

## 8

# Reproduction and Genetic Selection

As discussed in Chapter 5, one of the major criteria in selecting a species for culture is the existence of either suitable techniques for controlled breeding or easy availability of spawn, larvae or juveniles from natural breeding grounds. Even when culture can be initiated using 'wild seed', it is essential to achieve controlled reproduction as early as possible, to ensure timely availability of young ones in adequate numbers for large-scale rearing. It is also a basic need in the domestication of the animal and for taking advantage of the benefits of genetic selection and hybridization that have contributed so much to terrestrial agriculture and animal husbandry.

Controlled breeding will obviously be possible only if there is adequate knowledge of the factors governing reproduction of the animal and its breeding behaviour. Lack of such knowledge has hampered the progress of aquaculture of several important species. The extensive culture of Chinese carps, Indian carps, mullets, milkfish, sea-bass, sea-bream, penaeid shrimps, oysters and mussels has been based until recently on 'seed' obtained from natural breeding. Despite advances made in techniques of controlled or semi-controlled breeding, the techniques have not been sufficiently perfected or adapted for large-scale production of seed, with the result that the aquaculturist has still to depend partially or entirely on natural seed resources. There are also species like the eels for which no propagation technique has so far been developed, even though some progress has been made in maturing and spawning under laboratory conditions.

Among the aquaculture species, finfish as a group has received greater research attention

in controlled reproduction. The reproductive cycles of almost all fish are regulated by environmental stimuli. Appropriate sensory receptors convey the environmental stimuli to the brain in the form of neural inputs. This neural information, on reaching the hypothalamus, causes the release of hypothalamic peptides known as releasing hormones, which in turn stimulate the pituitary gland to release the gonadotropic hormone(s), which act on the gonads. The gonads in turn produce the sex steroid hormones which are responsible for the formation of gametes, as well as for the regulation of secondary sexual characteristics, nuptial coloration and breeding behaviour. This pattern of reproductive mechanism provides the basis for methods of induced reproduction, namely the provision of appropriate environmental stimuli and the administration of hormones for maturation and release of gametes.

### 8.1 Reproductive cycles

The large majority of aquaculture species are seasonal breeders, although some breed intermittently or continuously. Seasonal breeding is generally related to climatic seasons. For example, most fresh-water fish of temperate zones spawn in spring and early summer, but the salmonids spawn in autumn. Rainy season and flood waters are associated with the spawning of fresh-water fishes of tropical and subtropical regions of Latin America and Africa. Obviously the fishes integrate their own reproductive functions with environmental cycles. The breeding season appears to coincide with environmental conditions that are most conducive to the survival of the offspring. These

favourable factors, that act as cues for a suitable breeding season, affect the central nervous system and through it the pituitary and the gonads. Photoperiod, temperature and rainfall are important factors involved in regulation of the reproductive cycles.

Mechanisms of reproductive timing vary very considerably among species. For example, in salmonids that spawn in the autumn, gradually increasing photoperiods followed by short photoperiods or decreasing photoperiods have a major role in regulating the cycle. Temperature has an important role in the reproductive cycle of cyprinid species. Gonadal recrudescence takes place in Indian carps during the period of the year when both photoperiod and temperature are increasing. Changes in the volume and velocity of water, flooding of shallow areas and dilution or replacement of water are also considered to be important factors. Warm temperatures and long photoperiods appear to affect also the reproductive cycle of Chinese carps. A review of available information would appear to show that in the majority of cases gonadal recrudescence is regulated chiefly by seasonal variations in photoperiod and temperature, while spawning may be controlled by temperature and/or rainfall.

The age of sexual maturity varies widely between species. For example, tilapia species become mature within a few months, whereas others may take a few years. The same fish may mature earlier in a warm climate and much later in colder climates; examples of this are the common carp and the Chinese carps. The common carp, which takes three to four years to mature in Europe, takes only a year to attain maturity in tropical regions. Chinese carps that take five to seven years to mature in Europe become mature in one to three years in tropical and subtropical conditions.

Some species have only one spawning season, during which they may spawn several times. Others may have two or more spawning seasons. Some species of finfish exhibit well developed parental care, which may consist of incubating fertilized eggs in the buccal cavity of the parent, or guarding the eggs and larvae during development. Many of the species that exhibit parental care lay eggs in nests made of plant or other available material or in hollows dug out on the bottom.

Some of the species like the Chinese and Indian carps that are essentially riverine spawners would not spawn in the confined waters of fish ponds or other enclosures. Their gonads develop only up to a certain stage and then remain dormant until resorption sets in. They have however, been observed to spawn in special types of ponds (called bundhs in India) that have a flow of fresh rainwater, inundating shallow marginal areas where the conditions are favourable for the fish to breed. The simulation of conditions in natural spawning grounds may serve to induce certain fish to breed in confined areas. The provision of nest-building material for nest-breeding species and the provision of artificial substrates for the attachment of eggs required for certain species are also believed to induce spawning.

## 8.2 Control of reproduction

In aquaculture, the main purpose of controlled reproduction is to achieve sexual maturation and spawning at the time of the year which is normal to that species. As mentioned earlier, some species will not breed in the confined waters of an aquaculture facility. In other cases, maturation and spawning are unpredictable, because of the culture conditions or environmental factors. Controlled reproduction can also be of considerable importance in advancing or retarding the spawning period as required. This can help in making available young ones at appropriate times or of appropriate sizes. A higher level of reproduction control would involve development of the capability to mature and spawn a species at any time of the year, in order to enable continuous production and marketing throughout the year.

The two major types of control that are possible, consist of (i) manipulation of the reproductive cycle and (ii) induction of gonadal gamete release (ovulation and spermiation). The reproductive cycle is manipulated so as to have gametes available when needed. This may be initiated in the juvenile stage, or advanced or retarded in the adult stage. Altered gonadal gamete release can be achieved by hormonal supplementation, manipulation of environmental factors or the use of special selected strains.

In oviparous animals, embryos are dependent on the egg yolk for their nutritional require-

ments. Vitellogenesis, or the process of yolk deposition in oocytes, is a seasonal or cyclic phenomenon. All stages of it, starting with the mobilization of lipid from storage sites, the synthesis in the liver of a female-specific glycolipophosphoprotein, vitellogenin, and its eventual deposition in oocytes are known to be gonadotropin-dependent.

The interaction between the brain, pituitary gland, testis and ovary largely mediates the influence of environmental factors on the reproductive development of finfish. The thyroid and interrenal may also have a less important role. The substance formed by the nucleus lateralis tuberis in the hypothalamus, which is responsible for such influence, is the gonadotropin-releasing factor or releasing hormone. In the case of mammalian luteinizing hormone (LH) and follicle stimulating hormone (FSH), the releasing activities for these two hormones have been shown to be present in the same peptide, which consists of a chain of 10 amino acids (Schally and Kastin, 1972). The molecule is referred to as LH-RH. The presence of LH-RH has been demonstrated in certain species of fish (Crim *et al.*, 1978) and it has also been demonstrated that in large doses mammalian LH-RH or its analogues brings about the release of gonadotropin.

Even though attempts have been made with salmonids, the induction of a completely new reproductive cycle has not yet been successful. Chronic administration of gonadotropic hormones can, however, initiate a normal reproductive cycle and assure its progress. By pellet implantation of hormones, it has been possible to advance normal spawning by one year in pink salmon. The release of gametes can be advanced by a single dose of hormone. Similarly, it has been demonstrated that hormone injections can induce late ovulations, as in brown trout, when maturity is blocked by adverse environmental conditions.

As mentioned earlier, the two major environmental factors that affect maturation and spawning are the photoperiodic regime and temperature. Although any definitive conclusions regarding the independent influence of photoperiodism have not been possible, there is enough evidence of the combined effect of these in several species. When, by manipulation of these factors, early maturation is achieved,

egg-laying can more easily be synchronized by hormonal injection. This helps in predicting ovulation more precisely and in avoiding ageing of ova, which may occur at high summer temperatures. There is considerable experimental evidence of the independent role of temperature in maturation and spawning. It is believed that spawning is timed to ensure that gametes are released into water whose temperature is within the appropriate stenothermal conditions for embryonic development. While the precise mechanisms by which temperature regulates reproductive development are not known, it is presumed that it acts as a triggering mechanism at the hypothalamic level or alternatively exerts a generalized stimulatory effect on metabolic rate. The influence of rainfall on the spawning of certain species, as referred to earlier, is also ascribed to the combined effect of temperature and photoperiod, plus the dilution of inhibitory elements in the water.

