Recent Advances in Biotechnology Applications to Aquaculture

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ABSTRACT : Biotechnological research and development are moving at a very fast rate. The subject has assumed greatest importance in recent years in the development of agriculture and human health. The science of biotechnology has endowed us with new tools and tremendous power to create novel genes and genotypes of plants, animals and fish. The application of biotechnology in the fisheries sector is a relatively recent practice. Nevertheless, it is a promising area to enhance fish production. The increased application of biotechnological tools can certainly revolutionise our fish farming besides its role in biodiversity conservation. The paper briefly reports the current progress and thrust areas in the use of synthetic hormones in fish breeding, production of monosex, uniparental and polyploid individuals, molecular biology and transgenesis, biotechnology in aquaculture nutrition and health management, gene banking and the marine natural products. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 455-462)***

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INTRODUCTION

Demand for fish is soaring worldwide. It appears unlikely that the increasing demand can be met through increased natural harvest. There is international recognition that many of natural ocean and freshwater fisheries are being harvested to their limit. Aquaculture could help to meet increasing demand, and biotechnology can make a great contribution to improve aquaculture yields. Aquaculture animals are particularly well suited for research in biotechnology. Experimentation is facilitated by the availability of large numbers of gametes (germ cells), use of external fertilization, and ease of *in vitro* rearing of embryos. In addition, many aquatic animals can be treated with hormones during development to induce sterility or functional sex reversal thus simplifying experimental procedures. The agenda for modern biotechnology in aquaculture seems very similar to that of livestock and agriculture. Remarkable achievements have been made in the recent past in increasing production of crops, livestock and poultry through genetic and bio-technological tools. The potential areas of biotechnology in aquaculture include the use of synthetic hormones in induced breeding, production of monosex, uniparental and polyploid population, molecular biology, transgenic fish, gene banking, improved feeds and health management and development of natural products from marine organisms.

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BIOTECHNOLOGY IN FISH BREEDING

Gonadotropin Releasing Hormone (GnRH) is now the best available biotechnological tool for the induced breeding of fish. GnRH is the key regulator and central initiator of reproductive cascade in all vertebrates (Bhattacharya et al., 2002). It is a decapeptide and was first isolated from pig and sheep hypothalami with the ability to induce pituitary release of luteinising hormone (LH) and follicle stimulating hormone (FSH) (Schally et al., 1973). Since then only one form of GnRH has been identified in most placental mammals including human beings as the sole neuropeptide causing the release of LH and FSH. However, in non-mammalian species (except guinea pig) twelve GnRH variants have now been structurally elucidated, among them seven or eight different forms have been isolated from fish species. (Halder et al., 1991; Sherwood et al., 1993; King and Miller, 1995; Jimenez-Linan et al., 1997). The most recent GnRH purified and characterized was by Carolsfeld et al. (2000) and Robinson et al. (2000). Depending on the structural variant and their biological activities, number of chemical analogues have been prepared and one of them is salmon GnRH analogue profusely used now in fish breeding and marked commercially under the name of "Ovaprim" throughout the world. In fact, most of the economically important culturable fish in land locked water do not breed until the hormone induces them. The induced breeding of fish is now successfully achieved by the development of GnRH technology.

CHROMOSOME ENGINEERING

Chromosome sex manipulation techniques to induce polyploidy (triploidy and tetraploidy) and uniparental

chromosome inheritance (gynogenesis and androgenesis) have been applied extensively in cultured fish species (Pandian and Koteeswaran, 1998; Lakra and Das, 1998). These techniques are important in the improvement of fish breeding as they provide a rapid approach for gonadal sterilization, sex control, improvement of hybrid viability and clonation.

Most vertebrates are diploid meaning that they possess two complete chromosome sets in their somatic cells. Polyploid individuals possess one or more additional chromosome sets, bringing the total to three in triploids, four in tetraploids and so on. Induced triploidy is widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management.

The methods used to induce triploids and other types of chromosome set manipulations in fishes and the applications of these biotechnologies to aquaculture and fisheries management are well described (Purdom, 1983; Chourrout, 1987; Thorgaard, 1983; Pandian and Koteeswaran, 1998). Tetraploid breeding lines are of potential benefit to aquaculture, by providing a convenient way to produce large numbers of sterile triploid fish through simple interploidy crosses between tetraploids and diploids (Chourrout et al., 1986; Guo et al., 1996). Although tetraploidy has been induced in many finfish species, the viability of tetraploids was low in most instances (Rothbard et al., 1997).

