1 Recent Developments

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1.1 INTRODUCTION

The first decade of the twenty-first century saw a remarkable growth in aquaculture production due to the surge in the development of new technologies and better understanding of the production biology of new aquaculture species. A worldwide interest in the production biology of new candidate species for aquaculture and associated technology is not only deemed to be environmentally friendly, but could also lead to an increase in the productivity of aquaculture. This rise in interest in the subject has led to a gap in the published information, as only a few comprehensive textbooks are available to meet the demand.

This chapter highlights the recent developments in biotechnology and the research attempts to extend aquaculture to non-traditional farming sites. The use of biotechnology during breeding strategy has been very impressive and has also been applied to deal with widespread disease issues through molecular genetics and through the use of specialised feed additives, which have a potential to enhance the immune-competence of the cultured species. Captive breeding is playing an increasingly important role, and has been commercialised while producing high-value freshwater ornamentals.

1.2 DISEASE RESISTANCE IN AQUACULTURE SYSTEMS *VIS-À-VIS* BREEDING STRATEGY

Disease outbreaks are major constraints in any intensive production system. Diseases that remain at a low level of incidence in natural populations may reach epidemic levels in intensive cultivation systems. Intensive management systems in livestock production encourage the unpredictable appearance of new diseases and changes in the characteristics of established diseases (Biggs 1985).

If elimination of pathogens or control of culture conditions is difficult, selective breeding for host resistance to the pathogen may be an attractive option for disease control. Host

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resistance should only be considered when (a) the disease causes severe damage, (b) there are no other existing simple, cost-effective control measures, (c) there is demonstrable genetic variation in resistance and (d) this is not coupled with an excessive level of negative associations with other desirable characteristics.

The principles and concepts behind breeding programmes are based largely on experiences with plants and terrestrial animals as information from aquatic animals is very limited. With catastrophic diseases, such as white spot syndrome virus (WSSV), which cause mortalities of 98% or more, the frequency of resistance is low and it is suggested that for theoretical reasons single-gene, rather than polygenic, resistance is likely to develop. The low frequency of resistance genes in breeding populations may cause genetic bottlenecks, which will greatly reduce the genetic variation in the populations. In order to maintain the genetic variation the genes from the small numbers of survivors should be introgressed into populations with broader genetic variability.

Genetic variation in resistance may be encountered either in the initial base populations or may arise spontaneously due to mutations. Once genetic variation has been detected, the most appropriate breeding methodology will depend on the nature of both the resistance and the disease(s) that are of interest to the producers. Most populations of farmed shrimp have only had a relatively short period to evolve and adapt to intensive cultivated production systems.

In India modern intensive shrimp production systems provide almost ideal conditions for the propagation of diseases. The conditions favour epidemics and the appearance of apparently new diseases in intensive shrimp production systems. In Central and South America, *Penaeus vannamei* was widely devastated by Taura syndrome virus (TSV) in the early 1990s (Brock 1997). Later WSSV appeared in Asia and rapidly devastated the shrimp industry in many parts of the world. Both of these diseases were previously unreported. In Asia, epidemics of white spot and yellow head virus (YHV) have reduced production of various Penaeid shrimp species, including the native species *P. monodon* and the introduced species *P. vannamei.* As concepts behind disease control in aquatic animal species have been developed from warm-blooded terrestrial species, the major differences in their environments indicate that transfer of technology from one to the other should be carried out with caution. Warm-blooded terrestrial land animals maintain a relatively constant body temperature, whereas aquatic organisms are ecto-thermal and their body temperature fluctuates with that of the water in which they live. Similarly, the composition of the medium in which land animals live, the air, varies little, with such vital aspects as oxygen and carbon dioxide content relatively constant on a global basis. On the other hand, shrimps face tremendous variability in the environment in which they live, with dramatic changes often occurring abruptly. Stress, which is closely related to the manifestation of disease (Biggs 1985), is often induced by changes in such parameters as temperature, oxygen, salinity and ammonia.

Vaccination is a common disease control measure in warm-blooded animals, protecting hundreds of millions of animals from disease and death (NOAH 2002). It is generally accepted that the crustaceans do not possess the capacity to acquire resistance and hence vaccination is not possible, although Witteveldt (2006) has questioned this assumption.

In domesticated animal populations simple avoidance of diseases and pests has long been one of the most important means of disease control, with eradication of Newcastle disease in poultry and rinderpest and foot and mouth disease in cattle being well known cases (Biggs 1985). Disease avoidance or eradication is only possible in certain circumstances. Exclusion of diseases has been attempted with some success in shrimp cultivation

(McIntosh 1999; Moss 1999), with various programmes emphasising the use of Specific Pathogen Free (SPF) stock in breeding programmes to minimise spread of diseases (Moss 1999; Moss *et al*. 2003; Lightner 2005a; Hennig *et al*. 2005). However, it is not easy to avoid or eradicate diseases in an open-air aquatic growout environment.

