Journal of the Indian Fisheries Association
18,1988 27-37

REPRODUCTIVE ENGINEERING IN CRUSTACEAN AQUACULTURE

T. SUBRAMONIAM

Department of Zoology, University of Madras, Life Science Building, Guindy Campus, Madras 600 025.

ABSTRACT

Crustacean aquaculture industry in India suffers very much from lack of technological developments. A major constraint in this enterprise is the limitation of seed stock availability. This paper gives a critical appraisal of the techniques used in the manipulation of reproductive processes in order to augment year-round production of seeds in the controlled condition. The occurrence of neutoendocrine centres (X-organ-sinus gland complex) in the eyestalk of the decapod crustaceans facilitates the easy extirpation of these hormonal sources by simply ablating the eyestalk. A new possibility of induced ovarian maturation in crustaceans is by administering steroid hormones of vertebrate source. Environmental factors are known to govern the gametogenic cycle of marine crustaceans. Alteration of photoperiod and day light intensity could accelerate the ovarian maturation, as demonstrated in ocypod crabs. Rematuration of prawns and mating in the laboratory condition have been shown to be difficult in many species. Cryopreservation of male gametes and artificial insemination by way of spermatophore transfer could solve some of the problems in this respect. More importantly, *in vitro* fertilization could be used in hatchery production and in hybrid production from species of desired characters.

INTRODUCTION

Decapod crustaceans comprising the culture-important species are generally fast growing but slow breeding aquatic arthropods, ideally suited for aquaculture. Thanks to an awareness of the vast aquaculture potential in the long coastline of the peninsular

India, and to the sizable foreign exchange the shrimps, lobsters and crabs fetch in international market, crustacean aquaculture in recent years has received increased public attention and funding from government agencies. Unfortunately, this development has not contributed much to the desired expansion of basic research on the growth and reproductive biology of these organisms. Introduction of modern methods of genetic engineering and other novel biotechnology, as applied in fish culture, to augment growth and production of quality seeds would require a better understanding of the growth and reproductive phenomena, as well as their mechanisms in crustacean's too.

ENDOCRINE MANIPULATION

Crustaceans, as other arthropods, periodically undergo molting to facilitate body growth. However, continued growth as manifested in molting even after attainment of sexual maturity poses problems of sharing the nutrient reserves for these two energy-demanding physiological processes. This situation, entailing a precise endogenous control, seems to be solved by a bihormonal mechanism operating on molting and reproduction. Thus, two inhibitory or restraining hormones namely molt and gonad inhibitory hormones (MIH and GIH) are purportedly produced from the eyestalk neurosecretory centres; whereas, the stimulatory hormones emanate from Y-organ and brain/thoraic ganglia respectively for molting and reproduction (Adiyodi and Adiyodi, 1970).

It is now possible to shorten the intermoulting and interbreeding periods by eyestalk ablation in a variety of crustaceans. However, the relative importance of eyestalk removal in inducing moulting or reproduction depends much on the season in which the operation is made, as many forms exhibit seasonality for somatic growth and reproduction (Adiyodi and Subramoniam, 1983).

Eyestalk ablation or unilateral eyestalk enucleation has been used by aquaculturists to induce ovarian maturation in several prawns. This technique is especially useful to induce ovarian maturation and spawning in those prawns that are incapable of maturing in captive conditions. It was Idyll (1971) who first carried out bilateral eyestalk ablation on *Penaeus duorarum* to successfully induce ovarian maturation in 1-2 weeks. Subsequently, several penaeid species have been induced to mature and spawn in captive conditions; unilateral eye ablation was invariably found to be adequate for it (Alikunhi *et al.*, 1975; Primavera, 1978; Lumare, 1979; Muthu and Lakshminarayana, 1981). The quality

of the spawn as well as the hatchability of the eggs have been reported to be low in such eyestalk ablated females. Lumare (1979) found that repeated spawning in the eye ablated female of *P. kerathurus* led to a significant decrease in egg numbers as well as hatchability in subsequent spawns. Obviously, spawning seems to be partial, together with incomplete gonadal recrudescence, as the number of spawning increases after eye ablation. Similar results have been reported by Muthu and Lakshminarayana (1981) in many Indian species. This method could, however, help in extending the reproductive season of shrimps especially in temperate climatic conditions. While so much is known about the gonad inhibitory hormones and their manipulative methods to augment prawn breeding, the effect of gonad stimulatory hormone, purportedly originating from the brain/thoraic ganglia is not well known (see Adiyodi and Subramoniam, 1983). Experimental data also indicates that the eyestalk inhibitory hormones could control female accessory gland activity in several crustacean species (Deecaraman and Subramoniam, 1983; Munuswamy and Subramoniam, 1987). Though the antagonistic activity of eyestalk hormones to the gonad stimulatory hormones is known, the mechanism of hormone action on ovarian tissues or on the stimulatory neuroendocrine centres is still not well-understood.