In teleosts, technique for inducing sterility include exogenous hormone treatment (Hunter and Donaldson, 1983) and triploidy induction (Thorgaard, 1983). The use of hormone treatments, however could be limited by governmental regulation and a lack of consumer acceptance of hormone treated fish products. Triploidy can be induced by exposing eggs to physical or chemical treatment shortly after fertilization to inhibit extrusion of the second polar body (For reviews see Purdom, 1983; Thorgaard, 1983; Ihssen et al., 1990). Triploid fish are expected to be sterile because of the failure of homologous chromosomes to synapse correctly during the first meiotic division. Methods of triploidy induction include exposing fertilized eggs to temperature shock (hot or cold), hydrostatic pressure shock or chemicals such an colchicine, cytochalasin-B or nitrous oxide. Triploids can also be produced by crossing tetraploids and diploids. Tetraploid induction involves fertilizing eggs with normal sperm and exposing the diploid zygote for physical or chemical treatment to suppress the first mitotic division.

Gynogenesis is the process of animal development with exclusive maternal inheritance. The production of gynogenetic individuals is of particular interest to fish breeders because a high level of inbreeding can be induced in single generation. Gynogenesis may also be used to produce all-female populations in species with female homogamety and to reveal the sex determination mechanisms in fish. It is convenient to use all female gynogenetic progenies (instead of normal bisexual progenies) for sex inversion experiments. Methodologies combining use of induced gynogenesis with hormonal sex inversion have been developed for several aquaculture species (Gomelsky et al., 2000).

Androgenesis is the process by which a progeny is produced by the male parent with no genetic contribution from female. Induction of androgenesis can produce all male population in fish which would have commercial application in aquaculture. It can also be used in generating homozygous lines of fish and in the recovery of lost genotypes from the cryopreserved sperms. Androgenetic individuals have been produced in a few species of cyprinids, cichlids and salmonids (Bongers et al., 1994).

SEX CONTROL

The use of sex control techniques to influence characteristics of economically desirable teleost species is becoming an important management tool to increase aquaculture production. Techniques that allow production of monosex population by sex manipulation are potentially useful in species where one sex is more useful than the other. There are basically two ways of sex manipulation i.e. hormonal and genetic. The hormonal or endocrine control involves the treatment of fish with sex steroids during the early phase before sex differentiation starts. The process of sex differentiation in teleost is protracted and labile rending the hormonal induction of sex reversal possible in gonochoristic and hermaphroditic species. The induction involves administration of an optimum dose of sex steroid during the labile period which reverses the phenotypic expression of a genetic female into a male but the genetic male remains a male. Presently, protocols for hormonal sex reversal have been described for 44 species of gonochores and hermaphrodites using one of the 31 steroids (Pandian and Sheela, 1995). The genetic approach to sex manipulation for production of all male, all female or all sterile populations is through the induction of ploidy.

In teleosts, some species have fully developed sex chromosomes. In others, a pair of autosomes act as sex chromosomes but their morphology is unspecialized. As a consequence the genders are difficult to recognize karyotypically although sex identification can be achieved using molecular genetic methods (Griffiths et al., 2000).

The phenotypic sex of gonochoristic fish is determined essentially by sex chromosomes, it can also be influenced by environmental factors (Baroiller et al., 1999). The most pervasive environmental factor governing sex determination in fish based on current knowledge is temperature. Indeed a complete change from strictly monosex male to strictly

monosex female progenies (or vice versa) has never been observed except for the atherinid *Basilichthys bonariensis* Val. In cichlids (*Oreochromis* spp.) monosex or almost monosex populations can be obtained after exposing juveniles to temperatures of 37°C (Nile tilapia) or 35°C (Blue tilapia *O. aureaus*) over 28 days after yolk sac resorption (Baras et al., 2000).

TRANSGENESIS

Transgenesis or transgenics may be defined as the introduction of exogenous gene/DNA into host genome resulting in its stable maintenance, transmission and expression. The technology offers an excellent opportunity for modifying or improving the genetic traits of commercially important fishes, mollusks and crustaceans for aquaculture. The idea of producing transgenic animals became popular when Palmitter et al. (1982) first produced transgenic mouse by introducing metallothione- in human growth hormone fusion gene (mT-hGH) into mouse egg, resulting in dramatic increase in growth. This triggered a series of attempts on gene transfer in economically important animals including fish.