With the exception of some diseases, such as yellow head virus (YHV) and monodon bacillus virus (MBV), most of the shrimp viruses have spread rapidly from the sites where they were first recognised (Lightner 1996, 2005b; Flegel *et al*. 2004). The recent epidemic of white spot syndrome indicates how rapidly an epidemic may spread in marine species. First detected in Taiwan in 1992 (Chou *et al*. 1995), WSSV spread rapidly to most Asian countries (Inouye *et al*. 1994; Wongteerasupaya *et al*. 1995; Flegel & Alday-Sanz 1998; Zhan *et al*. 1998) and by 1996 most shrimp-farming regions in Southeast Asia were affected (Flegel & Alday-Sanz 1998). In the western hemisphere the first outbreak of WSSV appeared in farmed *P. vannamei* and *P. stylirostris* in South Carolina (USA) in 1997 and it was associated with 95% of cumulative losses (Lightner 1999). By early 1999, WSSV had spread to farmed *P. vannamei* in Central America (Jory & Dixon 1999), reaching the Colombian Pacific coast in May of that year. The disease devastated most of the major shrimp-producing areas of the world. Attempts to eradicate or exclude it were mostly unsuccessful. WSSV appeared to have been successfully excluded from a few shrimp-producing regions, particularly the Atlantic coast of South America; however it has recently been reported in the cooler regions of southern Brazil (Anon 2005). It now appears that conditions on the Atlantic coast of South America were in general not conducive to the development of full-scale white spot due to the high water temperatures (Vidal *et al*. 2001). The absence of a white spot virus epidemic in this area appears to be related to the virus's inability to replicate at the higher temperature rather than a temperature-mediated response by the shrimp (Reyes *et al*. 2007). Some areas in South and Southeast Asia may have escaped or have a low incidence of WSSV due to higher water temperatures. Shrimp farmers have in some cases reduced water exchange and appear to have achieved some level of control of WSSV with this practice, which probably both increases water temperatures and also reduces the chances of pathogens entering the ponds. In Thailand the use of specific pathogen free (SPF) stocks and biosecurity measures have reduced WSSV incidence dramatically.

In many animals, disease resistance is both innate and acquired. Innate immunity is rapid, non-specific and acts as a first line of defence, while acquired resistance involves antigen-specific responses (Bishop *et al*. 2002). Shrimps possess an innate immune system that protects them from foreign organisms. Recently Witteveldt (2006) indicated that vaccination of shrimp against WSSV might be possible, which would open the way for the design of new strategies to control WSSV and other invertebrate pathogens. In addition, there may be possibilities to stimulate the immune system and a series of non-specific responses against invading organisms. Genetically controlled behavioural characteristics may also provide resistance to disease: for example genetically controlled hygienic behaviour in bees prevents chalk brood disease (Milne 1983). It is possible that genetic control of cannibalistic behaviour may be involved in providing a measure of resistance to infection (Gitterle *et al*. 2005). With diseases that are difficult to eradicate, control measures have been developed based on stimulation or enhancement of the natural defence mechanisms of the host organism, including selection for host resistance or tolerance to diseases and modification of the environment so that the disease is not favoured.

Genetics-based host resistance is an attractive proposition from the point of view of the grower of improved stock. An advantage of host resistance is the minimal negative impact on the environment. On the other hand, development of genetically based host resistance is often costly and may be impossible to achieve in the absence of useful levels of resistance. Selective breeding programmes inevitably lead to slower progress in other desirable characteristics in the breeding goal. Added to this, disease resistance may be negatively associated with other desirable characteristics. The genetic control of disease resistance in shrimps is not well understood and little research has been done in this area. Shrimps appear to have no acquired immune response, and in this sense they are perhaps somewhere between plants and mammals in their response. Inferences on various aspects of genetic control of disease resistance are mainly drawn from other species, particularly plants and mammals, of which there is a vast stock of knowledge. Selective breeding for disease resistance in plants has a longer history than in mammals. Vertical resistance provides effective immunity, normally through hypersensitivity, and is controlled by a single gene. Horizontal resistance does not provide total immunity but slows the spread of the disease and is controlled by many genes. In a selection programme, the selection protocol itself may affect the type of resistance that is encountered: selection procedures with a limited range of genetic variation and dosages or inoculum pressure that ensure more survivors are likely to lead to uncovering and selection for polygenic resistance, whereas natural selection in the field with larger genetic variation and extremely high mortalities (well over 99%) are likely to uncover single-gene resistance which will normally be dominant.

In most of the animals studied, disease resistance is controlled quantitatively by multiple genes and breeding programmes are based on this assumption. However, breeders should not ignore the possibility of single-gene resistance, which has also been observed in animals and humans (Hills 2001).

Fjalestad *et al*. (1993) suggest that in the fish farming environment, resistance to a given pathogen will normally develop slowly. However, resistance to serious pathogens may develop through natural selection in aquaculture populations where the animals have been exposed continuously to the pathogen for only a few generations, as in the case of TSV and with the QX disease in the Sydney rock oyster *Saccostrea glomerata* (Nell & Hand 2003). In shrimp, which has only recently been bred in captivity, most of the genes that control resistance will probably have come from the original native populations, although their frequency may have been radically altered as populations encountered vastly different conditions.

Selection for disease resistance is directly related to its effect on growth and survival: the objective is not disease resistance *per se* but rather the impact that disease resistance will have on the desired performance characteristics of the selected stock. Diseases can directly affect both growth and survival. Diseases such as TSV and WSSV cause severe damage through mortality, although animals that survive may have reduced growth rates. Other diseases, for example *Vibrio*, may cause high mortality under some conditions, whilst in other conditions their main effect may be to reduce growth. Until now the main focus in selection for disease resistance in shrimps has been to improve survival in the face of epidemics of diseases such as TSV, which may cause mortalities of 70% or greater, and WSSV with mortalities close to 100%.

The white spot case highlights the importance of having a broad genetic base so as to identify sources of resistance: the frequency of resistance genes appears to be very low and there may be sources of resistance that are not included in the initial populations. This case also highlights the difficulties encountered when there is a negative correlation between two or more desired traits. Selection procedures are needed to ensure selected stock will perform well commercially: this normally means having the ability to survive an epidemic.

At present, selection for disease resistance in designed breeding schemes is normally carried out based on survival recorded in controlled challenge tests.

In principle, the simplest programme of genetic stock improvement is to choose superior animals as breeders so that as generation succeeds generation the variation in the original population is translated into improved production. This straightforward approach can be guaranteed to work only if certain conditions are met:

- the variation must be heritable so that the superior qualities of the parents are passed on to their offspring
- the qualities designated 'superior' must be easy to recognise so that large numbers of animals can be classified quickly
- traits under selection must not be correlated in a way which is counterproductive
- it must be physically convenient to induce the selected individuals to mate and to keep the selected offspring separate from the rest of the population
- the progress of the selection programme must be carefully monitored to maintain the integrity of the experimental design over many generations.