Another means of reproductive control, oriented to spreading egg production over the year, is through the use of selected strains for early or late spawning. Strains have been developed that spawn for much longer periods than normal for the species. There is also the possibility of using in a farm several strains, reproducing at different times of the year, in order to ensure the availability of young throughout the year.

### 8.3 Induced reproduction

As explained in the previous section, the hypothalamus regulates the reproductive functions of the pituitary gland. The correct combination of environmental factors required for maturation, ovulation and spawning, brings about an accelerated release of gonadotropin from the pituitary into the bloodstream. Ng and Idler (1978 a, b) and Idler and Ng (1979) have isolated two gonadotropic hormones: one with a low carbohydrate content that induces vitellogenesis and the other, rich in carbohydrates, inducing maturation and ovulation. The surge of gonadotropins that occurs brings about maturational changes culminating in the act of spawning. Environmental conditions required for the initiation of oocyte maturation, ovulation and spawning are much more complex than those for gametogenesis.

Very often under culture conditions, the required environmental conditions may not be available, or may not persist for a sufficient length of time for spontaneous maturation to occur. This has led to the development of induced reproduction or hypophysation techniques (Houssay, 1931; Von Ihering, 1935, 1937). By the injection of pituitary homogenates

(fig. 8.1), the natural gonadotropin surge is simulated, by-passing to some extent the environmental variables of temperature, rainfall, photoperiod, etc. Besides the advantage of regulating the time of spawning, this enables the adoption of other methods of artificial propagation, including hand-stripping (fig. 8.2), fertilization, incubation, hatching and larval



Fig. 8.1 Injection of pituitary homogenates to induce spawning.



Fig. 8.2 Hand-stripping of a mature female fish.

rearing. While hypophysation has been demonstrated to be effective in a large variety of fish species, its major contribution in respect of aquaculture technologies, since its first field application in Brazil in 1935, has been in the inducement of spawning in fishes that do not ordinarily breed under conditions of confinement or do so only under specific environmental conditions. It has now become a common practice in many countries and is utilized widely in the reproduction of finfish, despite the fact that the relevant mechanisms are not fully understood and little standardization of the techniques has been achieved.

Vitellogenesis in decapod crustacea, particularly Penaeid shrimps and lobsters, has been shown to be mediated by hormones. Male shrimps mature fully under captive conditions and spermatophores can be seen through the carapace. Female shrimps often do not mature fully, even though maturing eggs can be found in the ovaries. The maturation process seems to be inhibited by a gonad-inhibiting hormone (GIH) secreted by the medulla terminalis ganglionic x-organ (MTGX) and stored in the sinus gland. The y-organ, which secretes the moulting hormone crustecdysone, also has an influence on maturation. The ablation (surgical removal) of eye stalks, which have the glands containing the inhibitory hormone, has been shown to accelerate vitellogenesis in many crustaceans. Besides environmental factors like temperature, photoperiod, salinity and pH, the state of nutrition of brood animals seems to be an important factor in the maturation and spawning of shrimps.

### 8.3.1 Hypophysation

A more detailed description of the techniques of induced spawning, including environmental control employed for the breeding of important aquaculture species, can be found in Part II. Only some of the common features of induced spawning, with special reference to finfish, will be discussed here.

The mammalian gonadotropic hormones, LH and human chorionic gonadotropin (HCG), are effective in inducing maturation and ovulation in fishes. Although a number of species have been induced to breed by the administration of HCG or a combination of HCG and mam-

malian pituitary extract, there are certain refractory breeders, like the Indian and Chinese carps, where fish pituitary homogenates or extracts are needed to induce spawning. There are reports of successful breeding of even these species, by using HCG under certain circumstances. The Chinese carps, which have been bred two or three times by administration of fish pituitary extract, will respond positively to injections of HCG. Bhowmick (1979) has reported on the use of crude HCG for induced spawning of one species of Indian carp, *Labeo rohita*. It has, however, been reported that repeated injections of HCG could induce a 'drug resistance effect' related to the production of antibodies against foreign proteins. Nevertheless, it would appear that homogenates and extracts of whole pituitary glands and partially purified fish gonadotropins are more potent in inducing maturation and ovulation in fishes than mammalian gonadotropins, and can be used extensively in commercial fish culture.

While the administration of the appropriate hormone is basic to the success of induced breeding, the condition of the brood fish and the environmental conditions are also equally important. The large number of failures in induced breeding can often be traced to poor condition of the brood fish, including their health and nutrition and stage of gonadal development, as well as to environmental conditions in spawning tanks or enclosures. Chinese farmers believe that it is more difficult to breed wild Chinese carps, as well as carp that have attained maturity for the first time. They prefer to rear spent fish in special holding ponds, fed on a special protein diet, for future breeding.

The identification of sex is another important requirement for successful induced breeding. Many species do not have distinctive and permanent sex characteristics. When there are no secondary sex characteristics, detailed morphometric characteristics will have to be used to separate sexes, particularly in the prepuberty stages. After sexual differentiation, it may be possible to distinguish the sexes through examination of the gonads. This will involve the use of endoscopy or biopsy, which is difficult to use on a large scale. Siphoning of eggs and their examination under a microscope, to determine the stage of maturity of females, have been

described by Chen *et al.* (1969) but the adoption of this method in large-scale breeding work is not always very practical. Other possible methods, such as the use of serum markers or detection of circulating vitellogenin, are also difficult to use in the field. Aquaculturists have therefore to depend largely on practical experience and field observations to distinguish the sexes and determine the stage of maturity of breeders. Brood female fish ready for spawning are identified by the well-rounded and soft abdomen and swollen genital opening. The male releases a few drops of thick milt when its abdomen is slightly pressed.

As indicated earlier, several species of fish respond to injections of HCG and other mammalian hormones, and these hormones are commercially available to aquaculturists. Many species, which are more difficult to spawn under confinement, need injections of fish pituitary for maturation and spawning. There are differences of opinion regarding the species-specificity of the pituitary, but aquaculturists generally prefer to use the glands of the same or closely related species. It is recommended that pituitaries from phylogenetically close donors should be used, when there is a choice. However, common carp is considered a universal donor and its pituitary is being used very widely for both experimental and commercial breeding purposes for several species. Salmon pituitary is also used for breeding a number of species. Though commercially available on a limited scale, a large majority of aquaculturists have to depend on local arrangements for the collection and preservation of the glands. Glands of the recipient species or of other proven donor species are used. Fractionation and purification of teleost gonadotropins are still in experimental stages. Though potent gonadotropic preparations have been made from fish pituitaries by means of chemical/ethanol fractionation, they have not been used widely in spawning refractory fish.

Glands extracted from catches of the selected mature donor species are preserved in alcohol or acetone or frozen for storage. Freshly collected glands are first desiccated in absolute ethyl alcohol (changing the preservative several times) and then stored in fresh alcohol at room temperature or under refrigeration. The glands remain active for a period

of about two years. Instead of alcohol, the glands can be desiccated in acetone, changing it several times as for alcohol. The desiccated glands are dried in vacuum and stored in that condition or sealed in vials and stored in frozen condition. Acetone-dried glands retain their activity for 6–10 years. The glands can also be preserved by quick freezing, but the most common method of preservation is acetone drying.

Though a number of methods of preparing pituitary homogenates and extracts have been tried, the most commonly accepted method is extraction with distilled water or saline solution. The glands are macerated in a small volume of water or saline solution and brought up to the desired volume. Distilled water, common salt solution (0.3–1 per cent) and physiological saline can be used, as they all seem to give equivalent results. The homogenate can be used as such for injection, or filtered or centrifuged to obtain filtrate or supernate which can be injected. Extraction with trichloroacetic acid (TCA) at low concentrations of 1.25–2.5 per cent for short time-periods of three to six hours, is reported to provide more complete extraction and better results. But this practice has not received wide acceptance, probably because of the specific requirements of concentration and extraction time. It is reported that higher concentrations and/or longer extraction, can result in denaturation of glycoproteins.

