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^{TM}) 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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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.

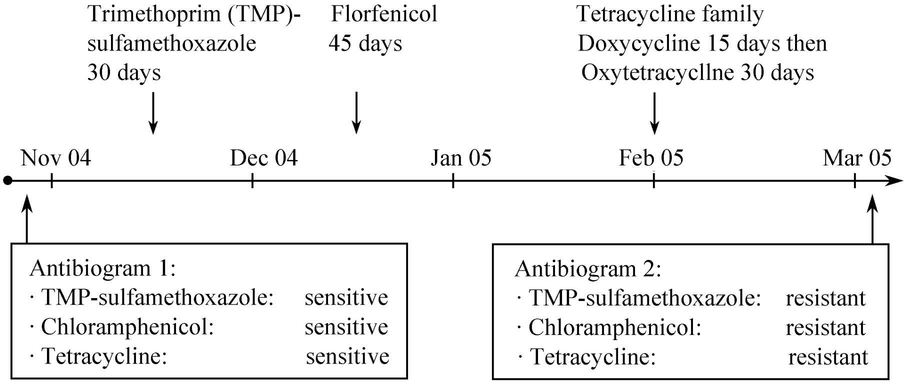

Figure 1. A real case on induction of antibiotic resistance in a fish farm.

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.

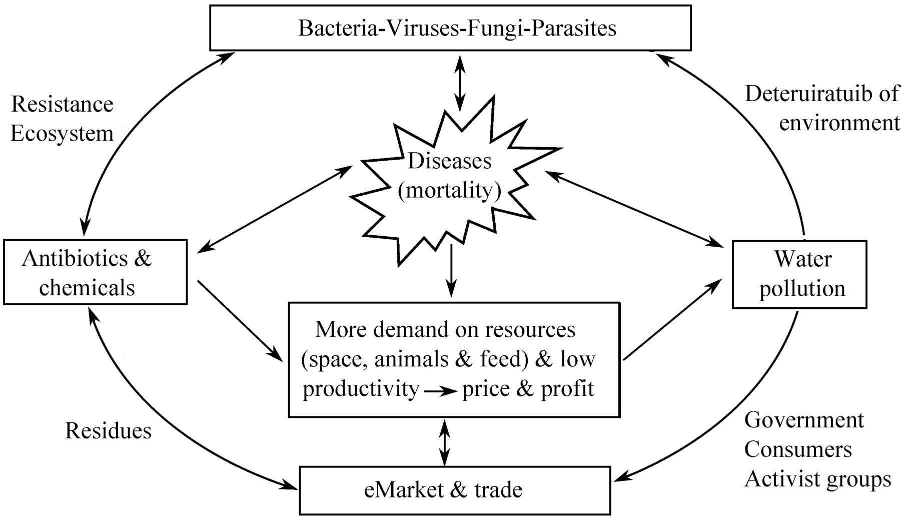

Figure 2. The negative impacts of infectious diseases on sustainability of the aquaculture industry.

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.

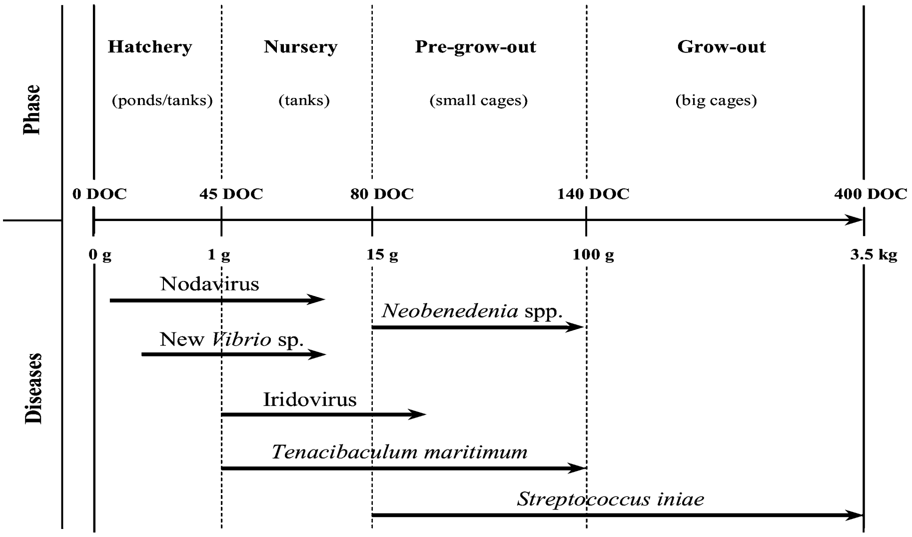

Figure 3. Major diseases affecting Asian seabass during the production cycle.

