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INTRODUCTION

Aquaculture, an ancient practice, is thought to have originated in China, over 3000 years ago, and Fan Li is credited for the first written account on aquaculture in 460 BC (Chinese Aquaculture Society, 1986). Aquaculture, for all intents and purposes, remained an 'art' until the second half of the last century, when the world began to realize that the increasing demand for aquatic food products could be met from the traditional supplies, in particular the marine capture fisheries. It is in this context that the science of aquaculture began to develop, and over the last 30 years this sector has recorded the highest rate of growth amongst all primary production sectors in the world (De Silva, 2001).

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Currently, the world aquaculture production is estimated at 51.4 million tons, valued at US$ 59.9 billion, and accounts for approximately 33% of all aquatic food products that are consumed (FAO, 2002). Over the last 20 years, aquaculture production has recorded a mean annual growth rate of approximately 10%, and all projections indicate that the sector is still growing, although the rate of growth has decreased, being only 6.5% between 2000 and 2001 (De Silva, 2001). Cultured finfish production accounts for over 70% of the world aquaculture production, and this is a trend that has been witnessed throughout its growth history, and it is expected to remain so to the foreseeable future. Also, the aquaculture industry is dominated by the developing world, with PR China being the main producer (FAO, 2002). Consequent to this is that the bulk of cultured finfish production is non-carnivorous species, feeding low in the food chain, and is dominated by species such as the silver carp, Hypophthalmichthys molitrix, bighead carp, Aristichthys nobilis, grass carp, Ctenopharyngodon idellus, common carp, Cyprinus carpio, rohu, Labeo rohita, catla, Catla catla and mrigal, Cirrhinus mrigla, all belonging to the Family Cyprinidae and the tilapias, notably Nile tilapia, Oreochromis niloticus, of the Family Cichlidae. These species are cultured in both tropical and sub-temperate climates. Amongst the cold-water species, Salmonid species account for the highest production, notably, Atlantic salmon Salmo salar and rainbow trout Oncorhynchus mykiss.

At present, the relevance of aquaculture as a main source of supply for aquatic food is becoming increasingly important for a number of reasons. Firstly, it is now generally accepted that the marine capture fisheries have reached a static state, and that its contribution to the world fish production is unlikely to exceed 100 million tons, at best, as nearly 50% of the commercially important fish stocks have been already overfished (Botsford et al., 1997). Secondly, and on the other hand, the aquatic fish food consumption in the world is growing (Ye, 1999; Delgado et al., 2003) and, coupled with human population growth the gap between supply and demand for these food resources is widening. It is in this regard that aquaculture will have an increasing role in narrowing this widening gap. The increase in consumption patterns for aquatic foods have been further exacerbated by relatively recent findings that finfish are a very good source of highly unsaturated fatty acids of the omega three and six series, commonly referred to as n-3 and n-6 fatty acids (HUFA), such as eicosapentaenoic (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6), amongst others. The fatty acids EPA and DHA in particular are known to have positive effects on human
health and well being, the foremost being their influence on coronary thrombosis (Stansby, 1990; Ulbricht and Southgate, 1991; De Deckere et al., 1998). The other functions thus far attribute to HUFA fatty acids include:

- as structural elements of cell membranes,
- act as precursors to eicosanoids, a heterogenous group of highly active 'local hormones',
- contribute to osmo-regulation,
- influences reproduction and egg quality,
- important for brain development, and
- important for development of vision.

Furthermore, the development of the human brain—and hence what we are today—and in particular from an evolutionary viewpoint, has also been linked to food sources rich in n-3 (EPA and DHA) and n-6 (AA) PUFAs. Indeed, a large quantum of evidence has been brought forward to show that Homo sapiens evolved not in a savannah habitat but in a habitat that was rich in fish and shellfish resources (Crawford et al., 1999). More and more medical studies are emerging on the positive aspects of fish in the diet on human health, growth and general well-being. However, no attempt will be made here to review these in detail, but suffice is to say that in all probability H. sapiens is what it is today because of its early dependence on fish.

From the above discussion it is increasingly evident that aquatic food supplies are becoming an increasingly important component in the human diet and consequently, the importance and reliance of aquaculture to fulfil some of these needs is also growing. On the other hand, the aquaculture sector cannot and will not be able to grow unabated, particularly so with limitations on land and water resources and environmental concerns. It is, therefore, important that all developments in aquaculture take into account the importance of artificial propagation and improvements to brood stocks and hence the special relevance here of this chapter.

**RELEVANCE OF FISH REPRODUCTION TO AQUACULTURE DEVELOPMENT**

The success of large-scale aquaculture production has been underpinned by access to large numbers of seed stock or juveniles for grow-out. Historically, few farmed fish species were capable of breeding naturally
in captivity (see Jhingran and Gopalakrishnan, 1974). Some species such as tilapias (Tilapia spp.), common carp, channel catfish (Ictalurus punctatus) and Asian catfish (Clarias batrachus) readily spawn in ponds in captivity. But often the ability to control spawning in these species was limited as the timing of spawning was difficult to predict and survival of eggs and larvae was highly variable (Harvey and Hoar, 1979).

In many traditional aquaculture industries, early pioneers relied on procuring stock directly from the wild with eggs, larvae, fry or juveniles being harvested. The only source of Chinese carps (grass carp, silver carp, bighead carp, black carp—Mylopharyngodon piceus and mud carp—Cirrhina molitorella) and major or Indian carps (catla, rohu, calbasu—L. calbasu and mrigal) for culture was from eggs, larvae or fry collected from streams or rivers during the spawning seasons (Pillay, 1990; Landau, 1992). Mullet (Mugil spp.) and milkfish (Chanos chanos) culture, which has occurred over centuries, was initially based on wild harvest of fry from coastal and estuarine areas (Nash and Shehadeh, 1980; Pillay, 1990; Su et al., 2002). Eel (Anguilla spp.) farming is a major industry in several Asian and European countries, yet even today farms rely solely on the capture of glass eels or elvers from the wild (Brusle, 1990; Heinsbroek, 1991; Gousset, 1992).

For other species, mature, running ripe fish were captured from the wild during the spawning seasons, and gametes were stripped and fertilized artificially. In the case of trouts (e.g., rainbow trout and brown trout—Salmo trutta) and salmons (Atlantic salmon and Pacific salmon—Oncorhynchus spp.), spawning adults were trapped and stripped during upstream spawning migrations, a technique that is still used today for some species. Adult striped bass (Morone saxatilis) were captured as they congregated during the spawning season (Landau, 1992). Until recently, seed stock of Tor spp., the mahseers, a group that is highly regarded as sport fish and considered to have a high aquaculture potential in Asia, could be obtained from hormone-induced spawning of mature females and males caught from the wild, during the reproductive season (Joshi, 1988; Ogale, 2002).

The inability to breed fish in captivity has hampered the progress of aquaculture of several important species. Relying on wild caught fish, either juveniles or spawning adults, as the sole source of seed stock for grow-out was problematic for many species and industries. Availability of spawning adults or seed stock from year to year was highly inconsistent and variable. Predicting when fish were spawning and when juveniles
could be caught was often difficult, and for many species this occurred either seasonally or for a short period only. The quality of seed stock collected was also often highly variable. Frequently demand outstripped supply, and impeded industry development. Further, dwindling stocks in the wild, associated with over-exploitation, habitat destruction, environmental degradation, etc., meant that such harvesting was unsustainable in the long term.