As pituitary extracts are subject to rapid enzymic deterioration, they have to be prepared fresh every time fish are to be bred. This is obviously inconvenient. Methods of preserving extracts have been tried with some success. One method involves the extraction of pituitary glands in a small volume of distilled water, and refrigerating it for 24–48 hours, after which glycerine is added to make a 2:1 ratio with water. The suspension is again refrigerated for 24–48 hours, centrifuged and the supernate stored under refrigeration in airtight vials. Another method consists of grinding acetone-dried pituitaries, sieving them through 40–60 mesh/mm<sup>2</sup> sieves and storing in sealed vials at 5°C. Both these techniques are aimed at achieving homogeneous preparations of uniform potency.

Despite its wide use, the dosage frequency and latency period of pituitary administration

remains more or less at a trial-and-error stage, and sometimes leads to poor results. This is mainly on account of the variations in the gonadotropic content of the pituitary material used and the stage of sexual maturity of the brood fish, besides the environmental conditions and the stress to which the breeders are subjected. The mode of injection (intraperitoneal or intramuscular) also appear to affect results. Development of an acceptable method of assessing gonadotropic content should greatly assist in determining practical dosages. Though several biological units have been proposed, none seems to have gained wide acceptance.

### **8.3.2 Gametes and fertilization**

Injection of pituitary homogenate or extract is usually given into the dorsal muscles above the lateral line and below the anterior part of the dorsal fin, or the dorsal part of the caudal peduncle. Injections into the body cavity are considered less efficient. The required quantity of the gland is generally administered in two to four doses (one or more preparatory injections followed by one or more final doses). As indicated earlier, suitable environmental conditions, besides pituitary injection, will be needed for ovulation to take place. Temperature, high dissolved oxygen levels and lack of stress are some of the important requirements. The process of ovulation takes some time, depending on the species and environmental conditions. Maturation of the ovum starts when its nucleus starts to migrate from the centre toward the micropyle and undergoes hydration by absorbing fluids. Ovulation starts with the disappearance of the nuclear membrane and ends with the first meiotic division. At the same time, the follicle which attaches the eggs to the wall of the ovary splits and releases the eggs into the cavity of the ovary, from where it can freely flow through the genital opening.

Many of the fish that are treated with gonadotropic hormones start to spawn in the presence of active males after normal ovulation. The eggs are fertilized by the male breeders and the fertilized eggs can be collected easily for hatching. Where such induced spawning does not occur, it becomes necessary to strip the sex products from the females and males

and artificially fertilize them. Ripe ova remaining unspawned for long periods after ovulation become over-ripe and do not develop normally. It is also not uncommon for the phenomenon of 'plugging' to occur in gravid females subjected to overdoses of hormone. In such cases, natural spawning cannot be accomplished, since a mass of ovarian eggs forms a plug at the urinogenital opening, preventing the free flow of eggs. Stripping will be necessary to obtain eggs from such fish. Stripping and artificial fertilization are necessary also for fish with sticky eggs like the common carp. Such eggs will have to be treated to dissolve the sticky layer, so that they can be incubated in suitable incubators. A quick washing with a weak tannin solution after the eggs have swollen will be effective in removing the stickiness of the eggs. Common salt and carbamide (urea) solution can also be used for removing the sticky layer.

The ovulated egg which has undergone the first meiotic division will have the second meiotic division when the sperm penetrates it, ending in the extrusion of the second polar body. Further embryonic development leading to the formation of the first somatic cell completes the process of fertilization. The time available for the ripe egg to become fertilized is rather limited in most fresh-water fish, as the eggs swell rapidly in water and this results in the closure of the micropyle. The time available for common and Chinese carps is about 45–60 seconds. In saline solution the eggs seem to remain fertilizable for longer periods, up to several minutes.

The sperm, which is immotile in the testis, becomes motile on contact with the medium in which fertilization takes place. The duration of the activity of spermatozoa varies with the species, but is generally not longer than a couple of minutes. In the males of most species, dense semen with highly motile spermatozoa can be obtained without hormone injection. Administration of pituitary extracts brings about thinning of the seminal plasma and would facilitate spermiation. Relatively large numbers of spermatozoa are needed to fertilize an egg. For example, the requirement of a trout egg is reported to be 10000–300000 spermatozoa and of a carp egg 13000–30000. This is due to the fact that the spermatozoon can penetrate at only one place, i.e. the micropyle, and the dis-

tance that can be covered by a trout spermatozoon during its life span (2 mm) is often less than the circumference of the ovum which is about 15–20 mm. the probability of its reaching the micropyle is therefore low, if the motility is less. The number of spermatozoa compensates for the low motility. It is necessary to take special care in regulating the quantity of water added to the sexual products during fertilization. If too much water is added, many of the sperms will not be able to reach the micropyle. On the other hand, if sufficient water is not added, the micropyle of an egg may get blocked by other eggs, due to crowding, preventing the sperm from entering it.

#### 8.4 Preservation of gametes

In many species, the maturation of gonads in the two sexes is not synchronous. Males often show testicular recrudescence earlier during the season. Because of this, ripe males occur during the beginning of the season, when the females are not yet mature and ready for spawning. The reverse situation occurs during the end of the breeding season. Under such circumstances, it will be most advantageous to have a suitable means of preserving the gametes for artificial fertilization, when needed. Methods of gamete preservation would also help in the initiation of genetic selection programmes, by providing easy access to a reserve of genetic material of known and desired qualities.

Cryopreservation with liquid nitrogen, used widely in the preservation of cattle and livestock sperm, has been tried for the preservation of a number of species of fish. Blaxter (1955) reported successful fertilization of fresh eggs with cryopreserved ( $-79^{\circ}\text{C}$ ) sperm of *Clupea harengus*. Sections of ripe testis were stored in 80 per cent sea water containing 12.5 per cent glycerol as a protector, and the mixture frozen quickly or slowly at  $1^{\circ}\text{C}/\text{min}$  to  $-30^{\circ}\text{C}$ , then quickly to  $-79^{\circ}\text{C}$  (using dry ice). Besides the sperm of rainbow trout, spermatozoa of the common carp, Chinese and Indian carps and grey mullet are among the cultivated species which have been subjected to cryopreservation, which consists of cooling and storing at subzero temperatures of liquid nitrogen ( $-196^{\circ}\text{C}$ ), using dimethyl sulphoxide, glycerine, ethyl glycol or

other cryoprotectants and diluents (Harvey and Hoar, 1979). Attempts at cryopreservation of ova have not been as successful as for sperm. Zell (1978) reported the first successful cryopreservation of unfertilized ova and zygotes of salmonid fish. Ova frozen in liquid nitrogen at  $-20^{\circ}\text{C}$  for five minutes proved to be fertile, and zygotes frozen at  $-50^{\circ}\text{C}$  survived the exposure. All subsequent attempts have failed. While it is difficult to predict possible advances in cryopreservation of fish gametes, it would appear that the results so far indicate only the feasibility of short-term preservation of semen or the prolongation of embryonation.

#### 8.5 Use of sex steroids for sex reversal

In certain situations and species, it will be advantageous to restrict fertility. A well-known example is the cichlid tilapia, which attains maturity at an early age and breeds repeatedly at short intervals, overpopulating ponds and other rearing facilities. This results in stunted populations, as energy is expended for reproduction rather than growth. Among the techniques that can be employed for restricting fertility is the application of hormones to produce monosex populations. Androgenic and oestrogenic steroids are used for masculinization of genotypic females and feminization of genotypic males (Jalabert *et al.*, 1974; Guerrero, 1975, 1979; Shelton *et al.*, 1978). Genotypic female fry of the species of *Sarotherodon* (= *Tilapia*), when fed on methyltestosterone and ethinyltestosterone have become males. Similarly, monosex female tilapia have been produced by treatment with oestrone, ethinyloestradiol and stilboesterol. While the feasibility of sex reversal by steroid administration has been demonstrated, the percentage of fish that underwent sex change in any treated group varied greatly. Since the presence of even a small percentage of the opposite sex in a population is sufficient to initiate uncontrolled breeding, the value of the results achieved so far becomes less significant. Similar experiments to produce monosex fish have been conducted with salmonids and other species. Sex inversion of the protogynous species of *Epinephelus* (*E. tauvina*) has been accelerated to produce male brood stock from three-year-old females, by oral administration of methyl-

testosterone. Production of all-female eggs is now a common practice in a number of rainbow trout hatcheries (see Section 17.1.2). The initial functional males required for fertilizing ova from normal female brood stock are obtained by sex reversal, by treating with 17 methyl-testosterone through immersion or incorporation in starter feed in the fry stage.

