EXPERIMENT ON ARTIFICIAL BREEDING
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INTRODUCTION

Chang and Sagi (2008) and Parnes et al. (2008) reported that in crustaceans the regulation of reproduction was highly diverse. In the crustacean industry the reproductive biology is playing a crucial role. In many crustaceans including crayfish, prawns, crab, shrimp, lobster etc reproduction is controlled by hormones. Chang et al. (2001), Fingerman (1997 a & b), Laufer et al. (1993 a & b), Mazurova et al. (2008), Nagaraju (2007) and Raviv et al. (2008) state that in controlling the gonad maturation the neuroendocrine organs play an important role. In crustaceans, the eyestalk ablation is the common method for the stimulation of gonads. Studied and reported by Fanjul-Moles (2006), Nagaraju and Borst, (2008) and Treerattrakool et al. (2008).

In regulating the gonadal maturation and moulting there are sufficient experiments available about neurosecretions of brain in various crustaceans (Adiyodi and Adiyodi, 1970 and Payen 1980). Ostu (1963) reported some experiments on thoracic ganglion in crab indicating an elaboration of growth stimulating hormone (GSH) in thoracic ganglion in both the sexes. Baid et al. (1967), Joshi and Khanna (1984 and 1985) observed that in crab morphologically both the brain and thoracic ganglion contain different types of neurosecretory cells. Similar observation was made on prawns by Ostu (1963), Gomez and Nayar (1965), Hinsch and Bennett (1979). Rao et al., (1981) supposed that these are the source of GSH in prawns. As brain and thoracic ganglion, the eyestalk also plays an important role in reproduction as they secrete growth inhibiting hormone (GIH) from X-organ as reported by Carlisle (1953 a and b) and Demeusy (1953). From the brain, thoracic ganglion and eyestalk X-organs, the axonal tracts transport the
neurosecretory material to a neurohaemal organ and sinus gland in the eyestalk for the storage and release. Agarwal (1985) demonstrated several changes in sinus gland with relation to gonadocycle.

For any economically viable culture program the successful domestication is the footstep of any candidate species. It lays controlled and enhanced reproduction of the brood stock prawns. The evolution of modern prawn culture demands captive reproduction and seed production in hatcheries. The growing demand from the farmers and entrepreneurs for quality seed from nature at particular time prompted to research for seed productions. The control of gonadal maturation in developing the commercial aquaculture is the major problem in the decapod crustaceans (Yano, 1992). According to Adiyodi and Adiyodi (1970) and Sastry (1983) in decapods crustaceans, the reproduction can be controlled by physiological (GIH) which prevents precocious maturation of ovaries and growth stimulating hormones (GSH) which promotes vitellogenesis. Bomirski et al. (1981), Quackenbush and Herrkind (1981) and Mohammed et al. (1991) suggested that the growth inhibiting hormone regulate the ovarian maturation from the X-organ of sinus gland complex of eyestalk.

In the decapods crustaceans the experimental studies in the young animals on eyestalk removal results in to precocious gonad development. Tan Fermin (1991) used a technique of eyestalk ablation in prawns for inducing precocious maturation and spawning in order to obtain prawn seeds for mass culture. Fingerman (1987) reported that for the induce maturation the eyestalk ablation is the major technique which involves site production and storage of growth inhibiting hormones. Rangnekar and Deshmukh (1968) and Rangnekar et al. (1971) state that in decapods crustaceans the experiments on eyestalk removal shows the precocious gonad development and the extract of eyestalk helps the stalkless animal to retain there gonadal maturity status. In eyestalk ablation of Penaeus species several studies were
done like Primavera (1985) investigate that no ovarian development takes place due to the eyestalk ablation similarly Emmerson (1980), Lumare (1979) and Millamena et al. (1985) observed that the values of GSI were lowered due to eyestalk ablation. Adiyodi and Adiyodi (1970) reported the presence of GIH in the eyestalk and presence of GSH in the brain and thoracic ganglion. In the testis of shrimp *Lysmata seticaudata* there is no effect due to the administration of eyestalk ablation as observed by Carlisle (1954). Panouse (1943) demonstrated for the first time the experiment on eyestalk removal on the female shrimp *Leander serratus* and observed a rapid increase in ovarian size and egg decomposition. In several crustaceans the technique of eyestalk ablation was used like *Pandalus kessleri* (Aoto and Nishida 1956), *Carcinus means* (Demeusy 1967), *Cragon cragon* (Bomirski and Klek 1974), *Barytelphusa cunicularis* (Nagabhushanam and Diwan 1974) and *Panulirus argus* (Quackenbush and Harrnkind 1981). Meeratana et al. (2006) reported the induced maturation in the giant freshwater prawn *Macrobrachium rosenbergii*. Several contributions are made in this field for Penaeid culture by Vaca and Alfaro (2000), Withyachumnarnkul et al. (2001), Wouters et al. (2001), Hoang et al. (2002), Coman et al. (2003, 2004, 2005, 2006, 2007), Toledo et al. (2005), Sellars et al. (2006).