The first transgenic fish was produced by Zhu et al. (1985) in China, who claimed the transient expression in putative transgenics, although they gave no molecular evidence for the integration of the transgene. The technique has now seen successfully applied to a number of fish species. Dramatic growth enhancement has been shown using this technique especially in salmonids (Devlin et al., 1994). Some studies have revealed enhancement of growth in adult salmon to an average of 3-5 times the size of non-transgenic controls, with some individuals, especially during the first few months of growth, reaching as much as 10-30 times the size of the controls (Devlin et al., 1994; Hew et al., 1995).

The introduction of transgenic technique has simultaneously put more emphasis on the need for production of sterile progeny in order to minimize the risk of transgenic stocks mixing in the wild populations. The technical development have expanded the possibilities for producing either sterile fish or those whose reproductive activity can be specifically turned on or off using inducible promoters. This would clearly be of considerable value allowing both optimal growth and controlled reproduction of the transgenic stocks while ensuring that any escaped fish would be unable to breed.

An increased resistance of fish to cold temperatures has been another subject of research in fish transgenics for the past several years (Fletcher et al., 2001). Coldwater temperatures pose a considerable stressor to many fish and few are able to survive water temperatures much below 0-1°C. This is often a major problem in aquaculture in cold climates. Interestingly, some marine teleosts have high levels (10-25 mg/ml) of serum antifreeze proteins (AFP) or glycoproteins (AFGP) which effectively reduce the freezing temperature by preventing ice-crystal growth. The isolation, characterization and regulation of these antifreeze proteins particularly of the winter flounder *Pleuronectes americanus* has been the subject of research for a considerable period in Canada. Consequently, the gene encoding the liver AFP from winter flounder was successfully introduced into the genome of Atlantic salmon where it became integrated into the germ line and then passed onto the off-spring F3 where it was expressed specifically in the liver (Hew et al., 1995).

The introduction of AFPs to gold fish also increased their cold tolerance, to temperatures at which all the control fish died (12 h at 0°C; Wang et al., 1995). Similarly, injection or oral administration of AFP to juvenile milkfish or tilapia led to an increase in resistance to a 26 to 13°C drop in temperature (Wu et al., 1998). The development of stocks harbouring this gene would be a major benefit in commercial aquaculture in countries where winter temperatures often border the physiological limits of these species.

The most promising tool for the future of transgenic fish production is undoubtedly in the development of the embryonic stem cell (ESC) technology. These cells are undifferentiated and remain totipotent so they can be manipulated in vitro and subsequently reintroduced into early embryos where they can contribute to the germ line of the host. This would facilitate the genes to be stably introduced or deleted (Melamed et al., 2002).

Although significant progress has been made in several laboratories around the world, there are numerous problems to be resolved before the successful commercialization of the transgenic brood stock for aquaculture. To realize the full potential of the transgenic fish technology in aquaculture, several important scientific break-through are required. These include i) more efficient technologies for mass gene transfer ii) targeted gene transfer technologies such as embryonic stem cell gene transfer iii) suitable promoters to direct the expression of transgenes at optimal levels during the desired developmental stages iv) identified genes of desirable traits for aquaculture and other applications v) informations on the physiological, nutritional, immunological and environmental factors that maximize the performance of the transgenics and vi) safety and environmental impacts of transgenic fish.

AQUACULTURE NUTRITION

Over the last decade, the world has witnessed spectacular growth in the aquaculture industry of many developing

countries. As a result, aquaculture has been contributing significantly to food security and poverty elevation. It is further anticipated that world aquaculture production will continue to increase and since nutrition and feeding play a pivotal role in sustainable aquaculture, use of nutritionally balanced and complete formulated feeds will, continue to play a dominant role in finfish and shellfish production. Hence, alternative and biotechnologically improved feed ingredients should be sought alongwith improvements in pond management and manipulation of pond productivity.

The idea of introducing exogenous enzymes into fish feed is not new but their efficacy in fish feeds is being reinvestigated. Addition of proteolytic enzymes to diets resulted in only small positive effects in common carp (Dabrowska et al., 1979; Srivastava et al., 1994) and in freshwater giant prawn (personal communication). The use of thermo stable bacterial α-amylase on growth and feed utilization in rohu (*Labeo rohita*) (Ghosh et al., 2001) has been reported.

Probiotics are probably one of the most important research developments in recent times. Probiotics have been successfully used in aquaculture to enhance both internal and external microbial environment and the current trend is to replace antibiotics by probiotics for ecological consideration (Gildberg et al., 1997). A classical example of the successful use of probiotics in shrimp culture in Indonesia is given by Moriarty (1996). Shankar (1996) advocated the use of probiotics as an efficient feed and also as a tool for the prevention of the viral attack in shrimp farming. Mohanty et al. (1996) used probiotic alongwith ground goat liver and starch to show the potential significance of probiotic supplementation in larval diet. Ravi et al. (1998) studied the use of commercial probiotics for maintaining water quality and thereby enhancing growth rate of *P. indicus.* They noticed that in addition to the better growth rate, experimental shrimps were also observed to moult faster than the control shrimps. Hence, probiotics could be a safe alternative to antibiotics for sustainable shrimp culture.

