; vitamin B_{12}, 0.1mg; inositol, 2000mg; choline, 5000mg; ascorbyl-2-polyphosphate, 2000 mg. Mineral mix (per kg feed): $FeSO_4•7H_2O$, 372mg; $CuSO_4•5H_2O$, 25mg; $ZnSO_4•7H_2O$, 120mg; $MnSO_4•H_2O$, 5mg; KI, 0.8 mg; $CoCl_2$ $6H_2O$ (1%), 50 mg; $MgSO_4$, 2475mg; NaCl, 1875mg; KH_2PO_4, 1000mg; $Ca(H_2PO_4)$, 2500mg.
[c] energy ($kJ•g^{-1}$ diet) computed: protein, 23.7 $kJ•g^{-1}$; fat, 39.5 $kJ•g^{-1}$; carbohydrate, 17.2 $kJ•g^{-1}$.
[d] Calculated from the determined values of raw materials.

Feeding management

Fish in three random tanks were assigned to each of the experimental diets and fed to apparent satiation twice daily (between 0800-0830 h and 1500-1530 h) for 50 days. During each feeding, feed was offered by hand three or four times until satiation was reached. The amount of feed consumed was determined daily by calculating the difference in the weight of feed before the first feeding and after the last feeding. Thirty percent of the water in the tank was exchanged by fresh seawater 30 minutes after each feeding

Sampling and data analysis

At the end of the feeding trial fish in each tank were counted and weighed collectively and final average weight, weight gain (WG), specific growth rate (SGR), survival rate (SR), protein efficiency ratio (PER), feed conversion ratio (FCR) and condition factor(CF) were determined using the following equations:

WG = Average final weight- average initial weight

SGR= 100 × [(ln (final average weight) - ln (initial average weight)]/ days fed.

PER= Weight gain (g)/protein fed (g).

FCR= Dry weight of feed fed (g)/wet fish weight gain (g).

CF = 100 × body weight (g)/[body length (cm)]^3

At the end of the experiment, five fish were randomly sampled from each tank, pooled and stored at -20°C, ground finely in a meat grinder for determination of whole-body proximate composition. Each sample was analyzed using the method of AOAC (1990). Moisture was determined based on the loss due to drying at 105°C; crude protein were determined using Kjeldahl's method; crude lipid were determined by ether extract method; crude ash were determined by heat 550°C.

All the data were analyzed by one-way analysis of variance (ANOVA) while Duncan's new multiple range test was used to determine the differences between treatment means. Differences were considered significant at $P<0.05$. All the analyses were carried out following the procedures of SPSS for Windows 11.0.

Results

Growth performance and feed utilization efficiency

Average final weight, weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate of fingerling black seabream are presented in table 2. Survival rates ranged from 93.33 to 98.30 %. No significant differences ($P>0.05$) were found among survival of fish receiving various treatments. Fish fed diet 2 (12.5 % FM protein replaced by 11.86 % SM) had significant lower ($P<0.05$) WG, PER and higher ($P<0.05$) FCR compared to fish fed the control diet (diet 1), but with similar SGR. Average WG, FCR, SGR and PER of fish fed diet 3 (25 % FM protein replaced by 23.72 % SBM) were not significantly different ($P>0.05$) from those of fish fed the control diet. Increasing FM protein substitution levels to 37.5 % or higher significantly decreased ($P<0.05$) WG, SGR (except that of diet 4), PER and increased FCR ($P<0.05$) relative to the group fed the control diet.

Table 2. Average initial and final weight, weight gain, specific growth rate, feed conversion ration, protein efficiency ratio and survival of fingerling black seabream fed diets containing various levels of soybean meal1.

Diet no.	Level of soybean meal (%)	Initial weight (g)	Final weight (g)	Weight gain(g)	Feed conversion ratio	Specific growth rate	Protein efficiency ratio	Survival (%)
1	0	5.35±0.21^a	14.12±0.73^a	8.77±0.53^a	1.98±0.16^a	1.98±0.04^a	1.17±0.1^a	96.29±4.85^a
2	12.5	5.18±0.10^a	12.95±0.1b	7.77±0.19^{bc}	2.41±0.15^b	1.87±0.05^{ab}	0.96±0.07^b	96.29±4.85^a
3	25.0	5.42±0.16^a	13.79±0.14^a	8.37±0.29^{ac}	1.95±0.10^a	1.91±0.08^{ab}	1.18±0.06^a	98.30±2.89^a
4	37.5	5.41±0.18^a	13.54±0.24^{ab}	8.14±0.14^c	2.38±0.22^b	1.87±0.04^{ab}	0.98±0.09^b	94.81±5.56^a
5	50.0	5.37±0.31^a	12.86±0.44^b	7.49±0.20^b	2.98±0.13^c	1.78±0.07^{bc}	0.79±0.06^c	93.33±6.67^a
6	62.5	5.15±0.13^a	11.94±0.14^c	6.78±0.01^d	2.44±0.44^c	1.71±0.03^c	0.78±0.04^a	97.67±0.00^a

1Values (means ± SD) in the same column having a common superscript are not significantly different ($P>0.05$).

Whole-body proximate composition and condition factor

Whole body proximate composition and condition factor are presented in table 3. There are no significant differences (*P*>0.05) among moisture, crude protein, ash and condition factor of the fish.in different treatments. Lipid contents significantly increased (*P*<0.05) in fish fed diets containing SBM as compared to that fed the diet without SBM (diet 1).

Table 3. Whole body composition (%) and condition factor of fingerling black seabream fed diets containing various levels of SM1

Diet no.	Moisture	Crude protein	Crude lipid	Crude ash	Condition factor
1	77.71±0.36[a]	14.37±0.18[a]	2.64±0.03[a]	4.81±0.09[a]	2.95±0.25[a]
2	77.42±0.43[a]	14.13±0.13[a]	3.13±0.20[b]	4.62±0.19[a]	2.89±0.42[a]
3	77.62±0.26[a]	14.27±0.15[a]	2.92±0.14[b]	4.67±0.06[a]	2.65±0.18[a]
4	77.81±0.14[a]	14.09±0.22[a]	3.79±0.03[c]	4.51±0.10[a]	2.66±0.29[a]
5	78.28±0.36[a]	14.33±0.20[a]	3.64±0.21[c]	4.65±0.13[a]	2.79±0.23[a]
6	78.85±0.26[a]	14.26±0.19[a]	2.96±0.13[b]	4.64±0.12[a]	2.76±0.24[a]

[1]Values (means ± SD) in the same column having a common superscript are not significantly different (*P*>0.05).

Discussion

Information from earlier studies indicate that optimum levels of SBM that can be incorporated in marine fish diets appear to vary based on fish species and size (Peres & Lim, *In Press*). Results of the present study demonstrate that 23.72 % SBM can be incorporated in diets of black seabream as a substitute for 25 % of protein from Peruvian FM (diet 3) without affecting their growth performance, feed utilization efficiency and survival. The poorer performance of diet 2 (11.86 % SBM) as compared to diets 1 and 3 could not be explained since fish fed diet 4 (35.58 % SBM) performed equally well or better than the group fed diet 2. Nevertheless, our results are in agreement with those of Robaina et al. (1995) and Nengas et al. (1996) who found that inclusion of soybean meal at levels of up to 24 % as replacements of fishmeal has no effects on growth , FCR and PER of gilthead seabream (*Sparus aurata*), compared with fish fed the all-fishmeal control diet. For the same species, Venou et al. (1997) concluded that 22 % was the acceptable level of SBM when used as a substitute for high quality FM. EL-Sayed (1994) reported that the diet in which 25 % of FM was replaced by 21 % SBM and 0.5 % methionine provided similar growth and feed efficiency of juvenile silver sea bream (*Rhabdosargus sarba*) to those obtained with the FM-control diet. A SBM level of 25 % as replacement for 33 % of Chilean brown FM has been reported to provide good growth performance of red sea bream, *Pagrus major* (Ukawa et al. 1994). Red drum (*Sciaenops ocellatus*), however, can tolerate relatively high levels of dietary SBM. Mcgoogan & Gatlin (1997) showed that red drum fed diets in which 90 % of anchovy FM was replaced by 66.6 % SBM gained as much weight as fish fed 100 % FM (53.4%) diet.

Increasing dietary levels of SBM to 35.58 % or higher adversely affected fish performance and feed utilization efficiency. Nengas et al. (1996) also obtained significantly decreased growth and feed efficiency of red sea bream when SBM levels were increased to 36 % or higher. Ukawa et al. (1994) showed that increasing the dietary SBM level to 40 % as a replacement for 50 % of FM resulted in significant reduction of weight gain and feed efficiency. The poor performance of diets containing high levels of SBM has been attributed to several factors, such as the presence of antinutritional factors and indigestible nutrients, imbalanced essential amino acids and minerals, and reduced diet palatability (Peres & Lim, *In Press*).

In our experiment, whole-body contents of moisture, protein and ash were found to be not affected by inclusion levels of dietary SM. Body lipid contents significantly increased in fish fed all diets containing SBM, even though our diets were isonitrogenous and isocaloric. However, there was no clear trend of increasing body lipid with increasing dietary levels of SBM. This may be due to essential amino acid imbalance leading to the conversion on a part of the dietary protein to energy.

Results of the present investigation reveal that approximately 24 % SBM can be incorporated in diets of fingerling black seabream as a replacement of 25 % of FM protein. Additional studies to increase the inclusion level of SBM in black seabream diets by supplementation of deficient nutrients (e.g. essential amino acids and minerals), enzymes and palatability enhancers, and inactivation of antinutritional factors, especially trypsin inhibitors, are needed.

References

Abdelghany, A.E. (2003) Partial and complete replacement of fish meal with gambusia meal in diets for red tilapia, *Oreochromis niloticus* × *O. mossambicus*. Aquaculture Nutrition 9, 145-154.

Allan, G..L., Parkinson S., Booth M.A. (2000a). Replacement of fishmeal in diets for Australian silver perch, Bidyanus bidyanus: I. Digestibility of alternative ingredients. Aquaculture 196, 293-310.

Allan G..L., Parkinson S., Booth M.A. (2000b) Alternative protein sources to fishmeal in aqua-feeds: plant proteins. International Aquafeed Directory and Buyers' Guide. Pp. 12-18.

AOAC. (1990) Official Methods of Analysis, 15th edn. Association of Official Analytical Chemists, Arlington, Virginia.

Catacutan, M.R. and Gregoria, E P. (2004) Partial replacement of fishmeal by defatted soybean meal in formulated diets for the mangrove red snapper, *Lutjanus argentimaculatus* (Forsskal 1775). Aquaculture Research 35, 299-306.

Chen L., Du, N. and Lai W. (1994) Evaluation of soybean cake as a substitute for partial fishmeal in formulated diets for Chinese mitten-handed crab juvenile. Journal of Fisheries of China 1, 24-30.

Dong, Y. and Niu, C. (2000) Effects of dietary protein sources on growth and activities of digestive enzymes of giant freshwater prawn (*Macrobrachium rosenbergii*). Journal of Beijing Normal University (Natural Science) 36 (2), 260-263.]

Hu, J., Chen, X. and Hong, H. (1995) Evaluation of soybean cake as a substitute for partial fish meal in artificial diets for Lateolabrax japonicus. Journal of Oceanography in Taiwan Strait 14 (4), 418-421.

Lei, C., Liu, C., Sun, J. (1998) Research of partial substitution of Peruvian fishmeal with animal protein in the rainbow trout diets. Chinese Journal of Fisheries 11 (2), 81-85.

Lim, C. and Dominy, W. (1990) Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp (*Penaeus vannamei*). Aquaculture 87, 53-63.

Liu J., Wang, K., Wang X, (1995). Studies on the optimum protein content and the ratio of animal to plant protein in the diet of the black seabream (*Sparus macrocephalus*). Oceanologia et Limnologia Sinica 26(4), 445-448.

Liu, J. (1996) Studies on the nutrient requirements and mixed feed of black seabream (*Sparus Macrocephalus*). Transactions of Oceanology and Limnology 27(3), 49-53.

McGoogan, B. and Gatlin, D.M. III (1997) Effects of replacing fish meal with soybean meal in diets for red drum, *Sciaenops ocellatus*, and potential for palatability enhancement. Journal of the World Aquaculture Society 28, 374-385.

Mendoza, R, Dedios, A. and Vazquez, C. (2001) Fishmeal replacement with feather meal -Eenzymatic hydrolyzates co-extruded with soya-bean meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture

Nutrition 7, 143-151.

Peres, H. & Lim, C. (*In Press*) Utilization of soybean products in diets of non-salmonid marine finfish. In: Lim C, Webster C.D. and Lee C.S. (Eds). Alternative Protein Sources in Aquaculture Diets. The Haworth Press, Inc, New York.

Rónyai, A.R., Csengeri, I and Va'radi L. (2002) Partial substitution of animal protein with full-fat soybean meal and amino acid supplementation in the diet of Siberian sturgeon (*Acipenser baerii*). Journal of Applied Ichthyology 18, 682–684.

Shimeno, S., Kumon, M., Ando, H. and Ukawa, M. (1993) The growth performance and body composition of young yellowtail fed with diets containing defatted soybean meal for a long period. Bulletin of the Japanese Society of Scientific Fisheries 59, 821-825.

Ukavwa, M., Takii, K., Nakamura, M. and Kumai, H.. (1994) Utilization of soybean meal for red sea bream diet. Suisanzooshoku 42, 335-358.

Venou, B., Alexis, M.N., Fountoulaki and Mattila, P. (1997) Partial Substitution of fish meal with extruded and non-extruded soybean meal in diets for gilthead seabream (Sparus aurata L.). Proceedings of the Martinique 1997 Island Aquaculture and Tropical Aquaculture. European Aquaculture Society. Pp. 314-315.

Ye, J., Chen, J., Wang Y. (2006) Study on dietary protein requirement of blac seabream *Sparus macrocephalus*,Zhejiang Freshwater Fisheries, 44(1):42-45.

Effects of Fishmeal Replacement by Plant Proteins on Growth and Body Composition of Juvenile Japanese Seabass, *Lateolabrax japonicus*

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Abstract

A 50-day experiment was conducted to find out the optimal replacement of Peru fishmeal protein by plant proteins (soybean meal:corn concentrated protein meal:1:1) in diets for juvenile Japanese seabass (mean weight 19.5±1.48g). Five isonitrogenous (44.0% crude protein) and isocaloric (about 19.50 $kJ•g^{-1}$gross energy) diets were formulated in which plant proteins replaced 0, 25, 50, 75 and 100% of the protein supplied by fish meal, respectively. The basal diet contains 51.50% Peru fishmeal, 8% rapeseed meal, 5% cotton seed meal, 22.5% wheat middling, 2% sleeve-fish meal, 2% α-starch, 5% fish oil, 1.5% lecithin, 2% Vitamin premix and mineral premix. The triplicate feeding trials were conducted in indoor experimental tanks. Water temperature and salinity in the laboratory were 28±1.0°C and 22 ‰, respectively. The results on the fish fed diets with more than 25% fishmeal replacement, are the following: the growth performance, including average final weight, weight gain and specific growth rate (SGR) and protein efficiency ratio (PER) significantly decreased (P<0.05) with an increase in the plant protein replacement, the feed conversion ratios (FCR) significantly increased (P<0.05) along with the increase in plant protein substitute. The fish fed diets containing 25% level of replacement by plant protein (diets 2) was statistically similar to the fish fed diet at 0% fishmeal replacement level (diet 1) in the growth performance and feed efficiency. Moreover, body composition of the fish was not significantly different (P>0.05) from that of the fish fed diets 1 or 2. The experiment results showed that 25% substitution of the fishmeal protein by plant proteins is optimal in diets of juvenile Japanese seabass.

Introduction

Japanese seabass *Lateolabrax japonicus* is a carnivorous species that has been widely cultured in Southeast Asia. Some studies on the requirement of protein for juvenile Japanese seabass have been conducted. Lin et al. (1994) considered that the most suitable content of protein in artificial feed was 42.73 to 43.04% for Japanese seabass. Qinghui et al. (2003) found that the diet containing 41% protein and 12% lipid with P/E of 25.9 mg protein kJ-1 was optimal for this fish. In our recent works, we found that the optimal protein requirements of juvenile fish (mean weigh = 4.20±0.14g) was 45.53%. All the results showed that diets of this fish have more than 40% protein content and most of the protein sources come from fishmeal. In traditional culture, large amount of minced trash fish or fishmeal have been used in its diets to maintain rapid growth and good palatability. Just recently, due to shortage in domestic fishery resources and the sharp rise in global fishmeal prices, available trash fish and fishmeal could not adequately meet the demand for aquaculture in China. Thus, it is necessary to seek for acceptable and available substitutes in marine fish feeds. In addition, excessively depending on the fishmeal supply can no longer meet the needs of the expanding aquafeed industry as a result of rapid aquaculture development (Abdelghany 2003).

A number of conventional oilseed meal and plant concentrated protein source have been evaluated, including soybean meal, sunflower meal, linseed meal, cottonseed meal, rapeseed meal, soybean protein concentrated and corn concentrated protein meal (El-Saidy & Gaber 2003). Those feed ingredients commonly singled or incorporated in fish feeds have been studied as plant protein replacement for fishmeal. Partial replacement of fishmeal by singled soybean meal has been studied for juvenile Japanese seabass (Pan et al. 2000). Although he considered that at least 40% fishmeal in the formulated feed is necessary, 40% fishmeal in the diets is still rather high. What is the optimum replacement level by plant proteins for the commercial formulated diets of Japanese seabass? Do the combinations of plant proteins to replace or reduce fishmeal in commercial fish diets affect the growth and feed efficiency? The aims of this study were to evaluate the growth, feed utilization and whole-body composition of Japanese seabass fed diets containing partial and total substitutions by the mixture of soybean meal and corn concentrated protein meal (1:1) protein (SCP) for the fishmeal protein (FMP).

Materials and Methods

Experimental fish

A total of 500 juvenile Japanese seabass were obtained from the hatchery of Xiangshan Damutu in Zhejiang Province. Prior to the start of the experiments, all of the fish were placed in an indoor concrete tank where they were fed a commercial diet for two weeks as an acclimation and training feeding period to laboratory conditions. Then the fish were divided into 15 groups, each stocking 20 fish (mean weight ± SEM = 19.5±1.48 g). Each group of fish was transferred at random into a 1.4 m^3 columniform concrete tank.