In crustaceans the influence of brain and thoracic ganglion governs the gonadal maturation and development and seems to be variable. Ostu (1963) and Gomez (1965) reported that in several crustaceans the stimulation of ovarian maturation was observed by hormonal secretion from neurosecretory of thoracic ganglion. The synthesis of gonad stimulation in male marine prawn, *Parapenaeopsis stylifera* was demonstrated by Nagabhushanam and Joshi (1984) on the neurosecretory cells of brain and thoracic ganglion. Rangnekar et al. (1971) investigated that in crab *Scylla serrata*, due to the brain and thoracic ganglion hormones the testicular activity remains unaffected. Deecaraman and Subramonian (1983) reported
induced ovarian maturation in stomatopod, *Squilla holoschista* due to aqueous thoracic ganglion extracts whereas due to brain extract no effect was found in same animal.

To the raising demands of seeds to the farmer and to re-establish the natural declining stock the induced breeding is the highly reliable techniques for used. The technique in which stimulation of artificial hormones administration i.e. Gonadotropin was done is known as inducing breeding. In India this practice was successfully administrated in the fishes. The present chapter deals with the influence of eyestalk ablation and injections of eyestalk extract, brain extract, thoracic ganglion extract, ovatide and ovaprim on the development of gonads and breeding of *Macrobrachium lamarrei lamarrei*.

**OBSERVATION**

**Experiments on influence of eyestalk extract, brain extracts and thoracic extracts**

The experiment consisted of four repeated injections of each tissue at the time interval of five days. Table 8.1 and 8.2 and Figs. 8.1 to 8.4 and Plate 8.1 to 8.8 shows the influence of extracts on gonadal material. Observation on gonadal material was done after the five days of the last injection.

Due to the injections of brain and thoracic ganglion there was a significant increase in GSI of male and female *M. lamarrei lamarrei*. The extent of increase in both the male and female animal was different with respect to control animals for both the injections. The GSI value for female animal was $2.30 \pm 0.4$ and $2.10 \pm 0.07$ and for male animal $0.60 \pm 0.09$ and $0.58 \pm 0.07$ with both the brain-extracts and thoracic ganglion extracts injections. Obtained result for GSI for both the injections shows that there
was a significant increase in the mean diameter of oocytes and testicular acini with little more increment in the degree with brain-extract injection (Table 8.1 & 8.2).

But in the experiment of eyestalk-extract, a reduction in mean diameter of acini 0.108 ± 0.04 and oocytes 0.167 ± 0.05 was observed with a fall in GSI of both male and female prawns 0.20 ±0.03 and 0.69 ± 0.01 and the GSI values were significant statistically from the control values of GSI 0.30 ± 0.05 and 0.92 ± 0.30 (Table 8.1 & 8.2) and (Fig. 8.1 to 8.4).

Histologically, in most of the prawns the control ovaries were found to be previtellogenic stage with small appearance possessing growing oocytes with very young oocytes (Plate 8.1) whereas the control testes consist of secondary spermatogonia along with degenerating sperms under with few seminiferous tubules (Plate 8.5).

The ovaries contained ripened oocytes with tertiary vitellogenic but after treatment with the brain-extract injections they were observed with small oocytes with dense accumulation in ooplasm with yolk vacuoles and globules and do not become vitellogenic during normal development (Plate 8.2) whereas in the testes after the brain-extract injection the testes stimulated the spermatogonial transformations. Various stages of cell division are exhibited in the seminiferous tubules as primary spermatocytes and secondary spermatocytes (Plate 8.6).

Administration of thoracic ganglion-extract on ovaries possesses advanced development during histological examination and reveals the presence of matured ova in them in comparison to the controlled prawns (Plate 8.3). Ovaries behave more or less same as in brain-extract injection whereas in testes, its seminiferous tubules mainly constitute secondary spermatocytes with spermatids and sperms occasionally (Plate 8.7).
Table 8.1: Observations on gonadosomatic index and mean oocytes diameter of female *M. lamarrei lamarrei* by the injections of eyestalk extract, thoracic ganglion extract and brain extract.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FEMALE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gonadosomatic index</td>
<td>Mean oocyte diameter (mm)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
</tr>
<tr>
<td>Eyestalk extract injections</td>
<td>0.92 ± 0.30</td>
<td>0.69 ± 0.01 (p&lt; 0.01)</td>
</tr>
<tr>
<td>Thoracic ganglion extract injection</td>
<td>0.90 ± 0.28</td>
<td>2.10 ± 0.07 (p&lt; 0.01)</td>
</tr>
<tr>
<td>Brain extract injection</td>
<td>0.93± 0.32</td>
<td>2.30 ± 0.4 (p&lt; 0.05)</td>
</tr>
</tbody>
</table>