1.3 FRESHWATER ORNAMENTAL AQUACULTURE – AN INDUSTRY VIEW FROM WESTERN AUSTRALIA

This section is based on a personal communication from Iain Mcgregor (2010), a leading freshwater ornamental aquaculturist in Western Australia. Some of the information also comes from leading magazines.

The freshwater ornamental aquaculture industry in Western Australia has many complexities and provides unique challenges for the people working in it. This industry can be seen as typical of ornamental freshwater industries in the developed economies where captive breeding and other forms of technologies are employed. Most species are cultured in field conditions; some are kept in intensive situations such as recirculating systems or aquariums to match optimum requirements. To capitalise on time and space, complementary species are grown together. Aggressive or predatory species may present unique problems such as cannibalism. The greater the number of species in a polyculture situation the greater the complexities involved in successful production.

Goldfish are probably the oldest species of ornamental fish and the shape, colour and physical mutations to choose from are mind-boggling, with new phenotypes appearing all the time. Other famous ornamental subjects such as discus fish and guppies have also been extensively developed, with many colour strains now available. These subjects have legions of avid admirers worldwide, to the extent that whole shows are now held for only one species.

Japanese coloured carp or koi have a long history of culture for the dual purpose of table and ornamental qualities. Initially this fish was a protein source for people across Europe and Asia. As it spread to remote areas human culture started to mould the genetics of common carp (*Cyprinis carpio*). It was selected for high growth rates and reduced numbers of scales to make it easier to prepare for consumption. But in rural China and Japan another pressure was to change the destiny of this fish and launch it worldwide. The parts of the country where this happened suffered from extreme winter conditions and the locals were snowed in for extended periods. Fish for food were placed in a pond inside the homes and used to get the snowbound inhabitants through this difficult season. This environment of isolation led to inbred genetics, leading to the mutation of colour. The unusual specimens must have attracted much attention, so that people began to take an interest in the fish beyond their value as a food source.

As colours appeared and were mixed, new combinations arose and breeders began to select exclusively for superior specimens. Soon this diversity needed order and a basic grouping of 13 colour varieties was settled on as a rough guide and standard. One of the more amazing of these mutations is the trait that gives the fish a 'metallic' look (Fig. 1.1); so completely divergent is this from the brown/grey fish that shape is all they seem to have in common. The lustre of the skin on 'Hikari muji mono' and 'Hikari moyo mono' seems to glow, almost generating its own light. This intense colour trait has not been tried in all the traditional patterns but only needs a motivated breeder to achieve this.

If the kaleidoscope of colour was not enough of a new factor, amazing reflecting scales looking like glitter appeared termed 'kin gin rin' (Fig. 1.2), and in a few years of breeding selected individuals, this trait could be expressed in five ways each illuminating the scale with a different facet. When combined with the metallic fish the the effect was very attractive.

Long-fin or butterfly koi (Fig. 1.3a, 1.3b) seemed to have originated in Southeast Asia during the 1970s and distributed worldwide a short time after. The long-fin trait expresses itself with fins about twice the length of those in normal carp and some fish show great individuality of fin shape amongst themselves, such as with pectoral fins having each ray longer than the surrounding webbing and appearing ragged – in complete contrast to smooth entire-finned fish (Fig. 1.4).

Fig. 1.1 Two metallic koi or ogons: one gold and one orange (with permission of David Prangell, from his thesis). (Please see plate section for colour version of this figure.)

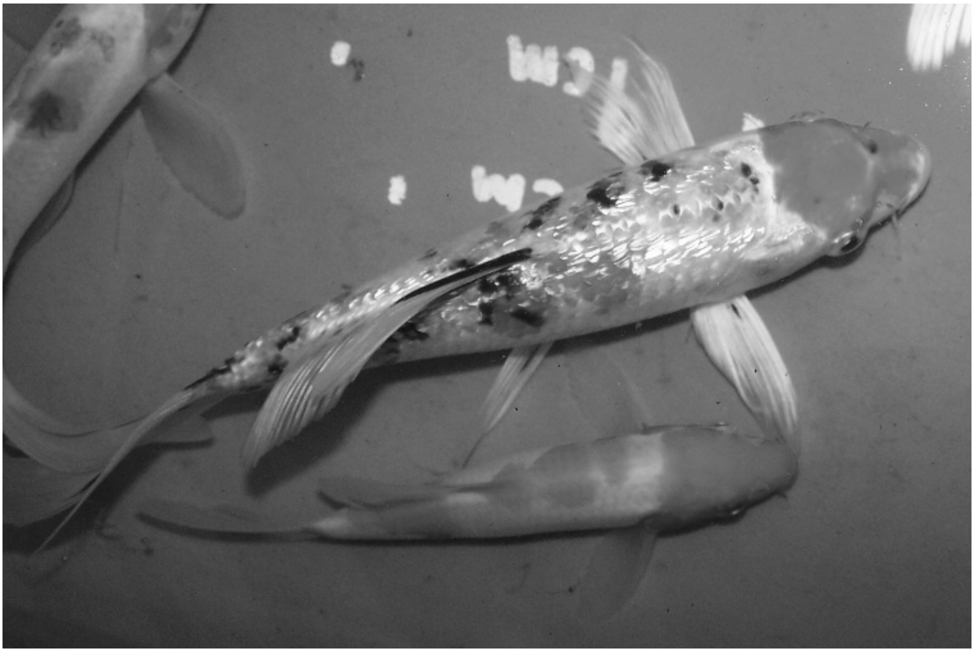

Fig. 1.2 Small butterfly koi. The larger fish shows the scale trait of 'kin gin rin'. (Please see plate section for colour version of this figure.)