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.

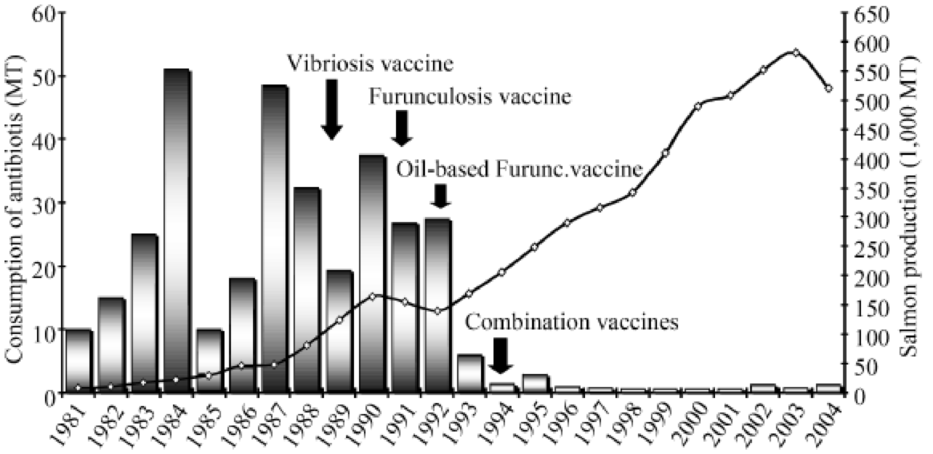

Figure 4. Norwegian salmon production, consumption of pure antibiotics and the effect of vaccines.

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.

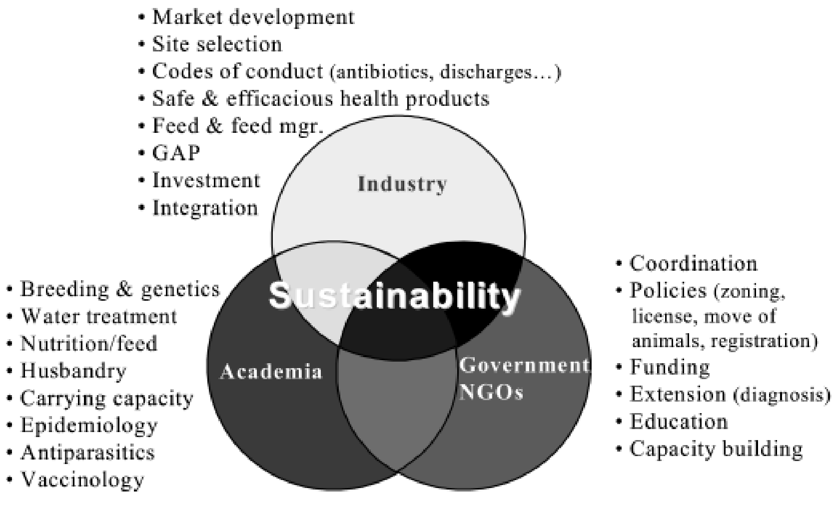

Figure 5. Sustainability is the shared responsibility of all stakeholders, including the private sector, governments and academia.

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.

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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.