## 8.6 Genetic selection and hybridization

The use of genetically selected strains and hybrids has contributed very substantially to modern agriculture and animal husbandry. But aquaculture is yet to gain much from efficient breeding and selection programmes (see also Section 8.8). Among the many reasons for this are the delays in the development of suitable techniques for controlled reproduction of many farmed species and the paucity of genetic expertise among aquaculturists. Genetic improvements usually require long-term experimentation with a large number of individuals and generations, and so considerable time may elapse before useful results become available. Moreover research on farming technologies has not reached that level in most cases, when the only way to improve production is by genetic improvement of the stocks. Except in a few cases, the present technologies are too inefficient to benefit from the use of selected stocks.

In traditional aquaculture, certain strains have evolved as a result of environmental or farming conditions without much conscious effort by the aquaculturist, as in the case of the common carp, or as a result of rule-of-thumb selection. These more or less accidental strains can seldom be used with confidence for commercial farming.

There is no doubt that effective selective breeding programmes are expensive and require more facilities than are presently available in most aquaculture farms or even institutions that can function as central stations for breeding and distribution of aquaculture species. Though the economic benefits of selection programmes have been worked out for domestic animals, comparable evaluations are few in aquaculture.

As pointed out earlier, the number of domesticated species used in aquaculture for food is

limited (unlike culture of ornamental species), but the number is steadily growing. Opportunities to increase the production properties and adaptation to a new environment of species through selection can therefore be expected to expand in the future. Kirpichnikov (1966) gives some of the main aims of fish selection as follows:

- (1) to increase the growth rate by better utilization of food (physiological decrease of food expenditure per unit of growth increment);
- (2) to increase the growth rate by fuller utilization of natural food in ponds and higher consumption of feed mixtures;
- (3) to increase resistance to oxygen deficiency, to high or low temperature, to higher salinity or to other deviations from the normal environmental conditions;
- (4) to improve resistance to infectious diseases and to infestation of parasites (to develop new breeds resistant to particular diseases);
- (5) to improve the nutritive properties of fish (to increase the calorie content, to decrease the proportional weight of non-edible parts, to decrease the bone content, to increase or decrease the fat content, etc.).

Other aims may include speeding up of sexual maturation, the ability to reproduce at relatively low temperatures and the slowing down of maturation to prevent early switching over of metabolism to develop sex products, affecting growth and resulting in prolific reproduction.

The relative advantages of a fish in genetic breeding schemes are brought out by Skjervold (1976), using salmonids as an example. Among the most important of these are:

- Very high fertility, leading to high 'litter' of considerable importance in selection work.
- External fertilization, which makes it possible to have several combinations of matings and the production of many 'litters' of large numbers of half-siblings.
- The high fertility of females, which enables:
  - (a) some types of family selection, even when the heritability of the selected trait is low, as large family groups will in



always been as a result of planned selection. Carp farming in different regions has led to the establishment of strains which appear to have adapted to the general climatic conditions under which they are grown, and which grow faster than the wild strains. In rainbow trout, the usual practice has been to pick the best-looking fish from a stock to be the parents of the next generation. These common-sense approaches cannot be depended upon in a cultivation programme to achieve genetic improvement.

Most economically important characteristics of cultivated organisms are measurable and their variation within a population usually takes the form of a 'normal' distribution (Purdom, 1972). Such a distribution of measurements occurs because the magnitude of a characteristic is determined by a large, often variable, number of factors, some of which are environmental and some genetic. The separation of environment and heredity has been one of the main aims of studies on population genetics. The reliable models of the inheritance of the measurable characteristics can be used for predicting and controlling the gains from genetic selection within cultivated species.

The variation of a character between individuals can be expressed as 'variance', the mean square deviation of individual values from the mean. This is called the phenotypic variance ( $V_P$ ) of a sample or population and is the sum of two components, the environmental variance ( $V_E$ ) and the genotypic variance ( $V_G$ ). Hence  $V_P = V_E + V_G$ . The proportion of phenotypic variance that is genetic ( $V_G/V_P$ ) is approximately equal to the value of 'heritability' which measures the proportion of additive genetic inheritance in the phenotypic variance. It is a measure of the degree to which multiple genes control resemblance between offspring and their parents in the face of a particular set of modifying environmental factors. Heritability can be used to predict selection gains in the formula  $R = h^2S$ , where  $R$  is the response, measured as the change in the mean from one generation to the next, and  $S$  is the 'selection pressure', or the difference between the mean of the selected parents and the mean of the population from which they were chosen.

As indicated, the ratio of  $V_G$  to  $V_P$  is only an approximate measure of  $h^2$ . More reliable

values can be obtained through parent/offspring correlations or by the use of the above formula in a selection. Though laborious and time-consuming, it is essential to have an indication of the magnitude of  $h^2$  before starting an extensive selection programme.

The primary reason for desiring estimates of heritability is to enable prediction of results expected from a given level of selection. The effectiveness of selection depends upon:

- (1) heritability of the attribute ( $h^2$ );
- (2) degree of variation in the attribute ( $\sigma_p$ ); and
- (3) intensity of selection applied in (1).

According to Falconer (1981), the anticipated response to selection ( $R$ ) can be stated algebraically as

$$R = i\sigma_p h^2$$

where

$R$  = mean of offspring from selected parents  
minus mean of all adults before selection  
mean of group selected minus

$$i = \frac{\text{minus mean of all adults before selection}}{\sigma_p}$$

$\sigma_p$  = phenotypic standard deviation of the attribute

$h^2$  = heritability estimate for a particular attribute

### 8.6.2 Mass selection

Mass selection, or individual selection, is based on characteristics of the individuals under selection as opposed to selection based on the performance of their relatives. It is one of the simplest and most common methods employed in breeding programmes, where the characteristic to be improved is easy to measure. It can be used efficiently in selection for growth rate and to some extent for age at maturity.

As stated above, response in mass selection ( $R$ ) is determined by the general equation

$$R = i\sigma h^2 = Sh^2$$

where

$S$  = selective differential (the difference in a certain trait between the individuals selected and the population as a whole),

$h^2$  = heritability of the differences (the share of additive genetic variation in the general variation of the character), and

$i$  = intensity of selection.

The high fecundity of cultivated fish causes high selective potential and intensity of selection compared to domestic animals and poultry. In performing mass selection in fish breeding, the selection severity coefficient or the rejection rigidity factor ( $V$ ) is calculated by the equation:

$$V = \frac{100n}{N} \%$$

where

$n$  = the number of individuals selected and  
 $N$  = the total number of fish grown.

In fig. 8.3 the intensity of rejection is plotted against its severity on a semi-logarithmic scale. The curve obtained shows that there is a sharp increase in the intensity of selection with decrease in the severity coefficient within the range 100–10 per cent. A further decrease in  $V$  (down to 1 per cent) results in a considerably lower increase in  $i$ ; with further decrease in  $V$  (0.1–0.01) there is hardly any increase in  $i$ . For fish with high fecundity, selection gives best results when the severity of selection is 1–0.1 per cent.

Response to selection is directly proportional to the heritability of the character ( $h^2$ ). In many cases, a rather accurate estimate of the value of heritability of the character can be obtained from the equation:

$$h^2 = \frac{R}{S}$$

To obtain the estimate, selection has to be conducted in several successive generations.

For increasing response in mass selection, the values of  $i$ ,  $\sigma$  and  $h^2$  have to be increased. The value of  $i$  can be raised in fecund fish by increasing the number of individuals and, through this, increasing the severity of selection. Variability should relate only to genetic and not environmental variation, as the latter is conditioned by the non-additive genetic variation. To increase  $h^2$  values, non-related individuals have to be crossed. Outbreeding increases the degree of heterozygosity, i.e. increase in genetic variation, but inbreeding results in higher homozygosity. A sufficient number of fish should be available every year for crossing, to enable selection of fish from different crossings for breeding purposes. Another method of increasing genetic variation is by speeding up the process of mutation through irradiation and chemical mutagens.