Undoubtedly finfish aquaculture production the world over has made great strides in the last three to four decades, and has become a stable source of finfish supplies to the global market place. It is relevant and appropriate to question how this sector managed to achieve such high production levels in a relatively short time period. Obviously, a number of factors, technical, managerial, financial, socio-economic and policy-related (Subasinghe et al., 2001), acting singly or in combination, have been responsible, and the impact of these factors being variable from nation to nation, and region to region, for the developments in the sector. However, amongst the technological factors, one of the foremost developments was the development of artificial propagation techniques, through hypophysation, which enabled the culture of finfish to be independent of the vagaries of natural seed supplies, and over the years also enabled selective breeding programs to be developed in order to improve the quality of seed stock.

Overall, presently, globally, about 50 finfish species are cultured commercially. Of these, perhaps with the exception of anguillid eels, almost all other finfish cultures are based on artificially propagated seed stocks. Therefore, artificial propagation and, hence, reproduction of all these finfish species is crucial to aquaculture.

**CONTROL OF REPRODUCTION**

In general, in cultured species a commonly used phrase is, 'closing the life cycle'. This phrase is used to denote those species in which the whole life cycle can be completed in a culture environment, and preferably when such a closing can be applied for purposes of commercial production. Closing of the life cycle enables culturists to be independent of wild stocks, except when they require genetic improvements, i.e., increase the genetic diversity, of the existing stock(s). The majority of cultured finfish species of commercial importance in the world fall into this category.

Development of fish reproduction techniques has largely been driven by aquaculture needs. Large-scale rearing is a requirement in the
domestication of animals and is needed to take advantage of genetic selection, as has been the case in agricultural industries. Achieving control of reproduction of captive fish of aquaculture importance is a major step in industry development. This eliminates the need to rely on collection of running ripe adults or seed stock from wild, provides for more predictable and reliable production, and is a substantial step forward in meeting seed stock demands of expanding industries.

However, this has not always been the case. In Australian native species, for example, development of captive breeding techniques for several species, were initially developed to produce juveniles for release into the wild to replenish stocks of species that had undergone dramatic declines in numbers and distribution in the wild and to enhance fisheries and stocking farm dams. Several of these species particularly silver perch (*Bidyanus bidyanus*) and Murray cod (*Maccullochella peeli* *peeli*), are now being farmed commercially for human consumption in Australian and other countries (Rowland and Bryant, 1995; Ingram and De Silva, 2004).

Several major reviews in reproduction cycles and control of reproduction for fish have been published (e.g., Harvey and Hoar, 1979; Lam, 1982; Shelton, 1989; Zohar, 1989, 1996; Mylonas and Zohar, 2001). The reproduction cycle of many fish species are regulated by environmental stimuli such as light, temperature, rainfall and habitat (Fig. 17.1). When the brain, through sensory receptors, receives appropriate environmental information, the hypothalamus is stimulated to produce releasing hormones (RH) which, in turn, stimulate the pituitary gland to release gonadotrophic hormones, which act on the gonads. In turn, the gonads are stimulated to produce sex steroids that are responsible for gamete development (gametogenesis) as well as secondary sexual characteristics such as nuptial colouration changes and breeding behaviour. In females, gametogenesis (oogenesis) involves multiplication and transformation of oogonia into oocytes, which then grow in size through accumulation of yolk (vitellogenesis). Following vitellogenesis, oocytes reach the final oocyte maturation (FOM) stage, and then undergo ovulation. In males, gametogenesis (spermatogenesis) comprises a phase of formation and multiplication of spermatogonia followed by accumulation of spermatozoa and their subsequent emission (spermiation). Understanding these mechanisms provides the basis for controlling and inducing reproduction, particularly through provision of appropriate environmental stimuli and administration of hormone treatments to facilitate gametogenesis, ovulation and spermiation.
Fig. 17.1 Schematic relationship of environmental and hormonal stimuli controlling reproduction in fish.
There are three general patterns of ovarian development in fish (Tyler and Sumpter, 1996). Synchronous fish reproduce only once in their lifetime (e.g., Pacific salmons and eels). Group-synchronous fish have two or more distinct populations of oocytes present and ovulate once in a season (e.g., rainbow trout, silver perch and Murray cod) or undergo multiple ovulations over a few days or weeks within the spawning season (sea bass—Dicentrarchus labrax and certain tilapias). Asynchronous fish, which have a population of primary oocytes and a heterogenous population of vitellogenic oocytes, are capable of ovulating on a regular or semi-regular basis.

In a culture environment, often in a confined space, the natural cues that trigger the reproductive process are often absent. The operative natural cues are mostly environmental, and these may be changes in temperature and day length, water quality, rainfall and associated flooding, and so on. These cues act either singly and/or in combination, and different fish species react to different cues. For example, most temperate fish species will respond to changes in day length and associated change in temperature, whereas tropical fish species may respond to flooding and associated changes in water quality. In the latter case, it is often difficult, if not impossible, to discern whether the fish respond to the flooding per se or the associated changes in water quality, such as increased turbidity.

In the absence of natural cues, the fish species can be induced to reproduce only through intervention of the hormonal cycle, which was initially achieved through hypophysation.

Early aquaculture industries relied on species that readily spawned naturally in captivity such as in ponds. Carp, were spawned and reared in China some 2,500 years ago (Landau, 1992). During the latter half of the nineteenth and early twentieth centuries, fish farms and hatcheries were established on several continents; and artificial fertilization techniques (e.g., hand stripping and mixing of gametes) were developed and used on several species such as salmonids.

Probably the most common type of reproduction dysfunction in captive fish is the failure of females to undergo final oocyte maturation (FOM) and thus fail to ovulate and spawn. Instead, oocyte development is arrested at the stage of vitellogenesis and oocytes became atretic and are resorbed. This may be due to an absence of appropriate environmental stimuli to stimulate release of hormones by the pituitary gland to, in turn, effect FOM and trigger ovulation. Another type of reproduction dysfunction
occurs when FOM and ovulation occurs but ovulated eggs are not released to the water. These fish must be manually stripped before the eggs become 'overripe' and artificially fertilized. However, the most severe type of reproduction dysfunction in captivity is exemplified by eels, which fail to undergo vitellogenesis (and spermatogenesis). In males, poor quality and/or small amounts of milt may be produced (Zohar and Mylonas, 2001).

**Environmental Manipulation**

Several species of farmed fish readily spawn in captivity, providing the appropriate environmental cues that trigger the reproductive cycle, are present. For example, some tropical grouper species, *Epinephalus* spp., tend to spawn on their own accord in culture ponds, provided that the brood stock is in good condition and the environmental conditions in the ponds, such as salinity and temperature are conducive. Other examples include tilapias, common carp and channel catfish and Murray cod. Environmental factors such as water temperature, photoperiod, light intensity, changes to volume and velocity of water, flooding, and access to spawning habitats influence the reproduction cycle of fish (Munro et al., 1990), and manipulation of environmental conditions has also been successfully used to control spawning in captive fish. Heating culture water by covering brood ponds with a hothouse structure may advance spawning events. A range of habitats and structures have been used provided to induce spawning. These include gravel for nest construction (e.g., bass —*Micropterus* spp. and some catfish species), and plants, spawning mats (e.g., strips of raffia of plastic) and spawning boxes for egg deposition or attachment (e.g., common carp, channel catfish and Murray cod). Increasing water level in ponds has been used to induce spawning in golden perch (*Macquaria ambigua*) (Lake, 1967). Environmental manipulation of temperature and photoperiod have been applied to induce spawning in fish on demand and out-of-season so as to provide a constant and reliable supply of seed stock year-round for aquaculture (Bromage et al., 1993; Carrillo et al., 1993; Bromage, 2001).