Experimental system

The experiment was conducted in an indoor aquaculture laboratory at the Zhejiang Institute of Freshwater Fisheries (ZIFF). Experiment water was natural seawater deposited and filtrated in sand with 200 mesh sieve. Each tank was also supplied with air provided by a central air compressor. During the experiment period, salinity, water temperature and pH were kept at 22‰, 28±1.0°C and 7.4 -8.2, respectively. Dissolved oxygen was maintained above 6.0 $mg \cdot L^{-1}$. Nitrite and ammonia (basically $NO_2^-N<0.02$ $mg \cdot L^{-1}$, total ammonia-N<0.03 $mg \cdot L^{-1}$) were measured weekly using Nitrite and Ammonia Reagent Box. At least, 50% of the water in the tank was changed by fresh seawater every day.

Feeding management

The fish were fed to near 100% satiation. Near 100% satiation was the amount of feed that fish consumed at each feeding before they stopped taking feed and swim down to bottom of the tank. Any feed not consumed when fish stopped taking feed was in excess of satiation. Fish were fed three times a day at 07:00, 12:00 and 17:00 hrs for a 50-day period. At 06.30 and 16:30 hrs every day, 30% of the water in the tank was changed by fresh seawater. The trials started on August 1 and finished on September 20.

Experimental diet preparation

By using fishmeal, soybean meal and corn concentrated protein meal mixture (1:1) as the main protein resource, five isonitrogenous (44.0% crude protein) and isocaloric (about 19.50 kj• g^{-1} gross energy) diets were formulated in which plant proteins replaced 0, 25, 50, 75 and 100% of the protein supplied by fish meal, respectively (Table 1). All the feed ingredients were ground and passed through a 60-mesh sieve, then mixed soundly with a small amount of water, the wet mixture was made into pellets in a meat grinder, diameter of the pellets was 2.5 mm. The wet pellets were air-dried at room temperature, then transferred into plastic bags and stored in a refrigerator (0°C) until such time that these were to be used.

Table 1. Ingredient composition and proximate analyses of the experiment diets (%)

	Diets				
	1(control)	2	3	4	5
	0%SCP1	25% SCP	50% SCP	75% SCP	100% SCP
Ingredients(%)					
Fishmeal (68 % CP)[a]	51.50	38.63	25.75	12.88	0.00
SCP (54.1 %CP) 1	0.00	16.50	33.40	49.72	66.20
Rapeseed meal (41.3 %CP)	8.00	8.00	8.00	8.00	8.00
Cottonseed meal (42.7 % CP)	5.00	5.00	5.00	5.00	5.00
Wheat middling (17.3 % CP)	22.50	19.30	15.70	12.67	9.50
Squid meal (47.6 % CP)	2.00	2.00	2.00	2.00	2.00
α-starch	2.00	2.00	2.00	2.00	2.00
Soya-lecithin	1.50	1.50	1.50	1.50	1.50
$CaHPO_4$	1.00	1.00	1.00	1.00	1.00
Fish oil	5.00	4.57	4.15	3.73	3.30
Mineral mixc					
Vitamin mixd	0.75				
0.75	0.75				
0.75	0.75				
0.75	0.75				
0.75	0.75				
0.75					
Proximate analysis					
Moisture	9.99	9.94	9.89	9.85	9.81
Crude protein	44.04	44.02	44.15	44.07	44.04
Crude lipid	10.82	10.18	9.55	8.93	8.29
Ash	8.24	6.87	5.52	4.14	2.77
Gross energy (kJ•g^{-1})	19.44	19.44	19.55	19.64	19.74

[a] sources: Peru fishmeal (68%), pesquera Hayduk S.A.,Peru; soybean meal (43%), Local vendor; corn concentrated protein meal (65.2%), Local vendor.

[b] SCP: Soybean meal : corn concentrated protein meal (1:1) protein mixture.
[c] vitamin mix (per kg feed): vitamin B_1, 25 mg; vitamin B_2, 45 mg; vitamin B_6, 20 mg; vitamin B_{12}, 0.1mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacinamide acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; vitamin A, 32 mg; vitamin D ,5 mg; vitamin E, 120 mg; ascorbyl-2-polyphosphate, 2000 mg.
[d] mineral mix (per kg feed): KI, 0.8 mg; $CoCl_2$ $6H_2O$ (1%), 50 mg; $CuSO_4$ $5H_2O$, 10 mg; $FeSO_4H_2O$, 80 mg; $ZnSO_4$ H_2O, 50 mg; $MnSO_4$ H_2O,60 mg; $MgSO_4$ $7H_2O$, 1200 mg; NaCl, 100 mg.
[c] energy(kJ•g^{-1} diet) computed: protein, 23.7kJ•g^{-1}; lipid, 39.5 kJ•g^{-1}; carbohydrate, 17.2kJ•g^{-1}.

Composition analysis

Feed and fish samples were homogenized using a high speed homogenizer for 3 min while lipid was determined by freezing and vacuum drying the sample. Dry matter was calculated by weight loss after 24 h at 105°C. Crude protein was measured using the Kjeldahl technique and crude lipid was measured after ether extraction. Ash was calculated from the weight loss after incineration of samples for 24h at 550°C in a muffle furnace. For detailed methods, please refer to "Official methods of analysis of AOAC International" 17th edition"Horwitz.W. 2000". All analyses were carried out in duplicate.

Statistical analysis

Final average weight, weight gain (WG), specific growth rate (SGR), survival rate (SR), protein efficiency ratio (PER) and feed conversion ratio (FCR) were determined at the end of the experiment. All the data were analysed using one-way analysis of variance (ANOVA) and Duncan' s New Multiple Range Test. The difference of the means was considered as significant at $P< 0.05$. All the analyses were carried out following the procedures of SPSS for Windows 11.0. WG, SGR and PER was calculated as follows:

WG = Final average weight-Initial average weight

SGR= 100×[ln (Final average weight) - ln (initial average weight)]÷ trial days.

PER= weight gain (g) ÷ protein fed (g).

FCR= dry weight feed intake (g) ÷ wet fish weight gain (g).

Results

Growth performance

The results of final weight, weight gain, FCR, SGR, PER and SR are presented in table 2. The fish survival rates ranged from 93.33 to 96.67 %. No adverse effect was observed with the increase in the dietary SCP contents. It was easy to notice that the final weight, weight gain, SGR and PER had the tendency to decrease with the increase in fishmeal protein substituted by SCP, while the FCR had the opposite impact. However, the results of Duncan's New Multiple Range Test indicated no significant differences (P>0.05) for final weight, weight gain, SGR, PER and FCR between the fish fed the diets containing 25 % SCP (diet 2) and the control containing 100 % FMP (diet 1). All the fish fed the diets containing more than 25 % SCP showed the significant lower (P<0.05) final weight, weight gain, SGR, PER and significant higher (P<0.05) FCR compared with the fish fed the control diet.

Table 2. Effect of partial and total substitution of fishmeal by SCP mixture in practical diets for juvenile Japanese seabass on SGR, FCR, WG, PER and SR after a 50•day feeding trial

Diets	Initial weight (g)	Final weight (g)	Weight gain (g)	FCR	SGR	PER	SR
1	19.33±1.07[a]	52.77±1.47[a]	33.63±2.82[a]	2.03±0.40[a]	2.07±0.16[a]	1.15±0.22[a]	95.00±5.00[a]
2	19.63±0.73[a]	49.61±1.95[a]	29.97±2.71[ba]	1.87±0.26[a]	1.89±0.10[ba]	1.23±0.17[a]	93.33±7.64[a]
3	19.52±1.07[a]	45.30±2.38[ab]	25.80±2.40[cb]	2.50±0.42[ab]	1.72±0.07[cb]	0.93±0.19[ab]	96.67±5.77[a]
4	19.93±1.83[a]	40.97±3.32[b]	21.07±2.70[c]	2.75±0.39[b]	1.47±0.08[c]	0.87±0.24[b]	91.67±5.77[a]
5	18.75±0.90[a]	39.25±1.69[b]	20.47±1.42[c]	2.73±0.38[b]	1.51±0.05[c]	0.90±0.29[b]	93.33±2.89[a]

Values (mean±SD) in the same column having a common superscript were not significantly different ($P>0.05$).

Fish composition

An increasing trend in the whole body moisture content was observed in fish fed diets containing various levels of SCP (Table 3). No statistical difference was found for whole body moisture content between the fish fed diets 1 and 2, but they had significantly lower ($P<0.05$) moisture contents than the other treatments. The fish fed diets 4 and 5 had significantly higher ($P<0.05$)moisture contents. The fish fed diet containing 100 % FMP or containing 25 % SCP and 75 % FMP showed a similar whole body crude protein, crude fat and ash. However, significantly lower ($P<0.05$) whole body crude protein, crude fat and ash were found in fish fed more than 50% FMP replaced with SCP.

Table 3. Whole body composition (% dry basis) of juvenile Japanese seabass fed experimental diets containing various levels of SCP

Diets	Moisture	Crude protein	Crude fat	Ash
1	79.16±0.70[a]	13.29±0.30[a]	3.34±0.25[ab]	4.19±0.11[a]
2	79.61±0.20[a]	12.79±0.35[ba]	3.55±0.10[a]	4.01±0.05[a]
3	80.92±0.60[b]	12.27±0.46[abc]	3.05±0.25[ab]	3.76±0.05[b]
4	81.63±0.30[bc]	11.98±0.55[cb]	2.68±0.20[b]	3.66±0.04[b]
5	82.45±0.30[c]	11.23±0.61[c]	2.71±0.51[b]	3.52±0.02[c]

Values (mean±SD) in the same column having a common superscript were not significantly different ($P>0.05$).

Discussion

The present study demonstrated the potential of plant protein combinations replacing fishmeal in commercial feeds for Japanese seabass. Under the experiment condition, the favorable level of plant protein substitution to fishmeal protein is 25 %. Obviously, the substitution of fishmeal by plant protein could reduce the feed cost, without causing significant effect on growth rate and body composition of the fish. The result will be of great value for us to develop least-cost formulation for Japanese seabass. With an increase in the substitution level, the weight gain, SGR, PER of the fish would decrease gradually. What is the reason? Is it due to the anti-nutritional factors present in the soybean meal or is it due to a shortage of limiting amino acids like lysine and methionine? A further experiment on this matter is to be conducted in the future.

Soybean meal and corn concentrated protein meal mixed at the ratio of 1:1 has more than 54% crude protein and relative balancing amino acids profile, although lysine and methionine are commonly easy to be the limited amino acids in fish feeds. More and more people consider that soybean meal and corn concentrated protein meals are the first choice plant proteins to replace fishmeal, the low lysine and methionine may be supplemented by using individual essential amino acids (Ronyai A. et al. 2000). Many similar works have reported on this, such as Viola, S et al. (1981;1982); Shiau, S.Y. et al.(1987) ;Mambrini et al. (1999); Ronyai et al. (2002) and EL-Saidy & Gaber (2002).

Conclusion

This experiment was conducted using isonitrogenous (44.0 % crude protein) and isocaloric diets. The diets contained various levels of SCP and various levels of digestible energy, despite the same gross energy. Since the digestibility of the fishmeal protein and SCP for Japanese seabass is obviously different, the difference might result in different growth performance. D. A. J. Stone et al. (2003) pointed out that high energy and isonitrogenous feeds could improve fish growth performance in fishmeal diets partially replaced by plant protein.

A change in body composition of Japanese seabass would occur when the substitution level of fishmeal protein by plant protein exceeds 25 %. The reason is to be explored further. Moreover, it is still to be confirmed in the future whether the high energy and isonitrogenous diets have better growth performance and feed utilization rate for Japanese seabass.

References

Abdelghany A.E. (2003) Partial or total replacement of fishmeal with gambusia meal in diets for red tilapia *Oreochromis niloticus* × *O.mossambicus*. Aquaculture Nutrition 9, 145-154.

D.A.J.Stone, G.L.Allan andA.J. Anderson.(2003) Carbohydrate utilization by juvenile silver perch, Bidyanus bidyanus (Mitchell). II. Digestibility and utilization of starch and its breakdown products. Aquaculture Research 34 (2).109–121.

El-Saidy, D.M.S.D. and M.M.A. Gaber. (2002) Complete replacement of fish meal by soybean meal with dietary Llysine supplementation for Nile tilapia *Oreochromis niloticus* (L) fingerlings. J. World Aquaculture Soc. 33, 297-306.

EL-Saidy, D.M.S.D. and M.M.A.Gaber. (2003) Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L) diets. Aquaculture Research 34, 1119-1127.

Horwitz, W. (Ed). (2000) Official methods of analysis of AOAC International, 17th edition, volume 1, AOAC International.

Lin L., Chen, X. and H. Huixin. (1994) The most suitable content of protein in the arti- ficial feed to culture *Lateolabrax japonicus* . *Journal of Xiamen Fisheries College* 16(1), 6-10.

Mambrini, M., Roem, A.J. and J.P. Craved. (1999) Effect of replacing fish meal with soy protein concertrate and of DL-Met supplementation in high-energy, extruded diets on the growth and nutritient utilizationof rainbow trout, *Oncorhynchus mykiss*. J.Anim.Sci. 77, 2990-2999.

Pan, Y., Wang, F. and L. Huanliang. (2000) Optimal proportion of fish meal and soybean cake in formulated diets of juvenile seaperch Lateolabrax japonicus . Journal of Dalian Fisheries University 15(3),157-163.

Qinghui, A., Mai, K. and H. Li. (2004) Effects of dietary protein to energy ratios on growth and body composition of juvenile Japanese seabass, *Lateolabrax japonicus*. Aquaculture 230, 507–516.

Ronyai, A., Csengeri, I. and L. Varadi. (2002) Partial subsititution of animal protein with full-fat soybean meal and amino acid supplementation in the diet of Siberian sturgeon (*Acipenser baerii*). J. Appl. Ichthyol. 18, 682-384.

Shiau, S.Y. and J.L. Chuang. (1987) Sun C.L. Inclusion of soybean meal in tilapia diets at two protein levels. Aquaculture 65, 251-261.

Viola, S., Arieli, J., Rappaport, U. and S. Mokady. (1981) Experiments in the nutrition of carp replacement of fishmeal by soybean meal. Bamidgeh 33, 35-49.

Viola, S., Mokady, S., Rappaport, U. and J. Arieli. (1981/1982) Partial and complete replacement of fishmeal by soybean meal in feeds for intensive culture of carp. Aquaculture 26, 223-236.

Study on Diet and Stocking Density in Net Cage Culture of Sex-Reversed Tilapia Fry (*Oreochromis niloticus*)

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Abstract

Two experiments (E1 and E2) were conducted to evaluate the effects of diets and stocking density (StD) on growth performance, feed conversion ratio (FCR), survival rate and benefit cost ratio (B/C ratio) of sex-reversed tilapia fry. Experiments were conducted in hapas (1m □ 1m) suspended within a fertilized earthen pond (1,000 m^2). Fish stocked (individual size of 0.33 ±0.01 g) were fed twice daily at 6% (bw•day^{-1}). In E1, fish were stocked at 100 fish•m^{-2}, and fed diets with combinations of commercial egg laying duck feed: fine rice bran (DR) at 2:1 (38.64% CP), fish meal:fine rice bran (FR) at 2:1 (37.83% CP), and commercial finishing swine feed:fine rice bran (SR) at 2:1 (28.34% CP). Water quality and fish growth were monitored biweekly for a period of 90 days. The fish were randomly divided into treatments of 100, 200 and 300 fish•m^{-2}, and fed with SR for E2. At the end of the E1, mean weight gain (MWG), specific growth rate (SGR), total length (TL), FCR and survival were unaffected by treatments ($P>0.05$). However, B/C ratio of fish fed SR diet was significantly ($P<0.05$) higher than the other groups. In E2, fish in StD of 300 fish•m^{-2} had significantly ($P<0.05$) lower MWG and SGR and higher FCR than those at 100 and 200 fish•m^{-2} but not significantly different ($P>0.05$) between 100 and 200 fish•m^{-2}. No differences ($P>0.05$) in survival and B/C ratios were found among treatments.

Introduction

At present, farmers encounter the problem of rearing small sex-reversed tilapia fingerlings in a net cage that has not been well-prepared. Therefore, the fingerlings often survive at a very low rate and, consequently, the production is lower than expected. To circumvent such problem and increase the production, farmers could nurse the fingerlings to a proper size before transferring them to a net cage. Most farmers usually buy fingerlings of 2-3 cm and feed them with commercial fingerling feed 2-3 times per day for about two weeks before switching to 25-30% crude protein (CP) commercial feed (Viwatanachaiset 1998).

To make this method more efficient, guidelines for practical and low cost management should be on hand, especially in the aspect of feed cost which is the largest variable cost in the operating expenses of aquaculture business (De Silva et al. 1986). Furthermore, the problem in finding appropriate raw materials is also important in developing a quality feed that can be acquired locally. Using fish meal as a source of protein in nursing feed is not practical in many areas due to quantity and quality problems. Nursing fingerlings in a hapa net cage, thus, can be done with a combination of egg laying duck feed or finishing swine powder feed and fine rice bran (2:1).

Therefore, the study on appropriate diets for nursing sex-reversed tilapia in a hapa net cage can provide information to help in reducing production cost and in maximizing return on investment, and at the same time, the guidelines toward effective net cage culture of tilapia could then be developed.

Materials and Methods

Preparation of experimental unit

A 1,000 m^2 earthen pond was prepared by adding 16-20-0 chemical fertilizer at the rate of 28 kg nitrogen•ha^{-1}•$week^{-1}$ to create a green water system. A 1m×1m×1.2m (width×length×depth) nylon net cage was set up by attaching nylon nets to staked bamboo poles. The net was suspended in water at 30 cm height above water level and the cage bottom was kept at least 0.5 cm above the pond floor. Every two weeks after the data were recorded, the net was replaced and cleaned before reuse.

Preparation of experimental animal

Sex-reversed tilapia fingerlings were purchased from a private farm at the Maetang District, Chiang Mai. The fish were soaked in NaCl solution (5 g of NaCl•L^{-1} of water) before releasing into the net cage. Commercial fingerling feed was used during the first week to help the fish adapt to its new environment before starting the experiment. The total number and average weight of fish were then obtained in each cage before 30 fish were randomly selected and the average total length was measured as the initial length of fingerlings.