Non-hereditary variation can be depressed by following special rearing methods such as growing spawners under favourable conditions for maturation, simultaneous crossings, incubation of all eggs under identical physico-chemical conditions, growing larvae and young ones under conditions that do not promote food competition, avoiding the mixing of stocks grown in different ponds or enclosures, and by selection at the age when the animal is more susceptible to improvement by selection.

It is necessary to point out here that a long period of one-way selection for certain characteristics may bring about changes in other morphogenetically or genetically correlated characters. There are many observed examples of correlated responses in selection of non-selected characters, such as physiological and biochemical factors (Steffens, 1964), growth rate (Moav and Wohlfarth, 1967), fecundity, etc.

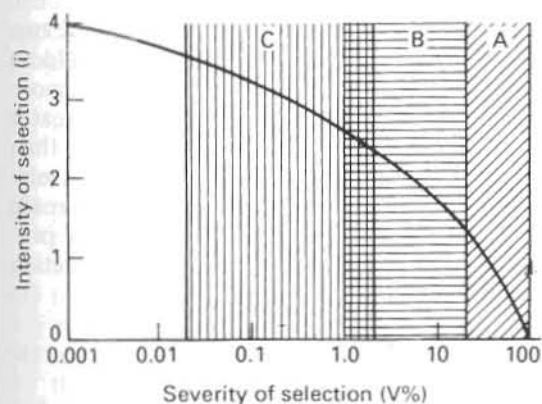


Fig. 8.3 Relation between intensity of selection and its severity in cattle (A), poultry (B) and fish (C). (Adapted from Kirpichnikov, 1971.)

### 8.6.3 Genotypic selection

Individual or mass selection can only be used on traits that can be recorded on live animals and is not very efficient for traits with low heritability. In such cases, other types of selection have to be resorted to. The two types of

genotype selection that have applications in aquaculture are family selection and progeny testing.

#### Family selection and sib-selection

Family selection is of special interest in selection for characteristics of low heritability, such as survival, meat quality and age at maturation. Use of full and half sib families in a selection programme has the advantage that the generation interval will not be increased, compared to individual selection. However, a disadvantage is that usually each family has to be reared in separate tanks, as it is generally difficult to mark newly hatched larvae or fry. This will introduce environmental and tank effects on characteristics, such as body weight, between families. Because of this, Falconer (1981) recommends a combination of individual and family selection.

In family selection, several families are grown under identical conditions to determine the ones to be maintained for breeding. To obtain separate progeny (family), either one male/female pair or a small group of spawners can be used. The response equation is essentially the same as in mass selection:

$$R_f = i_f \sigma_f h_f^2$$

The intensity of selection appears to be lower than in mass selection, as it is not possible to grow such a large number of families. Similarly, a reduction can be observed in the standard deviation, as this denotes the variation in the family and not individual variation. However, the heritability is much higher.

If the individuals have to be sacrificed for examination, the brothers and sisters of the individuals from the best families can be maintained for breeding. This is known as sib-selection. Kirpichnikov (1971), in his description of the methods of family selection, underlines the importance of carrying out crossings, egg incubation, larval rearing and grow-out of families separately, under as identical timings and conditions as possible. The main disadvantage of family selection is the practical difficulty in simultaneously growing many families under identical conditions. Marking of individuals will reduce some of the problems, as communal growing will then become possible. Fin clipping

and cold or hot branding have been used in many large-scale selection programmes. Molluscs can be marked more easily on their shells, whereas in crustaceans moulting habits make marking difficult.

#### 8.6.4 Progeny testing

Progeny testing enables the assessment of the breeding qualities of separate spawners or pairs of spawners and the selection of the best for further selection work. However, progeny testing will increase the generation interval very markedly. For example, in carp breeding it requires one or two years, which would mean a slowing down of selection work by 20 to 30 per cent.

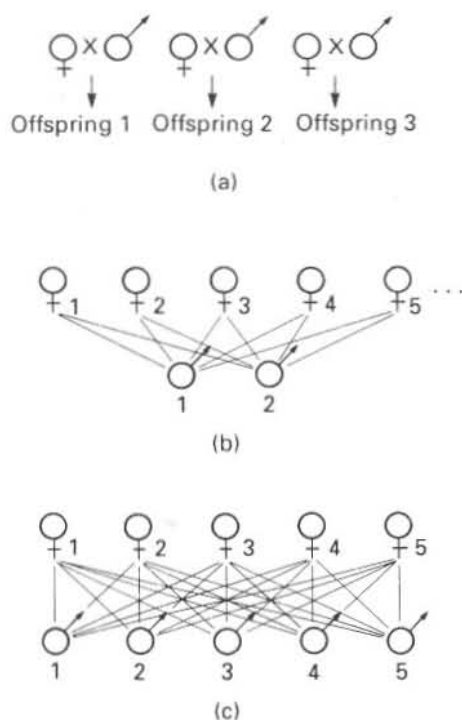
Three methods of progeny testing are applicable in aquaculture. The first method is testing of pairs, without testing males and females separately (fig. 8.4a). The second is to test spawners belonging to one sex, as for example females only as shown in fig. 8.4b, and the third is the testing of both females and males (complete diallele crossing) (fig. 8.4c).

The equation to measure selection response is the same as in family selection. Intensity of selection is limited by the number of families. Variability of family means ( $\sigma_f^2$ ) is also the same in both cases. Heritability of family means ( $h_f^2$ ) in progeny testing is high, as in family testing. This may occur only if the breeding conditions of all progenies are practically identical or if breeding proceeds with a three- or four-fold iteration.

By comparing the response values in the two equations ( $R = Sh^2$  and  $R_f = S_f h_f^2$ ), it should be possible to determine which method is more efficient in a given programme. If  $Sh^2$  is greater than  $S_f h_f^2$ , mass selection is more effective than selection for relatives. The selection for relatives is only advantageous when the increase in heritability is not accompanied by a proportionally greater drop in the selection differential.

#### 8.6.5 Combined selection

Even though mass selection has been found to be more efficient than selection for relatives in fish, the advantages of the latter in selection for certain characteristics like fat content has been



**Fig. 8.4** Progeny testing in fish breeding a = Comparison of pairs without testing sexes separately, b = Testing spawners belonging to one sex only, c = Testing of both sexes (diallele crossing). (From Kirpichnikov, 1971.)

demonstrated. For characters like weight, it appears possible to follow a combined selection programme, which may consist of:

- (1) performing mass selection among fingerlings or two-year-old fish with a great severity coefficient,
- (2) progeny testing of males through to maturity, since males often mature earlier than females,
- (3) family selection with a simultaneous breeding of five to ten families and
- (4) repeated performance of mass selection in the best families.

Such a scheme allows summing up of the efficiency of mass selection and selection for relatives in a relatively short time.

### 8.6.6 Cross-breeding

Cross-breeding is another well-known means of genetic improvement which has application in aquaculture. Heterosis or hybrid vigour enables an offspring to surpass its parents for one or more traits. On the other hand, inbreeding depression caused by mating of closely related parents has a deleterious effect on the progeny.

The inbreeding measure is the coefficient of inbreeding, incorporating the degree of the animal's homozygosity. It shows what part of the genes in a group of individuals are in the homozygous state. Close inbreeding, especially sib mating (brothers and sisters and parents with offspring), causes homozygosity to increase rapidly, to as much as 0.9–0.95 (expressed as fractions of one) or even more. In most animals, inbreeding results in inbreeding depression, characterized by a drop in viability, growth rate and often fertility. Such depression has been observed by many workers in common carp, brook trout and other fish species. Outbreeding, on the other hand, is accompanied by heterosis in growth rate and viability, especially when fish from different high inbred groups are crossed. The main types of crossings undertaken are:

- (1) Commercial crossing, directed towards breeding of the first-generation hybrids for commercial purposes. Only the first generation, that has the heterosis of productive qualities or incorporates the advantageous characteristics of both the parental forms, is used. They are maintained for further reproduction.
- (2) Synthetic or distant crossing, in which distant parents, including those of intergeneric origin, are crossed to develop a new breed, in the course of long selection. It may attempt to combine the qualities of parents of several breeds, species or even genera. Such crossings to produce new breeds should ensure the preservation and perfection of the productive qualities of the breed, the preservation of genetic variability and the prevention of inbred depression.