**Hormonal Manipulation**

Where environmental manipulation has had limited success or failed to induce spawning, or it has been impractical to simulate complex environmental conditions required for spawning in the hatchery, efforts have instead shifted to the application of hormones to control the
reproduction cycle. The focus of induced breeding involves the hormone induction of final oocyte maturation (FOM) and/or ovulation in female fish, induction or enhancement of spermiation in males, followed by either natural spawning or artificial fertilization. In some species, hormonal treatments have been found to be the only means of inducing maturation and ovulation in captivity. Hormone dosage regimes (number of injections, dose rates and timing of injection(s)) vary quite considerably between species. Some types of hormones and treatments may be more effective on some species than others.

Consequently, a considerable body of work has been undertaken on the major aquaculture species to identify appropriate hormone treatments and dosage regimes to optimize production of gametes.

**Hypophysation**

Hypophysation refers to induction of ovulation by injection of ground fish pituitary glands or crude extracts of pituitary glands. Houssay (1931) first demonstrated the effectiveness of this technic to induce spawning in fish, with later successes being reported by von Ihering (1937). Injection of pituitary gland extracts simulates the natural gonadotropin surge, which by-passes to some extent the effects of environmental stimuli, such as temperature or photoperiod, on gonadotropin production.

Initial problems in hypophysation related to supply and dosage, mainly due to the crudity of collection, refinement, preservation, storage and administration techniques. However, these techniques have since been developed and refined and today, carp pituitary glands (CPG), either fresh or refined and preserved, are widely used for many species, particularly for carps. Methods for use of CPG in carps have more or less been standardized and typically involve an initial injection of a small priming dose (10-20% of total dose) followed by a larger resolving dose 12-24 hours later (Lam, 1982). CPG with known purity of active ingredients and quality are now readily available from a number of suppliers. There are, however, several drawbacks to the use of pituitary glands. The potency (hormone content) varies considerably due to the weight, sex and age of the donor fish, time of year and time between collection and preservation. Continued handling of fish to administer several does of CPG may have a negative effect on spawning due to stress, and there is a risk of disease transmission between donor fish and recipient fish.
HCG

Mammalian gonadotropin and human chorionic gonadotropin (HCG), typically administered in a single dose (100-4,000 µ/kg), have been found to be effective in inducing maturation and ovulation (and spermiation) in numerous fish species (Lam, 1982; Zohar and Mylonas, 2001). Mammalian gonadotropins act directly at the level of the gonad and do not require the existence of either stores of releasing hormones or activation of the pituitary gonadotropins. Highly purified mammalian gonadotropins of standardized biological activity are widely used in domestic animal industries (pets and livestock) and as such are readily available through commercial suppliers. Yet in some species such as carps, mammalian gonadotropins are ineffective and fish pituitary extracts are more potent in inducing ovulation. Further, recent research indicates that some fish which have been regularly treated with HCG develop antibodies against HCG, which may reduce the effectiveness of this hormone in subsequent applications (Zohar and Mylonas, 2001).

GnRHa

Research in the early 1970s showed that release of gonadotropins from the pituitary gland was controlled by a hypothalamic hormone, gonadotropin-releasing hormone (GnRH) (Schally, 1978). Since then, numerous studies have shown that GnRH agonists (GnRHa), synthetic decapetides that are 30-100 times more potent in inducing gonadotropin release rather than native GnRH, are highly effective in inducing FOM and ovulation of many species. Consequently, the application of various GnRHa peptides in aquaculture has had a significant impact on controlling and improving the reliability of reproduction. In particular, the use of GnRHa has been especially effective in spawning of cyprinids.

GnRHa is most often administered by injection with either a saline solution or in the form of a sustained-release delivery system. Dopamine antagonists such as domperidone and pimozide that inhibit negative steroidal feedback on gonadotropin secretion and enhances the stimulatory effect of subsequent GnRH doses have often been used in combination with GnRHa (Zohar, 1989). A number of GnRHa peptides are now commercially available for use in finfish, such as Ovaprim (Syndel Laboratories Ltd., Canada), a synthetic salmon GnRHa combined with domperidone suspended in propylene glycol, Gonazon (Azagyl-nafarelin) (Intevet International, The Netherlands) and Ovapel (Interfish, Hungary), a synthetic GnRHa combined with metoclopramide.
With many species multiple injections of hormones (CPG, HCG or GnRHa) may be required to induce FOM, ovulation and spermiation (Zohar and Mylonas, 2001). However, the repeated handling associated these treatments can be stressful to broodstock and may adversely affect progression of FOM and spermiation, and even result in mortalities of valuable stock. Repeated hormone treatment is also laborious and time consuming. These problems may be alleviated by administration of hormones in a sustained manner, which will improve the efficacy of treatment and reduce the amount of bloodstock handling.

A range of sustained-release delivery systems have been developed for GnRHa to control FOM, ovulation and spermiation (Mylonas and Zohar, 2001). Various preparations of cholesterol and cellulose have been used to produce implantable pellets that are 'slow' (weeks) or 'fast' (days) releasing, depending on composition (Crim, 1985; Lee et al., 1986; Sherwood et al., 1988). Cholesterol implants for use in fish are commercially available (e.g., Ovaplant (Syndel Canada), which contain salmon GnRHa in a cholesterol matrix). Other hormone delivery systems include microspheres (95-500 µm diameter) of co-polymers of lactic acid and glycolic acid, wherein hormone release is immediate and can last for months depending on the ratio of lactic acid:glycolic acid and the length of the polymer, solid monolithic implants using non-degradable co-polymer of ethylene and vinyl acetate and emulsions of lipophilized gelatin with various lipid anhydrites (Mylonas and Zohar, 2001).

Although GnRHa delivery systems have been used to induce FOM, ovulation and spermiation in fish, they have also been effective in advancing and synchronising ovulation, especially in fish with multiple-batch group-synchronous or asynchronous ovarian development, and in some instances have also enhanced vitellogenesis and spermatogenesis (Crim and Glebe, 1984; Mylonas and Zohar, 2001).

While application of appropriate environmental stimuli and/or administration of an appropriate hormone treatment(s) have been shown to be important factors in inducing FOM, ovulation and spermiation in fish, the condition of broodstock also plays a major role in the reproductive cycle. Disease, poor nutrition and other stressors associated with captivity may impede FOM, ovulation and spermiation and even affect the quantity and quality of eggs and fry.

Despite the significant developments in fish reproduction, there are still major aquaculture species for which captive techniques are yet to be developed. Notably, while progress has been made in maturing and
spawning eels under laboratory conditions, large-scale seedstock production has not been achieved and production of *A. anguilla*, *A. japonica*, *A. rostrata* and *A. australis* is still based entirely on wild catches of glass eels and elvers, during their migration into freshwaters (Tachiki and Nakagawa, 1993; Ohta *et al.*, 1997; Tanaka *et al.*, 2001; Pedersen, 2003; Tanaka *et al.*, 2003). Nevertheless, the advent of induced spawning techniques has led to greater control over production through reliability of supply and quality of seedstock, and has also made it possible to apply a range of other reproduction techniques aimed to improve and enhance production. These include out-of-season spawning to provide for year-round supply of juveniles, genetic improvement by selective breeding, hybridization between closely related species, and other chromosome manipulation techniques such as ploidy induction by heat or pressure shock shortly after fertilization.