Analysis of feeding combination and water quality

Thoroughly mixed feeding materials were packed in plastic bags. These experimental feeds were then kept in a refrigerator at -18°C throughout the experiment. The Micro-Kjeldahl method was used to analyze the protein content in feeds according to AOAC (1990). Each diet was fed to fingerlings twice daily (9.00 and 15.00 hrs) at 6% body weight (BW•day^{-1}), and the feeding rates were adjusted every two weeks.

Water quality in the pond was monitored every two weeks by recording the water temperature, dissolved oxygen, total ammonia, and pH level. Oxygen meter (YSI model 59), spectrophotometer (Hach DR/2000) and pH meter (Schott-Gerate CG 840) were utilized in acquiring such data.

Experimental design

Experiment 1 (E1): Study on appropriate diets in fingerling nursing

Fingerlings with the initial weight of 0.33 ± 0.01 g and total length of 2.53 ± 0.15 cm were released into a net cage at a density of 100 fish•m^{-2}. Three different feeding combinations/diet formulations were made as follows:

Formula 1 (DR): The combination of commercial egg laying duck feed and fine rice bran at a ratio of 2:1 (38.64% CP).

Formula 2 (FR): The combination of fish meal and fine rice bran at a ratio of 2:1 (37.83% CP).

Formula 3 (SR): The combination of commercial finishing swine feed and fine rice bran at a ratio of 2:1 (28.34% CP).

Statistical analyses were performed on the data obtained from CRD with three replications after 90 days of treatment.

Experiment 2 (E2): Study on an appropriate stocking density in fingerling nursing

Fingerlings with the initial weight of 0.33 ± 0.01 g and total length of 2.53 ± 0.15 cm were released into net cages at three different stocking densities (100, 200 and 300 fish•m^{-2}). The experiment was conducted in CRD with three replications. Data were recorded after 90 days of feeding with SR diet (28.34% CP).

Statistical analyses were performed on the data obtained after the experiment period.

Data collection and statistical analysis

Weight, length and number of fish in each cage were recorded every two weeks throughout the period of 90 days. Mean weight gain (g), survival rate (%), feed conversion rate (FCR), specific growth ratio (SGR,% per day) and benefit:cost ratio of feeding cost (B/C ratio) were analyzed using analysis of variance (ANOVA) to statistically assess the treatment effects. Turkey's tests were then used for treatment comparisons at a statistically significant level of 0.05. All statistical analyses performed were done using SPSS version 9.0.0. Statistical analyses for ratio and percentage data (e.g. B/C, FCR, SGR and survival rate) were performed on data after arcsine transformation (Sokal and Rohlf 1981; Rohlf and Sokal 1981).

Results

Experiment 1 (E1): Study on appropriate diets in fingerling nursing

Effects of three diets on mean weight gain (MWG), specific growth rate (SGR), final total fish length (TL), feed conversion ratio (FCR), and survival rate of sex-reversal tilapia fingerlings were not significantly different at the statistical significance of 0.05 (Table 1).

Results of all three diets investigated, the feed cost of SR diet (swine feed mixed with fine rice bran) were statistically significant and the lowest which led to the highest B/C ratio of 36.56 ± 3.26 (P<0.05) is shown in figure 1.

Table 1. Growth, feed conversion ratio, survival rate and B/C ratio of sex-reversed tilapia after 90 days

Indicator	Feed formula (%Protein)		
	DR (38.6%)	FR (37.8%)	SR (28.3%)
INITIAL			
Average weight (g)	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.01
Average total length (cm)	2.52 ± 0.17	2.54 ± 0.24	2.52 ± 0.15
FINAL			
Average weight gain (g)	2.26 ± 0.22	2.11 ± 0.16	1.93 ± 0.22
Average total length (cm)	6.15 ± 0.41	5.88 ± 0.31	5.48 ± 0.24
Specific growth rate, SGR (%•day^{-1})	2.28 ± 0.09	2.22 ± 0.07	2.13 ± 0.01
Feed conversion ratio, FCR	2.18 ± 0.14	2.35 ± 0.16	2.55 ± 0.09
Survival (%)	76.00 ± 2.52	75.67 ± 2.40	73.33 ± 1.33

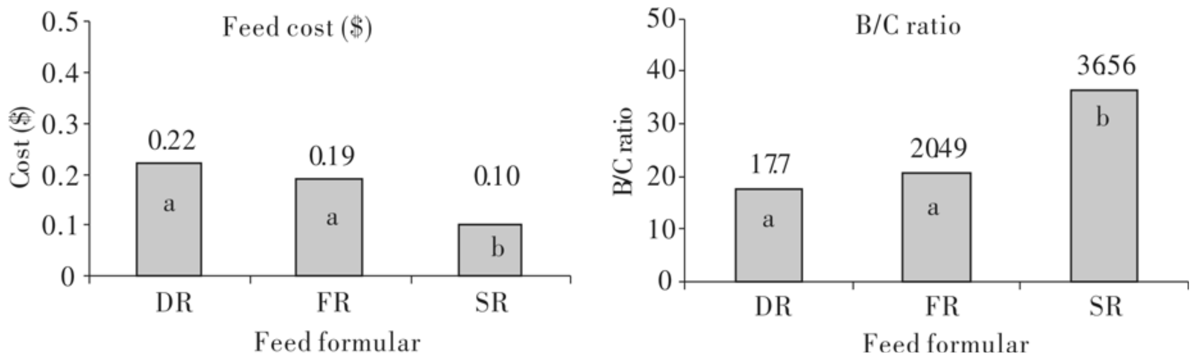

Figure 1. Feed cost and B/C ratio of sex-reversed tilapia fed on different diets after 90 days

Note: Feed costs were calculated on the following variable costs: costs of egg laying duck feed=US\$0.82•kg^{-1}, fish meal=US\$0.69•kg^{-1}, finishing swine feed=US\$0.36•kg^{-1}, fine rice bran=US\$0.14•kg^{-1}, and price of fingerling=US\$0.06•kg^{-1} (at 5-6 cm in length).

Experiment 2 (E2): Study on an appropriate stocking density in fingerling nursing

The sex-reversed tilapia nursed at a stocking density of 300 fish•m^{-2} showed significantly lower MWG, SGR, and FCR than those nursed at 100 and 200 fish•m^{-2} (P<0.05). Final total fish length was also lower than those nursed at 100 fish•m^{-2} although no difference was found when compared with those nursed at 200 fish•m^{-2}. However, all the three stocking densities were not significantly different in survival rates at a significant level of 0.05.

Feed costs of nursing the fingerlings were found to be in accordance with stocking densities (P<0.05), but the effect of stocking density on B/C ratio was not detected (Table 2).

Water quality conditions in both experiments (Table 3) were within the acceptable range for rearing tilapia fingerlings (Bhujel 2000; Boyd 1990).

Table 2. Growth, feed conversion ratio, survival rate and B/C ratio of sex-reversal tilapia nursed at different stocking densities after 90 days.

Indicator	Stocking density (per m^2)		
	100	200	300
INITIAL			
Average weight (g)	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.01
Average total length (cm)	2.52 ± 0.19	2.53 ± 0.19	2.53 ± 0.18
FINAL			
Average weight gain (g)	2.09 ± 0.10 a	1.83 ± 0.07 a	1.52 ± 0.01 b
Average total length (cm)	6.17 ± 0.09 a	5.91 ± 0.05 ab	5.63 ± 0.07 b
Specific growth rate, SGR (%•day^{-1})	2.21 ± 0.04 a	2.09 ± 0.04 a	1.91 ± 0.07 b
Feed conversion ratio, FCR	2.55 ± 0.10 a	2.77 ± 0.14 a	3.48 ± 0.08 b
Survival (%)	73.33 ± 1.33	75.67 ± 1.85	74.00 ± 2.15
Feed Cost (per Cage) US$	0.114 + 0.003 a	0.225 + 0.009 b	0.344 + 0.014 c
B/C ratio	33.30 ± 0.38	35.08 ± 3.80	33.51 ± 1.63

Note: Mean ± se in rows with the different alphabets were statistically different at the significant level of 0.05 when compared by Tukey's test. Feed costs were calculated on the following variable costs: costs of egg laying duck feed=US$0.82•kg^{-1}, fish meal=US$0.69•kg^{-1}, finishing swine feed=US$0.36•kg^{-1}, fine rice bran= US$ 0.14•kg^{-1}, and price of fingerling=US$0.06•kg^{-1} (at 5-6 cm in length).

Table 3. Water quality in the pond after 90 days of experiment

Indicator	Experiment 1		Experiment 2	
	morning	afternoon	morning	afternoon
Temperature (°C)	22.8 + 0.3	23.8 + 0.4	22.8 + 0.4	25.3 + 0.2
DO (mg•l^{-1})	4.9 + 0.2	10.8 + 0.2	5.8 + 0.7	13.0 + 0.6
pH	7.6 + 0.1	8.9 + 0.1	7.9 + 0.1	9.1 + 0.1
Total Ammonia (mg•l^{-1})	-	0.5 + 0.1	-	0.5 + 0.0

Discussion and Conclusion

From Experiment 1, it can be concluded that a diet with 28.3 – 38.6% CP was just as effective in nursing sex-reversed tilapia fingerlings because no statistical difference in growth was observed in the present study. This finding was supported by those reported in Wee and Tuan (1988) which concluded that diets with 27.5-35% CP had similar effect on growth. Abdelghany (2000) also suggested the appropriate protein in nursing at 30-40% (CP) since no significant differences in growth rates were observed. However, the similar growth rates could due to the presence of natural food in the pond that was fertilized to generate a green water system. Turker et al. (2003) found that small tilapia filtered significantly more phytoplankton than larger individuals. Tilapia fingerlings in this study recovered nutrients by utilizing natural foods derived mainly from fertilizers applied for a green water system. Diana et al. (1994) indicated that feeding at 0.5 satiation ration plus fertilization could produce similar growth to 100% feeding over 155 days of culture.

Feed cost per cage of SR diet (swine feed mixed with fine rice bran) was the lowest. As a result, B/C ratio was the highest. Therefore, this diet can potentially be used as a substitution of protein source in concentrated feed, especially in areas where fish meal is not available to farmers. Using this diet as feed also enables farmers to reduce production cost, and eventually increase the return of investment as compared to the traditional use of fish meal.

From Experiment 2, fingerlings nursed at 300 fish•m^{-2} showed lower MWG and SGR than those nursed at 100 and 200 fish•m^{-2}. The TL of the fingerlings decreased as the stocking density increased. The lowest TL was obtained at a density of 300 fish•m^{-2}, but no difference was observed at 100 and 200 fish•m^{-2} densities. This was relevant to the report made by Gall and Bakar (1999) that stocking densities had no significant effect on total fish length after nursing the fingerlings in a concrete tank at 100 and 200 fish•m^{-2} for 56 days.

Several studies have reported that the increase in stocking density of fingerlings resulted in the decrease in growth and appetite of fish. Refstic and Kittelsen (1976) stated that fish nursed at a high density would eat less since competition for food among fish increased. When the association between MWG and stocking density was considered, it was found that MWG tended to decrease as the density increased. The FCR also increased as the density increased in the study on red tilapia (*Oreochromis mosambicus*×*O. urolepis hormorum*) by De Silva and Maughan (1995). A constant feeding rate of 6% BW was used throughout the experiment, therefore, accessibility to food would be limited when nursed in a high density environment.

Study on feed cost per net cage of fingerlings showed that feed cost positively increased with stocking densities. A density of 200 fish•m^{-2} showed the highest B/C ratio although the B/C ratios among all three densities were not significantly different. Thus, selection of an appropriate stocking density would depend upon the purpose of production, whether it is for sale or to promote growth (Gomes et al. 2000) because sex-reversed tilapia fingerlings (5-6 cm in length) are sold on a per piece basis.

It is interesting to note that MWG and SGR in Experiments 1 and 2 were relatively lower than those reported by Souza et al.(2000) which carried out an experiment on sex-reversed tilapia (Thai strain) fed on 28% protein diet and by Abdelghany (2000) who nursed 35 gram tilapia and fed with 30-40% protein diet. The experiments in this study were carried out during the cold season and the drop in water temperature to 22.8 ± 0.3°C in the morning led to slow growth rate. However, Dan and Little (2000) reported that despite the slow growth of fingerlings nursed during the cold season in northern Vietnam, it could still resume normal growth when the cold season was over.

From this experiment, it is concluded that commercial animal feeds can be used as a substitute for fish meal in the net cage culture of sex-reversed tilapia fingerlings. The diet with the lowest feed cost and highest return on investment is the SR diet (finishing swine feed mixed with fine rice bran at a ratio of 2:1), and the stocking density at 200 fish•m^{-2} could give the highest return on investment and growth rate.

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References

Abdelghany, A.E. (2000) Optimum dietary protein requirements for *Oreochromis niloticus* L. fry using formulated semi-purified diets. In K. Fitzsimmons and J.C. Filho (eds.) Tilapia Aquaculture in the 21st century. Proceedings from the fifth international symposium on Tilapia aquaculture, pp. 101-108, RJ, Brazil.

Bhujel, R.C. (2000) A review of strategies for the management of Nile tilapia (*Oreochromis niloticus*) broodfish in seed production systems, especially hapa-based systems. Aquaculture 181, 37-59.

Boyd, C.E. (1990) Water quality in pond for aquaculture. Birmingham Publishing, Alabama, pp. 482.

Dan, N.C. and Little D.C. (2000) The culture performance of monosex and mixed –sex new-season and overwiintered fry in three strains of Nile Tilapia (*Oreochromis niloticus*) in northern Vietnam. Aquaculture 184, 221-231.

De Silva, A.M. and Maughan, E. (1995) Effects of density and water quality on red tilapia (*Oreochromis mossambicus* × *O. urolepis hornorum*) in pulsed-flow culture systems. J. Appl. Aquacult. 5, 69-76.

De Silva, S.S., Gunasekera, R.M. and Keembiyahetty, C. (1986) Optimum ration and feeding frequency in *Oreochromis niloticus young*. pp. 559-564. In J.L. Maclean, L.B. Dizon and L.V. Hosillos (eds.) The First Fisheries Forum. Asain Fisheries Society, Manila, Philippines.

Diana, J.S., Lin, C.K., Jaiyen, K. (1994) Supplemental feeding of tilapia in fertilized ponds. J. World Aquaculture Soc. 25(4), 497-506.

Gall, G.H.E., Bakar, Y. (1999) Stocking density and tank size in the design of breed improvement programs for body size of tilapia. Aquaculture 173, 192-205.

Gomes, L.C., Baldisserotto, B., Senhorini, J.A. (2000) Effect of stocking density on water quality, survival, and growth of larvae of the matrinxa, *Brycon cephalus* (Characidae), in ponds. Aquaculture 183, 73-81.

Refstie, T., Kittelsen, A. (1976) Effect of density on growth and survival of artificially reared Atlantic salmon. Aquaculture 8, 319-326.

Rohlf, S.J. and Sokal, R.R. (1981) Statistical Tables. W.H. Freeman and Company, New York, pp. 219.

Sokal, R.R. and Rohlf, S.J. (1981) Biometry. W.H. Freeman and Company, New York, pp. 859.

Souza, V.L., Silva, P.C., Padua, D.M.C., Dalacorte, P.C. (2000) Comparison of productive performance of sex reversed male Nile tilapia, *Oreocheomis niloticus* (Thai strain) and Tetra hybrid red tilapia, (Israeli strain). *In* K. Fitzsimmons and J.C. Filho (eds.) Tilapia Aquaculture in the 21st century. Proceedings from the fifth international symposium on Tilapia aquaculture, pp. 83-87, RJ, Brazil.

Turker, H., Eversole, A.G. and Burne, D.E. (2003) Effect of Nile tilapia, *Oreochromis niloticau* (L.), size on phytoplankton filtration rate. Aquaculture Research, 34(12), 1087-1091.

Viwatanachaiset, Y. (1998) Tilapia cage culture in Khonkaen province. Thai fisheries gazette 51(2), 167-177.

Wee, K. L. and Tuan N. A. (1988) The effect of dietary protein levels on growth and reproduction of Nile tilapia (*Oreochromis niloticus*). In: Pullin, R.S.V., Bhukaswan, T., Tonguthai, K., Maclean, J.L., (Eds.), The Second International Symposium on Tilapia in Aquaculture, ICLARM Conference Proceedings, 15, Department of fisheries, Bangkok. Thailand, and International Center for Living Aquatic Resources, Manila Philippines, pp. 623.

The effect of red kwao kreua (*Butea superba*) and 17-α-methyltestosterone (MT) on some growth parameters and in inducing sex reversal on Ghana strain Nile tilapia (*Oreochromis niloticus* L.) raised in hapas

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Abstract

The first part of the study aimed to investigate the effect of red kwao kreua and 17-α- methyltestosterone (MT) feeding regimens on Nile tilapia (Ghana strain) in terms of inducing sex reversal, survival (SR), feed conversion ratio (FCR) and gain in weight (GW). Treatment diets were composed of different mixtures of red kwao kreua pounded roots and fishmeal (100 $g·kg^{-1}$ (T3), 200 $g·kg^{-1}$ (T4) and 300 $g·kg^{-1}$ (T5)) and 40 (T1) and 60 mg $MT·kg^{-1}$ fishmeal (T2). The control treatment was 100% fishmeal (T0). These diets were fed to first feeding fry in hapas in pond for a period of 28 days.

Results revealed that male sex ratio in the control or T0 (47.2 + 21.0%) was statistically significant from T1 (83.3 + 15.3%) and T2 (93.3 + 5.8%), whereas it was not significantly different from T3 (62.2 + 3.8%), T4 (72.2 + 25.5%) and T5 (68.1 + 6.4%). Comparison between red kwao kreua treatments revealed no significant difference. T4 and T5 were also statistically comparable with MT treatments. Moreover, the MT dosage in the MT–treated feeds did not statistically affect the male sex ratio between MT treatments in the earthen pond. However, the incidence of intersex was common. Survival rate in T0 (81.3 + 13.4%) was significantly different from T1 (98.0 + 3.5%) but was not statistically significant with T2 (92.3 + 13.3%), T3 (92.3 + 2.5%), T4 (92.3 + 2.5%) and T5 (93.3 + 1.5%). The FCR in the control (1.0 + 0.3) was statistically significant when compared with T3 (1.4 + 0.0) but not with T1 (0.9 + 0.0), T2 (0.8 + 0.2), T4 (0.9 + 0.0) and T5 (0.9 + 0.0). Regardless of the dosage, MT-treated feed was not observed to provide better FCR in the earthen pond. In terms of GW (grams), the Control (53.9 + 13.0) was statistically different from T2 (76.3 + 14.3) and T3 (36.0 +1.0) and comparable with T1 (57.8 + 2.0), T4 (57.5 + 5.3) and T4 (58.2 + 6.3).