Table 8.2: Observations on gonadosomatic index and mean acinar diameter of male *M. lamarrei lamarrei* by the injections of eyestalk extract, thoracic ganglion extract and brain extract.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MALE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gonadosomatic index</td>
<td>Mean acinar diameter (mm)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
</tr>
<tr>
<td>Eyestalk extract injections</td>
<td>0.30 ± 0.05</td>
<td>0.20 ± 0.03 (p&lt; 0.01)</td>
</tr>
<tr>
<td>Thoracic ganglion extract injection</td>
<td>0.32 ± 0.04</td>
<td>0.58 ± 0.07 (p&lt; 0.01)</td>
</tr>
<tr>
<td>Brain extract injection</td>
<td>0.34 ± 0.06</td>
<td>0.60 ± 0.09 (p&lt; 0.01)</td>
</tr>
</tbody>
</table>
Histologically, the sections of testis of eyestalk extract on prawns comprises secondary spermatogonia as in control prawns (Plate. 8.8) similarly in the ovaries of eyestalk extract on prawns, the sections obtained are same as control one. Plate 8.4 shows the rare observations of yolk vacuoles presence and previtellogenic oocytes.

**Experiment on influence of eyestalk ablation**

**After 10 days observation**

The influence of both the eyestalk removal of the prawn on the diameters of testicular acini, oocytes and gonadosomatics indices (GSI) are given in table 8.3 and 8.4, figure 8.5 to 8.8 and plate 8.9 to 8.12. In the controlled animals only increment was observed in diameter of oocyte which was statistically insignificant (p>0.05) (Table 8.3 & 8.4). And in the operation which was done after the time interval of 10 days gives a significant increase in the diameters of testicular acini and oocytes of the two sexes.

**After 25 days observation**

There was again a significant increase in the diameters of acini and oocytes of the experimental prawns values 0.173 ± 0.03 and 0.478 ± 0.04 whereas the increase in GSI of both the sexes were also observed, as for experimental male 0.421 ± 0.07 and in controlled male 0.220 ± 0.05 similarly for experimental females 4.16 ± 0.6 and controlled females 2.05 ± 0.5. The values found are significant statistically (Fig. 8.5 to 8.8).

**After 45 days observation**

Same trend of increment was followed as above now the values of diameter of acini and oocytes became 0.201 ± 0.03 and 0.530 ± 0.03 and the GSI value became 0.553 ± 0.06 for male and 6.89 ± 1.31 and female (Table 8.3 & 8.4). This is also statistically significant.
Table 8.3: Following the eyestalk ablation in female *M. lamarrei lamarrei* the changes observed in gonadosomatic index (GSI) and mean oocyte diameter

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Eyestalk ablation</th>
<th>Control</th>
<th>Eyestalk ablation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.02 ± 0.50</td>
<td>2.11 ± 0.6 (p &lt; 0.05)</td>
<td>0.267 ± 0.04</td>
<td>0.310 ± 0.04 (p&lt; 0.05)</td>
</tr>
<tr>
<td>25</td>
<td>2.05 ± 0.46</td>
<td>4.16 ± 0.6 (p &lt; 0.01 )</td>
<td>0.309 ± 0.03</td>
<td>0.478 ± 0.04 (p&lt; 0.05)</td>
</tr>
<tr>
<td>45</td>
<td>2.63 ± 0.50</td>
<td>6.89 ± 1.3 (p&lt; 0.01)</td>
<td>0.349 ± 0.04</td>
<td>0.530 ± 0.03 (p&lt; 0.05)</td>
</tr>
</tbody>
</table>

Table 8.4: Following the eyestalk ablation in male *M. lamarrei lamarrei* the changes observed in gonadosomatic index (GSI) and mean acinar (seminiferous tubule) diameter

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Eyestalk ablation</th>
<th>Control</th>
<th>Eyestalk ablation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.148 ± 0.02</td>
<td>0.297 ± 0.04 (p&lt; 0.05)</td>
<td>0.130 ± 0.01</td>
<td>0.159 ± 0.02 (p&lt;0.05)</td>
</tr>
<tr>
<td>25</td>
<td>0.220 ± 0.05</td>
<td>0.421 ± 0.07 (p&lt; 0.05)</td>
<td>0.135 ± 0.02</td>
<td>0.173 ± 0.03 (p&lt;0.01)</td>
</tr>
<tr>
<td>45</td>
<td>0.252 ± 0.04</td>
<td>0.553 ± 0.06 (p &lt; 0.05)</td>
<td>0.142 ± 0.02</td>
<td>0.201 ± 0.03 (p&lt;0.01)</td>
</tr>
</tbody>
</table>
Histologically the testes of control animal comprises of secondary spermatogonia and resting sperms (Plate 8.9) whereas the eyestalk ablated prawns after 10 days, 30 days and 45 days exhibits the rapid transformation and the seminiferous tubules comprises primary and secondary spermatocytes with occasional sperms and spermatids (Plate. 8.10). Similarly the ovaries of control animals exhibited the early primary vitellogenic oocytes in which yolk vacuoles start appearing with peripheral ooplasm (Plate. 8.11) whereas the 10 days of eyestalk ablated prawns comprises slightly larger oocyte than control females which turns due to the deposition of yolk globules into secondary vitellogenic oocytes and the 40\textsuperscript{th} day fully ripe ova was observed with yolky material in their ooplasm (Plate 8.12).