Unlike the female prawns, the males normally mature in captive conditions. However, Alikunhi *et. al.* (1975) found that the eye ablation of males was necessary to induce maturation of *P. monodon* in brackishwater. Similarly, for *P. vannamai*, Chamberlain and Lawrence (1981 a & b) found an increase in gonad size and mating frequency in ablated males. Furthermore, unilateral ablation in *P. vannamai* increased spermatophore weight and sperm count, without diminishing sperm quality (Leung Trujillo and Lawrence, 1985).

Recently, attempts have been made to induce ovarian maturation in the European sand shrimp *Crangon crangon* by administering hypophysial gonadotropins such as FSH and LH (Areridarezyk, 1981). The efficacy of a variety of mammalian steroid hormones has also been tested to accelerate ovarian maturation in the field crab *Paratelphusa hydrodromous* (Sasikala and Subramonian., 1987 b) with encouraging results. By such testings on various aquaculture species, there is good promise for evolving a technique to accelerate ovarian maturation and spawning in crustaceans.

ENVIRONMENTAL CONTROL

Physiological adaption of crustaceans inhabiting various

marine habitats to changing environmental factors is well known. Of them, water temperature, salinity, photoperiod and availability of food materials are shown to act as effective regulators on reproductive activities in crustaceans. Increasing day length as well as elevating light intensity may serve as fruitful manipulation technique for enhancing the reproductive activities of crustaceans, but experimental data is needed in this respect. Altering the day light intensity in the laboratory, shortening of ovarian maturation period in the ghost crab, *Ocypoda macrocera*, has been found (Natarajalingam and Subramoniam, 1987). The neuroendocrine centres influencing reproduction were also found to respond positively to this treatment. This experimental data should bear important implication for extending this result to other crustaceans too. In short, by controlling temperature, photoperiod and probably diet, it appears to be possible to increase production of larvae during all times of the year in species which breed seasonally in nature.

CRYOPRESERVATION AND SPERM BANKING

Cryopreservation and sperm banks are widely used in animal husbandry. It provides a ready source of sperm from desirable males and permits the transport of sperm to various places. A constraint in adopting sperm preservation techniques for crustaceans is the non-motile nature of crustacean spermatozoa. Further, the spermatozoa are packed into spermatophores in which form they are transferred to the females. Information is now available on spermatophore biochemistry and its functional role in sperm survival, maintenance and transport to female during mating (Uma and Subramoniam, 1979; Subramoniam, 1977, 1984; Radha and Subramoniam, 1985; Jeyalectumie and Subramoniam, 1987 a; Sasikala and Subramoniam, 1987 a).

Previous attempts to preserve spermatozoa in king crabs and spermatophores in lobsters and freshwater shrimps have yielded only limited success (Behimer and Brown, 1984; Chow *et al.*, 1985; Ishida *et al.*, 1986). Standardized techniques are now available to preserve the spermatophores as well as seminal plasma of the edible crab, *Scylla serrata*, in liquid nitrogen (at -197°C) for a period upto 30 days, and the sperm survival rate is found to be 95% (Jeyalectumie and Subramoniam, 1987b and 1988). The potency of various cryoprotectants has also been tested in the experiment. A significant finding of this study is the similarity of non-motile crustacean sperm to mammalian sperm in the utilization of carbohydrate substrates from seminal plasma that carries the spermatophores in a fluid medium during

mating (Jeyalectumie and Subramoniam, 1987 b).

At present seed stocks are obtained by capturing fertilized females from their natural environment and spawning them in captivity. Since many prawn species reproduce seasonally, females are difficult to obtain all through the year. Cryopreservation of sperm together with improved methods of artificial insemination in prawns, lobsters and crabs will be a potential alternative to improve seed stock availability.