The cost of feed ingredients and other inputs are increasing, while market costs for the major cultivable finfish and shellfish species have remained static or are decreasing. It is, therefore, likely that increased aquaculture production will be from herbivorous/omnivorous fishes in developing countries of Asia and other parts of the world. Aquaculturist can reduce the current dependence on natural marine resource to farm carnivorous finfish and shellfish through the use of the low cost, locally available, alternative feed ingredients (Hasan, 2001). The use of biotechnologically improved products and appropriate use of locally available feed ingredients in semi intensive aquaculture is still needed. A reliable database of agricultural feed resources is thus an essential prerequisite, for planning sustainable aquaculture development. This database will give projection of major agricultural by-products throughout the world that may benefit the aquaculture feed industry.

Finally, improvement of nutrition and feeding for sustainable aquaculture development can be achieved through: i) clear understanding of the dietary nutrient requirements of cultivable species including their application to practical culture conditions, ii) developing species specific diet for maximal reproduction and larval quality, iii) increased understanding of larval nutrition, iv) improving the efficiency of resources use in aquaculture using agriculture and fishery by-products wastes and also employing biotechnological approach to breakdown the complex products to simpler and easily digestible forms, v) developing feeding strategies based on renewable feed ingredients and employing biotechnological techniques specially the use of microbes and/or heat stable microbial enzymes (Ghosh et al., 2001), iv) better understanding of nutrient modulation of disease resistance, vii) improved strategies to minimize toxicity of nutrients, viii) promotion of biotechnological approaches to improve feed quality by using microbial stable digestive enzymes and use of probiotics, ix) recognizing the importance of feed and quality concerns over food safety issues and x) considering the effects of biotechnologically manipulated diets on product quality and the improved nutritional characteristics of the final product in terms of human nutrition, e.g. omega-3 fats, iodine, selinium, vitamin A and D.

It is hoped that biotechnology will play a promising role in the fish nutrition in future. One day, genes that enable fish to digest and utilize nutritionally poor feedstuffs will be transferred. Once this is accomplished fish will be able to utilize chitin and will be able to efficiently utilize poor sources of protein such as chicken feathers. This will lower feed & production costs.

BIOTECHNOLOGY AND FISH HEALTH MANAGEMENT

Disease problems are a major constraint for development of aquaculture. Biotechnological tools such as molecular diagnostic methods, use of vaccines and immuno stimulants are gaining popularity for improving the disease resistance in fish and shellfish species world over. For viral diseases, avoidance of the pathogen is very important. In this context, there is a need to have rapid methods for detection of the pathogens. Biotechnological tools such as gene probes and polymerase chain reaction (PCR) are showing great potential in this area. Gene probes and PCR based diagnostic methods have been developed for a number of pathogens affecting fish and shrimp (Karunasagar and Karunasagar, 1999).

In case of finfish aquaculture, a number of vaccines against bacteria and viruses have been developed. Some of these have been conventional vaccines consisting of killed microorganism but new generation of vaccines consisting of protein subunit vaccines, genetically engineered organisms and DNA vaccines are currently under development.

In the vertebrate system, immunization against disease is a common strategy. However, the immune system of shrimp is rather poorly developed. Biotechnological tools are helpful for development of molecules, which can stimulate this immune system of shrimp. Recent studies have shown that the non specific defense system can be stimulated using, microbial products such as lipopolysarcharides, peptidoglycans or glucans (Itami et al., 1998). Among the immunostimulants known to be effective in fish, glucan, chitin and levamisole enhance phagocytic activities and specific antibody responses (Sakai, 1999).