**Experiment on effect of ovaprim and ovatide on breeding of female prawns**

In order to examine the effect of synthetic hormones (ovaprim and ovatide) on the development of gonads in male and female prawns were administrated separately with the single dose i.e. 0.1 ml for male and 0.2 ml for female (Table 8.5 & 8.6, Figure 8.9 to 8.12 and Plate 8.13 to 8.16).

Female prawns injected with ovaprim shows an increased GSI (2.60±0.02) from the control prawns (0.40 ± 0.05) with increase in oocyte diameter (0.247±0.05). Similarly female prawns injected with ovatide show a slight less increase in GSI in comparison to ovaprim (2.49 ± 0.01) from the control prawns (0.38 ± 0.04). Histologically ovary in both the cases shows the presence of secondary vitellogenetic and tertiary vitellogenetic with matured oocytes (Plate. 8.13) whereas ovary of control prawns shows only the presence of very young oocyte with previtellogenetic (Plate 8.14).
Table 8.5: Observations on gonadosomatic index and mean acinar diameter of male *M. lamarrei lamarrei* by the synthetic injections of ovaprim and ovatide.

<table>
<thead>
<tr>
<th>Synthetic hormones tested</th>
<th>MALE</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gonadosomatic index (GSI)</td>
<td>Mean acinar diameter (mm)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
<td>Control</td>
</tr>
<tr>
<td>Ovaprim</td>
<td>0.25 ± 0.02</td>
<td>0.69 ± 0.04 (p&lt; 0.05 )</td>
<td>0.129 ± 0.01</td>
</tr>
<tr>
<td>Ovatide</td>
<td>0.24 ± 0.02</td>
<td>0.51 ± 0.04 (p&lt; 0.05 )</td>
<td>0.131 ± 0.03</td>
</tr>
</tbody>
</table>

Table 8.6: Observations on gonadosomatic index and mean oocyte diameter of female *M. lamarrei lamarrei* by the synthetic injections of ovaprim and ovatide.

<table>
<thead>
<tr>
<th>Synthetic hormones tested</th>
<th>FEMALE</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Gonadosomatic index (GSI)</td>
<td>Mean oocyte diameter (mm)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Experimental</td>
<td>Control</td>
</tr>
<tr>
<td>Ovaprim</td>
<td>0.40 ± 0.05</td>
<td>2.60 ± 0.02 (p&lt; 0.05)</td>
<td>0.139 ± 0.01</td>
</tr>
<tr>
<td>Ovatide</td>
<td>0.38 ± 0.04</td>
<td>2.49 ± 0.01 (p&lt; 0.05)</td>
<td>0.141 ± 0.02</td>
</tr>
</tbody>
</table>
Male prawns injected with ovaprim shows an increase in GSI as in female prawns (0.69 ± 0.04) from the control prawns (0.25 ± 0.02) with increased acinar diameter (0.170 ± 0.03). Similarly GSI obtained from the ovatide injected prawn is 0.51 ± 0.04 with mean acinar diameter 0.159 ± 0.03. In comparison to ovatide, an ovaprim injection shows more increment in GSI and mean diameter of acinar. Histologically, the testes of ovaprim and ovatide contain mostly secondary spermatocytes with spermatids (Plate 8.16) whereas control testes contain resting spermatogonia with few secondary spermatogonia and primary spermatocytes (Plate 8.15).

DISCUSSION

In the decapods crustaceans the physiological process are controlled by the hormonal secretions of different neuroendocrine cells located at different centers of ganglion is reported by Fingerman (1970). Ostu (1960), Oyama (1968), Hinsch and Bennet (1979), Eastman-Recks and Fingerman (1984), Takayanagi et al. (1986), Yano (1992), Yano et al. (1988) reported the evidence to show the presence of gonad stimulating hormone which stimulates the ovarian development and maturation of vitellogenic crab, lobster and shrimp in the thoracic ganglion. Kulkarni et al. (1984) demonstrated an experiment on male Parapenaeopsis hardwickii with the injections of brain and thoracic ganglion extracts and observed a precocious development of testes. Rangnekar et al. (1971) reported that in marine crab Scylla serrata there was no effect observed in brain and thoracic ganglion extracts. Several variability have been observed in the crustaceans due to brain and thoracic ganglion extracts. In the present study in M. lamarrei lamarrei both the treatments shows a significant increase in testicular maturation in which maturation degree was slightly higher in thoracic ganglion extract than brain extracts.
In the adult female *P. monodon* the extract of thoracic ganglion was effective for ovarian maturation. Yano and Wyban (1992) state that brain and thoracic ganglion comprises growth stimulating hormone and growth stimulating releasing hormone which enhanced the ovarian maturation. Ostu (1963) demonstrated a reproductive physiology of crab and state the extracts of thoracic ganglion stimulate the oocytes growth, GSI, precocious vitellogenesis. Kulkarni *et al.* (1981) reported that in female prawn *Parapeneaeopsis hardwickii*, a precocious increase in ovarian index and oocytes maturation due to the extracts of both brain and thoracic ganglion. The present study shows that both brain and thoracic extracts stimulate the vitellogenesis due to which ovaries change in to fully ripe matured ova.