ARTIFICIAL INSEMINATION AND SPERM-EGG INTERACTION

Following the procedures adopted in animal husbandry, attempts have been made to obtain spermatophores by electro-ejaculation in various prawn and lobster species (Sandifer and Lynn, 1980; Kooda-Cisco & Talbot, 1983). The spermatophores are sticky and are easily attached to the sternum of the females as the males do during the natural mating process. In our laboratory , a technique has been standardised for extruding intact spermatophores in the sand lobster, *Thenus orientalis*, by applying 12 volts current (Silas and Subramoniam, 1987).

In general, a single mating is enough to supply sperm for several spawnings in penaeid prawns. However, many authors have indicated a decline in the reproductive performance of male prawns when kept under long captivity. Leung Trujillo and Lawrence (1987) recently reported in *P. setiferus* significant decline in spermatophore weight, sperm count and percentage sperm survival, together with appearance of sperm abnormalities, when the prawns were kept under laboratory conditions up to a period of 7 weeks. Such a decline in male fertility is also associated with deterioration of spermatophores. Quality maintenance of spermatophoric components is an important prerequisite for effective impregnation of the females (see Sasikala and Subramoniam, 1987 a). Brown *et al*, (1979) reported a *Vibrio sp* infection of the terminal ampoules and enclosed spermatophores of *P. setiferus* when kept under captivity. When the bacteria were present in large numbers, the majority of the sperm were deformed and fragmented. Interestingly, the whole reproductive tract and the testis are melanised under extreme conditions leading to failure of spermatophore extrusion. Recently, Chamberlain *et al.* (1983) isolated a melanin producing bacteria (*Pseudomonas sp*) from the infected spermatophores of *P. setiferus*. However, the melanised spermatophores of the freshwater prawn *Macrobrachium rosenbergii* did not have any bacterial infection and the sperms inside the spermatophores were also not affected

(Harris and Sandifer, 1986). We have also observed such melanization in the gonopore and other parts of the male reproductive tract including testis of the sand lobster *Thenus orientalis* (Silas and Subramoniam, unpublished observation). However, such conditions were more common after repeated electrical extrusion of spermatophores. In most decapod crustaceans melanisation has been demonstrated as a wound response. Therefore, it is logical to conclude that electric shock may cause the reproductive tissues to become melanised, which in extreme conditions may lead to failure of spermatophore extrusion, thus bringing about impotency in males.

Artificial impregnation of the unmated females using electrically extruded spermatophores from healthy males could be thought of as a solution to the infertility of males under captivity (Lumare, 1979; Muthu and Laxminarayana, 1981). However, the percentage of fertilized eggs is much higher in the naturally inseminated shrimp than in the artifically inseminated ones, as indicated by Lumare (1979) in *Penaeus japonicus*.

Although the mode of fertilization is understood fairly well in many crustaceans, the sequential events of fertilization is poorly known. Ridgeback prawn, *Sicyonia ingentis*, has received some attention in this respect (Clark *et al.*, 1986). When this prawn spawns, sperm are externally mixed with eggs and attached to an ovum's vitelline envelope via the anterior tip of the spike. Immediately, the spike undergoes a rapid depolymerisation, pulling the sperm to the surface of the ovum. Following this, the acrosomal reaction occurs as the sperm traverses the vitelline envelope. During this period, the ovum releases its jelly precursors, mechanically lifting the vitelline envelop off the surface of the oolemma. A secondary acrosomal reaction occurs at this period by the formation of a "fertilization process" which helps in the fusion of fertilizing sperm with ova.

In spite of the complicated sequence of sperm-egg interaction mentioned above, the *in vitro* fertilization in the laboratory conditions may prove to be a simple process for the penaeid prawns. For example, Clark *et al.* (1973) accomplished *in vitro* fertilization in the brown shrimp, *Penaeus aztecus*, by mixing the mature eggs removed from female shrimp with sperm suspension in seawater and agitating in a manner simulating natural spawning. This experiment involved sacrificing the male and female prawns; but the recent development in the reproductive manipulation of females to spawn in captive conditions as well as the feasibility of extruding the spermatophores using electrical stimulation should facilitate *in vitro* fertilization without sacrifi-

cing the animals.

Further work on the physiological aspects of sperm-egg interaction is important to delineate the factors controlling initial sperm-egg interaction and fusion, especially for hybridization experiments involving two different species of prawns. *In vitro* fertilization studies on crustaceans and other marine invertebrates have not been initiated in India, although we have a rich marine fauna. Such studies are not only important in the context of carrying out successful hybridisation of aquaculture-important organisms, but also in the field of cellular interaction and fusion.

