CMFRI

Course Manual

Winter School on

Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish

30.12.2008 -19.1.2009

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BROODSTOCK DEVELOPMENT AND CAPTIVE BREEDING OF SAND LOBSTER *THENUS ORIENTALIS* LUND, 1793

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Introduction

Lobsters are among the largest marine arthropods, with a long life span. They are slow growing animals with a complicated and prolonged life cycle, which greatly increases the risk of stock depletion through indiscriminate fishing. Sand lobsters are known to inhabit the open unconsolidated sediments between the coast and adjacent reefs and further beyond. These animals prefer soft, muddy beds. They are nocturnal feeders, with specific predatory feeding habits. Unlike the spiny lobsters, they swim actively and cover large distances while foraging for food. Gross morphological modifications including dorsoventral compression of the body, lateral expansion of carapace and extreme reduction of antennae and body spines have resulted in a well streamlined, energy-efficient swimming shape. An insight into the reproductive biology of an animal is a preliminary requisite for broodstock development and breeding in captivity. The intricate processes underlying the actual phenomenon of propagation of a species usually begin at the formative phase of the individual. They however manifest as visible changes at a later phase of growth, commonly acknowledged as the "adult" or "sexually mature" phase. These processes include a number of anatomical, physiological, morphological and behavioral changes.

Lobsters are bisexual animals exhibiting sexual dimorphism, and conform to the general decapod crustacean reproductive pattern which has been extensively studied and described (Rahman, 1967; Ryan, 1967; Haefner, 1976 and Zuckner, 1978). Sand lobsters exhibit sexual dimorphism and show marked variations in behaviour particularly during the mating and spawning periods. The development of secondary sexual characteristics is an important aspect of study in understanding the reproductive biology of lobsters. An assessment of the reproductive potential of a species is usually made from:

- Morphological and histological changes on gonadal structure and development
- Maturation-associated changes in secondary sexual characters
- Mating and spawning behaviour
- Gonadosomatic Index and fecundity
- Size at maturity

Sexual Dimorphism

The males and females can be easily identified by the following distinct morphological characteristics –

Male	Female
Smaller in size	Larger in size
Abdomen narrow	Abdomen considerably broad
Genital opening on coxa of fifth pair of pereiopods	Genital opening on coxa of third pair of pereiopods
Gonopore is a relatively big simple aperture	Gonopore is a simple aperture but much smaller than the gonopore of males
Dactylus of fifth pereiopods bluntly cylindrical and club-shaped, relatively slender and sparsely setose. It ends with a very small inwardly curved spine	Dactylus of fifth pereiopods bluntly cylindrical, more stout, with a longitudinal groove on the dorsalr side against which lies the claw. Dense setae seen in maturing and mature females
Pleopods comparatively small	Pleopods much larger; pleopodal endopods of mature females bear ovigerous setae

Secondary sexual characters

Male

(a) Gonopore: The gonopore is the only distinct secondary sexual character marking the onset of sexual maturity in male *T. orientalis*. It is situated at the base of the fifth walking leg and is seen to increase in size as the animal grows. There is no distinct protrusion or extension of the gonopores into penile processes and the gonopores exist as simple apertures

Female

(a) Ovigerous setae on abdominal pleopods: In *T. orientalis* the endopods of the abdominal pleopods in females bear ovigerous setae which are used for attaching spawned eggs until they are hatched. In juvenile female, the pleopods are devoid of setae. As the female enters into the sub-adult phase, the pleopods enlarge and the leaf-like endopods bifurcate and develop long ovigerous setae. Development of the ovigerous setae marks the onset of sexual maturity in females. There is a small club-shaped process midway along the inner margin of the endopod of the first pair of pleopods. The endopods of other three pairs of pleopods are ovoid at the proximal end and narrow and tubular at the distal end.

(b) Gonopore: The female gonopore, situated in the form of a simple aperture at the base of the third walking leg, is seen to increase in size as the animal grows but is relatively much smaller than the male gonopore.