In less than 150 years of culture, ornamental carp or 'Nishikigoi' have graced garden ponds and been collected and exhibited by enthusiasts across the globe. This appreciation of art placed on a fish reaches its peak at the Japan show, a place where fortunes are made or maintained by having the grand champion fish.

Other genetic traits are the longer mouth barbels and larger nostrils that extend outside the head cavity (Fig. 1.5). They are also very robust individuals that grow strongly. As butterfly koi became a component of ornamental koi, the governing body was faced with a decision as to whether these fish had a place in the exhibition circuit. The Japanese group, the ZNA, made a decision against recognising the longer-finned fishes. This decision may have reduced their attractiveness to serious hobbyists. However, when a person who had no interest in this aspect was given a choice between short- and long-finned fish for their garden pond they usually chose the latter. These average pond keepers began creating an undeniable market for the illegitimate style. In recent years American koi shows have begun to give butterfly koi a class, and even in Japan, fish farms aim to service this part of the market.

The butterfly koi looks set to become more popular than ever, despite traditional Japanese values and tastes in fish. Fish growers are responding to the demand and the genetics of this strain of koi are strengthening as each growing season passes. Higher quality stocks are being produced in greater numbers and each day novice koi keepers enter the market.

What future can genetics hold for the appearance of butterfly koi? Soon they will be available in all colours with 'kin gin rin' scales. When this is achieved and all varieties are being supplied in large numbers, maybe the next horizon will be xanthic or albino fish with an underlying pattern of the major colours from traditional koi. Can the long-fin phenotype be exaggerated to be extra long, as has been bred into poultry or guinea pigs? Is body shape the next frontier, following down the same road as goldfish? Will some view short stumpy

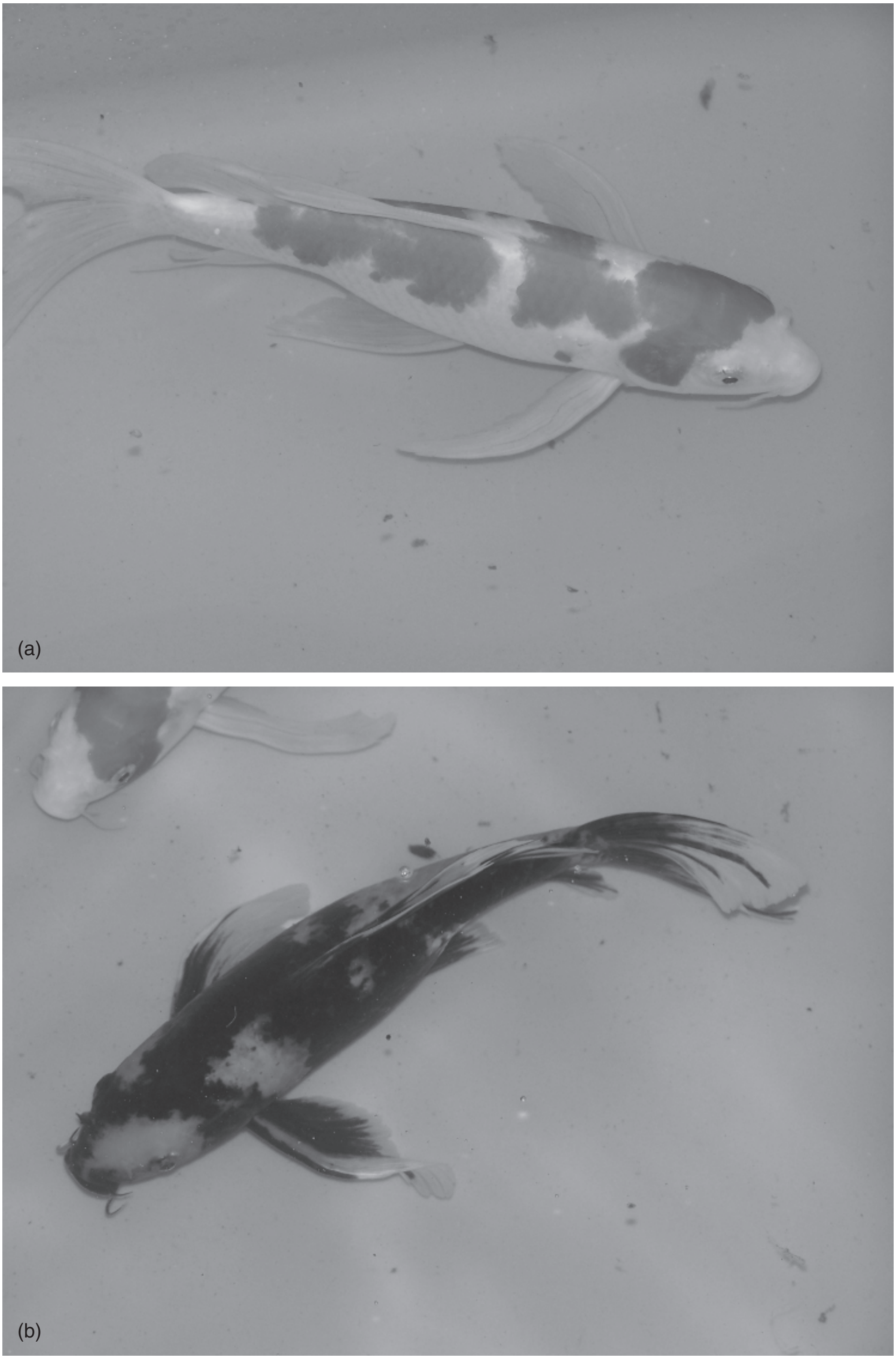

Fig. 1.3 **(a)** Long-finned kohaku. **(b)** Long-finned 'Hi utsuri'. (Please see plate section for colour version of this figure.)

Fig. 1.4 Long-finned golden ogon and hariwake showing pectoral fin ray diversity. (Please see plate section for colour version of this figure.)