For the second part, T1 (90%) was significantly comparable with all the treatments in terms of male sex ratio but lower than expected >95%. The rest of the treatments were statistically comparable. Highest male sex ratio among red kwao kreua treatments was T2 (58.2%). Highest SR was also in T1 (89%) followed by T4 (84.7%) which were statistically significant comparable with T0 (64.7%) and T5 (66.7%). Treatments T2 (82%) and T3 (74.3%) were comparable among all treatments. The FCR and GW were comparable among all treatments.

Introduction

The more common method of generating mostly male population is through the use of steroids fed to sexually undifferentiated fry. Typically the aim is to produce all males, so 17-α-methyltestosterone (MT) is included in the diet for several weeks when the fish start eating.

During the development of sex-reversal treatment procedures, concern was expressed on health and environmental issues. Feeding fish with food exposed to steroids is recognized as a controversial issue; more so, the treated feed's fate in the environment. The possible loss of this tool for sex reversal is serious. Fortunately, the development of the genetically male tilapia (GMT) technology provides a very good alternative. However, the technology might not be applicable on all strains of tilapia (Tuan et al. 1998).

Research on practical and convenient methods to produce an all male population should be continued especially using extracts from plant sources with reproductive endocrine disrupting mechanisms. Cherdshewasart et al. (2004) found that red kwao kreua (*Butea superba*) plant extract might either have an anti-estrogen mechanism or a potent cytotoxic effect because of its anti-proliferation effects on the growth of MCF-7 cells. They further reported on the presence of a phyto-androgen which is yet to be identified. Red kwao kreua (RKK) is endemic in Thailand and has been used by the Thais due to claims of various medicinal benefits especially sex invigoration in males.

Red kwao kreua obtained from Chiang Mai was analyzed for some bioactive compounds using standard flavonoids, namely daidzein, genistein and puerarin at 37.2, 4.5, and 1.9 mg·kg^{-1}, respectively, in the material (Manosroi and Manosroi 2005). Daidzen and genistein are believed to be aromatase inhibitors (AI) i.e. compounds which reduce the level of estrogen in the body (www.dadamo.com). Genistein is found to have both weak estrogen and anti-estrogen effects (www.genistein.com). The Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP, pdacrsp.oregonstate.edu) suggested that paradoxical feminization might be due more to aromatization than inhibition of *in vivo* synthesis of androgens. Flavonoids such as genistein act as estrogen agonists via estrogen receptors in cultured cells. Following genistein treatment of rats during early pregnancy, the number of males was higher than females among progenies although the sex ratios were not different from the control.

Therefore, it was deemed necessary to investigate the potential of this herbal plant for sex reversal and its effect on survival rate (SR), feed conversion ratio (FCR) and gain in weight (GW), and as well as evaluate the two MT dosages using the indicated parameters.

Materials and methods

The study was divided into two parts. Part 1 involved two MT dosages and red kwao kreua crude powder, while Part 2 involved one MT dosage and red kwao kreua ethanol extract.

Part 1

Experimental fry

A total of 18 (1m×1m×1m) hapas were suspended in a 30m×20m earthen pond. The water in the hapas was maintained at 0.6 m depth. Random distribution of recently hatched Ghana strain tilapia fry was done, according to the following treatments: pure fishmeal as control (T0); 40 mg•kg^{-1} MT (T1); 60 mg•kg^{-1} MT (T2); 100 g•kg^{-1} RKK (T3); 200 g•kg^{-1} RKK (T4) and 300 g•kg^{-1}

RKK (T5). Each treatment was replicated thrice with 100 fry per replication. After 28 days, SR, FCR and GW were obtained.

Feed preparation

Forty (40) and 60 mg•kg^{-1} MT doses were prepared by dissolving the MT separately in 200 ml ethanol (95%), which was then sprayed onto one kg fishmeal and air dried for 24 hours before feeding. Red kwao kreua roots were processed by drying and pounding to a powdery consistency, then mixed with fishmeal to produce 100, 200 and 300 grams•kg^{-1} dosage levels.

Part 2

Experimental fry

Eighteen (1m×1m×1m) hapas were suspended in a 30m×20m earthen pond. Water depth was maintained at 0.6 m. Random distribution of recently hatched Ghana strain tilapia fry was done according to the following treatments: pure fishmeal as control (T0); 60 mg•kg^{-1} MT (T1); 200 g•kg^{-1} RKK (T2); 2 ml•kg^{-1} RKK (T3); 4 ml•kg^{-1} RKK (T4) and 6 ml•kg^{-1} RKK (T5). Each treatment was replicated thrice with 100 fry per replication. After 28 days, SR, FCR and GW were obtained.

Feed preparation

A 60 mg•kg^{-1} MT dose was dissolved in 200 ml ethanol (95%) which was then sprayed onto one kg fishmeal and air dried for 24 hours before feeding. A 200 g•kg^{-1} pounded red kwao kreua roots were processed by drying and pounding to a powdery consistency and mixed into one kg fishmeal. The RKK extract was prepared by soaking one kg of the crude powder with 95% ethanol for 24 hours then concentrating the extract by a rotary evaporator (Rotavap R-114, Switzerland). Two, four and six milliliters from the crude extract obtained (40 ml) were sprayed onto one kg fishmeal and air dried for 24 hours.

Feeding regimen

The amount of feed (g) given in this study was fixed based on the assumed average biomass (g) per fry per week (week 1= 0.01; 2= 0.06; 3= 0.20; 4=0.30). The treatment period was 28 days. During the first week the fry were fed at 30% of their biomass five times per day. On the second week they were fed at 20% of their biomass four times per day. On the third week they were fed at 15% of their biomass three times per day and on the 4th week they were fed at 10% of their biomass two times per day. After the treatment period the feeding level was adjusted to 5% of fish biomass.

Gonad examination and computation of SR, FCR and GW

Ten percent of fish from each treatment replicate was randomly selected (Parts 1 and 2) after 60 days for sex reversal rate computation. The gonads were surgically removed, prepared by squash method (Nuanmanee et al. 2004) and examined microscopically (40x). The remained fish were fed with farm-formulated tilapia pellets at 5% of their biomass.

SR, FCR and GW were calculated according to the following formula:

$$\text{Survival Rate (SR) (\%)} = \frac{\text{\# of live fish at harvest}}{\text{total \# of fish at stocking}} \times 100$$

$$\text{Feed Conversion Ration (FCR)} = \frac{\text{total feeds consumed}}{\text{net weight of fish}}$$

Gain in Weight (GW) = total final weight – total initial weight

Statistical analysis

One-way analysis of variance (ANOVA) and Duncan's multiple range test through SPSS 9.0 were used for statistical analysis.

Results

Part 1

Table 1 presents male percentage, SR, FCR and GW. In the male percentage, T1 (83.3% + 15.3) and T2 (93.3% + 5.8) were statistically comparable but were significantly higher than that in the control (47.2% + 21.0). The percentage of intersex was also consistently observed (T0=3.7%; T1=10.0%; T2=3.3%; T3=3.3%; T4=13.0%; T5=7.9%), while the male percentage in T3 (62.2% + 3.8), T4 (72.2% + 25.5) and T5 (68.1% + 6.4) were statistically comparable and higher than that in the control. The dosage did not affect the male percentage between RKK and between MT treatments. Although the highest sex reversal was observed in treatment 4, they all fell below the required percentage for male population, which should be > 95%.

Table 1. Comparison of means (per treatment) of male (%) + SD, SR + SD (%), FCR + SD and GW + SD (g) on the Ghana strain of *O. niloticus*.

Treatments	Male (%)	SR (%)	FCR	GW
T0	$47.2 + 21.0^{a}$	$81.3 + 13.4^{a}$	$1.0 + 0.3^{a}$	$53.9 + 13.0^{b}$
T1	$83.3 + 15.3^{bc}$	$98.0 + 3.5^{b}$	$0.9 + 0.0^{a}$	$57.8 + 2.0^{b}$
T2	$93.3 + 5.8^{c}$	$92.3 + 13.3^{ab}$	$0.8 + 0.2^{a}$	$76.3 + 14.3^{c}$
T3	$62.2 + 3.8^{ab}$	$92.3 + 2.5^{ab}$	$1.4 + 0.0^{b}$	$36.0 + 1.0^{a}$
T4	$72.2 + 25.5^{abc}$	$92.3 + 2.5^{ab}$	$0.9 + 0.0^{a}$	$57.5 + 5.3^{b}$
T5	$68.1 + 6.4^{abc}$	$93.3 + 1.5^{ab}$	$0.9 + 0.0^{a}$	$58.2 + 6.3^{b}$

* Means that do not share the same superscript in the same column are significantly different (P<0.05).

In terms of survival rate, only T1 (98.0% + 3.5) was statistically comparable with the control (81.3% + 13.4) which had the lowest SR. For FCR, the highest was observed in T3 (1.4 + 0.0) and was significantly lower than those in other treatments. The highest GW (76.3 + 14.3) was observed in T2 (P< 0.05), while the lowest (36.0 + 1.0) was observed in T3 (P<0.05).

Part 2

As shown in Table 2, T1 has the highest male percentage (90%) among all treatments (P<0.05).

The highest SR was also observed in T1 (89%) which was comparable with T2 (82%), T3 (74.3%) and T4 (84.7%), while the lowest SR was in T0 (64.7%). There were no significant differences in FCR and GW among all treatments ($P>0.05$).

Table 2. Comparison of means (per treatment) of male (%) + SD, SR + SD (%), FCR + SD and GW + SD (g) on Ghana strain of *O. niloticus*.

Treatments	Male (%)	SR (%)	FCR	GW
T0	45.2 + 5.0[a]	64.7 + 5.0[a]	0.79 + 0.0[a]	66.9 + 6.0[a]
T1	90.0 +10.0[b]	89.0 +10.1[c]	0.79 + 0.2[a]	69.7 + 16.5[a]
T2	58.2 +10.5[a]	82.0 +12.1[abc]	0.76 + 0.1[a]	70.1 + 15.1[a]
T3	31.2 +28.5[a]	74.3 + 8.3[abc]	0.71 + 0.0[a]	74.3 + 4.9[a]
T4	50.0 +26.4[a]	84.7 + 7.4[bc]	0.73 + 0.0[a]	71.9 + 6.6[a]
T5	56.7 + 5.8[a]	66.7 +11.9[ab]	0.74 + 0.0[a]	71.0 + 7.0[a]

* Means that do not share the same superscript in the same column are significantly different ($P<0.05$).

Discussion

The use of 40 mg•kg^{-1} MT dosage effected male sex reversal which was not significantly different from that of 60 mg•kg^{-1} dosage hence, using either dosages could yield comparable effects when done under the earthen pond environment (Part 1). Cagauan et al. (2004) mentioned that male sex reversal using MT has less control of reversal efficiency when done in the natural environment where food is present. This result plus the low stocking density (Phelps et al. 1995) suggest that the earthen pond provides a source of food aside from the artificial feed being offered, thus less treated feed may be taken in. Macintosh et al. (1986) and Abucay and Mair (1997), on the other hand, were consistently successful in sex reversal of tilapia using 40 mg•kg^{-1} MT dosage when application was done under a closed water system. Pongtana et al. (2004) utilized 60 mg•kg^{-1} MT to achieve 97.9 +1.5% male tilapia with 83.5 + 15.7 SR for 21 days. This practice is currently used in commercial mass production of sex reversed tilapia fry. In Part 1, MT treatment at 60 mg•kg^{-1} provided the highest GW and the best FCR. The incidence of intersex was also consistently observed especially in MT treatments (Part 1), which further suggests that the presence of readily available food in an earthen pond affected the efficiency of sex reversal using MT. The addition of kwao kreua except in T1 of Part 1 did not reduce the efficiency of feed utilization of the tilapia fry. RKK's components may have had other beneficial effects.

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References

Abucay, J. S. and Mair, G.C. (1997) Hormonal sex reversal of tilapias: implications of hormone treatment application in closed water systems. Aquaculture Research 28: 841-845.

Cagauan, A. G., Baleta, F.N. and Abucay, J.S. (2004) Sex reversal of Nile tilapia *Oreochromis niloticus* L. by egg immersion technique: the effect of hormone concentration and immersion time. Proceedings 6th International Symposium on tilapia in Aquaculture. 127-136.

Cherdshewasart, W. and Nimsakul, N. (2003) Clinical trial of *Butea superba*, an alternative herbal treatment for erectile dysfunction. Asian Journal of Andrology 5:243-246.

Cherdshewasart, W., Cheewasopit, W. and Picha, P. (2004) The differential anti-proliferation effect of white (Pueraria mirifica), red (*Butea superba*), and black (Mucuna collettii) kwao krua plants on the growth of MCF-7 cells.

Manosroi, A. and Manosroi, J. (2005) Determination of bioactive compounds in roots of different ages Pueraria mirifica, Airy shaw Suvatabhandhu and *Butea superba*, Roxb. from various locations in Thailand. III WOCMAP Congress on Medicinal and Aromatic Plants - Traditional Medicine and Nutraceuticals.

Macintosh, D.J., Singh, T.B., Little, D.C. and Edwards, P. (1986) Growth and sexual development of 17-α- Methyltestosterone and progesterone-treated Nile tilapia (*Oreochromis niloticus*) reared in earthen ponds. Proceedings the second International Symposium on Tilapia in Aquaculture 15:(623) 457-463.

Nuanmanee, P., Siangwan, S. and Nitham, J. (2004) Effects of androgens on sex reversal of Nile tilapia. Fisheries Magazine 57: p 251.

Pongtana, N., Siangwan, S. and Nitham, J. (2004) Effects of androgens on sex reversal of Nile tilapia. Fisheries Magazine 57 number 3.

Phelps, R. P., Salazar, G.C., Abe, V. and Argue, B.J. (1995) Sex reversal and nursery growth of Nile tilapia, *Oreochromis niloticus* (L.) free-swimming in earthen ponds. Aquaculture Research 26: 293-295.

Tuan, P. A., Mair, G.C., Little, D.C. and Beardmore, J.A. (1998) Sex determination and the feasibility of genetically male tilapia production in the Thai-chitralada strain of *Oreochromis niloticus* (L.). Aquaculture 173: 257-269.

Digestibility of Selected Feed Ingredients for Tiger Grouper, Epinephelus fuscoguttatus

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Abstract

The apparent digestibility (AD) of eight feed ingredients widely available in Indonesia was determined. In each of two 5×5 latin-square experiments, tiger grouper *Epinephelus fuscoguttatus* juveniles (100-150 g) were fed a reference diet and four test diets in accordance with the latin-square design. Test feed ingredients were substituted at rates of 40% for animal meal or 30% for plant meal. Chromic oxide was used as the digestibility marker. In determining the ingredient AD, the substitution ratio was calculated as the proportion of the nutrient (or energy) contributed by the test ingredient on an ‘as-is' basis. Digestibility tanks were steeply sloped 200 L cylindro-conical tanks with a bottom outlet to facilitate faecal collection, which was carried out at three hour intervals throughout the day. Each collection period took 5-7 days with a similar acclimatization time between diets. A combined ANOVA of the data for both experiments showed no difference ($P>0.05$) in the ADs for each reference diet. Thus for comparative purposes, the derived ADs of the test ingredients were analysed by a single ANOVA. The digestibility of animal meals was generally high (>59% for dry matter, >83% for protein, >65% for lipid, and >70% for gross energy) while that of plant meals was slow (<53% for dry matter, <53% for protein, <66% for lipid, and <46% for gross energy). This information will enable the formulation of grow-out feeds for tiger grouper based on least-cost digestible nutrients.

Introduction

Marine feed ingredients, especially fish meal, are in high demand as protein sources for fish feed. Fish meal is regarded as the best protein source for fish feed, being high in protein, has a close to ideal profile of essential amino acids and has few, if any, anti-nutritional factors (Allan et al. 2000). The world demand for fish meal keeps increasing. However, world production of fish meal has not increased beyond 6.5 million tons per year since the early 1990's (Shepherd et al. 2005).

Tiger grouper, *Epinephelus fuscoguttatus,* requires a high dietary protein of 47-50% (Laining et al. 2003a; Giri et al. 2004), and manufactured diets typically contain from 40 to 62% of expensive fish meal. Replacement of fish meal with a cheaper, high-quality source of protein could reduce the production costs of culturing high trophic level species such as tiger grouper. The assessment of different sources of protein for fish feed is an international and increasing research priority (Manzi 1989; Hardly & Kissil 1997 as cited in Allan et al. 2000).

Apart from considerations of palatability and anti-nutritional factors, the apparent digestibility of an ingredient determines how much of the nutrients in the ingredient can be absorbed by the animal. The higher the digestibility of the ingredient, more of its nutrient content will be available to support growth and other metabolic functions of the animal. Consequently, digestibility is an important measure of an ingredient's nutrient quality.

The potential sources of protein available in large quantities in Indonesia are poultry offal meal, golden snail, green mussel and mysid meal. Yellow and white corn meal, rice bran and sorghum meal are carbohydrate sources that are in plentiful supply in Indonesia. This paper reports a digestibility study to determine the apparent digestibility of these feed ingredients because of their potential to be used in the manufacture of cost-effective feeds for tiger grouper.

Materials and methods

Experiment overview

The study had two independent 5×5 latin square experiments with five diets i.e. a reference and four test feed ingredient diets, being examined in each experiment. The reference diet was the same for each experiment with four test feed ingredients of animal origin-poultry offal meal, mysid meal, golden snail meal and green mussel meal-being examined in Experiment 1and four of plant origin-fine rice bran, corn meal (yellow and white) and sorghum meal-being examined in Experiment 2. Test feed ingredients were substituted into the reference diet at the rate of either 40 or 30% for ingredients of animal or plant origin, respectively (Table 1). Chromic oxide was added at an inclusion rate of 1% of the diet as the digestibility marker (Takeuchi 1988; De Silva & Anderson 1995).

All test feed ingredients were obtained locally. Each set involved five faecal collection periods with all fish, randomly, being fed each of the five diets in accordance with the latin-square design of the experiment. The proximate nutrient and gross energy composition of the test ingredients are shown in table 2.