Reproductive System

Lobsters conform to the generalized decapod reproductive pattern (MacDiarmid and Sainte-Marie, 2006) with paired ovaries or testes lying dorsally in the body cavity leading via paired oviducts in females or vasa deferentia in males, to reproductive apertures or gonopores on the coxa of the third pair of pereiopods in females and the fifth pair in males.

Male

The testes, situated dorsal to the alimentary tract, are a pair of highly convoluted white tubular structures joined by a transverse bridge, giving it an H-shaped appearance. The lobes extend backwards into the abdominal region. The vas deferens arise posterior to the transverse bridge. The proximal vas deferens is highly convoluted while the distal vas deferens is straight and opens through the genital pore on the coxa of the fifth pair of pereiopods. The vas deferens in a mature male holds the spermatophoric mass which is seen as a white gelatinous substance. The spermatophoric mass is expelled from the terminal tubular part of the vas deferens and through a pair of gonopores on the coxa of the fifth pair of pereiopods at the time of copulation.

Female

The ovaries, like the testes, are a pair of tubular structures connected by a transverse bridge, giving an H-shaped appearance. The ovaries are also placed dorsal to the alimentary tract. A pair of thin oviducts arise from the ovary posterior to the transverse ridge and open through the genital pores on the coxa of the third pair of pereiopods.

Maturation

Male

There is no external indication of gonadal maturation processes. The testes and vas deferens undergo alterations in size and form. The colour tends to remain white, but as maturation progresses, the gonads become less translucent and more opaque. Immature gonads in juveniles of *T. orientalis* also appear as translucent membranes and do

not extend into the abdomen. In sub-adults, the gonads become slightly whitish and the median vas deferens become opaque. In active adults, the entire testis becomes milky-white and the median vas deferens becomes very thick. The posterior lobes of the testis extend beyond the carapace, into the abdomen.

Female

The ovary undergoes a series of colour and size variations in tandem with the maturity cycle. The immature ovary is translucent and becomes white as the animal enters into the early stages of maturation. At this stage the posterior lobes of the ovary do not extend into the abdominal region. As maturation progresses the ovary becomes creamish to dark yellow and finally dark orange, when it is ready for spawning. The oviducts however, remain translucent. In the mature stage, the posterior lobes of the ovary extend to the abdominal region. Ovarian development can be classified into six stages - Immature, Early Maturing, Late Maturing/Mature, Ripe, Spawning and Spent/ Recovery.

The onset of maturity in females is marked by the development of ovigerous setae. The setae once developed lose their density after the first spate of egg bearing, but regains it during the next phase of breeding. This continues as cyclic phenomenon, coinciding with the breeding activity of the female. There are no mating windows in female *T. orientalis*.

Size at maturity

The size at first maturity is an important indicator of the reproductive capacity of populations. The size at first maturity is defined as the minimum size at which 50% of the population has entered into a state of advanced maturation and is usually estimated by plotting the percentage of mature animals in different body size classes in a sampled population against size, and directly reading the size at which 50% are mature. In decapods, the size at first maturity is usually studied with reference to physiological maturation (the size at which the gonads attain maturity) and physical maturation (the size at which the animal is capable of mating and spawning). There is often a gap in time between the occurrence of physiological and physical maturity and hence the two phenomenon may not occur at the same size (Jones, 1988), and both must be known to determine the size at true sexual maturity (Stewart et al., 1997).

The size at first maturity in female lobsters is usually estimated using functional criteria of egg-bearing (Kensler, 1967; Aiken and Waddy, 1980), the presence of fresh or spent spermatophores and resorbing ova, physiological criteria of ovary colour and size, oocyte size and development of cement glands on the pleopods and morphological criteria of abdomen and pleopod development (Mac Diarmid and Sainte-Marie, 2006). Staging based on histological examination of gonads provides more detailed information than any other indicator. One of the most important external indicators of sexual maturity in females is the presence of fully developed ovigerous setae on the abdominal pleopods, to which spawned fertilized eggs remain attached till the larvae are hatched. The appearance and development of the ovigerous setae for the first time coincides with the onset of sexual maturity in *T. orientalis* (Kizhakudan, 2007). The number and size of the setae reach a maximum at the time of egg bearing. After the first batch of eggs are completely hatched/removed from the pleopods, there is a reduction in the density of the ovigerous setae, which later increases during the successive breeding cycle.