**Egg Incubation**

Egg incubation techniques are many and varied and depend mainly on the type of egg and number of eggs being incubated. Buoyant or pelagic eggs are more common to marine fish, such as sea bass and bream and porgy, though some freshwater species also produce pelagic eggs (golden perch and silver perch). Non-buoyant or demersal eggs are more common in freshwater stream-spawning fish such as salmonids, carps and catfish. Most pelagic eggs are non-sticky, but demersal eggs have varying degrees of stickiness, which allows them to adhere to various surfaces. Fecundity varies according to many factors including age, size and species. Relatively low fecund fish such as rainbow trout (2,200 eggs/kg) and channel catfish (7,000 eggs/kg) produce just a few thousand eggs per/kg, whereas as high fecund fish can spawn in excess of 100,000 eggs/kg (e.g., silver perch — 250,000 eggs/kg, common carp — 150,000 eggs/kg). Fecundity is broadly related to the level of care accorded to the eggs by the parent. High-fecund fish may simply broadcast their eggs into the environment and provide no care whatsoever. Low-fecund fish may provide varying levels of care and protection to eggs, such as laying eggs in a protected ‘nest’ and guarding the eggs from predation. Species that produce fewer and larger eggs tend to have larger and, therefore, hardier larvae than species that produce many and smaller eggs.

Pelagic eggs are typically incubated in large tanks that are hydraulically designed to ensure that the eggs remain in suspension during incubation. This may involve the use of tanks with a conical or hyperbolic base and
sufficient water flow and/or aeration to prevent the eggs from settling out of suspension (Fig. 17.2a). Incubation techniques for demersal eggs depend on the degree of stickiness. Non-sticky eggs may be incubated in a mono-layer in flat trays or baskets suspended in troughs that are provided with a constant flow of well-aerated water (Fig. 17.2b). Alternatively, demersal and semi-demersal eggs may be incubated in specially designed hatching jars such as MacDonald or Zuger jars (Fig. 17.2c). In upwelling jars such as used for trout and salmon, constantly flowing water enters the base of the jar and passed up through the eggs without disturbing them (Fig. 17.2d). It should be noted that at certain times of embryonic development, some eggs may be very susceptible to disturbance. In MacDonald jars water is injected onto the conical surface at the bottom of the jar, which causes the eggs to continually roll or tumble. This movement helps to keep the surface of the eggs clean and prevents fungal infection. For some species, when hatching is imminent or commences, the eggs are transferred from the jars to trays, baskets for hatching. Trout and salmon eggs are moved to trays when pigmented eyes become visible through the chorion (eyed stage).

Due to their nature, sticky eggs tend to be more difficult to incubate. In species where the broodstock are allowed to spawn naturally in earthen ponds, such as common carp and channel catfish, the eggs may simply be left alone to incubate and larvae or fry are harvested from the ponds following hatching. For some pond-spawning species, artificial spawning structures, such as spawning boxes or spawning mats (e.g., raffia rafts) are provided in the ponds, as for example in the case of the Australian native species the Murray cod (Fig. 17.3a). In some cases, once spawning has occurred and eggs detected, these structures may be removed from the ponds and the eggs incubated in hatchery facilities under more controlled conditions (Fig. 17.3b). In fish that are induced to spawn and hand-stripped in hatchery facilities, following artificial fertilization, the eggs may be simply transferred to flat trays or baskets, where they adhere to the sides or bottom. However, for some species, techniques have been developed to remove the adhesive layer so that eggs can be incubated as for non-sticky demersal eggs (Kowtal et al., 1986; Krise, 1988; Michaels, 1988).

In some cases with extended incubation periods, eggs may need to be treated with a fungicide to prevent fungal growth, which can greatly reduce hatch rates.
Fig. 17.2  Egg incubators: (a) 10,000 L halibut egg incubator; (b) Ewos incubation trough; (c) MacDonald jar; and (d) trout upwelling jars.
Fig. 17.3  (a) Artificial spawning structure for Murray cod. (b) Murray cod eggs adhered to mesh being placed into an incubator.
BROODSTOCK MANAGEMENT

Broodstock is the commonly used term for a group of individual fish that are used for producing the seed stock for grow-out purposes. As such, the health and well being of the broodstock is crucial for the production of viable, good quality seed stock, and especially so as aquaculture is becoming more and more independent of natural seed stock supplies, except in a few cases such as for anguillid species. As such, the management of broodstock in aquaculture is crucial to the success and well being of the sector.

Very often in aquaculture—as in the case of most terrestrial animal husbandry practices—broodstock rearing and larval and fry/fingerling production are often conducted separately from grow-out facilities (Fig. 17.4). This is to be expected for a number of reasons. Firstly, the capital layout will be higher if an operation is to have all stages of production in one entity, and secondly and more importantly, the expertise needed for maintaining and managing broodstock, and associated production of the young are widely different to those needed for grow-out operations.

Broodstock management entails a number of facets, each distinct from the other. Therefore, it is appropriate that these are dealt with separately.

Fig. 17.4 Stages in aquaculture operations (modified after De Silva and Anderson, 1995).
Broodstock Procurement and Rearing

In general, most cultured finfish species are relatively highly fecund, apart from a few exceptions such as catfishes and the tilapias. Also, broodstock of most cultured species are relatively large. For example, the average size of broodstocks of the Chinese major carps could range from 6 to about 20 kg and salmon in excess of 6 to 8 kg. Grouper broodstock can be as high as 25 kg. On the other hand, that of Nile tilapia may be in the region of 0.75 to 1.5 kg. In general, broodstock are maintained in large, indoor or outdoor facilities, depending on the species, and are rarely kept in high densities.

More often than not, the original broodstock of a species, for a facility, are obtained from the wild or from an ongoing aquaculture operation. In most operations dealing with species for which the life cycle has been closed, the original broodstock are replenished from progeny from the operation itself. However, continued reliance for replacement of broodstock from the same original stock, over a few generations, could bring about loss in genetic quality of the progeny.

Broodstock Nutrition and Egg Quality

Nutrition of the broodstock is the single most non-genetic factor that would potentially determine the viability and quality of the eggs and pre-feeding larvae (yolk-sac larvae). In fact, broodstock nutrition is a subject by itself, and has been dealt with comprehensively in the past (see for, e.g., Bromage and Roberts, 1995; Sargent, 1995). However, it is important to consider, in brief, some of the more important aspects pertaining to this area as the whole purpose of the reproductive process is lost if unviable or poor quality seed are the final result of spawning.

In finfish, the fertilized egg will have to carry all the nutrients required for its development, and the subsequent larval stages until the larvae change over from an endogenous food supply, the yolk, to an exogenous food supply. During this phase of development, crucial morphological changes occur and vital organ development, such as the brain, eyes and the skeletal system begin to take form. Also, during these stages of development, the metabolic rate is relatively high (per unit weight) and consequently for development to proceed the embryo will need to have adequate energy supplies. All of the above means that the spawned egg needs to have all the energy and other nutrient requirements needed for growth and development; in essence the mother will have to be properly
nourished to enable her to deposit all the developing embryo requirements prior to ovulation, and hence the importance of broodstock nutrition in aquaculture.

In general, most good grow-out diets used in aquaculture practices fulfil the broodstock requirements. Indeed, Sargent (1995), in his synthesis on origins and functions of egg lipids and their nutritional implications, stated very candidly, 'Fortunately, the theoretical problems of fish lipid nutrition become largely academic in practice, especially in broodstock nutrition, where, in the light of our present understanding, currently available fish oils closely approach what may be regarded as very satisfactory balance of the four fatty acid families...'. However, this may be an oversimplification, especially with regard to some diets available in the developing world, which may not be effective. A case in point was the inability to artificially propagate two mahseer species (Cyprinidae), *Tor tambroides* and *T. douronensis* in Sarawak, Malaysia, using long-term pond reared broodstock for a number of years (due to non-ripening ova), and the final success was attributed, amongst other factors, to a change in the diet, which had a suitable fatty acid profile (Ingram *et al.*, 2005).