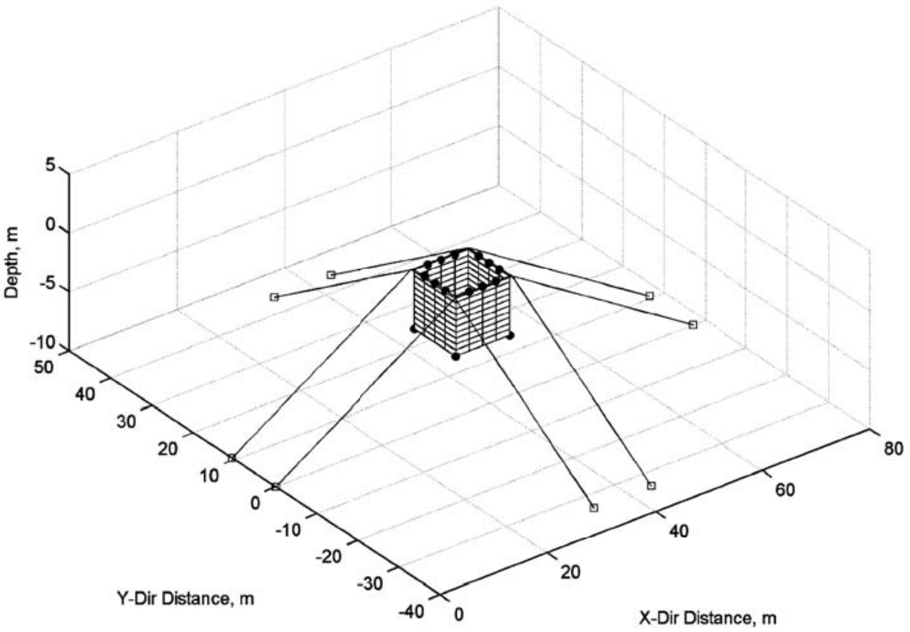

Fig.1. An aquaculture net cage system with bottom weights

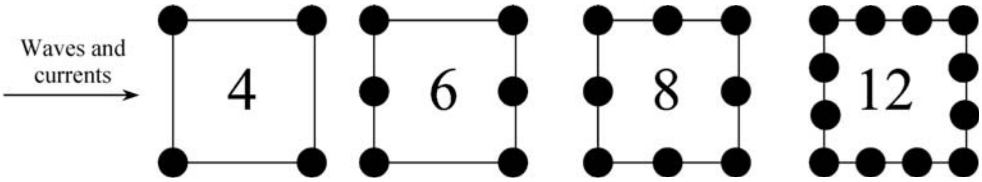

Fig.2. Four kinds of bottom weights

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.

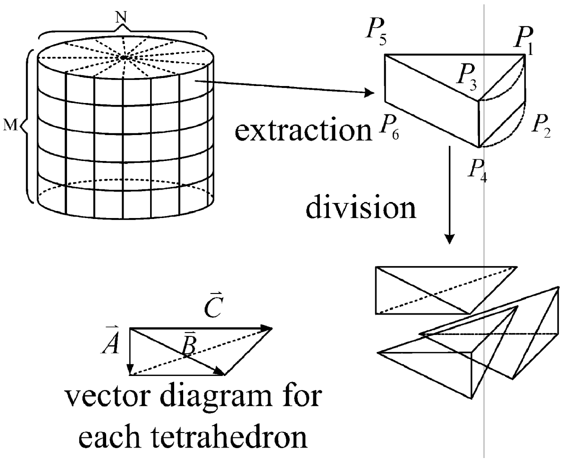

Fig. 3. Cage net volume divisions and its method of computation

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.

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

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 $\mathrm{m \bullet s^{-1}}$, 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.

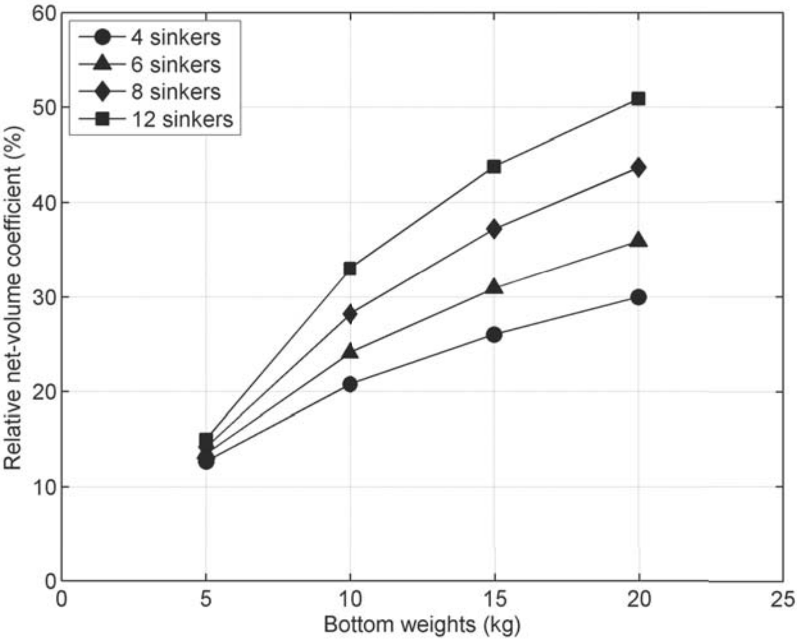

Fig. 4. Bottom weights and the corresponding relative net-volume coefficient

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.