There is not much time frame between the attainment of morphological, physiological and functional maturity in *T. orientalis.* The size at first maturity is attained at 51 - 55 mm CL in male *T. orientalis* and at 61 – 65 mm CL in female *T. orientalis.* The critical maturation phase in *T. orientalis* is not as extended as in spiny lobsters. The size at onset of sexual maturity, judged from the 25% success rate in development of different sexual characters is in the range of 46 – 50 mm CL for males and 51 – 55 mm CL for females (Kizhakudan, 2007).

Fecundity

Fecundity is a measure of fertility of an animal. It is usually expressed in terms of the number of eggs capable of being produced by an individual during a breeding cycle. Fecundity in lobsters is greatly influenced by external factors

like water temperature, pH and nutritional factors. The number of eggs produced is also directly related to the size of the lobster. In semi-enclosed areas and indoor experiments, density and competition for food is also an important factor determining the reproductive status and fecundity of lobsters. The total number of eggs produced by an animal during its life time is the net result of the interplay between two variable factors – the breeding frequency and the number of eggs produced in each breeding cycle. Since both these factors are independently subject to alterations in the environment, a measure of the total observed fecundity deviates greatly from the actual fecundity capacity of the animal. A better picture of the animal's fecundity can be obtained by taking into account the fecundity per breeding cycle. The fecundity of *T. orientalis* ranged from 19600 eggs (60 mm CL) to 59500 eggs (102 mm CL), with an average fecundity of 39300 eggs for reproductively active females in the size range of 60 – 102 mm CL (Kizhakudan, 2007).

Breeding in captivity

Maturation and breeding in captivity remain to be major challenges in the evolution of husbandry packages for lobsters. The establishment of culture conditions in which reproduction can be controlled to produce larvae all year round is one of the requirements for successful larval rearing of spiny lobsters (Vijayakumaran *et al.*, 2005). Compared to spiny lobsters, captive breeding of scyllarid lobsters is an area less explored. Kizhakudan *et al*. (2004) observed high incidence of maturation and breeding in scyllarids held in captivity. Water quality and photoperiod were found to play a major role and the animals were reared in larger tanks with increased water depth. Broodstock maintenance and development in *T. orientalis* can be done in a Closed Recirculatory System with fluidized bed filter and minimum light exposure (LD 1:23). Food is a major factor determining the performance of the animals in captivity. Booth and Kittaka (1980) mention the preference of shellfish, particularly mussels, over finfish by juvenile spiny lobsters. This was found to be true in the case of T. orientalis also as the animals show good reception to fresh clam meat. Juvenile (<30 mm CL) and sub-adult (30 – 40 mm CL) lobsters collected from the wild and reared in recirculatory systems developed into mature adult lobsters (65 - 70 mm CL) in a period of about 6 – 8 months. Regulation of light exposure and feeding @ 5% of body weight in two divided doses daily give good results.

Mating and spawning

Mate selection, courting and copulation in a lobster species are intricately related to the distinctive morphology of the sexes. Olfactory, visual, auditory and tactile stimuli have been known to play a role in the attraction, recognition and choice of mates in different lobster species (MacDiarmid and Sainte-Marie, 2006). Copulation in lobsters is usually very brief, typically lasting less than a minute in nephropid (Framer, 1974; Talbot and Helluy, 1995) and in palinurid lobsters (MacDiarmid and Kittaka, 2000). Copulation in *T. orientalis* was found to be slightly prolonged, lasting for five minutes. Males are smaller than females and are generally more active. Courtship lasts for a few days prior to mating, when the males actively move around in the tank often flipping over while swimming. During the courtship period, the males are very active at night and are often seen swimming even during the day, chasing the females, which are less active. Mating usually takes place during late night hours. In the course of mating, the male crawls on top of the female and overturns it. Copulation takes place with the animals in reverse positions and they swim away in opposite directions after the process. There is no compulsory premate moult in this species.