Distant outbreeding is indispensable in the selection of aquaculture species. The aims of

such crossings are as follows (Kirpichnikov, 1971):

- (1) an overall increase in genetic variability, resulting in an increase in selection response;
- (2) achievement of a combination of characteristics of two or three breeds or two (rarely three) species;
- (3) improvement of the productive quality of the local breed by making use of the few valuable traits of another breed;
- (4) increase in the viability of the breed by introducing genes responsible for resistance to environmental factors and diseases.

Kirpichnikov (1971) describes different cross-breeding patterns to achieve these aims.

*Reproductive crossing* is suggested when valuable properties from both parents are to be combined in the hybrids. It can be done with complete fertility of the hybrid and requires only meticulous selection in the subsequent generations.

*Introductory crossing* will be advantageous when one or only a few characteristics from a breed have to be incorporated in the hybrid. Each generation of the hybrid has to be crossed with the local breed and so there is the risk of losing the useful characteristics of the improved breed in back crossing, particularly in the case of polygenic inheritance of properties selected. This type of crossing is of considerable use in selection for increased resistance to certain diseases, which is often dependent on the presence of one or a few genes. These genes can be preserved by means of proper selection in each generation.

*Absorptive crossing* differs from introductory crossing only in that its purpose is a nearly complete substitution of the local breed of genotype by the genotype of the improved breed. Only some features of the local breed, such as viability, are preserved.

*Alternate crossing* is the most complicated system which is most useful when a combination of many characteristics from two breeds with polygenic inheritance is required. It allows the preservation of high genetic variability through a number of generations. Selection efficiency is kept at a high level owing to this variability and does not reach a plateau. The main

problem of obtaining new hybrid breeds by means of crossing (interspecific or intergeneric) is their complete or partial sterility, which takes a lot of time-consuming work to overcome.

A number of breeding systems have been proposed to utilize completely the advantages associated with heterogeneous crossings. Parallel breeding of two or more groups within a breed is possible when working with slowly maturing species, without intermingling, allowing a moderate inbreeding among each and carrying out selection in each generation. In breeding in groups for family selection, a large number of crossings from different groups are carried out for each generation. The parents producing the best offspring are used for subsequent commercial crossings. This system suffers from the drawback that the genetic variability gradually decreases during family selection. Moav and Wohlfarth (1967) recommend that a reserve group of a sufficient number of individuals should be kept for each group when selecting two groups marked by certain genes. In case of a drop in genetic variability, additional gene pools can be introduced into the exhausted group.

Another possible system is alternate inbreeding and outbreeding. After two or three generations of close inbreeding, the evaluation of hybrids from different inbred lines is performed. The best combinations are used for commercial rearing and among the offspring new inbred lines are established. Linear selection involving inbreeding for superior ancestors and top cross, where crossing is done between the best inbred individuals (say males) and individuals from the outbred population (say females) to preserve the genetic variability, are other methods that have applications in aquaculture.

One of the most complicated techniques of breeding is reciprocal recurrent selection (RRS), where the combining capacity of the parents from each of the two groups is evaluated by means of a cross with parents from the other group. The individuals thus selected are reproduced without recrossing and their offspring again tested for combining potential.

The basic feature of all the systems described is the utilization of heterosis in crossing individuals from different groups, lines and breeds. Along with this, moderate to very close

inbreeding is employed. The most appropriate system would naturally depend on the species and the traits that are of importance in commercial culture. Gjedrem (1985), however, proposed a cross-breeding scheme for fish farming as summarized below:

- (1) Test all possible crosses between different strains or species for the economic traits in question and select crosses that are likely to give useful results. It may be better to use strains with very different origins and which, in combination, have favourable traits.
- (2) Develop inbred lines and test the crosses under natural conditions to find the most valuable cross for farming.
- (3) Start an RRS programme to ensure continuous genetic improvement, utilizing both general and specific combining abilities simultaneously.

#### 8.6.7 Chromosomal manipulation

As mentioned earlier, the sex of fish that are not differentiated into males or females at hatching can be controlled by the use of sex steroids at the time the gonads are differentiating. While direct masculinisation is frequently easier, feminisation has also been possible in some species. Methods employed for commercial production of all-female rainbow trout by using sex-reversed functional males and genetic females is summarised in Section 17.1.2. An alternate method of producing monosex stock is to induce sterility, and this can be done by the administration of high doses of sex steroids or by chromosomal manipulation.

Chromosomal manipulation for inducing polyploidy, gynogenesis and androgenesis has been studied with a view to controlling sex, as well as for rapid inbreeding. Manipulation becomes feasible during the nuclear cycles of cell division, and since fertilisation in fish is external, artificial means can be employed to either gamete before fertilisation, or to the fertilised egg at any period during the formation of the zygote.

The chromosome number can be increased by subjecting the egg to a pressure or temperature shock shortly after fertilization. The normal expulsion of one set of maternal chro-

somes is prevented, and so after fusion of the chromosomes from the sperm, the developing embryo contains three sets of chromosomes instead of the normal two sets. The extra set of chromosomes in the triploid individuals interferes with gonad development. Such induced triploidy is also useful for producing individuals with increased heterozygosity.

Gynogenesis, the parthenogenetic development of eggs after activation with genetically inert spermatozoa, is a very effective means of achieving relatively rapid inbreeding. It can be used to generate all-female stocks and for gene-transfer.

By exposing milt to a very high dose of atomic radiation (for example by using the radioactive isotope cobalt-60), the chromosomes of the sperm cells are destroyed. The milt is kept on ice at 0°C during radiation and can be stored thus for several days without loss of vitality. When the irradiated milt is mixed with eggs, the sperm cells penetrate the eggs but play no further part in the development of the egg. The embryo develops from the egg material only, without any male chromosomes. Since the egg is haploid (with only a single set of chromosomes), the developing embryos are also haploid. Though most of them die at or soon after hatching, there will usually be some gynogenetic diploids (with a double set of chromosomes), as a result of spontaneous diploidization of the female chromosome complex. To increase the frequency of diploidization of the female chromosome complex, temperature shock can be used. Treatment of eggs with low and high temperatures at the time of meiotic divisions results in disturbances in the process, such as disintegration of the spindle, due to which none of the chromosome sets can form the polar body, or the return of the polar body into the plasma of the ovum. The output of genetic diploids varies greatly, being high under favourable conditions according to the strength and duration of the temperature shock and the stage at which it is administered (see also Section 8.8).

#### 8.7 Transgenics in aquaculture

The best genotypes for aquaculture in the future may be developed using traditional selective breeding and the new biotechnologies

(Dunham *et al.*, 2001; Hew & Fletcher, 2001). Initial experiments show good potential for this combined approach, using mass selection and cross-breeding, genetic engineering and selection, genetic engineering and cross-breeding, and sex reversal and polyploidy; all work more effectively in combination than alone. Genetic enhancement is an increasingly important component of aquaculture management and, if used properly, has the potential to enhance aquaculture production. The constraints of property rights, food security and consumer perceptions have, however, to be solved.

Transgenic fishes could be as beneficial as transgenic plants and land animals and could be more effective than those traditionally bred in producing improved fish strains (Hew and Fletcher, 1997). The transgenic technology can be helpful not only in producing faster growing fish, but also in regulating maturation/reproduction, enhancing resistance to diseases (Leong *et al.*, 1999) and to environmental extremes (Fletcher *et al.*, 1988), improving nutritional and other qualities of meat, and modification of metabolic pathways to enhance food conversion efficiency (Doupe and Lymbery, 2003).

The first form of gene transfer has been successfully accomplished in China. Due to lack of fish gene sequences, initial transgenic research employed mammalian growth hormone (GH), which enhanced growth in some species, but not all species were examined. Salmonids showed no effect (Guyomard *et al.*, 1989) in spite of their being very responsive to growth stimulation by exogenously administered GH protein, though gene constructs using fish GH sequence had shown some lower growth enhancement in common carp, catfish and tilapia (Martinez *et al.*, 1996). This is probably the first evidence that growth enhancement in fish can be achieved by transgenics.