The influence of dietary fatty acids in broodstock nutrition is the one area that has received most attention. The earliest studies, in this regard were conducted by Watanabe and his co-workers on the red seabream (*Pagrus major*), and were then reviewed by Watanabe and Kiron (1995). These studies showed the importance of fatty acid fractions in different ingredients, such as krill oil, in broodstock diets, and also the importance of Astaxanthin (3,3′-dihydroxy-β, β-carotene-4,4′-dione) on egg quality. Other studies, for example, have demonstrated the influence of the level of n-3 HUFA in broodstock diets on egg quality in the Japanese flounder, *Paralichthys olivaceus* (Furuita *et al.*, 2000); and European sea bass (Bell *et al.*, 1997); effects of 20: 4n-6 (arachidonic acid) on growth, survival, resistance to handling in gilthead seabream, *Sparus aurata* (Koven *et al.*, 2001); and the reproductive performance in male European sea bass (Asturiano *et al.*, 2001); and Bruce *et al.* (1999) emphasized the importance of n-3 and n-6 HUFA in broodstock diet development in for this species. Czesny and Dabrowski (1998) demonstrated that in walleye (Stizostedion vitreum) deficiency of n-3 fatty acids was associated with impaired development, and thus poor larval and juvenile viability. In contrast to fatty acid influences on spawning performance, egg quality and larval viability, little attention has been paid to influences of amino acid influences on these aspects. It may be that in most instances a good
quality grow-out diet more often than not satisfies the amino acid requirements needed. In this regard, Gunasekera et al. (1998) suggested that the 'swollen yolk sac syndrome (SYSS)', in Murray cod was related to broodstock nutrition, resulting from a cumulative dietary deficiency of some of the essential amino acids.

In general, it can be concluded that a good, effective grow-out diet, with the appropriate amino acid and fatty acid balance can be used for broodstock. However, there could be exceptions and regular monitoring will ensure that ovarian development progresses as expected.

Egg quality determinants could be physical (diameter, weight, shape, specific gravity and colour), structural (membrane stability, quantity of yolk, location of the yolk sac) and chemical. For all intents and purposes, physical and structural determinants could be considered to be genetically determined to a very great extent. For example, the egg diameter of cultured finfish species could range from as much as small as 0.2 to 0.5 mm to a few mm, as in the case of most cyprinids and salmonids, respectively. Within each species, the egg diameter will have a narrow range, genetically determined. The specific gravity of the eggs would determine whether they are free floating and or demersal, each of these also being a species characteristic. Most cultured cyprinids, except common carp, lay free-floating eggs, or mildly adhesive eggs, often less dense than water, whereas salmonids lay demersal eggs, which are denser than water.

The most important aspect of the egg quality, particularly from a broodstock nutritional viewpoint, is the chemical composition. The gross chemical composition often does not vary to a great extent amongst species; in all species the eggs tend to have a protein content (by dry weight) ranging from about 60 to 70%, and the biggest difference occur in the amount of water in the egg. Numerous studies on the fatty acid composition have been conducted on eggs of different species of fish, and all such studies indicate the relatively high proportion of n-3 and n-6 HUFA in the lipid component, indirectly indicating their importance to egg development and larval well-being. Obviously, and as expected, there are species differences the most notable being the n-3 to n-6 ratio in fresh water and marine species, it being higher in marine species and vice versa, reflecting the basic nutritional requirement differences in the two groups of fish.
Genetic Aspects

Selection of broodstock

Selection of broodstock is the initial requirement for any breeding program and often depends on the objectives of the programs. For example, if hatchery production is for the purpose of restocking or stock enhancement then it is often encouraged that broodstock should be acquired from the local populations in the environment where the juveniles are being released. The main reason for this is that introduction of exotic populations into a new environment may have adverse effects on the native/local gene pool. Also, it may capitalize on any adaptation to local conditions (Shaklee, 1983).

If the objective of the breeding program is for food fish production, many factors should be taken into account in broodstock selection. Firstly, the species of interest must be fully domesticated, e.g., full life-cycle is controlled in captivity. Secondly, selective breeding program should be undertaken only when aquaculture of species under consideration is sustainable. Thirdly, there should be genetic variations associated with traits of commercial interest. With the advanced developments in the field of molecular genetics, it is encouraged to measure levels of genetic variation within broodstock, if possible, at the initial stage of domestication of a new species for aquaculture, as it would help to develop an appropriate management strategy and to monitor genetic changes between generations. It is important to establish a base-line broodstock population with a significantly wide level of genetic variation. However, if there is no or little genetic variation in the initial founder broodstock, one possible solution is to introduce new genetic material by bringing in individuals from alternative populations or broodstocks. In such instances, care needs to be taken to ensure this does not risk the inadvertent introduction of alien individuals into the wild.

Broodstock management

From a genetic perspective, broodstock management is mainly aimed to avoid problems associate with inbreeding and random genetic drift. Inbreeding is defined as the mating of closely related individuals and random genetic drift is random changes in gene frequency that occur as a result of sampling error (e.g., broodstock selection). It is common that deleterious recessive alleles are hidden in heterozygous individuals, and inbreeding will provide an opportunity for these alleles to combine together in offspring, where potentially lethal phenotypes will begin to be expressed.
Negative impacts on productivity, such as reduced fecundity, reduced
disease resistance, reduced survival of seed stock and increased incidence
of abnormalities, can also be brought about by limited genetic variability
resulting from inbreeding and genetic drift.

Avoiding inbreeding and random genetic drift is critical for the
maintenance of genetic variance in cultured stocks. It is a problem that
aquaculture of highly fecund species, such as Indian and Chinese carps,
as well as marine species such as grouper are likely to encounter. Because
of the high fecundity of these species, generally, there is a tendency to
use a fewer number of broodstock to meet production targets. Furthermore,
as considerable volumes of fry and fingerlings are produced in backyard
hatcheries—as often practiced in developing countries—there is more
likelihood for the broodstock numbers maintained and used in such
practices to be less than desirable, an almost unavoidable consequence
of the practices. Consequently, genetic problems associated with small
gene pools, such as inbreeding, have a greater probability to occur.

Inbreeding is measured by the 'inbreeding coefficient', \( F \), and the
objective is to prevent \( F \) from reaching 0.25—the level where inbreeding
depression is likely to occur in fish (Dunham, 2004). The simplest method
to calculate the accumulation of inbreeding per generation with random
mating is as follows:

\[
F = \frac{1}{8N_{em}} + \frac{1}{8N_{ef}}
\]

where \( N_{em} \) and \( N_{ef} \) are numbers of males and females that successfully
breed, respectively.

Avoidance of inbreeding is often primarily resolved around population
size. Maintaining effective population size \( (N_e) \) together with avoiding
mating among closely related individuals of a hatchery stock are important
measures that are generally recommended for controlling genetic erosion
in hatchery produced seed. Genetic variability decreases rapidly if the
effective population size of the broodstock is small.