CONCLUDING REMARKS

Aquaculture technology should be developed on an interdisciplinary effort in the areas of water quality, nutrition, genetics, physiology, engineering and economics. As seed production is the principal constraint in aquaculture production, attention is focussed here on research relating to reproductive engineering to augment seed production under controlled conditions. In India, most of the basic research on crustaceans important in aquaculture are confined to Universities. However, the research projects taken up here do not reflect the specific problems confronted by culture scientists. Similarly, the aquaculture specialists also do not seem to recognise the work that is being carried out at the academic institutions. Thus, there is no integration of research information obtained by the aquaculture specialists and those working on basic problems. It is important to remember here that problems such as induced maturation and spawning and artificial insemination in decapod crustaceans could be tackled by the research scientists working on basic problems.

In India, aquaculture is a new enterprise and in many respects, crustacean aquaculture remains at an experimental stage. Private organisations have not taken up aquaculture practice very seriously in view of the high production cost. Aquaculture in India can be accelerated only by encouraging private entrepreneurs to invest in coastal aquaculture projects, besides persuading the government to offer more substantial financial aid. To make this work more firmly founded on theoretical knowledge available, it is highly desirable to efface the dichotomy between fundamental researchers in academic institutions and aquaculture practitioners.

REFERENCES

Adiyodi K.G., and R.G. Adiyodi, (1970). Endocrine control of reproduction in decapod crustacea, Biol. Review 45 : 121-165.

Adiyodi R.G. and T. Subramoniam (1983). Arthropoda - crustacesa, In : Adiyodi, K.G. and Adiyodi, R.G. (Eds) "Reproductivity Biology of Invertebrates" Vol.I Oogenesis, oviposition and oosorption, John Wiley & Sons Ltd., pp. 443-495.

Alikhuni, K.H., A. Poernomo, S. Adisukersno, M. Budiono, Busman (1975). Preliminary observations on induction of maturity and spawning in *Penaeus monodon* Fabricius and *Penaeus merguiensis* de Man by eyestalk exterpation, Bull. Shrimp Cult. Res. Cent. 1 : 1-11.

Areridarezyk, M.Z. (1981). Effect of hypophysial gonadotropins (FSH and LH) on the ovaries of the sand shrimp (Crustaces : Decapoda) Marine Biology, 63 : 241-247.

Behlmer, S.D. and G. Brown (1984). Viability of cryopreserved spermatozoa of the horse-shoe crab, *Limulus polyphemus*. Int. J. Invert. Reprod. Dev. 7 : 193-199.

Brown A. Jr., J. Mc Vey, B.S. Middledirch and A.L. Lawrence, (1979). Maturation of white shrimp *Penaeus setiferus* in captivity. Proc. World Maricult. Soc. 10 : 435-444.

Chamberlain and A.L. Lawrence (1981 a). Maturation, reproduction and growth of *Penaeus vannamei* and *P. stylirostris* fed natural diets. J. World. Maricult. Soci. 12 : 209-224.

Chamberlain and A.L. Lawrence (1981 b). Effect of light intensity and male and female eyestalk ablation on reproduction of *Penaeus stylirostris* and *P. vannamei*. J. World Maricult. Soci., 12 : 357-372.

Chow S., Y. Taki and Y. Ogasawara, (1985). Cryopreservation of spermatophore of the freshwater shrimp *Macrobrachium rosenbergii*. Biol. Bull., 168 : 471-475.

Clark, Jr. W.H., P. Talbot, R.H. Neal, C.R. Mock and B.R. Salser (1973). *In vitro* fertilization with non-mobile spermatozoa of the brown shrimp *Penaeus aztecus*. Mar. Biol. 22 : 353-354.

Clark, Jr. W.H., A.I. Yudin, J.K. Shigekawa and F.J. Griffin, (1986) Cellular events of fertilization in the penaeidae,

Sicyonia ingentis. In Biology of Benthic Invertebrates (M.R. Thomson, B. Sarojini and R. Nagabhushanam (Eds) Oxford & IBH Publishers Co., New Delhi. pp. 143-149.

Deecaraman, M. and T. Subramoniam, (1983). Endocrine regulation of ovarian maturation and cement gland activity in stomatopod crustacean, *Squilla holochista*. Proc. Indian Acad. Sci. (Anim Sci.) 92 : 399-408.

Haris, S. and P.A. Sandifer, (1986). Sperm production and the effects of electrically induced spermatophere expulsion in the prawn *Macrobrachium rosenbergii* (De Man). J. Crust. Biol., 6 : 633-647.