In the *M. lamarrei lamarrei* the experiment on eyestalk removal in intact prawns with repeated injection of eyestalk extract shows a inhibition in testicular maturation (reduced in acini diameter) with fall in GSI similarly inhibition in ovarian development (reduced in oocytes diameter) with fall in GSI. A factor reveals from this eyestalk removal contains a gonad-inhibiting hormone (GIH). Withdrawal of this hormone cause precocious development of gonads in both male and female prawns due to eyestalk removal. Histologically same pictures were observed as in control prawns. This result has been earlier observed by Kulkarni *et al.* (1984), Fingerman (1997) and Haihui *et al.* (2006)

Adiyodi and Adiyodi (1970) reported that a gonad inhibiting hormone was present in male crustaceans. Ostu (1963) reported that in freshwater prawn *Potamon dehaani* the inhibition of testicular maturation due to the removal of eyestalk. Similar results have been found in *Parapeneaeopsis stylifera* (Nagabhushanam and Joshi 1984) and *Macrobrachium kistnensis* (Sarojini *et al.*, 1982 b).
Carlisle (1953) demonstrated that in crustaceans the eyestalk is the source of ovary inhibiting hormone which is stored in the sinus gland and produced from X-organ (medulla terminalis ganglionic). Panouse (1944) reported the inhibition of ovarian development in the shrimp *Leander serratus* due to the implantation of sinus gland and rapid maturation of ovaries due to eyestalk ablation. In prawns and crab several workers Brown and Jones (1947), Nagabhushanam and Diwan (1947 a) investigated the same observation.

Nagaraju and Borst (2008) reported that the eyestalk of *C. maenas* results in the increased testicular index with 2-fold higher than of intact green crab. Chamberlain and Lawrence (2009) reported the increase in testicular size with doubled mating success in eyestalk ablation of male white shrimp *L. vannamei* and suggested that eyestalk ablation stimulates the spermatogenesis.

Carlisle (1954) reported that the eyestalk removal and eyestalk ablation does not give the similar result for male and female animals like in female shrimp *Lysmata seticeudata* shows induced precocious development in ovaries in relation to their volume and weight due to eyestalk ablation whereas in male shrimp it does not shows any effect. Aoto and Nishida (1956) investigated that in female hermaphrodite prawn *Pandalus kessleri* a precocious development of ovaries was observed but in males no effect was found in testicular tissue.

As the result given by Ostu (1963) and Sarojini et al. (1982 b) in male decapods for bilateral eyestalk ablation similar observation was observed in *M. lamarrei lamarrei* the experiment on bilateral eyestalk ablation influenced the increase in diameter of seminiferous tubules and Gonadosomatic index. In conformation to the results of Panouse (1944), Brown and Jones (1947), Nagabhushanam and Diwan (1974) the female
prawns *M. lamarrei lamarrei* shows the increase in Gonadosomatic index and diameter of oocytes. After 10 days several ovarian changes have been observed and the values were found to be statistically significant. In the present study after 40 days of eyestalk ablation the ovaries shows fully ripe oocytes and testes shows a full maturation containing secondary spermatocytes with few spermatids and sperms histologically. Several abnormalities have been found in *Penaeus* species like *P. monodon* showing reduction in GSI due to eyestalk ablation reported by Millamena et al. (1985). Similarly Primavera (1985) observed a decline in reproductive performance with ovarian development in *Penaeus* species. Drach (1944) reported that in crustaceans, removal of eyestalk does not result in to gonadal development but speed up the moulting. In prawn *M. lamarrei lamarrei* no abnormalities have been observed in the ablated animals due to the proper supply of food throughout the experiment.

Although very less information is available on the influence of synthetic hormones like ovatide and ovaprim on prawns but several studies were proceed in fishes to induce the fertilization rate, hatching rate and ovulation by the synthetic hormones. In India, states like Karnataka, Tamil Nadu and Andhra Pradesh several Indian major carps are in practice with synthetic hormones to increase the seed production. Induced breeding with synthetic hormones are highly effective because they are in liquid form, consistent potency with reliable results, ready to use do not require any refrigeration, cost-effective and proved as an effective ovulating agent.
Fig. 8.1: The graph showing gonadosomatic index values of female *M. lamarrei* by the injections of eyestalk extract, thoracic ganglion extract and brain extract.

Fig. 8.2: The graph showing mean oocyte diameter values of female *M. lamarrei* by the injections of eyestalk extract, thoracic ganglion extract and brain extract.
Fig. 8.3: The graph showing gonadosomatic index values of male *M. lamarrei* by the injections of eyestalk extract, thoracic ganglion extract and brain extract.