Sperm transfer from male to female during mating in crustaceans is effected by means of a spermatophore, which is a specialized sperm packet serving as a vehicle for sperm transport. The spermatophore essentially contains the sperms surrounded by protective layers of cellular secretions produced in the vas deferens (Aiken and Waddy, 1980, Kooda-Cisco and Talbot, 1982). The spermatophoric mass of *T. orientalis* lacks an external gelatinous matrix. The spermatophores remain embedded in a fibrillar mucoid matrix, which does not harden on exposure to sea water. It breaks open in a few hours after exposure to seawater. When copulation is over, the impregnated female curves its abdomen inwards and holds it at a slight elevation from the tank bottom. It is not very active and tends to crouch in some dark corner above the substratum. It carries the impregnation till the early morning hours. The freshly released spermatophores are milky white in colour, soft, delicate and embedded in a gelatinous matrix of mucoid fibrils. The impregnation is seen on the ventral side of the first abdominal segment as two parallel lines, usually

extending from the coxa of the fifth pair of pereiopods to the posterior tip of the second abdominal segment. The spermatophore packets are ovoid in shape and microscopic in size. They hold the active spermatozoa within. The gelatinous matrix in which the spermatophores are embedded holds a fibrillar network to which the spermatophores are attached. When the spermatozoa are released for fertilization, the empty spermatophore packets are left attached to the fibrils in the matrix.

Egg laying commences in about two hours and the eggs released are guided from the sternal region to the abdominal brood chamber where they are attached to the ovigerous setae on the endopod of the pleopods. The abdomen remains completely curved inwards, forming a 'U'-shaped cup-like brood chamber laterally sealed by the exopodites and the teeth-like extensions of the abdominal tergites. The endopodal brances and their setae spread like a floor on the ventral side of the chamber when all the eggs and spermatozoa mixed with water have been pushed in. Almost 90% of the eggs get attached to the pleopodal setae, irrespective of whether they are fertilized or not. The fertilized eggs are dark yellow or orange in colour while the unfertilized eggs turn pale cream or pinkish. The unfertilized eggs are shed off in 3 – 5 days. The incubation period in *T. orientalis* ranges from 32 to 37 days, during which embryonic development takes place inside the eggs. The abdomen continues to be curved inwards and the eggs are constantly fanned with the exopods of the pleopods and cleaned with the setal brushes on the dactyli of the pereiopods. Locomotor activity and feed intake are very much reduced during the incubation period and the female tends to remain in isolation.

At the time of hatching, the female holds the inwardly curved abdomen at a slightly elevated angle and the phyllosoma that hatch out are fanned away with the pleopods. Hatching takes place in batches only during the early morning hours and is usually completed in 1 – 3 days. After hatching, the empty egg capsules are seen attached to the ovigerous setae. The capsules are shed, along with a part of the setae, about 48 hours after hatching. Water quality, tank bottom quality and handling stress, particularly during the incubation period, greatly influence the success rate of hatching.

During the breeding period, the intermoult phase is highly extended in the female. After the first brood of eggs have been hatched, the female begins preparing itself internally for the next breeding, within the same intermoult phase. Interestingly, the ovigerous setae that developed before the first breeding continue intact for the next breeding also, in a single intermoult phase. The incubation period lasts for about 35 to 37 days and hatching occurs over an extended duration of 30 to 36 hrs. The rate of egg pruning by the brooder and the length of the incubation period is dependent on the quality of the water in which the animals are held. The phyllosoma that hatch from the eggs of laboratory-developed broodstock are found to be more viable than the ones that hatch from berried females collected from the wild.

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