Idyll, C.P. (1971). Induced maturation of ovaries and ova in pink shrimp. Comm. Fish. Rev., 33 : 20-27.

Ishida, T., P. Talbot and M. Kooda-Cisco, (1986). Technique for the long term storage of lobster *(Homarus)* spermatophores. Gamete Res., 14 : 183-195.

Jeyalectumie, C. and T. Subramoniam (1987 a) Biochemical composition of seminal secretions with special reference to LDH activity in the reproductive tissues of the field crab, *Paratelphusa hydrodromous* (Herbst). Exp. Biol., 46 : 231-236.

Jeyalectumie, C and T. Subramoniam, (1987 b) Cryopreservation techniques to store spermatophores and seminal plasma of the edible crab, *Scylla serrata*. Abstract, First Indian Fisheries Forum, Mangalore, Dec. 1987.

Jeyalectumie, C and T. Subramoniam, (1988). Cryopreservation of spermatophores and seminal plasma of the edible crab *Scylla serrata*. Aquaculture (Communicated).

Kooda - Cisco, M.J. and P. Talbot, (1983). A technique for electrically stimulating extrusion of spermatophores from the lobster, *Homarus americanus*. Aquaculture 30 : 221-228.

Lumare, F. (1979). Reproduction of *Penaeus kerathurus* using eyestalk ablation. Aquaculture, 19 : 203-214.

Leung-Trujillo, J.R. andf A.L. Lawrence (1985). The effect of eyestalk ablation on spermatophore and sperm quality in *Penaeus vannamei*. J. World Maricult. Soc. 16 : 258-266.

Leung-Trujillo, J.R. and A.L. Lawrence (1987) Observations on the decline in sperm quality of *Penaeus setiferus* under laboratory conditions. Aquaculture 65 : 363-370.

Muthu, M.S. and A. Lakshminarayana, (1981). Induced maturation and spawning of Indian penaeid prawns. Indian J. Fish 24 : 172-180.

Munuswamy, N. and T. Subramoniam (1987). Neuroendocrine activity during ovarian maturation in a fresh water fairy shrimp *Streptocephalus dictotomus* Baird (Crustacea : Anostraca) crustaceana 52 : 303-313.

Natarajalingam, K. and T. Subramoniam (1987). Influence of light on the endocrine system and ovarian activity in two Ocypodid crab, *Ocypoda playtarsis* and *O. marocera* Mar.Ecol. Prog. Ser. 36 : 43-53.

Primavera, J.H. (1978). Induced maturation and spawning in five month-old *Penaeus monodon* Fabricius by eyestalk ablation, Aquaculture 13 : 355-359.

Radha, T and T. Subramoniam (1985). Origin and nature of spermatophoric mass of the spiny lobster, *Panulirus homarus* Mar. Biol.86 : 13-19.

Sandifer, P.A. and J.W. Lynn, (1980). Artificial insemination of caridean shrimp. In : Advances in Invertebrate Reproduction (Eds. W.H. Clark and T.S. Adams) Vol. II, pp.271-288. Elsevier-North-Holland, New York.

Sasikala, S.L. and T. Subramoniam, (1987 a). On the occurrence of acid mucopolysaccaharides in the spermatophores of two marine penaeid prawns, *Peneus indicus* (Miline Edwards) and *Metapenaeus monoceros* (Fabricius). J. Exp. Mar. Biol. Ecol. 113 : 145-153.

Sasikala, S.L. and T. Subramoniam, (1987 b). The effect of vertebrate steroid hormones on the vitellogenesis of freshwater field crab *Paratelphusa hydrodromous*. Symposium on freshwater aquaculture. Nagarjuna University, Dec. 21-24.

Silas, M.R. and T. Subramoniam, (1987). A new technique to electroejaculate spermatophores of the sand lobster *Thenus orientalis*. Abstract, First Indian Fisheries Forum, Mangalore. Dec. 1987.

Subramoniam, T. (1977). Aspects of secual biology of the anomuran crab *Emerita asiatica*. Mar. Biol. 43 : 369-377.

Subramoniam, T. (1984). Spermatophore formation in two intertidal anomuran crabs *Emerita asiatica* and *Albunea symnista*. Biol. Bull. 166 : 78-95.

Uma, K and T. Subramoniam (1979). Histochemical characteristics of spermastophore layers of *Scylla serrata* (Forskal) Int. J. Invert. Reprod. 1 : 197-199.