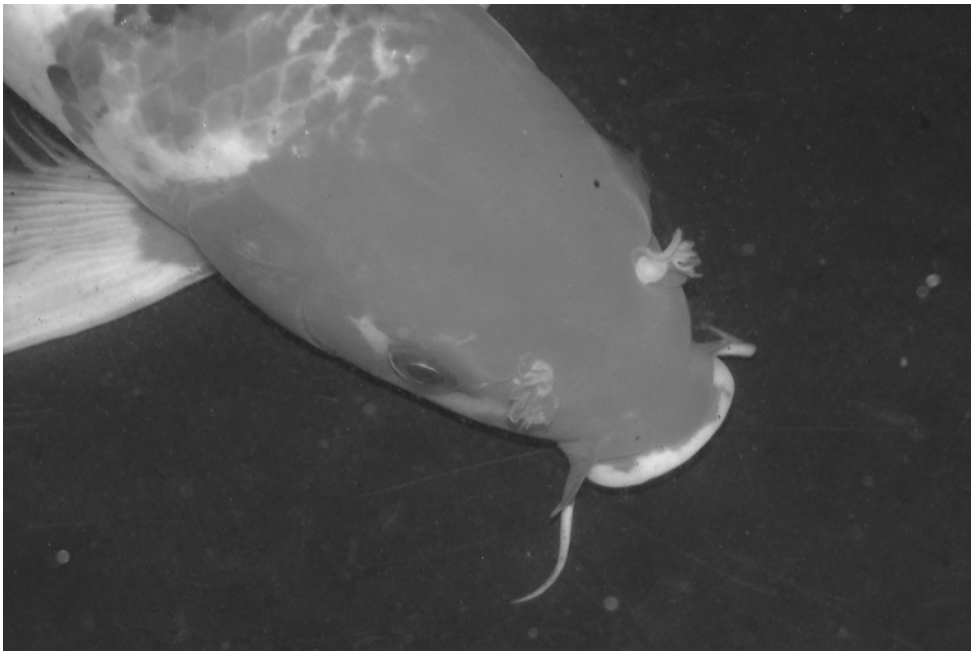

Fig. 1.5 Koi with long mouth barbels and extended nostril phenotype. (Please see plate section for colour version of this figure.)

koi as beautiful? The technology for cloning carp currently exists and this procedure may provide a stimulus for new mutations. The possibilities that genetic engineering can offer are bound only by the imagination; gigantism may be the goal, or even salt tolerance! Indeed genetic manipulation may even be able to have a direct influence on where the colour on a fish is placed.

As new markets unfold, new trends gain interest; these elegant fishes will continue to flourish. And for the breeders of butterfly koi each growing season holds new promise.

1.4 USE OF IMMUNOSTIMULANTS AS FEED ADDITIVES

Intensification of aquaculture has led to gradual and chronic environmental degradation, loss of biodiversity and eventually loss of productivity. In order to overcome the loss of productivity, the use of chemicals and antibiotics is an easy solution but not without drastic consequences on sustainability and the health of consumers of aquatic products. Recently, the use of environmentally friendly feed additives, namely probiotics and prebiotics, has become popular in the aquaculture industry (Vine *et al*. 2006; Soltanian *et al*. 2007). These specialised feed additives act as immunostimulants, which either enhance innate defence responses prior to exposure to a pathogen, or improve survival after the actual infection by pathogens (Bricknell & Dalmo 2005).

Research on innate immune systems has revealed new insights into the management and control of diseases in aquaculture (Bachère 2003). It is believed that understanding the immune criteria as enhancement of non-specific defence responses against bacterial and viral injections is the most effective way for sustainable aquaculture production (Chang *et al*. 1999; Bachère 2003; Chang *et al*. 2003), but the benefit of immunostimulants is still doubtful in invertebrates (Marques *et al*. 2006). Immunostimulants fed to animals may not be effective against all diseases (Sakai 1999). However, Bricknell and Dalmo (2005) claimed that a great deal can be done to improve larval survival against bacterial and viral pathogens by the judicious use of immunostimulants. Recently, immunological techniques have identified several distinct types of collagen in *P. japonicus* (Mizuta *et al*. 1992), but the genetic control of the production of these techniques has not been investigated (Benzie 1998).

Immunostimulants are obtained from various sources such as bacteria, brown and red algae and terrestrial fungi (Bricknell & Dalmo 2005), bacteria from aquatic habitats (Rengpipat *et al*. 1998) and marine yeast (Sajeevan *et al*. 2006). Immunostimulants can be divided into several groups, depending on their original sources such as bacteria, algae-derived, animal-derived, nutritional factors and hormones or cytokines (Sakai 1999). Probiotics are defined as ‘live microorganisms which when administered in adequate amounts confer a health benefit to the host’ (FAO/WHO 2002) and prebiotics are defined as ‘nondigestible food ingredients that beneficially affect the growth and health of the host’ (Gibson & Roberfroid 1995). According to Kesarcodi-Watson *et al*. (2008), certain suggested immunostimulants (Itami *et al*. 1998; Smith *et al*. 2003) such as peptidoglycan (PG) and lipopolysaccharides can be considered as probiotics. In addition, a number of chemical agents, polysaccharides, plant extracts or some nutritional additives, act as immunostimulants (Sakai 1999; Gannam & Schrock 2001), are adjuncts to vaccination and provide a potential route to reduction of the widespread use of antibiotics (Burrells *et al*. 2001). Herbal immunostimulants, namely methnolics extracted from five different herbal medicinal plants, were shown to increase *P. monodon* resistance against viral pathogenesis caused by WSSV (Citarasu *et al*. 2006). The use of immunostimulants in penaeid prawn aquaculture is described in Chapter 5.