Hew and Fletcher (2001) list 13 instances involving eight fish species (common carp, crucian carp, catfish, loach, tilapia, pike, Atlantic salmon and Pacific salmon), where growth increases have been obtained using GH genes, as evident from published reports between 1986 and 1996. Growth enhancement was in the lowest range for common carp (1.1-fold) and catfish (1.2-fold) and highest for two salmonids

tested (Atlantic salmon 10-fold; sockeye 11-fold).

Recently GH gene constructs have been used in obtaining precocious smoltification of Atlantic Salmon (Du, 1992) and of Coho salmon (Devlin *et al.*, 1995a,b). When a gene is transferred with the objective of improving a specific trait, it may affect another trait, causing positive or negative 'pleiotropic' effects. So it is important to evaluate all major traits of transgenic fish. Transfer of growth hormone genes have been observed to affect body shape and composition, feed conversion efficiency, disease resistance, reproduction and tolerance of low oxygen concentration, carcass yield, swimming ability and predator avoidance.

Most studies in aquaculture genetics to date are concerned with the improvement of growth rate of selected fishes (see above). It is also possible to genetically improve the food conversion efficiency (FCE, which is another expression of FCR) (see Chapter 7), but there are gaps in knowledge in these interacting aspects of genetics and nutrition in aquaculture, which have to be bridged, taking cues from terrestrial livestock farming.

More recent approaches to inbreeding, cross-breeding, hybridisation, genetic selection, correlated responses, polyploidy, sex manipulation, gynogenesis, androgenesis and cloning, as well as applications to molecular techniques in aquaculture genetics are explained in Section 8.8.

## 8.8 Molecular techniques

The production challenges facing aquaculture include disease resistance, tolerance of handling, enhanced food conversion and spawning manipulation. These areas concern wild animals adopted for productive 'domestication', which has begun causing changes in gene frequencies and performance of fish under domestication. Directed breeding programmes did not develop until comparatively recently. Fish genetic programmes became more common with greater knowledge of breeding inheritances. Molecular-based knowledge, developed in the 1980s, has continued to gain prevalence. It is now well established in the selective breeding, biotechnology and molecular genetics of finfish, and

rapidly developing for aquatic invertebrate domestication.

When wild fish are moved to aquaculture settings, an organism better suited to aquaculture environment begins to evolve as a result of domestication. Domestication effects can be observed in some fish within a few generations after removal from the natural environment (Durham, 1996a). In channel catfish (*Ictalurus punctatus*) an increased growth rate of 3–6 per cent per generation has been observed. The oldest domestic strain usually performs better in the aquaculture environment than wild strains, though there are exceptions. Channel catfish strains differ in growth, disease resistance, body conformation, dressing percentage, vulnerability to angling and seining, age of maturity, time of spawning, fecundity and egg size (Dunham and Smitherman, 1984; Smitherman and Dunham, 1985). Strains of rainbow trout (*Oncorhynchus mykiss*) show similar variability (Kincaid, 1981).

#### 8.8.1 Inbreeding and maintenance of genetic quality

Losses due to inbreeding should be prevented, in order to maximise increased production resulting from genetic enhancement. This applies particularly to species with high fecundity where few brood stock are necessary to meet demands for fry and brood stock replacement. The effects of inbreeding are well documented and can result in a decrease of 30 per cent or more in terms of growth, survival and reproduction (Kincaid 1976a, 1976b, 1983a; Dunham, 1996b).

#### 8.8.2 Intraspecific crossbreeding

Crossing of different strains may increase growth rate but heterosis may not be obtained in every case. Increases of 55 per cent and 22 per cent growth rate of channel catfish and rainbow trout cross-breeds respectively were achieved using this technique (Dunham and Smitherman, 1983; Dunham, 1996b). Chum salmon cross-breeds, however, have shown no increase in growth rates compared with parent strains (Dunham, 1996a). Common carp cross-

breeds usually show low levels of heterosis (Moav *et al.*, 1964; Wohlfarth, 1993; Hulata, 1995); however, those that exhibited positive heterosis are the basis for carp aquaculture in Israel, Vietnam, China and Hungary. During the last 35 years, crossbreeding has been tested in more than 140 crosses. Three were chosen, based on 20 per cent improvement in growth rate and other features. Now approximately 80 per cent of common carp production comes from these cross-breeds. Production of gynogenetic female lines and gynogenetic sex-reversed inbred male lines from common carp with the best continuing ability form an important part of the Hungarian crossbreeding programme. The growth rate of F1 cross-breeds was only 10 per cent higher than controls (Bakos and Gorda, 1995).

Kirpichnikov (1981) successfully produced a new strain of cold-resistant carp for cold zones in northern Russia using local carps and Siberian wild carps from the river Amur. In Israel, over 20 years of crossing common carp strains revealed that crosses using the strain 'DOR-70' (Wohlfarth *et al.*, 1980) and the Croatian line 'Nawica' produced fast growth, and this is one of the most popular crosses for Israeli carp production (Wohlfarth, 1993).

In Indonesia, strain development using artificial gynogenesis and sex-reversal resulted in 10 common carp inbred lines, which were used for cross-breeding (Sumantadinata, 1995). In Vietnam, eight local varieties of common carp along with 'Hungary', 'Ukraine', 'Indonesia' and 'Czech' strains are maintained, with significant heterosis observed in F1 generations of cross-breeds. Hungarian and Indonesian strains have subsequently been used for carp selection and cross-breeding programmes throughout Vietnam (Thien and Trong, 1995).

Under various rice field conditions, growth rates of different strains of Nile tilapia and their crosses were superior to pure strains (Circa *et al.*, 1994). Breeding programmes are also under development for Java or silver barb, another economically important fish species in Southeast Asia. The growth rate of females from six crosses was 23 per cent higher than average growth rate of the parent strains.

Cross-breeds of different strains of European catfish, *Silurus glanis*, have outstanding adapt-

ability under warm water holding conditions (Krasznei and Marian, 1985). Studies on domestic channel catfish also showed greater heterotic growth rates in domestic × wild crosses (Dunham and Smitherman, 1983). The same was found in rainbow trout crosses (Gall, 1969; Gall and Gross, 1978; Kincaid, 1981; Ayles and Baker, 1983). Strain mating incompatibilities can, however, occur and can impede fry output.

### 8.8.3 Interspecific hybridization

Interspecific hybridization has been used to increase growth rate, manipulate sex ratios, produce sterile animals, improve flesh quality, increase disease resistance, improve tolerance of environmental extremes and improve a variety of other traits that make aquatic animal production more profitable. Although interspecific hybridization rarely results in an F1 suitable for aquaculture application, there are a few significant exceptions. Channel catfish (*Ictalurus punctatus*) females × blue catfish (*I. furcatus*) males is the only cross that exhibits superior growth rate, growth uniformity, disease resistance, tolerance of low oxygen levels, dressing percentage and harvestability (Smitherman and Dunham 1985). However mating problems between the two species have prevented commercial utilization.

The 'Sunshine' bass is cross between white bass (*Morone chrysops*) and striped bass (*M. saxatilis*), which grows faster and has better overall culture characteristics than either parent species (Smith, 1988). In addition, crosses of the silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristis nobilis*) (*Hypophthalmichthys nobilis*); black crappie (*Pomoxis nigromaculatus*) and *P. annularis* (Hooe *et al.*, 1994); and African catfish hybrids, *Clarias gariepinus*, and *Heterobranchus longifilis* and *H. bisorsalis* (Salami *et al.*, 1993; Nwaduwe, 1995), all show faster growth than the parent species. Numerous crosses of common carp with rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), catla (*Catla catla*) (Khan *et al.*, 1990); tambaqui (*Colossoma macropomum*) and *Piaractus brachypoma* and *P. mesopotamicus* (Senhorini *et al.*, 1988) and green sunfish (*Lepomis cyanellus*) with bluegill (*L. macrochirus*) (Tidwell *et al.*, 1992), have

enhanced performance of aquaculture production systems.

Hybrids between *Sparus aurata* and *Pagrus major* (both belonging to *Sparidae*) developed vestigial gonads when two or three years old and were sterile (Knibb *et al.*, 1998). Similar vestigial gonads were observed in offspring of the reciprocal crosses.