MOLECULAR BIOLOGY

Recent advances in molecular biology have provided unlimited number of genetic markers which have multiple application in aquaculture and fisheries (Lakra, 2001). Molecular genetic approaches began to be used in fisheries in the 1950s. Their use in aquaculture and fisheries has increased dramatically over the past few years. The genetic identification of aquaculture stocks is a fundamental requirement in any culture programme. Mitochondrial DNA has provided a wealth of genetic markers to answer questions on the phylogeny, evolution and population structure of fishes. Mitochondrial DNA has an effective population size one quarter that of nuclear genes and thus might be expected to show greater population divergence than nuclear genes. Billington and Hebert (1991) reviewed patterns of mtDNA variation in 40 fish species where considerable divergence of local populations was reported. Among the DNA markers, multi-locus VNTR analysis (DNA fingerprinting) can be used to assess the amount of inbreeding in cultured populations. Marker based approaches can be used to increase the efficiency of breeding programmes based on biometrical methods. Genetic markers can be used to identify individuals and family groups so that they can be reared together thus simplifying experimental designs. One very powerful application of the new DNA based technologies is to identify marker loci which are associated with nuclear loci that control economically important traits (quantitative trait loci or QTLs). Once such markers have been identified they can be used in selection programmes. An approach towards his marker assisted selection (MAS) in fish has been made in rainbow trout by Herbinger et al. (1995).

During the past few years efforts have been devoted to the development of microsatellite markers for a variety of aquaculture species (Ward and Grewe, 1994). Highly polymorphic microsatellites allow the parents of superior progeny to be identified in mixed family rearing environments, thus enabling selective breeding to occur on commercial fish farms (Wright and Bentzen, 1994). Microsatellite markers are based on length variation of tandem repeats of usually 2-5 base pairs. They are abundant in genome, thus the number of markers is potentially unlimited. Microsatellite loci display varying levels of polymorphism. The highly polymorphic loci are of use in parentage studies, the less variable loci are more useful in discriminating populations. The assay of microsatellite variation is based on the PCR technique, thus only small amounts of tissue, for example from fish scales, are needed as a source of DNA. A number of recent studies have assessed the utility of microsatillite markers in aquaculture genetics (Herbinger et al., 1999).

CRYOPRESERVATION OF GAMETES OR GENE BANKING

Cryopreservation is a technique, which involves long-term preservation and storage of biological material at very low temperature, usually at -196^{o}C, the temperature of liquid nitrogen. It is based on the principle that very low temperatures tranquilize or immobilize the physiological and biochemical activities of cells, thereby, making it possible to keep them viable for very long period.

The technology of cryopreservation of fish spermatozoa (milt) has been adopted from animal husbandry. The first success in preserving fish sperm at low temperature was reported by Blaxter (1953) who fertilized herring (*Clupea harengus*) eggs with frozen-thawed semen. The spermatozoa of almost all cultivable fish species have now been cryopreserved (Lakra, 1993). Cryopreservation overcomes the problem of males maturing before females, allows selective breeding and stock improvement and enables the conservation of genomes (Harvey, 1996). One of the emerging requirements for undertaking gene banking of aquatic resources is the need to build a genetic base collection that can be used by breeders for evolving new strains. Most of the plant varieties that have been produced are based on gene bank collections. Aquatic gene banks however, suffer from the fact that at present it is possible to cryopreserve only the male gametes of finfishes and there in no viable technique for finfish eggs and embryos. However, the recent reports on the freezing of shrimp embryos by Subramoniam and Newton (1993) and Diwan and Kandasami (1997) look promising. Therefore, it is essential that gene banking of cultivated and cultivable aquatic species be undertaken expeditiously.

MICROBIAL TECHNOLOGY FOR AQUACULTURE

Aquatic habitats contain diverse microbial communities and their role in detrital food webs and organic mineralisation, particularly through bacteria has been well studied. Microbiological studies delineate the trophic interactions and define the nutrient and energy flow patterns providing specific tools for environmental modifications as also products that could be substitutes for chemical inputs or mechanical devices.

Microbial technology for aquaculture comprising aspects of biofertilization, microbial processing of organic matter, use of probiotics and enhancement of feed digestibility, detritus enrichment and shortening of food chains for better energy transfer rates, genetic upgradation of bacterial strains, biofiltration and waste recycling as also techniques pertaining to post-harvest technology hold great promise in improving aquaculture productivity, on a sustainable basis (Ayyappan, 1994).