One cohort of juvenile tiger grouper bred at the Gondol Research Institute for Mariculture's, Bali hatchery was air-transported to South Sulawesi and acclimated to the floating net cage facility at Awerange Bay, Barru Regency, South Sulawesi. Prior to starting the experiment, fish were held for several months in 2m×2m×2m floating net cages to acclimatize. They were fed with a moist reference diet. Afterwards, the fish were selected on the basis of freedom from defects, initial weight of 100-150 g and then distributed into 5 floating net cages of 1m×1m×2m at a density of 25 fish·cage^{-1}. For each collection period, the fish were acclimated to their test diet for five days and fed twice daily, morning and late afternoon. On the 6th day, 20 fish from each cage were carefully transferred into 200 L conical, 35% slope, bottom tank for faecal collection. In each period, faeces were collected over 5-7 days, which enabled a sufficient quantity of faeces to be collected for chemical analysis.

Table 1. Formulation of diets used in determining the apparent digestibility of feed ingredients in Experiments 1 and 2.

Ingredient	Diet type		
	Reference	40% substituted1	30% substituted2
As used product (g)			
Trash fish (fresh)[3]	90	54	63
Fish meal	30.5	18.3	21.4
Shrimp head meal	8	4.8	5.6
Squid liver meal	9	5.4	6.3
Soybean meal	8	4.8	5.6
Wheat flour	12	7.2	8.4
Animal test ingredient	0	40	0
Plant test ingredient	0	0	30
Fish oil	3	1.8	2.1
Soybean oil	1.5	0.9	1.1
Vitamin premix[4]	3	1.8	2.1
Mineral premix[5]	1.5	0.9	1.1
Chromic oxide	1.0	1.0	1.0
Total (as used)[6]	167.5	141.0	147.7

[1] For poultry offal meal, mysid meal, golden snail meal and green mussel meal
[2] For fine rice bran, yellow and corn meal and sorghum meal
[3] Dry matter content of 25%
[4] Vitamin mix provided (mg·kg^{-1} diet): Thiamin-HCl, 50; riboflavin, 50; Ca-panthothenate, 100; niacin, 20; pyridoxine-HCl, 40; biotin, 6; folic acid, 15; inositol, 2000; para-aminobenzoic acid, 50; astaxanthin, 150; menadione, 40; calciferol, 19; ∞-tocopherol, 200; ascorbic acid, 1500; cyanocobalamin, 1; and choline, 1000.
[5] Trace mineral provided (mg·kg^{-1} diet): $FeCl_3.4H_2O$, 1660; $ZnSO_4$, 100; $MnSO_4$, 67.5; $CuSO_4$, 20; KI, 1.5; and $CoSO_4.7H_2O$, 1.0.
[6] Equates to approximately 100 g on a dry basis.

Table 2. The dry matter (DM), crude protein (CP), total lipid (TL), crude fibre (CF), ash and gross energy (GE) composition of air-dry test feed ingredients.

Test feed ingredient	DM (%)	CP (%	TL (%)	CF (%)	Ash (%)	GE (kJ·g^{-1})
Poultry offal meal	94.5	59.2	16.2	1.8	5.0	22.5
Mysid meal	93.9	57.6	9.1	3.0	14.7	18.8
Golden snail meal	94.3	53.7	4.9	2.6	10.6	18.5
Green mussel meal	92.8	52.9	12.4	1.9	9.0	20.2
Rice bran	93.8	13.7	14.9	5.8	8.8	18.0
Corn meal (yellow)	92.4	10.2	3.8	2.2	1.7	16.8
Corn meal (white)	91.3	10.2	4.6	2.1	1.6	16.8
Sorghum	91.7	9.4	1.4	1.1	1.9	16.4

Chemical analyses and calculations

A representative sample of feed or dried faeces was homogenized using a mortar and pestle and analysed essentially by AOAC International (1999) procedures: dry matter (DM) by oven drying at 105ºC for 16 h; ash by ignition in a muffle furnace at 550ºC for 24 h and crude protein by micro-

Kjeldahl analysis with distillation into 4% boric acid and titration with sulphuric acid using methyl red indicator for end point determination. Total lipid was determined gravimetrically following chloroform:methanol extraction of the sample (Bligh & Dyer 1959); and, energy by bomb calorimetry and chromium after acid digestion using a Shimadzu UV-VIS 2401 PC spectrophotometer.

The apparent digestibility coefficient (ADC) of DM, crude protein (CP), total lipid (TL) and gross energy (GE) for the reference and test diets were calculated using the equation:

$$ADC(\%) = 100 * \left\{1 - \left(\frac{M_D * A_F}{M_F * A_D}\right)\right\}$$

Where, MD and MF are the concentrations (on a DM basis) of the marker in the ingested diet and faeces, respectively and AD and AF are the concentrations (on a DM basis) of the nutrient (or analyte) in the ingested diet and faeces, respectively. The AD of the test feed ingredient was calculated using Pfeffer et al. (1995) equation:

$$ADC_{NI} = \frac{1}{\alpha}\left[AD_{NT} - (1-\alpha)AD_{NR}\right]$$

Where, ADNI,, ADNT and ADNR are apparent digestibility of the nutrient in the ingredient, the test diet and the reference diet, respectively. The substitution rate (α) was calculated as the proportion of the nutrient (or energy) contributed by the test ingredient in the reference diet on an ‘as-fed wet' basis (Bureau et al. 1999).

The ADC data for diets and those derived for the test feed ingredient in each experiment were subjected to a least squares analysis of variance for a latin square design, isolating effects due to column (fish) and row (collection period). A combined analysis of the ADC's for diets for the two experiments found no significant difference between the reference diets in each experiment (P> 0.05). Thus, the derived ADC for the test feed ingredients was analysed as a single factor ANOVA with differences between treatments (ingredients) tested for significance using Fisher's protected t-test (Snedecor & Cochran 1989) wherein differences between means were examined only when the F-test of the ANOVA was significant (P< 0.05).

Results

Digestibility coefficients for the diets fed in Experiments 1 and 2 are detailed in Tables 3 and 4, respectively. A statistical analysis of the data for both experiments, isolating the effects of experiment, diet and associated interactions, showed that the observed ADCs for each of the reference diets were not significantly different (P>0.05) from one another. Thus, the ADCs derived for each of the test feed ingredients in each experiment were analysed as a combined ANOVA and the results presented in Table 5.

Table 3. The dry matter (DM), crude protein (CP), total lipid (TL) and gross energy (GE) apparent digestibility coefficients of the diets examined in Experiment 1.

Diets label	DM	CP	TL	GE
	Apparent digestibility coefficient (%)			
Reference	66.1^{B}	80.4^{CD}	88.4^{B}	79.7^{AB}
Poultry offal	65.8^{B}	82.4^{B}	86.1^{C}	80.1^{AB}
Mysid	68.3^{A}	85.0^{A}	90.0^{A}	81.0^{A}
Golden snail	63.4^{C}	78.6^{D}	84.2^{D}	76.2^{C}
Green mussel	60.1^{B}	82.0^{BC}	88.6^{B}	79.1^{B}
± sem	0.63	0.66	0.42	0.57

A,B,C,D Within columns, means without a common letter differ (P<0.05)

The apparent digestibility of the protein of animal meals was high (77 to 89%) and not significantly different from each other but significantly higher than for the plant meals (41 to 52%), which were statistically not different from each other. TL digestibility tended to follow a similar pattern as for CP digestibility but differed in that golden snail meal was lower than for the other animal meals (62 cf 84-94%, respectively) while the rice bran was higher than for the other plant meals (66 cf 24-31%, respectively). DM and GE digestibilities of poultry offal meal, mysid meal and green mussel meal were not significantly different from each other and significantly higher in the case of the mysid and mussel meals than the golden snail meal, which was significantly higher than the plant meals. For the plant meals, rice bran had the lowest, and sorghum the highest, DM and GE apparent digestibilities.

Table 4. The dry matter (DM), crude protein (CP), total lipid (L) and gross energy (GE) apparent digestibility coefficients of diets examined in Experiment 2.

Diet label	DM	CP	TL	GE
	Apparent digestibility coefficient (%)			
Reference	65.0^{A}	79.4^{A}	86.7^{A}	78.9^{A}
Rice bran	56.5^{C}	75.9^{D}	79.2^{C}	67.4^{C}
Maize – yellow	59.9^{B}	76.7^{C}	81.6^{B}	68.8^{BC}
Maize – white	60.3^{B}	76.7^{C}	80.6^{BC}	68.9^{BC}
Sorghum	61.4^{B}	77.6^{B}	84.6^{A}	70.0^{B}
± sem	0.57	0.22	0.53	0.61

A,B,C,D Within columns, means without a common letter differ (*P*<0.05).

Table 5. The dry matter (DM), crude protein (CP), total lipid (TL) and gross energy (GE) apparent digestibility coefficients of the test feed ingredients derived from diets examined in Experiments 1 and 2.

Test feed ingredient	DM	CP	TL	GE
	Apparent digestibility coefficient (%)			
Poultry offal meal	65.4^{AB}	84.0^{A}	84.1^{AB}	80.5^{A}
Mysid meal	69.9^{A}	88.9^{A}	93.9^{A}	82.8^{A}
Golden snail meal	59.4^{B}	76.9^{A}	62.0^{C}	70.8^{B}
Green mussel meal	66.0^{A}	83.4^{A}	88.9^{A}	78.3^{A}
Rice bran	36.3^{D}	43.7^{B}	$65.8B^{C}$	39.1^{D}
Maize – yellow	47.8^{C}	41.9^{B}	30.4^{D}	41.3^{CD}
Maize – white	48.8^{C}	41.3^{B}	30.9^{D}	41.7^{CD}
Sorghum	52.5C	52.1^{B}	23.7^{D}	45.1^{C}
± sem	1.64	5.23	6.90	1.72

A,B,C,D Within columns, means without a common letter differ (*P*<0.05).

Discussion

Tiger grouper digested the animal feed ingredients more efficiently than the plant feed ingredients with DM, CP, TL and GE ADCs generally being significantly higher. Of the animal meals, golden snail meal was the least digestible, while the fibre-rich rice bran had the lowest DM, CP and energy digestibilities among the plant meals. However, the lipid digestibility of rice bran was comparatively high, much higher than all the other plant meals and equivalent to that of the golden snail meal. Similar results wherein animal meals have high digestibility and plant meals much lower digestibilities have been reported for other carnivorous fish such as rainbow trout, *Oncorhynchus mykiss* (Austreng et al. 1977), red drum, Sciaenops ocellatus (Gaylord & Gatlin 1996; McGoogan & Reigh 1996), yellow tail, *Seriola quinqueradiata* (Masumoto et al. 1996), humpback, *Cromileptes altivelis* and gold-spot, *Epinephelus coioides* groupers (Laining et al. 2003b; Eusebio et al. 2004), catfish, Clarias spp. (Usmani et at. 2003) and cobia, *Rachycentron canadum* (Zhou et al. 2004). The low ADCs of these plant ingredients were probably due to their high nitrogen free extract (NFE) content, particularly the complex carbohydrate components of seeds which are poorly digested by piscivorous fish (Lupatsch et al. 1997; Cowey & Walton 1989).

The low protein ADC of the plant meals was not unexpected as studies with other grouper species have shown similar low digestibilities. For instance, tiger grouper digested only about 47% of the protein of rice bran in this study, which was in the range of values of 43% for gold-spot grouper (Eusebio et al. 2004) and 59% for humpback grouper (Laining et al. 2003b). However, higher protein digestibilities for rice bran were reported by Sullivan & Reigh (1995) for hybrid striped bass, *Morone saxatilis*×*M. chrysops* (71%) and by McGoogan & Reigh (1996) for red drum (77%). Such effects may indicate differences between species in their capacity to digest the fibre matrix of rice bran although differences in how the rice bran was handled and processed might equally have played a role. In our study, the rice bran originated from a local rice mill and was used without any further refining. Importantly, all diets were fed as moist pellets and manufactured using a cold extrusion process. Cooking of the meal, either that which results from steam conditioning of the mix prior to pelleting or that which occurrs during hot extrusion processing, could increase starch gelatinization and improve digestibility (De Silva & Anderson 1995; Davis & Arnold 1995). The rice bran examined in the above cited hybrid striped bass and red drum studies was finely ground prior to mixing and dried after pelleting. This processing may have improved the digestibility of the rice bran.

The digestibility of unsaturated lipids, including those from plants, is usually high for carnivorous fish with estimates typically between 90 and 100% (Caballero et al. 2002; Carter et al. 2003; Usmani et al. 2003; Zhou et al. 2004). In the present study, lipid digestibility was high (>84%) for each of the animal meals except golden snail meal, which at 62% was slightly lower than rice bran (66%). The comparatively low lipid digestibility of the golden snail meal was surprising since about two thirds of its lipid comprised unsaturated fatty acids, and, of this, one third were fatty acids with three or more unsaturated bonds (Bombeo-Tuburant et al. 1995). It is well established that the digestibility of lipid in fish, and animals generally, is influenced by the chain length and saturation of the constituent fatty acids, decreasing with increasing length and increasing with increasing degree of unsaturation (Olsen et al. 1998; Johnsen et al. 2000; Menoyo et al. 2003; Ng et al. 2004). Perhaps the quantitatively low content of lipid in the snail meal (4.9%) might explain the apparent anomalous result although the comparatively low DM and CP digestibility values for snail meals suggested that other factors might also have had a role in reducing overall digestibility. The low lipid digestibility of cereal grains (24 to 31%) is also an anomalous result but probably due to their low lipid contents (1 to 5%) and the associated mathematical errors in deriving the estimate rather than the lipid in these meals being poorly digestible.

There was a clear distinction between animal and plant meals in the amount of digestible energy they provided: 71 to 83% for animal meals and 39 to 45% for plant meals. This was to be expected given the low CP, DM and TL digestibility of plant meals and the comparatively high digestibility of animal meals. A similar result has been reported for other carnivorous fish (Sullivan & Reigh 1995; Burel et al. 2000; Booth et al. 2005).

In conclusion, the study has shown that tiger grouper could efficiently digest animal feed ingredients, indicating that the ingredients have a potential to be used as dietary replacements for fish meal. Although caution has to be taken for golden snail meal since its overall digestibility is inexplicably poor, perhaps indicating that unknown factors may be affecting its nutritional value. While plant meals were not easily digested, nevertheless they are an integral part of compounded diets and data on their digestibility are important when formulating diets to satisfy the animal's requirements for digestible nutrients. However, steam conditioning of these meals prior to pelleting or using hot extrusion manufacturing procedures may increase their digestibility for tiger grouper. The high digestibility of green mussel suggests its polyculture with fish may provide an additional local source of protein for the fish while helping to alleviate nutrient output from farms. Until more eco-friendly pelleted feeds become commercially available, such integrated systems may be an effective way of reducing the environmental impacts of cage aquaculture.

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References

Allan, G.L., Parkinson, S., Booth, M.A. et al. (2000) Replacement of fishmeal in diets for Australian silver perch, *Bidyanus bidyanus:* I. Digestibility of alternative ingredients. Aquaculture 186, 293-310.

AOAC International (1999) Official Methods of Analysis, 16th edn. Association of Official Analytical Chemists International, Gaithersberg, Maryland, USA. 1141 pp.

Austreng, E., Risa, S., Edwards, D.J. & Hvidsten, H. (1977) Carbohydrate in rainbow trout diets: II. Influence of carbohydrate levels on composition and feed utilization of fish from different families. Aquaculture 11, 39-50.

Bligh E.G. & Dyer W.J. (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911-917.

Bombeo-Tuburan, I., Fukumoto, S. & Rodriguez, E.M. (1995) Use of the golden apple snail, cassava, and maize as feeds for the tiger shrimp, *Penaeus monodon,* in ponds. Aquaculture 131, 91-100.

Booth, M.A., Allan, G.L. & Anderson, A.J. (2005) Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources. Aquacult. Res. 36, 378-390.

Bureau, D.P., Harris, A.M. & Cho, C.Y. (1999) Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 180, 345-358.

Burel, C., Boujard, T., Tulli, F. & Kaushik, S.J. (2000) Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture 188, 285-298.

Caballero, M.J., Obach, A., Rosenlund, G. et al. (2002) Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 214, 253-271.

Carter, C.G., Lewis, T.E. & Nichols, P.D. (2003) Comparison of cholestane and yttrium oxide as digestibility markers for lipid components in Atlantic salmon (*Salmo salar L.*) diets. Aquaculture 225, 341-351.

Cowey, C.B. & Walton, M.J. (1989) Intermediary metabolism. In: Halver, J.E. (ed) Fish Nutrition, 2nd edn. Academic Press, New York. NY. pp. 259-329.

Davis, D.A. & Arnold, C.R. (1995) Effects of two extrusion processing conditions on the digestibility of four cereal grains for *Penaeus vannamei*. Aquaculture 133, 287-294.

De Silva, S.S. & Anderson, T.A. (1995) Fish Nutrition in Aquaculture. Chapman & Hall, London, UK. 319 pp.

Eusebio, P.S., Coloso, R.M. & Mamauag, R.E.P. (2004) Apparent digestibility of selected ingredients in diets for juvenile grouper, *Epinephelus coioides* (Hamilton). Aquacult. Res. 35, 1261-1269.

Gaylord, T.G. & Gatlin III, D.M. (1996) Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus)*. Aquaculture 139, 303-314.

Giri, N.A., Suwirya, K. & Marzuqi, M. (2004) Optimum level of dietary protein and lipid for rearing juvenile tiger grouper (*Epinephelus fuscoguttatus*). In: Rimmer, M.A., McBride, S. & Williams, K.C. (eds.) Advances in Grouper Aquaculture. Australian Centre for International Agricultural Research, Canberra, pp. 92-94.

Johnsen, R.I., Grahl-Nielsen, O. & Roem, A. (2000) Relative absorption of fatty acids by Atlantic *salmon Salmo* salar from different diets, as evaluated by multivariate statistics. Aquacult. Nutr. 6, 255-261.

Laining, A., Kabangnga, N. & Usman. (2003a) Pengaruh protein pakan yang berbeda terhadap koefisien kecernaan nutrien serta performansi biologis kerapu macan, *Epinephelus fuscoguttatus* dalam keramba jaring apung. Jurnal Penelitian Perikanan Indonesia 9(2), 29-34.

Laining, A., Rachmansyah, Ahmad, T. & Williams, K. (2003b) Apparent digestibility of selected feed ingredient for humpback grouper, *Cromileptes altivelis*. Aquaculture 218, 529-538.

Lupatsch, I., Kissil, G.W., Sklan, D. & Pfeffer, E. (1997) Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. Aquacult. Nutr. 3, 81-89.