### 8.8.4 Genetic selection

Very little was done in the genetic selection of fish prior to 1970, but it has grown significantly since then (Dunham, 1996a). Response to selection for growth rate in aquatic species is very good (7 to 10 times in farmed aquatic species) (see Section 8.7). Fecundity is also higher.

Selection of body weight and disease resistance in salmonids has been particularly successful (Embry and Hyford, 1925). With respect to body weight, a 30 per cent increase in rainbow trout was achieved within six generations of selection (Kincaid, 1983b).

Responses can differ depending on the direction of selection. Body weight of common carp in Israel was not improved over five generations, but could be decreased (Moav and Wohlfarth, 1976).

Several authors have reported that mass selection improved body weight in tilapias (*Oreochromis mossambicus*, *O. aureus* and red tilapia). However, selection was less successful. Body weight of common carp appears unresponsive to selection; but body conformation can be dramatically changed (Ankorian, 1996).

### 8.8.5 Correlated responses

Although selection for body weight has generally been associated with positively correlated responses (e.g. increased survival and disease resistance), there are examples of long-term selection resulting in decreased bacterial resistance, possibly due to genetic correlation changes or inbreeding.

### 8.8.6 Polyploidy

Triploid fish are generally sterile. Females produce less sex hormones and although

triploid males may develop secondary sexual characteristics and exhibit spawning behaviour, they are generally unable to reproduce.

Channel catfish triploids become larger than diploids at about nine months of age (90 g) when grown in tanks (Wolters *et al.*, 1982). In tank experiments, the triploids converted feed more efficiently than diploids, had 6 per cent greater carcass yield at three years of age (Chrisman *et al.*, 1983) and were darker than diploids.

Polyploidy in the Asian catfish, *Clarias macrocephalus*, was induced by cold shock and resulted in 80 per cent triploidy (Na-Nokorn and Legrand, 1992). The effects on survival were not significantly different from diploid controls during first two months, but in the third to fifth month, triploid fish showed lower survival rates and body weight compared to the diploid group.

Polyploidy is not commercially feasible for all species. Bramick *et al.*, (1995) suggest that the use of triploid tilapia would reduce unwanted reproduction and stunting and would significantly increase yields from pond culture.

#### 8.8.7 Sex manipulation and breeding

Various strategies utilizing sex reversal and breeding progeny testing, gynogenesis and androgenesis can lead to the development of predominantly or completely male or female populations or a 'supermale' genotype (YY). The primary aim is to take advantage of sexually dimorphic characteristics (including flesh quality).

Sex reversal and breeding have allowed production of YY channel catfish males that can be mated to normal XX females to produce all-male XY progeny.

#### 8.8.8 Gynogenesis, androgenesis and cloning

Gynogenesis and androgenesis are techniques to produce rapid inbreeding and cloned populations. Androgenesis or all-male inheritance is more difficult to accomplish than gynogenesis, since diploidy can only be induced in androgens at first cell division, a difficult time to manipulate the embryo. Also, since androgens are

totally homozygous, a large percentage with deleterious genotypes probably die.

Fully inbred clonal lines have been produced in Zebrafish, ayu, common carp, Nile tilapia and rainbow trout (Komen *et al.*, 1991; Sarder *et al.*, 1999).

#### 8.8.9 Interspecific nuclear transfer

Interspecific nuclear transfer has been accomplished for cyprinids in China, resulting in embryos with the cytoplasm and mitochondrial DNA of the host species and the nuclear DNA of the donor species. As a result these fish may later serve as key for the application of transgenic technology.

Compared to the thousand years of aquaculture and its genetic improvement programmes, aquaculture genomics and gene mapping are truly in their infancy. Molecular genetics is less than 30 years old, although DNA was only discovered about 50 years ago. However, the late 1990s have seen an explosion in genomics and gene mapping of aquatic organism DNA sequences.

The first successful form of gene transfer – genetic engineering – was accomplished in China in 1985 and has subsequently been achieved in other countries. Most of this work focused on hormone enhancement of growth (size and rate), with results ranging from zero per cent up to an incredible 300 per cent enhancement under some conditions. Due to the lack of fish gene sequences, initial transgenic research in the mid-1980s employed mammalian growth hormone (GH) gene constructs, which enhanced growth in some but not all species examined (Zhu *et al.*, 1986; Enikolopov *et al.*, 1989; Zhu, 1992; Gross *et al.*, 1992; Wu *et al.*, 1994). Salmonids showed no effect (Guymard *et al.*, 1989a,b; Penman *et al.*, 1991), despite the fact that carp, catfish, zebrafish and tilapia are very responsive to growth enhancement (Martinez *et al.*, 1996), providing the first convincing evidence that this can be achieved in fish by transgenesis.

When a gene is inserted with the objective of improving a specific trait, it may affect another trait. Such 'pleiotropic' effect can be positive or negative; thus it is important to evaluate all important traits in the transgenic fish, not just the trait under active alteration. Transfer of

growth hormone genes has been documented to affect body shape, feed conversion efficiency, disease resistance, reproduction, tolerance of low oxygen concentrations, carcass yield, swimming ability and even predator avoidance.

Rainbow trout growth hormone (rt GH) transgene reduces survival of common carp and the number of F2 progeny inheriting the transgene is less than expected. Differential mortality or loss of the recombinant gene during meiosis is a likely explanation, since transgenesis was evaluated after the fish reached fingerling size. Remaining transgenic individuals, however, showed higher survival than controls when subjected to a series of stressors such as low dissolved oxygen (Chatakondi, *et al.*, 1995).

#### 8.8.10 Linkage mapping

Aquaculture genomics has seen dramatic progress over the last 10 years (Kocher *et al.*, 1998; Liu and Dunham, 1998; Waldbieser *et al.*, 1998). This includes progress in construction of framework genetic linkage maps for catfish (Li *et al.*, 2000) tilapia (Lee and Kocher, 1996; Kocher *et al.*, 1998; Agresti *et al.*, 2000; McConnel *et al.*, 2000) and oysters, *Crossostrea* and *Ostrea* spp. Genomic mapping of these phyletic groups was done recently (USDA) as a regional project.

Much work is ongoing on production of framework linkage maps with greater numbers of markers, particularly type I markers of known genes.

In the last few years, large numbers of molecular markers have been developed and evaluated for application in the culture of catfish as well as other commercially important species. Of the several types of markers evaluated, microsatellites and AFLP (amplified fragment length polymorphisms) were most reliable, efficient and reproducible for genetic linkage mapping in catfish (Liu *et al.*, 1999a,b,c,d).

#### 8.8.11 Marker-assisted selection

In aquaculture species, much effort is devoted to quantitative trait loci (QTL) mapping. QTL markers for growth, feed conversion efficiency, tolerance of bacterial disease, spawning time,

embryonic developmental rates and cold tolerance have been identified in channel catfish, rainbow trout and tilapias (La Patra *et al.*, 1993, 1996).

Molecular genetics through gene mapping have been used for studying genetic variation among different groups. DNA analysis is preferred over conventional protein analysis for determining genetic affinities and differences, though protein analysis is faster and less costly. While earlier linkages were studied using isozyme markers (Liu *et al.*, 1999a,b), recent catfish mapping has used microsatellite and AFLP markers.

#### 8.8.12 Combining genetic enhancement programmes

The best genotypes of aquaculture applications in the future will be developed by using a combination of traditional selective breeding and the new biotechnologies. Initial experiments indicate good potential for this combined approach, with examples using mass selection and cross-breeding, genetic engineering and cross-breeding and sex reversal and polyploidy, showing that all work more effectively in combination than alone. Several studies in aquaculture transgenics have dealt with improvement of growth rate of selected species. It has been indicated that it is possible to genetically improve the food conversion efficiencies (FCE); more precisely, it is an enhanced ability to convert measured parameters, such as (dry) weight/energy in food, into growth in the individual organism (Brett, 1995). This has also been referred to as 'effectiveness with which food is converted to saleable fish product' (Doupe and Lymbbery, 2003). According to these authors, if it is assumed that there is considerable genetic variation in FCE as found in terrestrial livestock, especially in pigs and poultry (Luiting *et al.*, 1994), improvements in FCE through genetic methods is possible subject to certain refinements in experimental methods. These would enable the determination of feed intake by individual fish and the optimal time over which the FCE is to be tested, and also the availability of information on genetic correlation between FCE and food intake conditions.

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