MARINE NATURAL PRODUCTS

In recent years the field of natural product research has emerged to generate large number of molecules with great structural diversity. Commercially valuable products such as pharmaceutical compounds, pigments, oils, sterols, alginates and agarose are being extracted from micro and macro-algae in many parts of the world. Joffe and Thomas (1989) estimated that about 25% of all pharmaceutical sales are drugs derived from plant natural product and an additional 12% are based on microbially produced natural product. Despite the belief that the biodiversity in the marine environment far exceeds the terrestrial environment, research into the use of marine natural product as pharmaceutical agents is still in its infancy. However, in the last 30 years, marine organisms-algae, invertebrates and microbes have provided key structures and compounds that proved their potential in several fields particularly as new therapeutic agents for a variety of diseases (Riguera, 1997). Among the recent notable products, novel compounds have been isolated from blood plasma and tissue extract of several marine animals particularly from horseshoe crab (*Limulus polyphemus*) which have the properties that destroy malignant melanoma cells, human colon carcinoma cells, stop the growth of marine phytoplankton and possesses strong spermicidal activity (Mukesh et al., 1998). Even the marine seaweeds are a good source of unique natural products with medicinal properties. Cell and tissue culture established from complex marine seaweeds have the potential to biologically synthesize these compounds in a controlled environment at a scale required for continued drug development or commercial production (Rorrer et al., 1998).

REFERENCES

Ayyappan, S. 1994. Microbial technology for aquaculture. Microbes For Better Living. MICON-94 & 35th AMI Conference 545-551.

Baras, E., C. Prignon, G. Gohoungo and C. Melard. 2000. Phenotypic sex differentiations of blue tilapia under constant and fluctuating thermal regimes and its adaptive and evolutionary implications. J. Fish Biol. 57: 210-223.

Baroiller, J.F., Y. Guiguen and A. Fostier. 1999. Endocrine and environmental aspects of sex differentiation in fish. Cellular and Molecular Life Sci.55: 910-931.

Bhattacharya S., S. Dasgupta, M. Datta and D. Basu. 2002. Biotechnology Input in Fish Breeding. Indian Journal of Biotechnology, 1: 29-38.

Billington, N. and P. D. N. Hebert. 1991. Mitochondrial DNA diversity in fishes and it implications for introductions. Can. J. Fish. Aquat. Sci. 48(5I): 80-94.

Blaxter, J.H.S., 1953. Sperm storage and cross-fertilization of spring and autumn spawning herring. Nature 172: 1189-1190.

Bongers, A.B. J., in't Veld EPC, K. Abo-Hashema, I. M. Bremmer, E. H. Eding, J. Komen and C. J. J. Richter. 1994. Androgenesis in common carp (Cyprinus carpio L.) using UV irradiation in a synthetic ovarian fluid and heat shocks.Aquaculture 122:119-132.

Carolsfeld, J., J. F. Powell, M. Park, W. H. Fisher, A. G. Craig, J. P. Chang, J. E. Rivier and N. M. Sherwood. 2000. Primary structure and function of three gonadotropin releasing hormones. Endocrinology 141: 505-512.

Chourrout, D. 1987. Genetic manipulation in fish: review of methods. In K Tiews (ed), Selection, Hybridization and Genetic Engineering in Aquaculture Bordeaux., Heenemann, Berlin, 2:111-126.

Chourrout. D., B. Chevassus, F. Krieg, A. Happe, G. Burger and P. Renard. 1986. Production of second generation triploid and tetraploid rainbow trout by mating tetraploid males and diploid females. Potentials of tetraploid fish. Theor. Appl. Gene. 72: 193-206.

Dabrowska, H., H. Grudniewski and K. Dabrowski, 1979. Artificial diets for common carp: effect of the addition of enzyme extract. Progressive Fish Culturist 41(4): 196-200.

Devlin, R.M., T. Y. Yesaki, C. A. Biagi, E. M. Donaldson, P. Swanson and W. K. Chan. 1994. Extraordinary salmon growth. Nature 371: 209-210.

Diwan, A.D. and D. Kandasami. 1997. Freezing of viable embryos and larvae of marine Shrimp *P. semisulcatus* de Haan. *Aquaculture Research* 28: 947-950.

Fletcher, G.L., C. L. Hew, P. L. Davies. 2001. Antifreeze proteins of teleost fishes. Annu. Rev. Physiol. 63: 359-390.

Ghosh, K., K. Chakraborty, S. K. Sen and A. K. Ray. 2001. Effects of thermo stable bacterial α-amylase on growth and feed utilization in rohu, *Labeo rohita* (Hamilton) fingerlings. *The Israeli Journal of Aquaculture* 53(3-4): 101-109.

Gildberg, A., H. Mikkelsen, E. Sandaker, E. Ringo. 1997. Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (Gadus morhua). Hydrobiologia 352(1-3): 279-285.

Gomelsky Boris, S. D. Mims and Richard. J. Onders. 2000. Induced gynogenesis in Black Crappie. North American

Journal of Aquaculture 62: 33-41.

Griffiths, R., J. Orr, A. Adam and I. Barber. 2000. DNA sex identification in the three spined stickleback. J. Fish Biol., 57: 1331-1334.