1.5 ALTERNATIVE SITES FOR AQUACULTURE

In terms of land availability, suitable sites for aquaculture are often not easily and readily available as most of them are expensive and have been occupied by other users (Allan *et al*. 2001). In addition, acquaculture activities may disturb other activities, or vice versa. In such conditions, aquaculture facilities often have to be built in areas that are essentially not suitable for aquaculture purposes as water and soil quality do not meet the aquaculture requirements. For example, in Asia, aquaculture ponds are built in mangrove areas, which have potential for acid sulphate released from the soils (Pillay 1993). This in turn can slow aquaculture production, as the cultured aquatic species are exposed to low water quality. In Thailand productive rice paddy fields have been used for inland saline prawn culture; the Thai authorities have now banned the practice as it has caused salinisation of agricultural land (Fegan 2001).

Inland saline water (ISW) has resulted from anthropogenic activities. It has adversely influenced the agricultural outputs and environment in the USA, Australia, India, China and Israel (Allan *et al*. 2001). Several actions have been taken for remediation of these problems, including an attempt to use ISW for marine aquaculture (Fig. 1.6). This has been

Fig. 1.6 A typical inland saline water purpose-built pond in Wannamal, Western Australia. (Please see plate section for colour version of this figure.)

seen as a remedial approach to reduce the cost of groundwater pumping and create economic opportunities for the farmers in the affected areas (Doupé *et al*. 2003a,b). In addition, most ISW is suitable for aquaculture purposes as it is located in remote areas where land is cheap and disease-free, so there is potential for integration between aquaculture and agriculture (Gong *et al*. 2004).

1.5.1 Inland saline water aquaculture in different countries

To date, studies on the use of inland saline water (ISW) as an alternative source for marine culture have been attempted in countries like the USA (Forsberg *et al*. 1996; McMahon *et al*. 2001; Treece 2002; Saoud *et al*. 2003; Gong *et al*. 2004), India (Rahman *et al*. 2005; Jain *et al*. 2006) and Australia (Allan *et al*. 2001; Fielder *et al*. 2001; Partridge & Furey 2002; Prangnell & Fotedar 2005, 2006a,b; Partridge *et al*. 2006, 2008; Tantulo & Fotedar 2006, 2007). If the use of ISW as a medium for marine culture can be proven cost-effective, then it should be considered as an alternative for marine aquaculture. It has some comparative advantages over the coastal areas in terms of cheap land availability, better quarantine capability and freedom from conflict over the same resources (Allan *et al*. 2001). The use of ISW as a medium for culture of marine fish and prawns can only be successful if the K^+ deficiency in ISW can be eliminated, e.g. by fortifying the K^+ concentration in ISW from 50 to 100% of K^+ concentration in ocean water (OW). This can be achieved by adding KCl in ISW, but high costs might be incurred when this method is used. Another method is by culturing hardy species like black tiger prawn and barramundi (*Lates calcarifer*) which have already been proven able to withstand the low salinities of ISW.

1.5.1.1 *Australia*

In Australia, most studies have been conducted with the considerations that ISW has caused a negative impact to the physical, social and economic systems of the affected area (Allan *et al*. 2001). Attempts have been made to minimise these problems by pumping the ground ISW to lower the water table and then storing it in earthen ponds. This has been seen as an alternative source for marine aquaculture purposes (Allan *et al*. 2001). The main cause of increase in ISW and dry land salinity is the clearing of deep-rooted native plants and replacing them with shallow-rooted grain and pasture crops that have less capability to catch the excess surface rainwater (Walker *et al*. 1999; George & Coleman 2001) and keep the groundwater table at constant levels. This in turn has increased the saline groundwater table and brought it to the surface.

In general, except for low K^+ concentration, ionic composition and concentration of ISW is similar to the ocean water (Nulsen 1999). As with ocean water, sodium and chloride are the major ions that determine the salinity of the ISW (Rayment & Higginson 1992) and the osmolality of the haemolymph of the cultured species (Pequeux 1995).

Low K^+ concentration in ISW occurs due to adsorption of the K^+ onto the clay (Allan *et al*. 2001). Although, K^+ concentration only contributes a small part of the total ions making up the ISW, it plays an important role in the functioning of the physiological systems of the aquatic animals (Burton 1995; Shiau & Hsieh 2001). In crustaceans, K^+ is very important to activate Na^+/K^+ ATPase (Skou 1957; Mantel & Farmer 1983), which is responsible for maintaining the ionic imbalance in the haemolymph. Therefore, alteration of Na^+ and K^+ ratio in the haemolymph may disturb entire physiological functions of the aquatic animals.

As ISW is K^+ deficient, most fish or prawns cannot survive in this type of water. Recent studies on prawn (Ingram *et al*. 2002; Saoud *et al*. 2003; Prangnell & Fotedar 2006a; Tantulo & Fotedar 2006) and fish culture (Fielder *et al*. 2001; Partridge & Creeper 2004) in this type of water have reported mortality. An approach to improve the survival rate of the cultured prawns is by fortifying K^+ in ISW through the supplementation of KCl (Rahman *et al*. 2005; Prangnell & Fotedar 2005, 2006a,b; Tantulo & Fotedar 2006; Partridge & Lymbery 2008) and also by adding 5% KCl in fish diets (Gong *et al*. 2004).