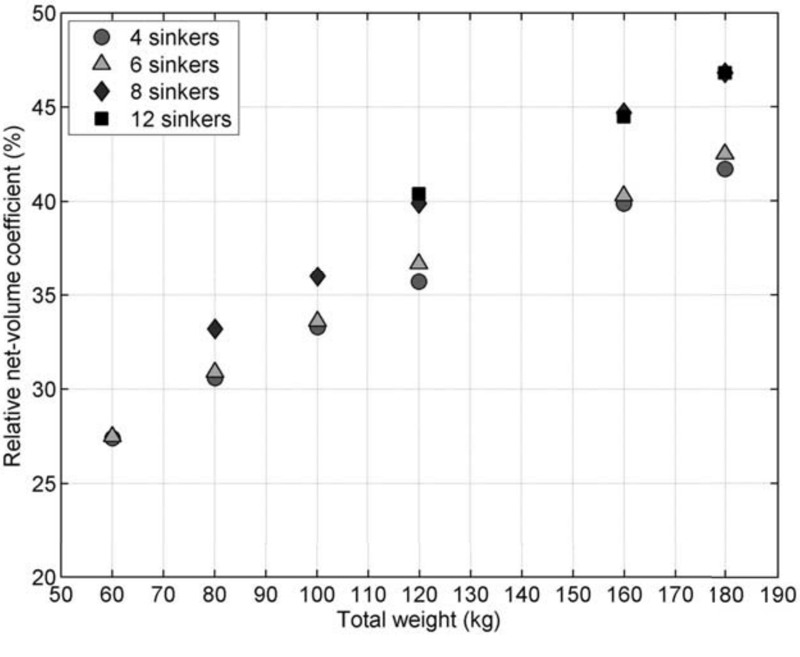

Fig. 5. Bottom weight and its corresponding deformation of cage net-volume

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.

Acknowledgment

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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).

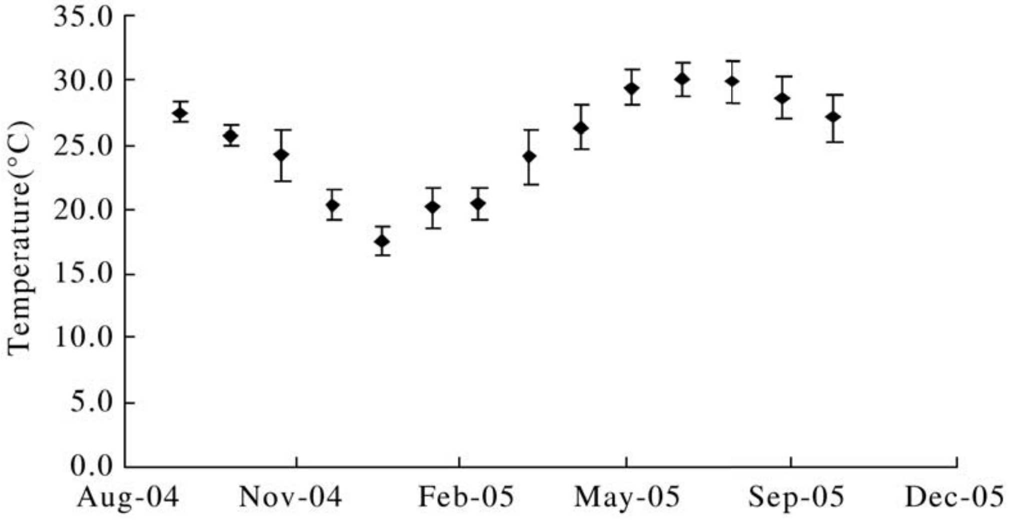

Figure 1. Sea water temperature during experimental period

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.