Guo, X., G. A. DeBrosse and S. K. Allen, Jr., 1996. All triploid Pacific oysters (Crassostrea gigas Thunberg) produced by mating tetraploids and diploids. Aquaculture, 142: 149-161.

Halder S, S. Sen, S. Bhattacharya, A. K. Ray, A. Ghosh and A. G. Jhingran. 1991. Induced spawning of Indian major carps and maturation of a perch and a catfish by murrel gonadotropin releasing hormone, pimozide and calcium. Aquaculture 97: 373-382.

Harvey, B. 1996. Salmon gene banking: a conservation opportunity. Publ. World. Fisheries Trust, Canada, 83 p.

Hasan, M. R. 2001. Nutrition and feeding for sustainable aquaculture development in the third millennium. In: *Aquaculture in the third millennium* (Eds. Rohana, P. Subasinghe, Pedro, B. Bueno, Michael, J. Philips, Coutney Hough, Sharon, E. McGladdery and J. Richard Arthen) NACA/FAO publications 193-219.

Herbinger, C. M., P. T. Oreilly, R. W. Doyle, J. M. Wright and F. O. Flynn. 1999. Early growth performance of Atlantic salmon full sib families reared in single family tanks versus in mixed family tanks. Aquaculture 173: 105-116.

Herbinger, C. M., R. W. Doyle, E. R. Pitman, D. Paquet, K. A. Mesa, D. B. Morris, J. M. Wright and D. Cook. 1995. DNA fingerprint based analysis of paternal and maternal effects on offspring growth and survival in communally reared rainbow trout. Aquaculture 137: 245-256.

Hew, C.L., G. Fletcher and P. L. Davies. 1995. Transgenic salmon: tailoring the genome for food production. J. Fish Biol. 47: 1-9.

Hunter, G. A. and E. M. Donaldson. 1983. Hormonal sex control and its application to fish culture. In: Fish Physiology, W.S. Hoar, D.J. Randall and E.M. Donaldson (eds). Academic Press, New York, 9: 223-291.

Ihssen, P.E., L. R. Mckay, I. McMillan and R. B. Phillips. 1990. Ploidy manipulation and gynogenesis in fishes: Cytogenetic and fisheries applications. Trans. Am. Fish. Soc. 119: 698-717.

Itami, T., M. Maeda, N. Suzuki, K. Tokushige, H. O. Nagagawa, M. Konto, J. Kosornchandra, I. Hirono, T. Aoki, R. Kusuda and T. Takahashi. 1998. Possible prevention of white spot syndrome (WSS) in Kuruma shrimp, *Penaeus japonicus* in Japan. In Flegel T. W. (Ed.). Advances in shrimp biotechnology. National Centre for genetic engineering and biotechnology, Bangkok, pp. 291-295.

Jimenez-Linan M., B. S. Rubnin and J. C. King. 1997. Examination of guinea pig luteinizing hormone releasing hormone reveals a unique decapeptide and existence of two transcripts in the brain. Endocrinology 138: 4123-4130.

Joffe, S. and R. Thomas. 1989. Phytochemicals, a renewable global resource, Ag. Biotech News Information, 1: 697-700.

Karunasagar, I. and I. Karunasagar. 1999. Diagnosis treatment and prevention of microbial diseases of fish and shellfish. Curr. Sci. 76: 387-399.

King, J. A. and R. P. Miller. 1995. Evolutionary aspects of gonadotropin-releasing hormone and its receptor. Cell Mol Neurobiol. 15: 5-23.

Lakra, W. S. 1993. Cryogenic preservation of fish spermatozoa and it application to aquaculture. Indian J. Cryogenics. 18(1-4): 171-76.

Lakra, W. S. and P. Das. 1998. Genetic engineering in aquaculture. Indian. J. Anim. Sci., 68(8): 873-879.

Lakra, W. S. 2001. Genetics and Molecular Biology in Aquaculture – Review. Asian-Aust. J. Anim. Sci. 14(6):894-898.

Melamed, P., G. Zhiyuan, G. Fletcher and C. L. Hew., 2002. The potential impact of modern biotechnology on fish aquaculture. Aquaculture 204: 255-269.

Mohanty, S. N., S. K. Swain, S. D. Tripathi. 1996. Rearing of catla (*Catla catla* Ham.) spawn on formulated diets. J. Aquacult. Trop., 11(4): 253-258.

Moriarty, J. W. D. 1996. Microbial biotechnology: a key ingredient for sustainable aquaculture. INFOFISH, 4/96, July - Aug., 29-33.