Fig. 8.4: The graph showing mean acinar diameter values of male *M. lamarrei* by the injections of eyestalk extract, thoracic ganglion extract and brain extract.
Fig. 8.5: The graph showing the changes observed in gonadosomatic index (GSI) values of female *M. lamarrei lamarrei* due to eyestalk ablation treatment.

Fig. 8.6: The graph showing the changes observed in mean oocyte diameter (mm) values of female *M. lamarrei lamarrei* due to eyestalk ablation treatment.
Fig. 8.7: The graph showing the changes observed in gonadosomatic index (GSI) values of male *M. lamarrei lamarrei* due to eyestalk ablation treatment.

Fig. 8.8: The graph showing the changes observed in mean acinar diameter values of male *M. lamarrei lamarrei* due to eyestalk ablation treatment.
Fig. 8.9: Graph showing effect on the values of gonadosomatic index of male *M. lamarrei lamarrei* by the synthetic injections of ovaprim and Ovatide.

Fig. 8.10: Graph showing effect on the values of mean acinar diameter of male *M. lamarrei lamarrei* by the synthetic injections of ovaprim and Ovatide.
Fig. 8.11: Graph showing effect on the values of gonadosomatic index of female *M. lamarrei lamarrei* by the synthetic injections of ovaprim and ovatide.

Fig. 8.12: Graph showing effect on the values of mean oocyte diameter of female *M. lamarrei lamarrei* by the synthetic injections of ovaprim and ovatide.
Plate 8.1: Ovary of control prawn showing previtellogenic oocytes and very young oocytes. ×200 (H & E).

Plate 8.2: Ovary of brain extract injected prawn showing dense accumulation with yolk vacuoles and globules. ×300 (H & E).
Plate 8.3: Ovary of thoracic ganglion extract injected prawn showing tertiary vitellogenesis with matured ova. × 300 (H & E).

Plate 8.4: T.S. ovary of eyestalk extract-injected prawn showing previtellogenic and few primary vitellogenic oocytes. × 200 (H&E).
Plate 8.5: Testis of control prawns showing spermatogenesis with degenerating sperms. × 300 (H & E).

Plate 8.6: T.S. testis of brain extract injected prawn showing the presence of few primary spermatocytes, secondary spermatocytes and spermatids. ×300 (H & E).
Plate 8.7: T.S. testis of thoracic ganglion extract-injected prawn showing secondary spermatocytes, spermatids and sperms. × 300 (H&E).

Plate 8.8: T.S. testis of eyestalk extract-injected prawns showing secondary spermatogonia with few primary spermatocytes and degenerating sperms. ×300 (H & E).
Plate 8.9: Testis in control (45 days) of eyestalk ablated showing secondary spermatogenesis and resting sperms. ×300 (H & E).

Plate 8.10: Testis of bilateral eyestalk ablated prawn (45 days) showing spermatocytes, spermatids and sperms. ×300 (H & E).
Plate 8.11: Ovary in control (45 days) of eyestalk ablation showing early primary vitellogenic oocytes. ×200 (H & E)

Plate 8.12: Ovary of eyestalk ablated prawn (45 days) showing almost ripe ova. ×200 (H & E).
Plate 8.13: T.S. of ovary showing previtellogenic oocyte in control. ×200 (H & E).

Plate 8.14: T.S. of ovary of hormone injected prawns showing the presence of tertiary vitellogenesis with some matured oocytes. ×300 (H & E).
Plate 8.15: Testis in control showing resting spermatogonia, secondary spermatogonia and primary spermatocytes. x200 (H & E).

Plate 8.16: Testis of hormone injected prawn shows the presence of secondary spermatocytes with spermatids. x400 (H & E).
SUMMARY
A Non-penaeid prawn comprises one of the most economically important products in international and national market due to its unique taste and high unit value. An important position has been occupied by them domestically and worldwide. Macrocrustaceans possess remarkable creatures in regard to their ability to inhabit and to reproduce in diverse ecological areas. From the academic point of view the utility of prawns is well understood. Prawn culture is now becoming an important field where many countries have been involved due to being a highly profitable food items which help them to earn foreign exchange in good amount. *Macrobrachium lamarrei lamarrei* (H. Milne Edwards) is a small freshwater prawn which is widely distributed in the tarai region of Kumaun Himalayas. In terms of availability size it competes well with other exploitable species of smaller prawns and constitutes an important local food resource. The present prawn grows to an average size of 80 mm in length. A disorganized fishing has been done by the poor peoples of tarai region. So in order to establish the prawn culture of the present species, it was of interest to study certain aspects of the ecology and reproductive potential of *Macrobrachium lamarrei lamarrei* (H. Milne Edwards) and the findings are expected to form a basis for the culture of protein rich species of the tarai region of Kumaun Himalayas, Uttarakhand.

The study site is located in Tehsil Kiccha, district Udham Singh Nagar named as Baigul reservoir with Altitude 211 masl, Latitude 79° 35’-79° 43’E, Longitude 28° 52’- 28° 57’N and with Height 13.7 M. Baigul reservoir is a small tributary of Ganga originating from the foothills of Kumaun himalayas. The climatic condition of district Udham Singh Nagar
is humid and characterized as extremely hot and dry summer with extremely cold winters. Fog generally appears from December continues till February.