Manzi, J.J. (1989) Aquaculture research priorities for the 1990s. World Aquacult. 20,29-32.

Masumoto, T., Ruchimat, T., Ito, Y. et al. (1996) Amino acid availability values for several protein sources for yellowtail (*Seriola quinqueradiata*). Aquculture 146, 109-119.

McGoogan, B.B. & Reigh, R.C. (1996) Apparent digestibility of selected ingredient in red drum (*Sciaenops ocellatus*) diets. Aquaculture 141, 233-244.

Menoyo, D., Lopez-Bote, C.J., Bautista, J.M. & Obach, A. (2003) Growth, digestibility and fatty acid utilization in large Atlantic salmon (*Salmo salar*) fed varying levels of n-3 and saturated fatty acids. Aquculture 225, 295-307.

Ng. W.K., Sigholt, T. & Bell, J.G. (2004) The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (*Salmo salar L.*) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. Aquacult. Res. 35, 1228-1237.

Olsen, R.E., Henderson, R.J. & Ringo, E. (1998) The digestion and selective absorption of dietary fatty acids in Arctic charr, *Salvelinus alpinus*. Aquacult. Nutr. 4, 13-21.

Pfeffer, E., Kinzinger, S., & Rodehutscord, M. (1995) Influence of the proportion of poultry slaughter by-products and of untreated or hydrothermically treated legume seeds in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum), on apparent digestibilities of their energy and organic compounds. Aquacult. Nutr. 1, 111-117.

Shepherd, C.J., Pike, I.H. & Barlow, S.M. (2005). Sustainable feed resources of marine origin. In: B. Howell & R. Floss (eds.) EAS Special Publication No. 35. pp. 59-66. European Aquaculture Society, The Hague, The Netherlands.

Snedecor G.W. & Cochran W.G. (1989) Statistical Methods, 8th edn. Iowa State University Press, Ames, Iowa, USA. 503 pp.

Sullivan, J.A. & Reigh, R.C. (1995) Apparent digestibility of selected feedstuff in diets for hybrid striped bass (*Morone saxatilis* ♀ ×*Morone chrysops* ♂). Aquaculture 138, 313-322.

Takeuchi, T. (1988) Laboratory work-chemical evaluation of dietary nutrients. In: Watanabe, T. (ed.) Fish Nutrition and Mariculture. JICA Kanagawa International Fisheries Training Centre, Tokyo, pp. 179-233.

Usmani, N., Jafri, A.K. & Khan, M.A. (2003) Nutrient digestibility studies in Heteropneustes fossilis (Bloch), *Clarias batrachus* (Linnaeus) and *C. gariepinus* (Burchell). Aquacult. Res. 34, 1247-1253.

Zhou, Q-C., Tan, B-P., Mai, K-S. & Liu, Y-J. (2004) Apparent digestibility of selected feed ingredients for juvenile cobia *Rachycentron canadum*. Aquaculture 241, 441-451.

Technical and Economical Evaluation of Small-scale Fish Cage Culture for Youth in the River Nile of Egypt: 2. Effect of Diet Form and Feeding Method of Nile Tilapia (*Oreochromis niloticus*) Monosex Fingerlings

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Abstract

The present study aims to evaluate the effects of three forms of feeds (wet form, sinking pellets, and floating pellets, Experiment 1) and two feeding methods (manual feeding twice daily or demand feeders, Experiment 2), on growth performance, survival rate, feed conversion ratio, yield and profitability of rearing Nile tilapia (*Oreochromis niloticus*) monosex fingerlings in floating net cages. Fingerlings with an average initial weight of 23.69 ± 1.46 g were cultured in 500 m^3 rectangular floating net cages for 90 days. Ten cages each measuring 10×10×5 m^3 (6 cages for Experiment 1 and 4 cages for Experiment 2) were used. Each treatment was replicated two times. Fish fingerlings were stocked at a rate of 30,000 fingerlings per cage (60 fish•m^{-3}). The cages were installed at the Demietta Branch of the Nile River, Egypt. The fish were fed ad libitum with a commercial diet (30% crude protein). Water quality parameters in the cages were within the desired levels for growing tilapia. The results of the first experiment revealed the following: a) fish fed with the floating pellets showed better average daily gain (0.99 g•$fish^{-1}$•day^{-1}), survival rate (93.56%) and feed conversion ratio (1.61) than fish fed with sinking or wet forms. The lowest daily gain and worst feed conversion ratio (FCR) were obtained from fish fed with sinking pellets, b) total production of fish fed with floating pellets was significantly higher (6.34 kg•m^{-3}) than fish fed with wet and sinking pellets (5.34 and 4.76 kg•m^{-3}, respectively); and, c) feeding cost (LE•kg^{-1} fresh body weight gain) for floating pellets (3.62 LE) was intermediate between wet and sinking forms (3.13 and 3.67, respectively).

The results of the second experiment showed that: a) the use of demand feeders improved fish growth performance (1.50 g•$fish^{-1}$•day^{-1}), survival rate (95.13%) and feed conversion ratio (1.52) as compared to the manual feeding twice daily (0.99 g•day^{-1}, 93.56% and 1.61, respectively); b) total production of fish fed using demand feeders was significantly higher (9.05 kg•m^{-3}) than that of fish fed manually (6.34 kg•m^{-3}); and, c) feeding cost of fish fed with demand feeders was higher (3.42 LE•kg^{-1} fish gain) than feeding cost of fish fed manually (3.14 LE•kg^{-1} fish gain) which is attributed to higher prices of floating pellets used in demand feeders.

Finally, the use of floating pellets and demand feeders as optimum feed form and feeding methods, respectively, for monosex tilapia fingerlings cultured in floating net cages could be recommended.

Introduction

Nile tilapia (*Oreochromis niloticus*) is distributed worldwide owing to its many desirable characteristics such as its general hardiness, fast growth rates, resistance to disease, ease of breeding and it's ability to adapt to a wide range of environmental and cultured conditions (Bromage 1995). Cage culture, the practice of rearing fish in cages, can be applied in existing bodies of water that cannot be drained (McGinty & Rakocy 1989). In Egypt, cage culture (semi-intensive or intensive) is a form of fish culture commonly practiced in the River Nile (Nour 2006). A special development was noted over the last few years in the culture of monosex Nile tilapia floating net cages in the River Nile. This development is apparent in the increase in protein needs in the diet of Nile tilapia. In 1988, there were only 54 cages in the River Nile; in 2005, it numbered 7,790 with fish production reaching 50,403 tonnes (GAFRD 2005). Feeding was mostly done manually with wet form diet. Feed waste is considered as one of the causes of eutrophication leading to algal blooms which use up oxygen from the water, and when these algal blooms die, the decomposing bacteria remove more oxygen. Essa et al. (2004) found a general decrease in dissolved oxygen and increase in BOD, nutrients (NH_3, NO_2 and PO_4) and total suspended solids in the water around the Nile tilapia cage culture area in the Damietta Branch of the River Nile in Egypt. To reduce the environmental impact, several interaction factors have to be optimized such as water quality, type of feed, feeding level, frequency and methods of feeding (McKinley 1994; Alvardo 1997; Dosdat 2001). The aim of the present study was to evaluate the effect of three forms of feeds (wet, sinking and floating) used in experiment 1 and two feeding methods (hand feeding twice daily and with demand feeders), used in experiment 2 on the performance, survival and profitability of net cage cultured monosex Nile tilapia fingerlings.

Materials and Methods

Two separate experiments were conducted on feeding monosex fingerlings of Nile tilapia cultured in floating net cages in the River Nile of Egypt, Demietta Branch from August to October, 2004 (90 days) to evaluate the effect of three feeding forms (wet, sinking and floating pellets, Experiment 1) and two feeding methods (manual and demand feeder, Experiment 2) on the growth performance, survival rate, feed conversion ratio, total production and profitability of rearing fish..

Both experiments were conducted in the same area, at the same time and with similar sources of Nile tilapia fingerlings (average initial body weights = 23.69 ± 1.46 g), purchased from a private hatchery located in Kafer El-sheik Governorate, Egypt. A total of 10 rectangular floating net cages measuring 500 m^3 in volume (10×10×5 m) were used (six for Experiment 1 and four for Experiment 2). Fish were stocked in each cage at a rate of 30,000 fingerlings•$cage^{-1}$ or at 60 fish•m^{-3}). There were two cages per treatment. The fish were fed *ad libitum* with a commercial diet containing 29.85% crude protein. Analysis of water samples obtained from fish cage area was carried out by collecting water samples weekly at 7 am from inside and outside of each cage for determination of dissolved oxygen concentration following the Winkler's technique. Methods described by Golterman et al. (1978) were used in the analysis of water quality for nitrite, ammonia, reactive phosphorus, biochemical oxygen demand (BOD) and total suspended solids (TSS). The pH was also measured *in situ* using Orion Research digital pH meter while water temperature (°C) was determined daily at 7:00 am using a thermometer.

The proximate analysis results of the commercial diet used in Experiments 1 and 2 are presented in Table 1. Fish were fed *ad libitum*. Analyses of the dietary dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash contents were conducted according to AOAC (1993). The Kjeldahl method was used to determine total nitrogen content in feed sample. The factor 6.25 was used to estimate crude protein according to ISO (1979).

Table 1. The percentage feed ingredients and chemical analysis (%) of the commercial diet used during Experiments1 and 2.

Feed ingredient (%)	(%)
Soybean meal	21.50
Fish meal	15.00
Yellow corn	15.00
Wheat milling by products	27.20
Corn gluten (60%CP)	10.00
Rice bran	10.00
Mineral mixture *	1.00
Vitamin mixture **	0.30
Chemical Analysis	
Dry matter	90.20
Crude protein	29.85
Crude fat	4.61
Crude fiber	4.20
Ash	10.03
Nitrogen free extract (NFE)	51.31

*Mineral mixture: Magnesium (mg), 300.00 ,Iron (mg), 50.00, Copper (mg), 5.00, Cobalt (mg), 0.20, Zinc (mg), 80.00, Manganese (mg), 40.00, Iodine (mg), 0.25, Selenium (mg), 0.30, Anti-oxidant (mg),150.00. **Vitamin mixture: A (IU) 20000, D_3 (IU) 25000, C (mg), 300, E. (mg), 10, K_3 (mg), 8, B1 (mg), 15, B_2 (mg), 13, B_3 (mg), 30, B_6 (mg), 15, Niacin (mg), 100, Folic acid (mg), 2.5, Biotic acid (mg), 1, Coline (mg), 1000.

Random fish samples from each cage were caught bi-weekly to determine total fish weights. Percent survival was determined for each cage by individually counting all live fish remaining at the end of each experiment.

Total weight gain, average daily gain, specific growth rate, feed conversion ratio were obtained according to Ricker (1975) and Castell & Tiews (1980) as follows:

1. Total Weight Gain ($g \bullet fish^{-1}$) = WT-WI
2. Average Daily Gain ($g \bullet fish^{-1} \bullet day^{-1}$): ADG = $W_T - W_I/n$
3. Specific Growth Rate ($\% \bullet fish^{-1} 1 \bullet day^{-1}$) = $100 \times (L_n W_T - L_n W_I)/n$
4. Feed Conversion Ratio = Dry matter intake (g)/Weight gain (g)
5. Survival Rate (%) =Final number of fish/initial number) ×100
6. Total Production Cost ($LE \bullet kg^{-1}$) = (Sum of variable costs$\bullet kg^{-1} \bullet sum^{-1}$ of fixed cost$\bullet kg^{-1}$) / number of kg(s)

7. Profitability = feed cost (LE•kg^{-1}) / (kg) body weight gain = FCR×cost per kg diet (Loverich 1996).

Data collected from the experiment was analyzed by using t-test (Steel & Torrie 1980) to analyze differences between the treatments means. Analysis of Variance (ANOVA) ($P\leq0.05$) and Duncan's Multiple Range Test were used to compare the means between individual treatments (Duncan 1955; Snedecor & Cochran 1967).

Results and Discussion

Results in table 2 show the values of water quality parameters obtained in the cages (El-Bostan, Damietta Branch, River Nile, Egypt). The average value of the water temperature was 26.80±2.08°C. The pH value averaged 7.71±0.15. The average value of total alkalinity was 310±3.46 ppm and dissolved oxygen was not less than 6.5 mg•L^{-1} while ammonia concentration was not more than 0.01 mg•L^{-1}. Concentrations of total nitrite (NO_2), nitrate (NO_3) and phosphate (PO_4) in the cages were moderate indicating that nutrients influx to the cages was still in balance with the biological activity of the different microorganisms. Results in table 2 clearly indicate the suitability of water quality for fish culture. Hepher & Pruginin (1981) reported that water pH ranging from 6.5 to 9.0 is quite reasonable for fish farming. Swingle (1969), Neill & Bryan (1991) and Daniel et al. (2005) considered that dissolved oxygen concentrations below 3.5 mg•L^{-1} and ammonia concentrations more than 0.2 mg•L^{-1} are undesirable for fish culture. Also, Maar et al. (1974) showed that water total alkalinity over 50 mg•L^{-1} $CaCO_3$ is beneficial for fish culture. Since water is the medium of all aquatic organisms, including fish, and serves to provide support, oxygen as well as enhances the availability of nutrients for the fish to utilize, maintaining good water quality is also maintaining a suitable environment for healthy fish growth.

Table 2. Water quality criteria in the floating cages.

Parameters	Average Values
Water temperature (°C)	26.80 ± 2.08
Dissolved oxygen (mg•L^{-1})	6.95 ± 0.18
pH	7.71 ± 0.15
Total alkalinity (mg•L^{-1} $CaCO_3$)	310.00 ± 3.46
Ammonia (mg•L^{-1})	< 0.01 ± 0.00
Nitrite (mg•L^{-1})	0.28 ± 0.02
Nitrate (mg•L^{-1})	3.61 ± 0.82

Tilapia is one of the few fish species which exhibits a wide range of tolerance to environmental conditions and has a low susceptibility to disease. However, in intensive rearing systems, due to the high density of fish, fish wastes may lead to deterioration in water quality and induce problems such as out-gassing and disease from parasitic, bacterial and fungal infections (Tonguthai 1995). Lethal temperature limits for Nile tilapia are 11 and 42°C (Philippart & Ruwet 1982) while temperatures up to 30°C have been found to promote growth rate.

Results in table 3 show the performance of monosex Nile tilapia fingerlings reared in floating net cages and fed on different feed forms (wet, sinking and floating pellets) for 90 days growing period. The results showed that, fish fed on floating pellets diet had significantly ($P\leq0.05$) the highest growth rate (0.99 g•fish^{-1}•day^{-1}), best feed conversion ratio (1.61:1) and total production (6.34 kg•m^{-3}). Evidently, not all of the wet form and sinking pellets diets were consumed, causing poor growth performance of the fish. Table 3 indicates that the differences in survival rates between the experimental fish groups varied (91.87 and 94.07% for fish fed with wet diet and with floating diet using demand feeder, respectively).

The present results clearly indicate that caged fish should be fed with floating diet that will stay floating in the water column. Floating feeds can be easily seen and eaten by the fish. It also allows the observation of the fish if they are feeding well. The behavior of the fish can be an indication of the quality of the feeds as well as the health of the fish. Sinking feed will only fall through the cage and will not be consumed by the fish which result in wasted feed (Sandfoss 2003). The result of Experiment 1 showed that the values of feed conversion ratio were 1.61, 1.74 and 1.88 for floating, wet and sinking feeds, respectively. These values were within the range reported by Sandfoss (2003) from 1.5 to 2.0. The same trend was observed in the profitability of the enterprise which varied between 2.52 to 2.91 LE•kg^{-1} gain. The results indicate that the average daily growth rate is a critical determinant of the financial performance of the cage culture operation. These results might be due to one or more of the following reasons: 1) Tilapia dislike hard pellets, such as sinking, because tilapia tend to chew pelleted feeds rather than swallow them whole (Jauncey & Ross 1982); 2) Floating pellets usually have a more palatable texture; and, 3) Using floating pellets has an advantage in intensive tilapia culture because it is easier to make an assessment of the behavior of the fish, its disease condition and appetite than if pellets of the sinking types were used.

Table 3. Effect of feeding form on growth performance, survival rate, feed utilization, yield and economical evaluation of monosex Nile tilapia fingerlings reared in cages for 90 days (1US$=5.75LE).

Items	Feed forms		
	Wet form	Sinking pellets	Floating pellets
Stocking			
Average initial weight (g•fish^{-1})	23.69	23.69	23.69
No. of fish stocked•m^{-3}	60	60	60
No. of fish stocked•cage^{-1}	30000	30000	3000
Harvesting			
Average final weight (g•fish^{-1})	96.84b	84.39c	112.98a
No. of fish harvested•cage^{-1}	27561	28221	28068
Survival rate (%)	91.87c	94.07a	93.56b
Growth and production			
Daily weight gain(g•fish^{-1}•d^{-1})	0.81b	0.67c	0.99 a
Feed conversion ratio (FCR)**	1.74 b	1.88 c	1.61 a
Total production (kg•m^{-3})	5.34 b	4.76 ab	6.34 a
Total yield (kg•cage^{-1})	2669.01	2381.57	3171.12
Profitability			
Feed price (LE•kg^{-1} fresh)	10	10	10
Feed price (LE•kg^{-1})	1.8	1.95	2.25
Feed cost (LE)•kg^{-1} gain	3.13c	3.67 a	3.62 b

*In the same row, means having different superscripts are significantly different ($P \leq 0.05$).
**FCR = g DM intake•g^{-1} fish body weight

The most important aspect of tilapia cage culture is providing good quality feed in the correct amounts to the caged fish. The diet should be nutritionally balanced and contain vitamins and minerals. Protein content should be 32 to 36% for 1 to 25 g tilapia and 28 to 32% for larger fish. Feeds and feeding are the major costs of production (McGinty & Rakocy 1989).

The results of Experiment 2 are shown in Table 4. It revealed that the best performance (daily growth rate, survival rate, feed conversion ratio and total production) came from fish fed through the demand feeder than those through manual feeding (1.5 g•fish^{-1}•day^{-1}, 95.13%, 1.52 and 9.05 kg•m^{-3} versus 0.99 g•day^{-1}, 93.56%, 1.61 and 6.34 kg•m^{-3}, respectively). The differences were statistically significant ($P \leq 0.05$). Also, the differences in feed conversion ratio between the experimental feeding methods resulted in a higher feed cost per kg for fish fed manually than those fed by demand feeder (Table 4). These results may be due to: 1) The demand feeder only releases feed when the bait or trigger is actually depressed; 2) The demand feeder gives the fish a chance to obtain the food it requires at all times (McGinty & Rakocy 1989; El- Ebiary & Essa 2002); and, 3) Floating feeds allows the observation of the feeding response and are effectively retained by a feeding ring. Since it takes about 24 hours for high quality floating pellets to disintegrate, fish may be fed once daily in the proper amount, but twice-daily feeding is better (McGinty & Rakocy 1989).