In a random mating population, effective population size is calculated
as follows:

\[
N_e = \frac{4N_{em} \cdot N_{ef}}{N_{em} + N_{ef}}
\]

As such, the effective population size can be increased in one of two
ways: (1) increase the number of breeding individuals; and (2) bring the
breeding population close to 1:1 sex ratio.
Effective population size is an important concept in broodstock management, as it is inversely related to both inbreeding and genetic drift. When $N_e$ decreases, inbreeding and variance in changes of allele frequencies resulting from genetic drift increase. The relationship between inbreeding coefficient $F$ and effective population size $N_e$ is described below:

$$F = \frac{1}{2N_e}$$

Genetic diversity of bottlenecked broodstock can be increased without bringing in new brooders, as described by Doyle et al. (2001). The mean relatedness of each potential breeder to the whole population is estimated using microsatellites, by the formula proposed by Ritland (2000). A subset of breeders is then selected to maximize the number of founder lineages, in order to carry the fewest redundant copies of ancestral genes. This approach is particularly effective when the available number of captive broodstock is small (e.g., endangered species).

To estimate relatedness between pairs of individuals, an indicator variable $\delta_{ij}$ ('Kronecker operator') is used. At each diploid locus, two paired individuals have four alleles, denoted by $A_i$ and $A_j$ for the first individual and $A_k$ and $A_l$ for the second individual. If allele $A_i$ and $A_j$ are the same then $\delta_{ij}=1$, otherwise $\delta_{ij}=0$. There are six $\delta$s among the four sampled alleles, one for each comparison between two alleles, both within and between individuals. Pairwise relatedness is estimated as:

$$\hat{r} = \frac{[\delta_{ik} + \delta_{il}]/p_i] + [(\delta_{jk} + \delta_{jl})/p_l] - 1}{4(n-1)}$$

The mean kinship of the $i^{th}$ individual, $m_{ki}$, is the average kinship values for that individual with every individual in the population, including it. A low mean kinship value indicates that an individual has few relatives in the population, and thus is valuable in maintaining genetic diversity.

Apart from genetic monitoring, some other strategies can be applied to maintain genetic diversity. For example, fertilization of a batch of eggs with sperms from several males can help to maximise $N_e$. The result of mixing of sperms from several males to fertilize eggs may not be desirable as sperms from one male may be more competitive and, thus, dominate the fertilization process. As such, it might be more practical to divide eggs from one female into sub-samples then fertilize each sample with sperms from different males (Tave, 1993). Recently, cryopreservation of
sperm has become routine for some species, which enables the hatcheries to use stored sperm from a large number of males.

**GENETIC MANIPULATIONS IN AQUACULTURE**

**Selective Breeding**

It is an accepted fact that selective breeding and consequent improvements have enabled the current production levels from husbanded terrestrial animals such as poultry, cattle (meat and milk), pigs, etc., to be achieved, the genetic improvements often contributing more than 25% to the overall increases in productivity. On the other hand, in respect of cultured aquatic organisms, significant and notable improvements in production through genetic improvements have been achieved in the case of Atlantic salmon and trout in Norway, and is reputed to be similar to those with livestock and poultry (Gjoen and Bentsen, 1997). Similarly, a multi-nation effort on selective breeding on Nile tilapia in Asia have resulted in the production of the GIFT Strain (Genetically Improved Farmed Tilapias), that is known to perform considerably better than those used previously in most Asian countries (Gupta and Acosta, 2004). Gupta and Acosta (2004) reviewed the development of the GIFT strain in Asia, using fresh germ plasm from Africa, and the socio-economic impacts and the future of the tilapia culture industry based on these improved strain.

In general, however, genetic improvement of cultured finfish species have lagged far behind that of husbanded terrestrial animals. Perhaps, one of the primary reasons is that the number of commercially important cultured species in the world exceeds 50, and to develop selective breeding programs for each of these species will take a considerable amount of time and effort.

Most of the economically important traits in relation to aquaculture are those described as being quantitative. These characteristics can be measured (e.g., body length, body weight, growth rate, etc.), in contrast to qualitative characteristics, which are descriptive (e.g., albino, black, spotted, unspotted, etc.). Common quantitative characteristics include body weight, length, fecundity, proximate composition, dressing percentage, etc. The nature of quantitative measurements means that such traits are continuously distributed throughout the population, or in other words, distribution of the frequency of these traits follows a normal distribution (Fig. 17.5). Quantitative traits show continuous distributions because of two reasons: (a) the large number of genes and alleles involved
and the various interactions they have with each other; and (b) the environment.

The population variation for a character is expressed as $V_p$, and this is a sum of the genetic variance ($V_G$), the environmental variance ($V_E$), and the variation contributed by the interaction between the environment and the genes ($V_{G-E}$). As such:

$$V_p = V_G + V_E + V_{G-E}$$

The genetic variance, $V_G$, is the one of most interest. $V_G$ or genetic variance can be further partitioned into three components: these are $V_A$, $V_D$ and $V_I$, which represent additive, dominance, and epistatic genetic variance, respectively.

$$V_G = V_A + V_D + V_I$$

Of these three components of $V_G$, $V_I$ is difficult to exploit; therefore the two important sources are $V_D$ and $V_A$. They are very different and are exploited in different ways: $V_D$ by hybridization, and $V_A$ by selection.

Selection is the process of selecting individuals or families, which have desirable characteristics, then breed from them to try and change the population mean for one or a number of quantitative traits in the next generation. Fish that do not meet the minimum selection criteria will not be crossed and their alleles will not contribute to future generations. One important parameter in this process is the heritability, which is an
indicator of the contribution to the total population variance ($V_p$), which is made by $V_A$. Heritability is designated by $h^2$:

$$h^2 = \frac{V_A}{V_p}$$

Values of $h^2$ range from 0 (where phenotypic variance is entirely the result of environmental effects) to 1.0 (where phenotypic variance is the result of genetic effects).

Heritability can be estimated by various methods. The first method is parent-offspring regression, which involves a series of matings and all families being reared under the same environmental condition. A scatter plot is carried out, each point presents one family (mean of parent mean versus mean of offspring of a particular trait), and the resulting slope of the regression gives a measure of the heritability of the trait. The second method involves the design of fill-sib and half-sib mating experiments, the full details of which can be obtained from Falconer (1981) and Kearsey and Pooni (1996). However, the only method available to estimate the true heritability is to carry out trial experiments and measure the actual response to selection is schematically represented in Fig. 17.6.

Several selection strategies are available for genetically improving performance of fish. These include:

- **Mass selection, or individual selection:** This is a strategy where the selection of breeding individuals is based on their phenotype, involving the selecting the best-performing individuals for the particular trait of interest from a population, and the next generation will be produced from these selected individuals. It is the simplest type and the most frequently applied selection strategy for fish. However, it can only be used to improve traits that are recorded on the breeding candidates while they are still alive (e.g., growth rate, body shape, colour), and it is not efficient to improve discrete traits with low heritability such as survival rate. Also, mass selection may encounter the problem of inbreeding, which can be prevented by using only a restricted number of individuals from each full and half-sib family to be tested as breeding candidates when applying mass selection.

- **Family selection:** In family selection, the breeding individuals are ranked on the basis of records taken on their full and half-sibs. This selection strategy can be applied to improve traits that cannot be recorded in the breeding individuals while they are alive (e.g., carcass quality traits),
traits that can only be recorded for groups of fish (e.g., feed utilization),
and discrete traits of high or low incidences (e.g., survival rate, age at
sexual maturity). Furthermore, family selection is more effective than
mass selection when the heritability of a trait is low (<0.30). However,
family selection requires that each family be produced and kept separately
and that all fish are tagged before testing in common test environment.
This may not be a major issue with the advanced developments in
molecular genetic techniques, from which molecular markers can be
generated and then used as tags for these purposes.