**Water parameters**

The temperature of Baigul reservoir seemed to be fluctuating during the year. The maximum temperature recorded was 37°C. During the monsoon period (June to September), the Baigul reservoir was over flooded due to the heavy rain. During the rainy season the juvenile (newly formed) along with the adult remains deep in the reservoir. The pH value of the reservoir did not show any significant fluctuation but the maximum value observed in the month of September (8.0) whereas the minimum month observed was April (7.0). Maximum DO concentration was found in the month of April (8.3 ppm) and minimum in the month of November (7.0 ppm). The monthly average water depth of Baigul reservoir was found to be fluctuating below 1.0 m and above ½ m except in the monsoon months.

**Food and Feeding**

The freshwater prawn *Macrobrachium lamarrei lamarrei* (H. Milne Edwards) is an omnivorous species in nature. But under the laboratory conditions it generally prefer for animals diet of soft musculature. The gut contents of prawn mainly consist of plant food, animal food, sand, debris and insect larvae during the different months of the year. During the monsoon season i.e. from June to September the gut of the prawn mainly found with sand and debris with less percentage of vegetation. The observation was recorded during the rainy season because there was significant decrease in the percentage of plant matter and significant increase in the percentage of microorganism. The fluctuations in feeding index are not related with the sex of the animal or any particular time of the day.
The increase in gastosomatic index (GSI) and feeding index (FI) was assessed the increase in feeding rate of both the sexes. During March to November in both the sexes the accomplishment of gonadal development and maturation takes place so it requires an energy demand for an animal for nutrient mobilization from hepatopancreas to ovaries and testes which results in increase of gastosomatic index (GSI) and feeding index (FI) which ultimately increases the feeding rate of an animal. The maximum GSI for females and male was found in May month (3.03 ± 0.02) and June (2.71 ± 0.02) and feeding index was found to be maximum in month of May (36.06 ± 1.13) for females and June (37.43 ± 2.03) for males.

**Food conversion efficiency**

Experiment on food conversion efficiency was conducted with soyabean meal, fish meal and compound diet (soyabean + fishmeal) which reflects the food conversion ratio as 9.71:1 for soyabean diet, 7.92:1 for fishmeal diet and 5.41:1 for compound diet. Hence we can say that artificially prepared food is nutritionally better for prawn’s growth because they contain important nutrient viz. proteins, lipids and carbohydrate for the growth of the species is to be cultured.

**Relative length**

Different graphs are plotted between carapace length / total length (CL/TL), abdominal length/ total length (ABL/TL) and carapace length /abdominal length (CL/ABL) and observed a positive allometric growth in the relationship between CL/TL i.e. $CL=0.2723 \, TL^{1.781} \quad (r=0.94; \, P<0.01)$, CL/ABL i.e. $CL=0.7012 \, ABL^{1.082} \quad (r=0.98; \, P<0.01)$ and ABL/TL relationship i.e. $ABL=0.3695 \, TL^{1.929} \quad (r=0.99; \, P<0.01)$ of male prawn. Similar positive allometric growth have been observed in relationship
between CL/TL i.e. CL=0.2676 TL^{1.435} (r= 0.95; P<0.01), ABL/TL i.e. ABL=0.3803 TL^{1.442} (r= 0.98; P< 0.01) and CL/ABL i.e. CL=0.6669 ABL^{1.037} (r= 0.92; P<0.01) of female prawn.

**Length-Weight relationship**

A strong and statistically significant relationship was observed between the length and weight parameter. The values calculated for female in log and parabola form are log W= 2.977+logL^{3.192} where r= 0.92; P<0.01 and W= 0.000096+ L^{2.977} where r= 0.93; P<0.01 and for male the log and parabola values are log W= 2.754+ logL^{4.054} where r= 0.90; P<0.01 and W=0.0000119 + L^{2.754} where r= 0.94; P<0.01. The values observed were statistically significant and proved that growth in relation to weight shows allometry.

**Condition factor**

Monthly fluctuation was observed in the relative condition factor (Kn). No special trend was found for reproductive cycle of both male and female prawns. Monthly estimation was done for one year (June 2009 to May 2010). In female prawns, the maximum value of relative condition factor was found in the month of August (2.5) and the minimum value was found in the month of January (0.6) while in the males, the maximum value recorded was in the month of May (2.6) and minimum in the month of December (0.7).

**Breeding pattern**

**Sex-ratio**

From the natural population the prawns were collected at monthly interval, the sex ratio of male and female prawn was observed as 1: 2.29 in which greater presence of females was observed from the natural population and this difference was statistically significant (p<0.05).
**Fecundity**

A positively strong relationship have been observed between brood fecundity with total length, brood fecundity with total weight and ovarian fecundity with ovary weight which was statistically significant p<0.05. A relationship between fecundity and month also observed. The mean brood fecundity count was 171 ± 30.66 with minimum value 80 and maximum value 264 similarly for ovarian fecundity minimum value was 90 and maximum value was 270 with mean fecundity 191±41.50.