The results also present the financial performance such as reducing labor costs and ensuring that a known quantity of food is released to the fish on a regular basis. McGinty & Rakocy (1989) reported that since floating pellets are round and uniform in size, they are best for demand feeders, while sinking pellets disintegrate rapidly and clog the feeder if they are splashed, and the less uniform size of sinking pellets makes adjustment of the trigger mechanism sensitivity more difficult.

Table 4. Effect of two feeding methods (manual and demand feeder) on growth performance, survival rate, feed conversion ratio, total production and profitability of monosex Nile tilapia fingerlings reared in cages for 90 days.

Items	Feeding Methods	
	Manual	Demand feeder
Stocking data		
Average initial weight (g•fish^{-1})	23.69	23.69
Initial fish number per cage	30000	30000
Harvesting		
Average final weight (g•fish^{-1})	112.98^{b}	158.64^{a*}
Fish harvested per cage	28068	28539
Survival rate (%)	93.56	95.13
Growth and production		
Average daily gain (g•fish^{-1}•day^{-1})	0.99^{b}	1.50^{a}
Feed conversion ratio (FCR)**	1.61^{b}	1.52^{a}
Production per cubic meter (kg•m^{-3})	6.34^{b}	9.05^{a}
Yield (kg•cage^{-1})	3171.12	4527.43
Profitability		
Fish price (LE•kg^{-1} fresh)	10	10
Feed price (LE•kg^{-1})	1.95	2.25
Feed cost (LE)•kg^{-1} fish gain.	3.14^{a}	3.42^{b}

*In the same row, means having different superscripts are significantly different ($P \leq 0.01$)

** Feed conversion ratio = Dry matter intake (g) / Weight gain (g)

Conclusion

Summing up the results of the present study, it could be concluded that:

● In practice, attention must be paid to floating pellets diets not only to increase monosex Nile tilapia cage culture production and profitability, but also for the protection of the River Nile aqueous environment and to maintain a balance between the cage fish culture activities;

● Using the demand feeder system enables fish to obtain its feed on demand by depressing a trigger, thereby ensure the optimum management conditions for fish growth, production and profitability.

Finally, the economical evaluation of both experiments (Tables 3 and 4) show that total production of caged fish fed on floating pellets was significantly ($P \leq 0.05$) higher (6.34 kg•m^{-3}) than the other feeding forms (5.34 and 4.76 kg•m^{-3}, wet and sinking, pellets, respectively). On the other hand, feeding cost for floating pellets was moderate (3.62 LE•kg^{-1}) between wet diet form (3.13 LE•kg^{-1}) and sinking pellets (3.67LE•kg^{-1}). However, total production of caged fish fed using demand feeder was significantly higher (9.05 kg•m^{-3}) than those fed manually (6.34 kg• m^{-3}). Feeding cost using demand feeder was higher (3.42 LE•kg^{-1} fresh fissile) than feeding fish manually (3.14 LE•kg^{-1} fresh fissile) which is attributed to increasing prices of floating pellets used in demand feeders.

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References

Alvardo, J. (1997) Aqua feeds and the environment. In: Feeding Tomorrow's Fish. Cahiers Options Mediterranean's. Vol.22, CIHEAM-FAO, pp. 275-290. Zaragoza, Spain.

AOAC (Association of Official Analytical). (1993) Official Methods of Analysis, 15th edn. AOAC. Arlington, VA.

Bromage, N.R. (1995) Broodstock management and seed quality-general considerations. *In*: Bromage, N.R. & Roberts, R.J. (eds.). Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford. p. 1-24.

Castell, J.D. & Tiewes, K. (Eds). (1980) Report of the EIFAC, IUNS and ICES Working Group on the Standardization of Methodology in Fish Research, Hamburg, FRG, 212-23 March, 1979. IFAC Tech. Paper 3, 24.

Daniel, S.B., Larry, W.D. & Joseph, H. S. Jr. (2005) Comparative oxygen consumption and metabolism of striped bass (*Morone saxatilis*) and its hybrid. J. The Journal of the World Aquaculture Society 36(4), 521-529.

Dosdat, A. (2001) Environmental impact of aquaculture in the Mediterranean: nutritional and feeding aspects, pp. 23-36. In: Environmental Impact Assessment of Mediterranean Aquaculture farms, cahiers options Mediterranean's, Vol.55, CIHEAM, Zaragoza, Spain.

Duncan, D.B. (1955) Multiple range and multiple F tests. Biometrics 11, 1-42.

El-Ebiary, E.H. & Essa, M.A. (2002) Culture of Florida red tilapia in Lake Mariut, Egypt. Delta J. Sciences 26, 1-12.

Essa, M. A., Nour, A.M., Zaki, M.A & Mabrouk, H.A. (2004) Culture of environmentally friendly fish species in cages. p. 60-70: *In*: Proc. The Second Conference on the Development of Fish Culture in Behera Governorate, Egypt. October 11, 2004.

GAFRD (General Authority for Fisheries Resources Development). (2005) Statistical Fishery Report, Cairo, Egypt.

Golterman, H.L., Clymo, R.S. & Ohnstad, M.A.M. (1978) Methods for Physical and Chemical Analysis of Fresh Waters.

Intern. Biol. Prog. Hand Books 8, 213 pp.

Hepher, B. & Pruginin, Y. (1981) Commercial Fish Farming. A Wiley Interscience Publication, New York.

ISO (International Standard Organization). (1979) Animal Feeding Stuffs Determination of Nitrogen Content and Calculation of Crude Protein Content. Switzerland Method no 5983.

Jauncey, K. & Ross, B. (1982) A Guide to Tilapia Feeds. Institute of Aquaculture, University of Sterling, Scotland. 111 pp.

Loverich, G.F. (1996) Offshore Aquaculture Harvest and Transport Concept Feasibility and Development. A Report of Ocean Spar Technologies. LLC Pursuant to National Oceanic and Atmosph. Admin., A. Word No. NA56 FD 0071, Bainbridge, Washington.

McGinty, A.S. & Rakocy, J.E. (1989) Cage Culture of Tilapia. Southern Regional Aquaculture Center (SRAC) Pub.281, Texas.

Neill, W.H. & Bryan, J.D. (1991) Responses of fish to temperature and oxygen, and response integration through metabolic scope. p. 30-57. *In*: D.E. Brune and J.R. Tomasso, (editors). Aquaculture and water quality (Advances in world aquaculture, volume 3), The World Aquaculture Society, Baton Rouge, Louisiana.

Neill, W.H., Oborny, E.L., Craig, S.R., Matlock, M. B. and Galtin, D.M. (2003). Estimating metabolism of fish in aquaculture production systems. Intern. J. Recirculation Aquaculture 4, 25-32.

Nour, A.M. (2006) The 2nd Annual Report on Development of Proposed Project for Fish Production for Youth. National Academy of Science, Cairo, Egypt, 144 pp.

Philippart, J.C.L. & Ruwet, J. (1982) Ecology and distribution of tilapia, p. 15-59. *In*: The Biology and Culture of Tilapias, ICLARM Conference Proceeding 7, Lowe-McConnel, R.H. (Ed.) Manila Philippines.

Ricker, W.E. (1975) Computation and Interpretation of Biological Statistics of Fish Populations. Fish. Res. Board Can. Bull. 191.

Sandfoss, M. (2003) Site selection and water quality for cage culture, North Carolina Cooperative Extension. NCCE Aquaculture Home Page, August, 2003 pp. 3.

Snedecor, G.W. & Cochran, W.G. (1967) Statistical Methods. Iowa State Univ., Ames, U.S.A.

Steel, R.G.O. & Torrie, J.H. (1980) Principles and Procedures of Statistics: A Biometrical Approach, 2nd ed. McGraw Hill, New York. 633 pp.

Swingle, H.S. (1969) Methods of Analysis for Water, Organic Matter and Pond Bottom Soils in Fisheries and Research. Auburn Univ., Auburn, Alabama, 119 pp.

Tonguthai, K. (1995) Fish Disease. Asian Institute of Technology, Bangkok, Thailand. 87 pp.

Assessment of Nitrogen Maintenance Requirement in Juvenile Red Sea Bream (Pagrosomus major)

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Abstract

Nitrogen balance method and nitrogen-free diets were used in the present study to determine nitrogen maintenance requirement (NM) and nitrogen maintenance requirement per unit metabolism body weight (NM') of Red Sea Bream (*Pagrosomus major*). Fish with body weights of 50, 100, 150, 200 and 250 g were fed with diets containing three graded levels of protein (380-460g kg^{-1} CP). The results from nitrogen balance experiment show that the amount of nitrogen deposition varied from 0.15 to 0.30 mg•g^{-1}•d^{-1}, accounting for total ingested nitrogen of 121 to 203 g•kg^{-1}. The amount of faecal nitrogen excretion varied from 0.20 to 0.32 mg•g-1•d^{-1}, accounting for total ingested nitrogen of 164 to 216 g•kg^{-1}.The endogenous nitrogen excretion, a main part of total ingested nitrogen consumption varied from 0.80 to 0.96 mg•g•d^{-1}, accounting for total ingested nitrogen of 628 to 677 g•kg^{-1}. A positive correlation was observed between NM and body weight, while a negative correlation was observed between NM of unit body weight and growth duration. There was no significant difference (P>0.05) among the NM' obtained from different growth stages. The average of NM' was 0.498 mg•g^{-1} per day. The results from nitrogen-free diet experiment show a negative correlation between NM and feed intake of nitrogen-free diet; with a decrease in feed intake of fish, NM increased. The average NM was 0.507 mg•g^{-1} per day with fast of fish. This value was close to 0.508 mg•g^{-1} obtained from fish with 150 g BW in the nitrogen balance experiment. The nitrogen balance method was recommended to be a better method for determining NM in consideration of fish stress and result stability. This study also supplied a calculated result about the protein content in diets, which are necessary for maintaining fish body protein at different growth stages. The calculation was based on the amount of nitrogen necessary for maintaining body protein per kg BW.

Introduction

The nutritive value of protein is a main indicator for feed formulation and evaluation of feed quality. Some parameters including protein efficiency ratio (PER), protein biological value (BV) and net protein utilization (NPU) can be applied to determine the content of crude protein (CP) in feeds required when the maximum growth rate is observed. The protein metabolism process is considered to be related to the income and expenses of nitrogen, a main structure element for protein. Thus, the determination of nitrogen retention (NR) is a basis of evaluation by applying the parameters of BV and NPU. Otherwise, NR directly depends on the expenses of maintenance nitrogen and nitrogen deposition for growth.

Fish metabolism nitrogen in faeces (MNF) and endogenous nitrogen (EN) are both produced as results of expense of maintenance nitrogen. It has been known that MNF originally came from spit, digestive enzyme and cell of enteron, while EN including urea and ammonia result in the degradation of body protein. At the state of maintenance, the amount of nitrogen requirement for skin metabolism could be ignored. The nitrogen maintenance requirement (NM) therefore could be regarded as the sum of NMF and EN. Currently, the methods of nitrogen balance, nitrogen-free diet and fast metabolism, by which NM is determined, are commonly employed.

Some researches concerning nitrogen balance method were reported (Bail et al. 2003; Carter & Brafield 1992; Chakraborty & Ross 1992; Kumpp & Von Westernhagen 1986; Li et al. 1998a; Porter & Krom 1987; Rychly 1980; Sun et al. 2004; Torres et al. 1996; Watanabe & Ohta 1984; Yang et al. 2003; Yu et al. 1998; Zhang et al. 1997). Other researches have also been conducted to study the nitrogen excretion rates of *Plectorhynchus cinctu* and *Liza haematocheila* (Wang & Qiu 2001; Xian & Zhu 2002). However, we found that the preliminary study for red sea bream, *Pagrosomus major*, was restricted to the income and expenses of nitrogen (Li et al. 1998b). Likewise, no research has ever been carried out to determine NM so far. The aims of present study therefore were: 1) to determine NM in juvenile of red sea bream following the methods of nitrogen balance and nitrogen-free diet. The expected results could be a basis for formulation of feed; 2) to compare the efficiency of the above mentioned methods, and find a better method for determining NM in fish nutrition study.

Materials and Methods

Nitrogen balance experiment

The experiment carried out in May and June 2005 was designed with five growth stages in accordance to variance in initial body weight (IBW). The fish were from Huangbi'ao marine fish farm. About 180-360 fish were selected for each growth stage. The average IBW (empty stomach) was approximately 50, 100, 150, 200 and 250 g, respectively. The fish were divided into three groups marked as A, B and C at each stage according to the protein content in the diets (Table1). Triplication was subjected to each group. Each experiment unit contained 20-40 fish. Forty fish with BW of 50 g and 30 fish with BW of 100 g were reared in the aquaria, wherein the number of fish with other BW was set at 20 for each BW level.

Table 1. Protein content in diets for red sea bream ($g \cdot kg^{-1}$)

BW stages (g)	ME ($MJ \cdot kg^{-1}$)	A group	B group	C group
50	15.41	460	420	380
100	15.41	460	420	380
150	15.41	460	420	380
200	15.41	460	420	380
250	15.41	460	420	380

BW=body weight; ME=metabolized energy

The experiment lasted for a period of 28 days, wherein 20 days were designed for adaptation and seven days was for pre-experiment. Sampling was done on the 28th day. The diets, prepared with a protein source of red fish meal, crude protein (CP) content required for the different experiment stages and corresponding metabolism energy (ME), were fed. The Cr_2O_3 was used as an indigestible marker to determine apparent digestibility. The fish were fed twice a day. The feeding amount, estimated using the eye survey method for satiation, was 2 g•100g^{-1} of BW. The exact feeding amounts were recorded for each day. The aquaria were supplied with oxygen adding water of 19±1°C. Siphoning method associated with renewed water (1/3) was employed to maintain stable water quality.

At the end of the experiment, samples of fish from each group were anaesthetized with MS222 (60 mg•L^{-1} in seawater). Before stripping fish were gently cleaned with soft tissue. Following this step, fish were individually stripped by using gentle pressure from the ventral fin to the anal region. Then the obtained faeces were pooled and stored in a freezer for subsequent analysis. Meanwhile, fish were weighted and 5 randomly-selected fish were analyzed for body nitrogen content. The diets and faeces samples also were analyzed for nitrogen and Cr_2O_3 content. Nitrogen-free diet experiment

A total of 100 fish with BW of approximately 150g were randomly distributed in five aquaria marked A, B, C, D and E (20 fish for each aquarium). The design of the experiment is shown in Table 2. A period of seven days is set for adaptation of fish, and a diet with 423 g•kg^{-1} protein content was fed to all five groups. The experiment lasted for six days, and the nitrogen free diets with feed attractant added were used in this period. Feeding amount was set at a graded level (the highest level was in the diet for group A while the lowest level was in the diet for group E). The fish of group E were kept diet-free. At the end of the experiment, faeces, obtained from the fish from groups A, B, C and D by stripping, were pooled and stored in a freezer for subsequent analysis. Sampling for Group E was conducted 48 hours after the experiment started. The subsequent calculations for NM and NM per unit metabolism body weight (NM') were based on the obtained results of initial and final body BW, as well as body nitrogen content. The average temperature of water was approximately 18°C during the experiment period.

Table 2. Design of nitrogen-free diet experiment

Groups	A	B	C	D	E	
	Days	7	7	7	7	7
Adaption period	Dietary (g•d^{-1})	3.0	3.0	3.0	3.0	3.0
	Feed	normal	normal	normal	normal	normal
	Days	6	6	6	6	6
Experiment period	Dietary (g•d^{-1})	2.8	2.1	1.4	0.7	0
	Feed	nitrogen-free	nitrogen-free	nitrogen-free	nitrogen-free	0

Chemical and statistical analyses

Nitrogen contents in diets, faeces and fish body were analyzed by employing the Kjeldahl method (AOAC 2000). Cr_2O_3 content was determined by the method described in 'Experimental Instruction of Livestock Thremmatology'. The results in present study were analyzed by ANOVA associated with significance test. The models utilized in this experiment are defined as follows:

NI =MDFI ×PCD / MBW

Where: NI is nitrogen intake mg•g^{-1}•d^{-1}; MDFI is mean daily feed intake (mg); PCD is protein content in diet ($g \cdot kg^{-1}$); and, MBW is mean body weight (g).

$$ND = [MBW_f \times NCB - MBW_i \times NCB] / [MBW_o \times EP]$$

Where: ND is nitrogen deposition ($mg \cdot g^{-1} \cdot d^{-1}$); MBW_f is final mean body weight (mg); NCB is nitrogen content of fish body ($g \cdot kg^{-1}$); MBW_i is initial mean body weight (mg); MBW_o is mean body weight over experiment period (mg); and, EP is experiment period (d).

$$NF = MDEF \times NCF/MBW$$

Where: NF is excretion amount of nitrogen in faeces ($mg \cdot g^{-1} \cdot d^{-1}$); MDEF is mean daily excretion of faeces (mg); NCF is nitrogen content in faeces ($g \cdot kg^{-1}$); and, MBW is mean body weight (g).

$$EAF = FI \times (dCr_2O_3) / (fCr_2O_3)$$

Where: EAF is excretion amount of faeces (g); FI is feed intake (g); dCr_2O_3 is Cr_2O_3 content in diet ($g \cdot kg^{-1}$); and, fCr_2O_3 is Cr_2O_3 content in faeces ($g \cdot kg^{-1}$).

Nitrogen balance: $NI = NG + NF + NU$

Where: NI is nitrogen intake; NG is nitrogen retention for growth; NF is nitrogen in faeces; and, NU is endogenous nitrogen

$$NM = NF + NU \ (NI = 0)$$

Where: NM is nitrogen maintenance requirement ($mg \cdot g^{-1} \cdot d^{-1}$)

NM per unit metabolism body weight: $NM' = NM / BW^{0.75}$

Where: BW represents fish body weight.