The physiological responses of the animals, including survival, growth, ionic and osmoregulation ability, have been analysed following rearing and culture in K^+ deficient ISW, K^+ fortified ISW and K^+ supplemented diet (Gong *et al*. 2004; Prangnell & Fotedar 2006a; Tantulo & Fotedar 2006). However, as survival and growth rate might not give clear explanations of the effect of K^+ deficient ISW on the cultured animals, detailed studies have been conducted on osmo- and iono-regulation of animals cultured in and exposed to ISW (Saoud *et al*. 2003; Prangnell & Fotedar 2006a; Tantulo & Fotedar 2007). The studies on the effect of K^+ deficient ISW on osmo- and iono-regulation of prawns have been conducted on the basis that K^+ is the principal intracellular cation in animals (Shiau & Hsieh 2001) and plays important role in creating differing electrical charges between inner and outer membranes (Burton 1995). K^+ indirectly affects the haemolymph osmolality through the Na^+/K^+ ATPase activity. Na^+/K^+ ATPase activity is a mechanism that establishes the Na^+ gradient in prawn haemolymph (Roer & Dillaman 1993). Tantulo and Fotedar (2007) revealed that a decrease in K^+ concentration led to increased Na^+ concentration in haemolymph of black tiger prawn (*Penaeus monodon*), which in turn increased the haemolymph osmolality and caused the death of the prawns. This is an indication that K^+ is not isolated in its effect on the physiological system of the prawns or fish, but works in conjunction with Na^+. Furthermore, the correct ratio of Na^+ and K^+ is very important for maintaining proper physiological functions of *Litopenaeus vannammei* (Zhu *et al*. 2004) and *P. monodon* (Tantulo 2007).

The low K^+ concentration in ISW can be increased either by supplementing ISW with KCl (Prangnell & Fotedar 2005, 2006a,b; Tantulo & Fotedar 2006, 2007) or spreading muriate of potash on the bottom of the earthen ponds (Collins *et al*. 2005; Partridge & Creeper 2004). Following addition of KCl, survival and growth rate of the prawns and finfish in ISW were similar to those cultured in OW (Collins *et al*. 2005; Prangnell & Fotedar 2005, 2006; Rahman *et al*. 2005; Tantulo & Fotedar 2006, 2007). In addition, K^+ fortification in ISW also increased the osmo-regulation capacity of western king prawns and black tiger prawns and led to higher survival and growth rate of both prawns, similar to those cultured in OW (Prangnell & Fotedar 2005; Tantulo & Fotedar 2006, 2007).

Exceptions to the negative effects of K^+-deficient ISW have been observed in black tiger prawns (Tantulo & Fotedar 2007) and barramundi juveniles (Jain *et al*. 2006; Partridge *et al*. 2008) that were cultured in low salinity ISW of 5 and 15 ppt respectively. As the black tiger juveniles can strongly osmo-regulate their K^+ concentratration, they exhibit similar survival and growth rates when cultured in ISW and OW of low salinities. Partridge *et al*. (2008) revealed that barramundi need more supplementation of the K^+ concentration at higher salinity (45 ppt) than the fish reared in salinity close to the isosmotic line. On the other hand, fish or prawns reared at lower salinities may not need supplementation of K^+.

To date, most marine fish and prawn ISW culture attempts have been on an experimental scale (see Table 1.1 for Australia). It has been reported that some fish such as mulloway (*Argyrosomus japonicus*) can survive and grow well in K^+-deficient ISW (Aquaculture SA 2003; Partridge & Lymbery 2009). However, barramundi died 10 days after stocking in

Table 1.1 Aquatic species cultured in ISW either on an experimental (E) or commercial (C) scale in Australia.

Species	**State(s)**	**Scale**	**Reference(s)**
Dunaliella salina	NT, SA, WA	E, C	Paust (1999); George & Coleman (2001); McFarlane & Christie (2002); Collins *et al*. (2005)
Giant clam (*Hippopus hippopus)*	NT	E	Lee (1999)
Greenlip abalone *(Haliotis laevigata*)	WA	E	Harris *et al*. (2005)
Pacific oysters (*Crassostrea gigas)*	Vic	E	Ingram *et al*. (2002)
Sydney rock oysters *(Saccostrea glomerata*)	Vic	E	Ingram *et al*. (2002)
Trochus (*Trochus niloticus*)	NT	E	Lee (1999)
Brine shrimp (*Artemia salina*)	SA, Vic	E, C	Hutchinson (1999); McFarlane & Christie (2002); Gooley *et al*. (1999); Gooley & Gavine (2003)
Banan prawns (*Penaeus merguiensis*)	Qld	E	Collins *et al*. (2005)
Kuruma prawns (*Penaeus Japonicus*)	Vic	E	Ingram *et al*. (2002)
Tiger prawns (*P. monodon*)	NSW, NT, Qld, Vic, WA		Ingram *et al*. (2002); Collins & Russell (2003); Doroudi *et al*. (2003); Collins *et al*. (2005); Rahman *et al*. (2005); Tantulo & Fotedar (2006)
Western king prawns (*Penaeus latisulcatus*)	WA	E	Prangnell (2006)
Western rock lobster (*Panulirus cygnus*)	WA	E	Tantulo *et al*. (2005)
Atlantic salmon (*Salmo salar*)	Vic	E	Ingram *et al*. (2002); Gooley & Gavine (2003)
Australian bass *(Macquaria novemaculeata*)	Vic	E	Ingram *et al*. (2002); Gooley & Gavine (2003)
Barramundi (*Lates calcarifer*)	NSW, WA, SA	E, C	Fielder & Allan (1997); Allan & Fielder (1999); Hutchinson (1999); Paust (1999); O'Sullivan (2003); Partridge & Creeper (2004); Partridge *et al*. (2006)
Black bream (*Acanthopagrus burcheri*)	SA, Vic, WA	E, C	Paust (1999); Walker *et al*. (1999); Ingram *et al*. (2002); Doupe *et al*. (2003a,b); Gooley & Gavine (2003)
European carp (*Cyprinus carpio*)	Vic	E	McKinnon *et al*. (1998)
Greenback flounder (*Rhombosolea tapirina*)	SA, Vic	E	Hutchinson (1999); Ingram *et al*. (2002)
King George whiting (*Sillaginodes punctatus*)	SA, WA	E	Hutchinson (1999); Partridge (2001)
Mulloway (*Argyrosomus hololepidotus*)	NSW, Vic, WA	E, C	Doroudi *et al*. (2003, 2006); O'Sullivan (2003); Dutney (2004); Flowers & Hutchinson (2004); Partridge *et al*. (2006)
Rainbow trout (*Onchorhynchus mykiss*)	NSW, Vic, WA	E, C	Ingram *et al*. (2002); McFarlane & Christie (2002); Doupe *et al*. (2003a,b); Gooley & Gavine (2003); Partridge *et al*. (2006)
Sand whiting (*Sillago ciliate*)	Vic	E	Ingram *et al*. (2002)