Mukesh, K., M. Z. Sahni, V. B. Wahrman and G. M. Sharma. 1998. New Developments in Marine Biotechnology (eds Le Gal, Y and H. O. Halvorson) Plenum Press, New York, 41-47.

Palmiter, R. D., R. L. Brinster, R. E. Hammer, M. E. Trumbauer, M. G. Rosenfeld. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein – growth hormone fusion genes. Nature 30: 611-615.

Pandian, T. J. and S. G. Sheela. 1995. Hormonal induction of sex reversal. Aquaculture 138:1-22.

Pandian, T. J. and R. Koteeswaran. 1998. Ploidy induction and sex control in fish. Hydrobiologia 384: 167-243.

Purdom, C. E. 1983. Genetic engineering by the manipulation of chromosomes. Aquaculture 33: 207-300.

Ravi, V., S. A. Khan, S. Rajagopal. 1998. Influence of probiotics on growth of Indian white prawn Penaeus indicus. J. Sc. Ind. Res. 57(10-11): 752-756.

Riguera, Ricardo. 1997. Isolating bioactive compounds from marine organisms. J. Mar. Biotechnol. 5: 187-193.

Robinson, T. C., S. A. Tobet, C. Chase, T. Waldron and S. A. Sower. 2000. Gonadotropin releasing hormones in the brain and pituitary of the teleost, the white sucker. Gen Comp Endocrinol. 117: 387-394.

Rorrer, G. L., W. H. Grewick and D. P. Cheney. 1998. New Developments in Marine Biotechnology (eds Le Gal, Y. and Halvorson, H.O), Plenum Press, New York, 65-67.

Rothbard. S., W. L. Shelton, Z. Kulikvosky, I. Rubinshtein, Y. Hagani and B. Moav. 1997. Chromosome set manipulation in the black carp. Aquaculture International 5: 51-64.

Sakai, M. 1999. Current research status of fish immunostimulants. Aquaculture 172: 63-92.

Schally A., A. Arimura and A. J. Kastin. 1973. Hypothalamic regulatory hormones. Sci.179: 341-350.

Shankar, G.S. 1996. Role of probiotics in shrimp farming. Industrial fish and fisheries, Andhra Layola College, Vijayawada, India, Fish-World 4(1): 21.

Sherwood, N. M., D. A. Lovejoy and I. R. Coy. 1993. Origin of mammalian gonadotropin-releasing hormones. Endor Rev. 14: 241-254.

Srivastava, P. P., M. N. Thagare, D. R. Rao., K. K. Jain and A. Sinha. 1994. Studies on the application of proteolytic enzyme, papain on dietary incorporation and its dose dependent influence in the digestion of protein ingredients and in increasing feed digestibility in *Cyprinus carpio*, *Second Asia Pacific Conference on Agricultural Biotechnology,* Madras,

India, 160: 243.

Subramonium, T. and S. Newton. 1993. Cryopreservation of penaeid prawn embryos. Current Sci. 65(2): 176-177.

Thorgaard, G.H. 1983. Chromosome set manipulation and sex control in fish. In W.S. Hoar, D.J. Randall & E.M. Donaldson (eds). Fish Physiology. Academic Press, New York, 9B: 405-434.

Wang, R., P. Zhang, Z. Gong, C. L. Hew. 1995. Expression of the antifreeze protein gene in transgenic goldfish (*Carassius auratus*) and its implication in cold adaptation. Mol. Mar. Biol. Biotechnol 4: 20-26.

Ward, R. D. and P. M. Grewe. 1994. Appraisal of molecular genetic techniques in fisheries. In: Reviews in Fish Biology and Fisheries. (Ed. R. Gary, Carvalho, J. Tony and Pitcher). Chapman and Hall, pp. 300-325.

Wright, J. M and P. Bentzen. 1994. Microsatellites: genetic markers for future. In: Reviews in Fish Biology and Fisheries. (Ed. R. Gary, Carvalho, J. Tony, and Pitcher). Chapman and Hall, pp. 384-388.

Wu, S. M, P. P. Hwang, C. L. Hew, J. L. Wu. 1998. Effects of antifreeze protein on cold tolerance in juvenile tilapia (*Oreochromis mossambicus* Peters) and milkfish (*Chanos chanos Forskaal*). Zool. Sci. 37: 39-44.

Zhu, Z., Li G, Je L. and S. Chen. 1985. Novel gene transfer into the fertilized eggs of goldfish (*Carassius auratus*). Z. Angrw. Ichthyol, 1:32-34.