Results and Discussion

Consumption of intake nitrogen in red sea bream

The NI, NG, NF and NU in red sea bream fed diets with different CP contents and at different growth stages are summarized in Table 3. The results illustrate that as CP content ranged from 380 to 460 $g \cdot kg^{-1}$ in diets, nitrogen deposition varied from 0.15 to 0.30 $mg \cdot g^{-1} \cdot d^{-1}$, accounting for total ingested nitrogen from 121 to 203 $g \cdot kg^{-1}$. The amount of faecal nitrogen excretion varied from 0.20 to 0.32 $mg \cdot g^{-1} \cdot d^{-1}$, accounting for total ingested nitrogen from 164 to 216 $g \cdot kg^{-1}$. The endogenous nitrogen excretion, a main part of total ingested nitrogen consumption, varied from 0.80 to 0.96 $mg \cdot g^{-1} \cdot d^{-1}$, accounting for total ingested nitrogen from 628 to 677 $g \cdot kg^{-1}$. A similar observation was obtained by Li et al. (1998b), when they conducted a study with 13 g red sea bream.

Relationship between nitrogen retention and protein content of diet or growth stage

The experiment on nitrogen balance for juvenile red sea bream with body weights of 50, 100, 150, 200 and 250 g was used in the present study to determine nitrogen requirement. The results showed that the significant correlativity between nitrogen retention rate and growth stage was presented (Figure1). The protein utilization rate was higher at an earlier growth stage than at a later growth stage. The results show that the decrease in nitrogen retention rate in unit BW was a result of the decrease in the protein content in the diets. Furthermore, nitrogen retention rate

(NG/NI) was also slightly affected by the protein content of diet. We observed that higher protein content is usually coupled with a lower nitrogen retention rate. Otherwise, there was no significant difference among the three groups at the same growth stage ($P>0.05$). The response of fish fed the diets with the same protein content demonstrated that the significant correlativity between nitrogen retention rate and growth stage was presented. The protein utilization rate was higher at an earlier growth stage than at a later growth stage. This also proved that the value of NG/NI decreased as the fish grew. A similar result was obtained in a previous study conducted by Gerking (1955). This author observed that protein conversion decreased from 390 to 100 g•kg^{-1} when fish grew from 14 to 85 g. A plausible explanation for this may be that growth rate was higher at an earlier growth stage, and protein accumulated.

Table 3. Nitrogen balance result of red sea bream in different growth stages

BW	Groups	CP (g kg^{-1})	NI (mg•g^{-1}•d^{-1})	NG (mg•g^{-1}•d^{-1})	NF (mg•g^{-1}•d^{-1})	NU (mg•g^{-1}•d^{-1})	NG/NI (%)	NF/NI (%)	NU/NI (%)
	A	462	1.48±0.08	0.30±0.02	0.25±0.05	0.93±0.02	20.3	16.9	62.8
50g	B	420	1.33±0.07	0.26±0.03	0.22±0.04	0.85±0.06	19.5	16.5	64.0
	C	381	1.22±0.03	0.22±0.03	0.20±0.02	0.80±0.02	18.0	16.4	65.6
	A	462	1.46±0.05	0.27±0.04	0.27±0.03	0.92±0.03	18.5	18.5	63.6
100g	B	420	1.34±0.04	0.23±0.02	0.25±0.01	0.86±0.04	17.1	18.6	64.3
	C	381	1.20±0.04	0.20±0.03	0.21±0.01	0.79±0.03	16.7	17.5	65.8
	A	462	1.50±0.05	0.24±0.01	0.29±0.02	0.97±0.02	16.0	19.3	64.7
150g	B	420	1.36±0.07	0.20±0.02	0.26±0.03	0.90±0.03	14.7	19.1	66.2
	C	381	1.25±0.02	0.17±0.02	0.23±0.01	0.85±0.06	13.6	18.4	68.0
	A	462	1.50±0.09	0.22±0.01	0.31±0.01	0.97±0.05	14.7	20.7	64.6
200g	B	420	1.35±0.03	0.19±0.02	0.27±0.01	0.89±0.02	14.1	20.0	65.9
	C	381	1.24±0.05	0.16±0.03	0.25±0.05	0.83±0.01	12.9	20.2	66.9
	A	462	1.48±0.06	0.20±0.01	0.32±0.04	0.96±0.03	13.5	21.6	64.9
250g	B	420	1.36±0.04	0.18±0.01	0.28±0.04	0.90±0.05	13.2	20.6	66.2
	C	381	1.24±0.03	0.15±0.03	0.25±0.02	0.84±0.04	12.1	20.2	67.7

Results are given as mean ± standard error of mean (SEM). BW=body weight; NI=nitrogen intake; NG=nitrogen retention for growth; NF=nitrogen in faeces; NU=endogenous nitrogen

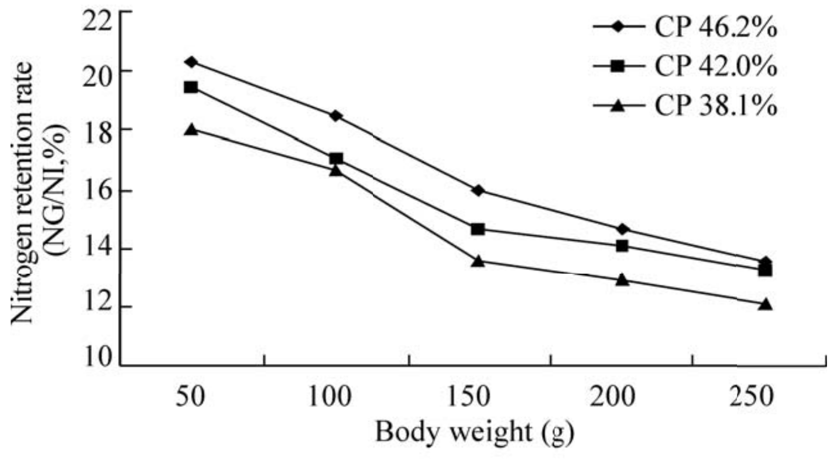

Figure 1. Nitrogen retention rate of red sea bream fed diets with different protein contents; NG=nitrogen retention for growth; NI=nitrogen intake; CP=crude protein; Statistical analysis used is ANOVA associated with significance test

Relationship between excreted nitrogen and protein content of diet or growth stage

As a result of decreasing protein content in diets, the values of NF and NI decreased. Whereas, an opposite pattern was illustrated by the result of endogenous nitrogen (NU) excretion rate, which was higher in the fish fed the diet with lower protein content (381 g•kg^{-1}) than in the fish fed the diet with higher protein content (462 g•kg^{-1}). The explanation could be that the decreasing protein intake has no effect on the metabolism of fish.

As fish weight increased, NF and NU excretion rates showed an increasing pattern (Figures 2 and 3). Furthermore, compared with NU rate, NF excretion increased more.

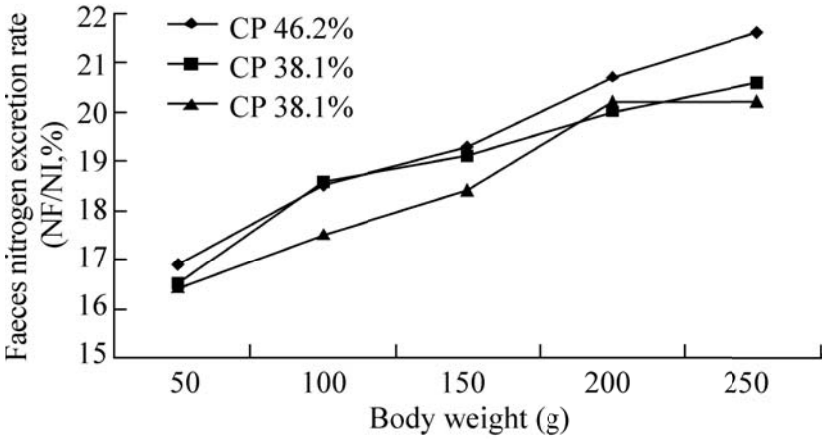

Figure 2. Faeces nitrogen excretion rates of red sea bream fed the diets with different protein contents; NF=nitrogen in faeces; NI=nitrogen intake; CP=crude protein

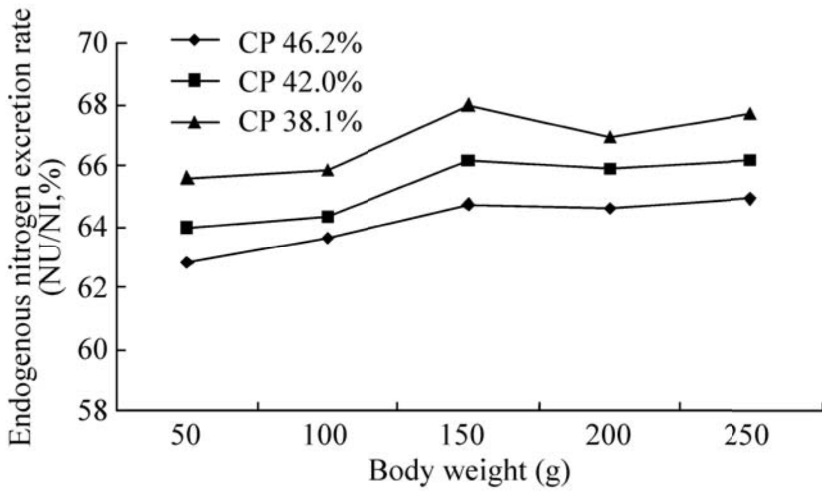

Figure 3. Endogenous nitrogen excretion rate of red sea bream fed the diets with different protein contents; NU=endogenous nitrogen; NI=nitrogen intake; CP=crude protein

Nitrogen maintenance requirements (NM)

The regression mode was applied to do the statistical analysis for the data in Table 3. The statistical results with regression equations are summarized in Table 4.

Table 4. Nitrogen maintenance requirement (NM) and nitrogen maintenance requirement per unit metabolism BW (NM') of red sea bream.

BW	Regression equation	R	NM ($mg\cdot g^{-1}\cdot d^{-1}$)	BW (g)	BW0.75 (g)	NM' ($mg\cdot g^{-1}\cdot d^{-1}$)
50g	$N_G=0.3138\ N_I-0.1698$	0.9998	0.1698	66.5	23.29	0.485
100g	$N_G=0.2566\ N_I-0.1490$	0.9450	0.1490	123.0	36.93	0.496
150g	$N_G=0.2610N_I-0.1383$	0.9639	0.1383	175.5	48.22	0.508
200g	$N_G=0.2346\ N_I-0.1297$	0.9991	0.1297	232.5	59.54	0.507
250g	$N_G=0.2049\ N_I-0.1189$	0.9642	0.1189	299.5	71.99	0.495
AVE			0.1411	179.4	47.99	0.498

BW=body weight; $BW^{0.75}$= metabolism body weight; R=relative coefficient; NM=nitrogen maintenance requirement; NM'=nitrogen maintenance requirement per unit metabolism body weight

The results of nitrogen-free diet experiment (Table 5) demonstrate that the increasing nitrogen excretion and NM were caused by the decreasing feed intake of nitrogen-free diet (From groups A to E).

Table 5. Results of nitrogen-free diet experiment.

Group	BW(g)	BW0.75 (g)	NG ($mg\cdot g^{-1}\cdot d^{-1}$)	NF ($mg\cdot g^{-1}\cdot d^{-1}$)	NU ($mg\cdot g^{-1}\cdot d^{-1}$)	NM' ($mg\cdot g^{-1}\cdot d^{-1}$)
A	150.8±5.3	43.0	-0.138	0.005	0.133	0.484
B	152.3±1.3	43.3	-0.140	0.005	0.135	0.492
C	149.2±3.4	42.7	-0.144	0.004	0.140	0.503
D	151.9±6.3	43.2	-0.145	0.003	0.142	0.509
E	153.1±6.9	43.5	-0.145	0.001	0.144	0.510

BW=body weight; BW0.75=metabolism body weight; R=relative coefficient; NG=nitrogen retention for growth; NF=nitrogen in faeces; NU=endogenous nitrogen; NM'=nitrogen maintenance requirement per unit metabolism body weight

Statistical analysis illustrates that a negative correlation between NM and feed intake (D) of nitrogen-free diet was significant:

$$Nm=0.5134 - 0.00986\ D \qquad (|r|=0.9758,\ P<0.01)$$

The value of NM was close to that in the fast group (E group) when the feed intake of nitrogen-free diets was reduced (D group). The mean daily NM was 0.518 $mg\cdot g^{-1}$, which is in agreement with the results from the nitrogen balance experiment. Mean NM was 141.1 $mgN\cdot kg^{-1}$ BW (Table 4). This is similar to the results achieved in two previous studies conducted by Carter & Brafield (1992) with common carp (100-140 mgN) and by Sadasivam & Teles (1985) with rainbow trout (95 mg N). However, it was slightly higher than the result of a study conducted with mullet (59 mgN; Xian & Zhu 2002). On the other hand, a study carried out by Cerking (1955) with 27.9 g Bluegill showed that NM could reach a level of 7.18 mg N (or 257.3 $mgN\cdot kg^{-1}$ BW), which was greatly higher than the result in the present study. The tendency of NM values over five growth stages demonstrates that there was a negative correlation between NM per unit BW and fish BW. That could be the reason for the observed increase in BW coupled with a decrease in NM. Usually, the energy maintenance requirement has a similar pattern showing that the maintenance requirement per unit BW was higher in smaller fish than in larger fish.

The relationship between NM obtained from nitrogen balance experiment and fish BW has been demonstrated. However, the significant difference can not be detected (P>0.05) when using the unit metabolism BW to replace the BW in statistical analysis. The daily NM' of red sea bream was 498 mg•kg^{-1}.

The NM per Kg BW was 0.1698 g in a 50g fish, equal to 1.061 g protein (x6.25); 0.1490 g in a 100 g fish, equal to 0.931 g protein (x6.25); 0.1383 g in a 150g fish, equal to 0.864 g protein (x6.25); 0.1297 g in a 200 g fish, equal to 0.811 g protein (x6.25); and 0.1189 g in a 250g fish, equal to 0.743 g protein (x6.25). The required protein contents in diets for the maintenance of fish reared at different stages are summarized in Table 6.

Table 6. Protein content in diets for maintaining fish body protein (g•kg^{-1}).

Diets•BW^{-1} (g•kg^{-1})	CP (g•kg^{-1})				
	50g	100g	150g	200g	250g
15	708	621	575	540	495
20	530	466	460	405	372
25	425	372	368	324	297
30	354	310	307	270	248
35	303	266	246	232	212
40	265	232	216	203	18.6

BW=body weight; CP=crude protein

The presence of a significant negative correlation, between the results in nitrogen-free diet experiment and the amount of feed intake in nitrogen-free diets, and the reliable performance of nitrogen balance experiment when comparing the results both from nitrogen balance and nitrogen-free diet experiments, make the nitrogen balance experiment a better method for determining the nitrogen maintenance requirement for red sea bream. In addition, this method has advantages in stress (slight as it in real culture environment) and experimental results.

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References

AOAC. (2000) Official Methods of Analysis. Association of Official Agricultural Chemists, Washington D.C., USA.

Bail, Z., He, X. and S. Li. (2003) Relationship between the nitrogen budget and diet types in juvenile of *Oreochromis niloticus*. J. Shanghai Fish Uni. 29, 43-49.

Carter, C.G. and A.E. Brafield. (1992) The bioenergetics of grass carp, *Ctenopharyngodon idella*: the influence of body weight, ration and dietary composition on nitrogenous excretion. J. Fish Biol. 41, 533-543.

Chakraborty, S.C. and B. Ross. (1992) The effect of dietary protein level and ration level on excretion of ammonia in common carp, *Cyprinus carpio*. Comp. Biochem. Physiol. 103A, 801-808.

Gerking, S.D. (1955) Influence of rate of feeding on body composition and protein metabolism of bluegill sunfish. Physiol. Zool. 28, 267-282.

Kumpp, D.W. and H. Von Westernhagen. (1986) Nitrogen balance in marine fish larvae: influence of developmental stage and prey density. Mar. Biol. 93, 189-199.

Li, J., Xu, C. and S. Xu. (1998a) Effect of ration levels on nitrogen budget in juvenile black porgy. Ocean. Limn. Sin. 21, 462-465.

Li, J., Xu, C. and S. Xu. (1998b) Study on energy budget, nitrogen and carbon budget in young red sea bream. Mar. Sci. 2, 46-48.

Porter, C.B. and M.D. Krom. (1987) Ammonia excretion and total N budget for gilthead sea bream and its effect on water quality conditions. Aquaculture 66, 287-297.

Rychly, J. (1980) Nitrogen balance in trout, nitrogen excretion and retention after feeding diets with varying protein and carbonhydrate levels. Aquaculture 20, 343-350.

Sadasivam, J.K. and A.O. Teles. (1985) Effect of digestible energy on nitrogen and energy balance in rainbow trout. Aquaculture 50.

Sun, Z., Yu, F. and H. Yao. (2004) Oxy consumption and Excretion of *Pseudosciaena crocea and Sciaenops ocellatus* in the net-culture. J. Zhejiang Ocea. Uni. 23, 207-217.

Torres, J.J., Brightman, R.l. and J. Donnelly. (1996) Energetics of larval red drum, *Sciaenops ocellatus*. *Part* I: Oxygen consumption, specific dynamic action, and nitrogen excretion. Fish Bull. 94, 756-765.

Wang, M. and S. Qiu. (2001) Studies on excretion rate of endogenous, exogenous and total nitrogen of juvenile *Plectorhynchus cinctus temminck*. J. Trop. Ocea. 20, 87-92.

Watanabe, T. and M. Ohta. (1984) Endogenous nitrogen excretion and non-fecal energy losses in carp and rainbow trout. Fish Sci. 41, 359-371.

Xian, W. and X. Zhu. (2002) Effect of body weight and temperature on nitrogen budget of bastard halibut. Studia. Mar. Sin. 44, 199-205.

Yang, Y., Cui, Y. and B. Xion. (2003) Comparative studies on nitrogen budget and energy budget of jian carp and gibel carp fed diets with different quality. Acta Hydro. Sin. 27, 572-579.

Yu, D., Han, A. and X. Liu. (1998) Study on energy source substance of red seabream juvenile. J. Fish Sci. Chn. 5, 108-110.

Zhang, Z., Zhang, M. and J. Li. (1997) Oxygen consumption and nitrogen excretion of *Paralichthys olivaceus* with different body weights at different water temperature. J. Qingdao Ocean Uni. 27, 483-489.