Usually, genetic breeding programmes are designed to obtain desired
traits such as fast growth, delay of early sexual maturity, disease resistance
and carcass quality, etc., and is mostly accomplished through traditional
genetic improvement methods (Tave, 1993). The more recent availability

Fig. 17.6 Response to selection (modified from Beaumont and Hoare, 2003).
of molecular genetic techniques offers several ways of improving the efficiency of these breeding programmes and is increasingly being incorporated into many of them. Phenotypic characteristics of fish species likely to be most important to genetic improvement programs in the aquaculture industry will almost always be inherited quantitatively (Falconer, 1981), making it difficult to determine the genetic basis of a desirable trait (Ferguson, 1995). The new DNA-based technologies are very powerful in identifying marker loci associated with nuclear loci that control commercially important traits (i.e., quantitative trait loci, or QTLs). Once such markers have been identified, they can be used in selection programs (marker-assisted selection, or MAS). Lande and Thompson (1990) used theoretical analyses and suggested that molecular genetic polymorphisms could be incorporated into traditional methods of artificial selection to achieve substantial increases in the efficiency of selective breeding.

**Hybridization**

In aquaculture (and in stock enhancement) inter-specific hybridization is utilized for many purposes, either singly and or in combination. These purposes include:

- Increase growth rate,
- Transfer and or combine desirable traits between species into a single group,
- Reduce unwanted reproduction through production of sterile or mono-sex progeny,
- Increase harvestability/production, and
- Increase environmental tolerance and general hardiness in culture environments (Bartley et al., 2001).

Bartley et al. (2001) listed 35 commonly used hybridizations in aquaculture and stocking programs, and included cyprinids (4), salmonids (5), tilapia (5), miscellaneous freshwater fish (6), miscellaneous marine and diadromous fish (10) and catfish (5). We will not attempt to expand on these hybridizations except to focus on selected examples. An instance of using hybridization to obtain a better growth/production and overall performance that exceeds that of either of the parent species, as well as better flesh quality is the use of hybrids of the Thai walking catfish *Clarias macrocephalus*, and the African catfish, *Clarias gariepinus*. This hybrid, the culture of which has became very popular with in a decade or so, is
almost exclusively used in Thailand catfish culture operations, and the hybrid is reputed to grow faster, to a larger size and be of superior flesh quality (Na-Nakorn, 2004). On the other hand, the continued use of hybrid catfish in Thailand has had negative consequences on the native catfish populations, in particular through its impact on the native gene pools (Na-Nakorn et al., 2004; Senanan et al., 2004).

The use of inter-specific hybridization between closely-related species carried out to produce progeny of a single sex, with better growth performance and also to avoid prolific, unwanted breeding resulting in overcrowding of the culture systems, and finally leading to stunting of the reared stock(s), is best exemplified in tilapia culture. Indeed, hybridization amongst tilapia species was one of the first instances when hybridization was utilized in aquaculture (Hickling, 1960, 1963). Most species of tilapias are prolific breeders, and also in general in most tilapia species males grow faster and perform better than the females. In order to avoid overcrowding and consequent stunting, as well as to utilize the faster growth trait of males, hybridization between tilapia species that results in preponderance of males was utilized, commencing in the early 1950s. For example, female O. mossambicus to male O. hornorum (Hickling, 1960, 1963) cross generated nearly all-male hybrids, and was followed by other crosses, some of which resulted in all male and/or nearly all male hybrids. Numerous crosses, mostly those that produced a preponderance of males, have been utilized in tilapia culture and are summarized in Table 17.1.

Table 17.1 Summary of different hybrid combinations that have known to produce monosex male progeny (from Mair, 2001).

<table>
<thead>
<tr>
<th>Female parent</th>
<th>Male parent</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. niloticus</td>
<td>O. aureus</td>
<td>Applied commercially but results inconsistent</td>
</tr>
<tr>
<td>O. niloticus</td>
<td>O. macrochir</td>
<td>—</td>
</tr>
<tr>
<td>O. niloticus</td>
<td>O. urolepis hornorum</td>
<td>Majority of broods are all male; some commercial application</td>
</tr>
<tr>
<td>O. niloticus</td>
<td>O. variables</td>
<td>All progenies monosex</td>
</tr>
<tr>
<td>O. mossambicus</td>
<td>O. aureus</td>
<td>—</td>
</tr>
<tr>
<td>O. mossambicus</td>
<td>O. urolepis hornorum</td>
<td>All progenies monosex</td>
</tr>
<tr>
<td>O. spilurus niger</td>
<td>O. macrochir</td>
<td>—</td>
</tr>
<tr>
<td>O. spilurus niger</td>
<td>O. urolepis hornorum</td>
<td>All progenies monosex</td>
</tr>
<tr>
<td>O. aureus</td>
<td>O. urolepis hornorum</td>
<td>—</td>
</tr>
<tr>
<td>T. zillii</td>
<td>O. andersonii</td>
<td>All progenies monosex</td>
</tr>
</tbody>
</table>
Overall, although a large number of inter-specific hybrids are used in aquaculture, those currently making an impact on production amounts to about 35, the most significant being Thai catfish culture and characid hybrids in Venezuela and tilapia in Israel, which in order contribute about 80, 29 and 100% to the production of these groups in each of these countries. Bartley et al. (2001) evaluated the 'status of hybridization' from a genetic viewpoint and concluded that hybridization does not fall within the realm of 'genetically modified organisms'. However, these authors stress the need for proper broodstock management, maintenance of correct parental lines, avoidance of inbreeding and inadvertent hybridization and or backcrossing to fully utilize this method of genetic improvement in aquaculture.

**Sex-reversal Using Hormones**

Initially, sex-reversal was conducted on progeny, through hormonal treatment (e.g., bath, feed or implant depending on developmental and culture characteristics of species) to utilize the faster growth rate of one of the sexes, e.g., males in tilapias, and also to avoid undesirable breeding amongst grow-out stocks, resulting in lower production and stunting of the stocks. Some androgenic hormones have been used to produce monosex male populations, and the most efficacious and widely used hormone is 17α-methyltestosterone (Yamazaki, 1983; Dunham, 1990). Several oestrogenic compounds are used to produce female monosex populations. An example of this is the use of hormone 3-oestradiol in producing all-females populations in salmonids species (Ashby, 1957; Donaldson and Hunter, 1982). The use of hormones on sex-reversal of progeny is beyond the scope of this chapter and the readers are referred to recent reviews on the subject (Pandian and Sheela, 1995; Penman and McAndrew, 2000).

**Chromosome Manipulation**

**Polyplody**

Polyplody has been well researched in fish. Individuals with extra sets of chromosome are called polyploid. The common individuals with two sets of chromosomes are diploid, while triploid refers to individuals with three sets of chromosomes and tetraploidy are those with four sets. Of these, triploidy is the major focus of aquaculture.

From the aquaculture point of view, culture of triploid fish is advantageous as triploid production has great potential to enhance
performance in fish. Potential benefits of triploidy include improved feed conversion ratio, higher survival rate and greater growth rate. Triploid individuals are sterile; as such reduced gonadal development may allow energy that are normally used in reproductive process to be used for growth of somatic tissues (e.g., the case of tilapia). Sterile characteristic of triploid also helps to minimize the potential risks to endemic wild populations of fish in areas where exotic species, hybrids or transgenic stocks are being cultured.