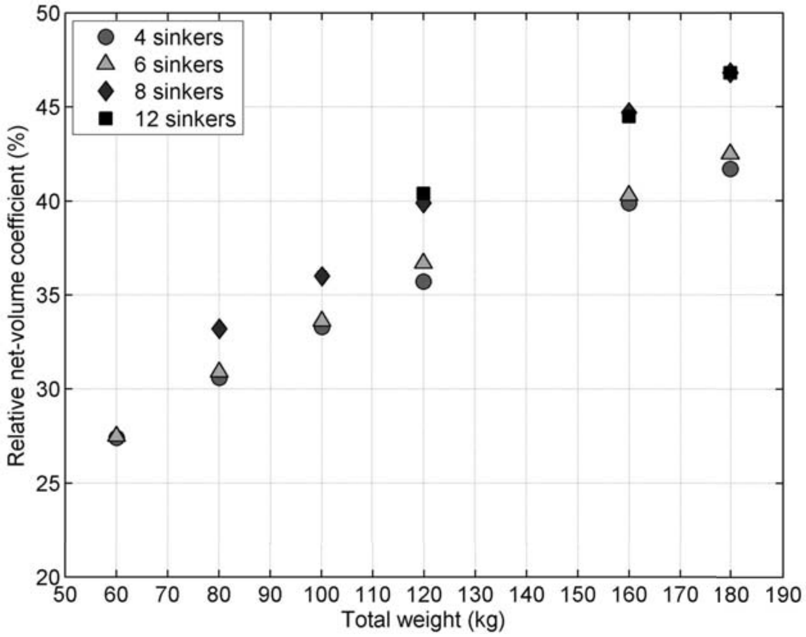

Figure 2. Weight increment of groups fed extruded feed and trash fish

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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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.

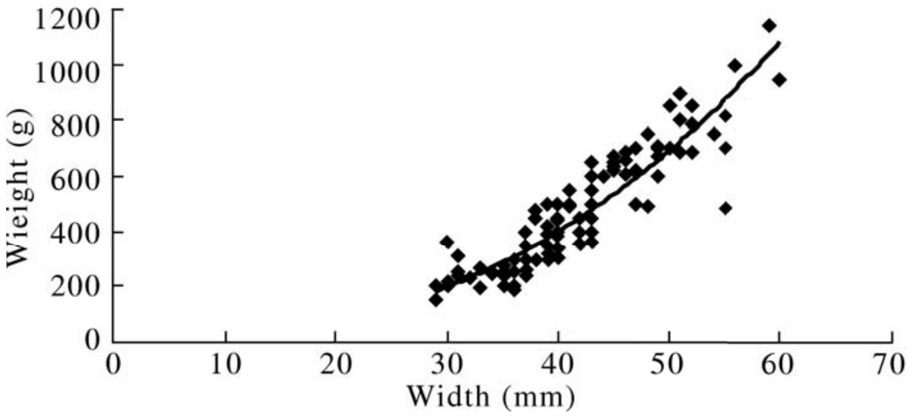

Figure 1. The correlation of weight and length of red seabream.

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.

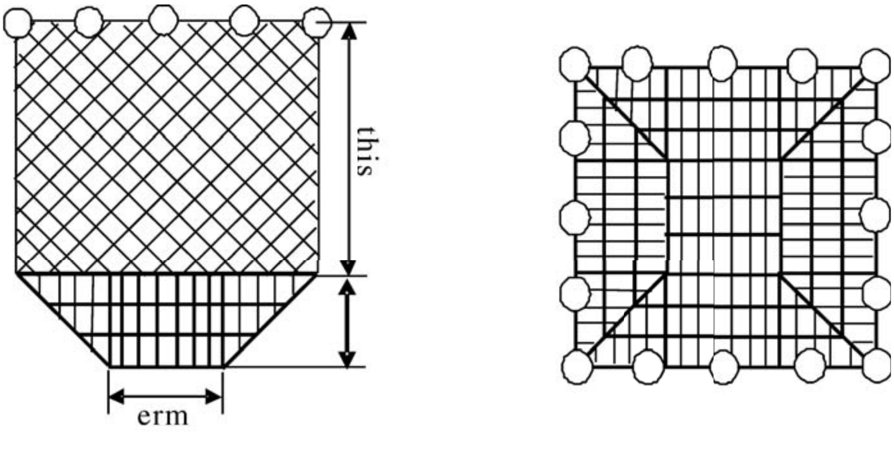

Figure 2. Side grid panel elevation (a) and platform of grids setting (b).

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.

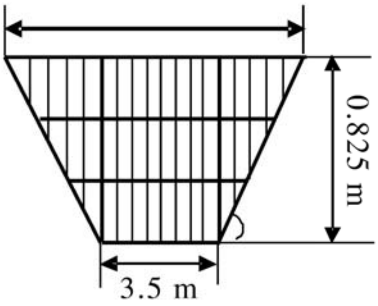

Figure 3. Side view of grid panel elevation.

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.

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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 $5\text{mg}\bullet\text{L}^{-1}$ and pH should range from 7.6 to 8.4 during hatchery stage. The normal acceptable level of un-ionized ammonia is less than $40\ \mu\text{g}\bullet\text{L}^{-1}$ 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), eurohaline (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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