Table 1.1 (*Continued*)

Species	State(s)	Scale	Reference(s)
Silver perch (*Bidyanus bidyanus*)	NSW, SA, Vic	E, C	Hutchinson (1999); Ingram *et al*. (2002); Doroudi *et al*. (2003); Gooley & Gavine (2003)
Snapper (*Pagrus auratus*)	NSW, SA, Vic, WA	E	Hutchinson (1999); Fielder *et al*. (2001); Ingram *et al*. (2002); Partridge & Furey (2002); O'Sullivan (2003)
Australian herring (*Arripis Georgiana*)	SA	E	Hutchinson (1999)
Yellow-fin whiting (*S. schomburgkii*)	SA	E	Hutchinson (1999)

NSW = New South Wales; SA = South Australia; Vic = Victoria; WA = Western Australia
Source: Adapted from Prangnell (2006, unpublished thesis)

K^+-deficient inland saline groundwater (Partridge & Creeper 2004; Partridge & Lymbery 2008). The fish survived and grew well when muriate of potash (KCl) was added to the ISW stocked in earthen ponds (Partridge *et al*. 2006, 2008), indicating that increasing K^+ concentration in ISW has a positive outcome. Similar result was exhibited by snapper (*Pagrus auratus*) when cultured in ISW containing 5% K^+ as in ocean water (Fielder *et al*. 2001). In this case, the mortality could be avoided by increasing the K^+ concentration to 40% of K^+ OW concentration (Fielder *et al*. 2001).

1.5.1.2 *USA*

The use of inland saline water for prawn culture in the USA was first introduced in 1973 in West Texas where prawn production ranged from 3.36 to 5.04 t/ha (Treece 2002). Recently, an increased interest in using ISW for marine prawn and fish culture has become evident due to widespread outbreaks of disease, perception of a cheap under-utilised resource and less conflict with other users (Samocha *et al*. 2002). In the USA, most inland saline water (ISW) for marine prawn and fish culture is extracted from shallow saline groundwater. In Texas, inland saline groundwater (ISGW) has higher SO_4^{-2} concentration when compared to ocean water at the same salinity (Forsberg *et al*. 1996). Boyd *et al*. (2009) did a study on the distribution of IGSW over the state of Alabama in order to map suitable areas of ISGW for aquaculture purposes. The suitability was assessed on the basis of chloride (Cl^-) concentration above 126 mg/L. It has been reported that 238 out of 2,527 wells in Alabama had a concentration of Cl^- above 126 mg/L.

Although different in ion concentrations compared to those in OW, the ISGW has a potential for aquaculture purposes. The ISGW, when used for culturing, has been able to support the survival and growth of marine diatom (*Phaeodacrylum tricomutum*), brine shrimp (*Artemia salina*) (Brune *et al.* 1981); Pacific white shrimp (*Penaeus vanname*) (Smith & Lawrence 1990; Saoud *et al*. 2003) and red drum (*Sciaenops ocellatus*) (Forsberg *et al*. 1996; Forsberg & Neill 1997).

Despite the great potential of ISGW for aquaculture purposes, some problems related to the osmo- and iono-regulatory capacity due to ionic imbalance of ISGW have been notified by Gong *et al*. (2004). However, a modification of diet by supplementing additional dietary magnesium, potassium, phospholipids and cholesterol to a commercial shrimp feed has been proven effective to improve the osmo- and iono-regulatory capacity of prawns (Gong *et al*. 2004).

1.5.1.3 *India*

In a similar manner to Australia, in the state of Haryana in India the extent of ISW is surging due to rising ground water tables, which bring saline water to the surface. The salinity of the ISW in the state ranges from 10–35 ppt with high Ca^{2+} and Mg^{2+}, which has resulted in high water hardness (Jana *et al.* 2004). As a consequence, productive agricultural land has been destroyed.

In India, research has focused on the use of ISW for culturing brackish water and freshwater species, such as black tiger prawns, milkfish (*Chanos chanos*), grey mullet (*Mugil cephalus*), barramundi and giant fresh prawn (*Macrobrachium rosenbergii*). Rahman *et al*. (2005) reported that black tiger prawns can experience high mortality when cultured in ISW, but survival and growth rates can be improved if the ISW is fortified with K^{+}, Mg^{2+} and Ca^{2+}. Grey mullet (Jana *et al*. 2004) and milkfish (Jana *et al*. 2006) can survive and grow well in ISW ponds. Similarly, Jain *et al*. (2006) reported that barramundi can survive and grow at lower salinity (15 ppt) compared to higher salinity (25 ppt) ISW.

Research funded by the Australian Centre for International Agricultural Research (ACIAR) and the New South Wales Department of Primary Industries (DPI), in partnership with Murray Irrigation Ltd (Partners 2009), has focused on producing prawn larvae in ISW. Using ISW to produce giant freshwater prawn larvae has presented a problem, which is related to the ionic imbalance of the ISW. Jain *et al*. (2007) reported that prawn larvae only survived until 11 days and developed to stage IV unless the concentration of potassium and magnesium was increased, whilst concentration of calcium was decreased to a similar level to ocean water.

1.6 FUTURE DIRECTIONS

Research in the area of molecular genetics and immunostimulants to prevent disease outbreaks is still in progress. However, the success of the research needs to be quantified by transferring it into the production of disease-free aquaculture products, which is yet to be witnessed and documented. The use of population genetics to enhance the value of ornamental species by improving their colour schemes is restricted to a few freshwater species. Similarly, research into the inland saline water aquaculture has had mixed commercial outcomes.

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