Triploids can be produced in two different ways. The most common method used in fish to produce 'meiotic' triploids involves applying either thermal, pressure or chemical shock to newly-fertilized eggs with the resultant disruption of the mechanisms that would otherwise force the second polar body out of the egg. Alternately, 'interploid' triploids can also be produced by crossing tetraploid (4N) individuals with normal diploids. Tetraploid fish produce diploid (2N) gametes, when fertilized with normal haploid gametes (1N) will form triploid (3N) zygotes.

Many attempts have been made to produce triploids of many fish species that are of importance in aquaculture. It is reported that the heat-shock induced triploid Oreochromis niloticus showed similar growth rates to the diploid counterpart up to the age of maturation, however, at the end of the experiment, triploid males and females exceed their diploid counterparts by an average of 95% and 66% on body weight, respectively (Brämick et al., 1995).

However, it is also noted that the results are not always desirable nor consistent. For example, in a study of meiotic triploid common carp, Cherfas et al. (1994) reported that overall survival of heat-shock treated triploids was only approximately 70% of that observed in diploid controls by one year of age. In addition, although triploids appeared to be functionally sterile, their mean body weight was approximately 85% of that of diploid controls.

**Gynogenesis and androgenesis**

With increasing opposition to use of androgens and oestrogens in animal husbandry, there has been an attempt to develop fish broodstock that would continue to produce monosex progeny, through the use of phenotypic males or females. As a sequel to the hormonal sex-reversal of tilapias, and the relative inconsistency in the production of single sex progeny through hybridization, and with a greater degree of understanding of the sex-determination systems in a number of the key species (Mair and Little, 1991; Mair et al., 1991), it became possible to develop
techniques for the production of genetically male tilapia (GMT), initially of *O. niloticus* (Scott *et al.*, 1989; Mair *et al.*, 1991). A schematic representation of the steps involved in the production of genetically all male tilapia is given in Fig. 17.7. This technique is known to produce all males, and at worst only a small percentage of females. Genetically male *O. mossambicus* have also been produced, but in this instance through gynogenesis of XY neofemales (Varadaraj and Pandian, 1989). The technique of producing GMT, particularly in the case of *O. niloticus*, is well established and is now commercially adopted, and has been achieved through several generations of breeding (Mair and Little, 1991).

Similarly, using a combination of sex-reversal and gynogenesis can also produce all female progeny. If the female is homogametic sex, the gynogenetic progeny are all XX. If the fry are sex-reversed to phenotypic males and if these are fertile, they can be mated with normal XX females to produce all XX female offspring. The technique has been applied to produce all female progeny in grass carp (Boney *et al.*, 1984; Shelton, 1986), and silver barb, *Puntius gonionotus* (Pongthana *et al.*, 1999).

**Gene Manipulation**

One of the most recent advances in genetic improvement of aquatic organism is the application of gene transfer technologies, or often referred

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**Fig. 17.7** Schematic representation of the steps involved in the production of supermale tilapia.
to as transgenesis. The technique involves the incorporation of genes from one individual into the nuclear DNA of another, which could be of the same or different species. Transgenesis was first successfully demonstrated in 1982 when a rat growth hormone gene was incorporated and expressed in mice (Palmiter et al., 1982). Not long after, production of transgenic fish were reported, e.g., rainbow trout (Maclean and Talwar, 1984) and goldfish, *Carassius auratus* (Zhu et al., 1985). To date, transgenic experiments have been performed on approximately 20 fish species worldwide.

Simply, transgenesis involves the introduction of foreign DNA into the nucleus of a host cell, which can also be a newly fertilized egg. The foreign DNA is often known as a DNA construct, which typically includes: (1) a promoter which acts to switch on and off the transcription and translation of the target gene, (2) a structural gene or target gene, (3) a reporter gene to enable to ascertain whether the target gene has combined successfully with the construct, and (4) a termination sequence. If the promoter does not precede the structural gene, there is possibility that the target trait may not be expressed in the transgenic individual.

Various techniques have been used to produce transgenic fish, and procedures are often straightforward. Details on these techniques are summarized as follows:

- Microinjection is the most widely used method in producing transgenic fish. A large number of transgenic fish from a wide range of species (e.g., Atlantic salmon, coho salmon, trout, tilapia, carp, catfish) have been produced using this method. The technique involves the use of a very fine glass tube to introduce a number of copies of constructs directly into newly fertilized eggs; the constructs are then incorporated into the chromosome before the first cell division.

- Electroporation involves the suspension of cells in a solution containing high concentration of DNA constructs, then high voltage pulses of electricity is applied to open pores in the cell membranes, through which foreign DNA can pass.

- Biolistic or biological ballistics, involves coating microscopic particles, usually of gold or tungsten, with DNA constructs and explosively firing these particles directly into the cell through the cell membrane.

- Lipofection or liposome-mediated transfection, involves the encapsulation of DNA constructs in synthetic lipid vesicles and
subsequently bringing the vesicles into contact with the target cell.

- Sperm-mediated transfer involves the binding of the DNA construct to the outer coat of spermatozoa before it fertilizes the egg. This technique has the potential to be one of the most favourable gene transfer methods because of the nature of the procedure, and counters the problem of limited number of DNA constructs that entering the egg as there is only one sperm can enter the egg at a time.

A number of genes with useful aquaculture traits have been successfully transferred into genomes of various aquaculture species. The gene that is of most interest in aquaculture is that of the growth hormone (GH). In fish, most work done to date has involved injecting a mammalian GH gene and mammalian metallothionein promoter into an embryonic fish. The overall conclusion is that growth rates of GH transgenic fish are significantly higher than that of non-transgenic controls. For example, GH transgenic salmonids were reported to grow 3-5 times faster than non-transgenic fish (Devlin et al., 1994). In addition, considerable effort has been expended to introduce the antifreeze protein genes (from winter flounder, Pleuronectes americanus) into other species, such as Atlantic salmon (Hew et al., 1999), goldfish (Wang et al., 1995), Nile tilapia, and milfoil (Wu et al., 1998). A number of other target traits such as salinity tolerance, sterility, disease resistance (Mialhe et al., 1995), and greater production of n-3 HUFA (Donaldson, 1997) also offer considerable potential for transgenic work. Although all of these still need a great effort, they have the potential to bring great benefits to the aquaculture industry.

One of the major issues relating to the expression of the transgene is its integration into a position on a chromosome. The factors determining sites of integration are still poorly understood. Transgene expression in fish is highly variable, even among fish independently transformed with the same construct. There is also no guarantee that primary transgenic fish showing strong transgene expression will give rise to progeny with the same characteristics.

Criticisms over the use of transgenesis, however, have been raised with concerns regarding the risks brought about to human health, biodiversity, animal welfare and poor communities (Dunham, 1999; Maclean and Laight, 2000). Transgenesis being a process that falls within the realm of genetically modified organisms (GMO) it has to encounter the ethical issues that confront all GMOs which are increasingly subjected
to public scrutiny and controversy. The final decisions on the wider application of transgenesis in aquaculture will finally be a political decision, and in this regard its applications in aquaculture will not be different to other food organisms.

In conclusion, it is evident that aquaculture, an important global food industry, has been able to sustain a very high growth rate, indeed the highest amongst all food-producing sectors (De Silva, 2001), primarily as a result of the developments of artificial propagation techniques for most of the important cultured species, thereby making it independent of wild seed stock. In the foreseeable future the developments in this regard are likely to mostly focus on increasing the efficacy of hormones and or their analogues used in hypophysation, and in larval rearing techniques, particularly of some marine fin fish species, in which the larval survival is very low. However, the most important developments will perhaps be related to the use of modern genetic techniques in improving broodstock management, which will contribute significantly to underpinning the overall sustainability of the aquaculture industry in the long term.

References