**Gonadosomatic index (GSI) and Hepatosomatic index (HSI)**

A temporally similarity between the development patterns of ovaries and testes of prawn was observed. The minimum and maximum values of gonadosomatic index were observed in the month of September (0.62 ± 0.02) and April (3.07 ± 0.11) in females whereas in the month of October (0.47 ± 0.03) and April (2.09 ± 0.18) in males. The minimum and maximum values observed for male hepatosomatic index were 0.76 ± 0.04 in the month of June and 2.83 ± 0.21 in the month of October similarly for female values were minimum in the month of the April (1.0 ± 0.05) and maximum in the month of October (6.5 ± 0.76). *M. lamarrei lamarrei* is the annual breeder. Between hepatosomatic index and gonadosomatic index an inverse relationship have been observed which suggested a nutrient mobilization during maturation from hepatopancreas to gonads. The first size related sexual maturity was attained at the length of 35 mm. During the seasonal cycle, moulting occurs before the females inseminated. Females show many changes but the male does not show much colour changes. Spawning starts from the month May to September where August month shows with 80 % berried animals. A potential of continuous breeding was also suggested for this species because after the spawning period the availability of berried animals in small numbers still occurs.
Seasonal histological changes in gonads

After spawning, by proliferation the young oocytes appear in the middle of the ovary from the germinal epithelium. The immature phase was marked by formation of previtellogenic oocytes mainly and lasted from July to September and during the maturing phase (November to April) the vitellogenesis was accomplished with the appearance of yolk vacuoles, yolk granules and yolk globules in the ooplasm. The ova diameter ranged from 0.450 to 0.520 mm. Three types of vitellogenic oocytes are present primary, secondary and tertiary. Maturation of ova can be observed in the month of May due to quick transformation of tertiary vitellogenic oocytes in to ripe ova.

In the testis the primary spermatogonia are formed by the proliferation from resting spermatogonia. In the immature stage (August to October) the testes either consisted of resting and primary spermatogonia or exhausted acini cells in fluid medium and during maturing phase (November to April) the maximum transformation of spermatogonia was observed with formation of secondary spermatogonia, spermatocytes and spermatids. In the month of June/July maturation of testes was observed by the presence of sperms.

Neither testis nor the ovary of *M. lamarrei lamarrei* exhibits any spell of gonadal quiescence.

Life History

Under the laboratory condition, the duration between brooding and hatching at 26 ± 3°C was for 25 to 30 days. Hatching could not occur at below the temperature of 20°C because below this temperature the brood gradually disintegrated. White transparent eggs were hatched with two black spots of eye externally visible before hatching. The larval stages consist of three advanced zoeal forms viz. zoea I, zoea II, zoea III and
The larval development was totally abbreviated type in *M. lamarrei lamarrei*. The first Zoea measured was 4.3 ± 0.06 mm in length. Rostrum was straight, extending beyond to sessile eyes slightly or equal. The second zoea measured was 4.6 mm in length. Rostral formula observed was 1/0. The third zoea measured was 5.0 ± 0.10 mm in length. Rostral formula observed was 6/5 and the rostrum reaching across the antenna length. The post larva was measured as 5.4 ± 0.012 mm in length. Rostrum turns upwards towards the tip and reaching almost to the doubled of antenna length. Rostral formula was 7/6. Feeding starts in the post larval stage when the animal becomes benthotropic in habit. Broadly triangular telson was seen with process formula of 7+7 observed in first zoeal stage. Whereas in post larval stage a rectangular telson with shallow and median notch on concave posterior margin was observed with process formula 5+5. Out of 5+5 process, the first 3 process are lateral and spine-like whereas the outer one was largest and spine-like and non-plumose.

**Influence of some ecological factors on the survival of juvenile and adult prawns**

For adult and juvenile the upper lethal thermal limit was found as 36°C and the lower lethal thermal limit was 4°C. The recovery extent for adults after 48 hrs shows the better recovery from hyperthermia stress to hypothermic conditions. The prawn tolerated a variable pH ranges for adult and juvenile from 6.50 to 9.00 and 6.75 to 8.75, respectively. In comparison to the adult, the juveniles were having poor tolerance of pH levels towards alkaline and acidic sides. The recovery extent for adults after 48 hrs indicates reductions with the increase in degree of acidity and alkalinity. Due to respiratory stress on DO concentrations of 3.5 ppm and below the adults and juvenile showed fast and jerky movements. There was almost no mortality in prawns tested by lowering the DO up to 4.5 ppm in juvenile and
adult. Only 10% mortality was shown after 24 hrs at DO concentration of 4.5 ppm. Juvenile were more resistant than adults. At 4.0 ppm juvenile shows no mortality but adult shows 20% mortality after 24 hrs. At 2.5 ppm, adult shows a mortality of 100% after 8 hrs whereas juvenile shows a mortality of 60% after 8 hrs and 100% after 16 hrs and 24 hrs. Nil mortality was shown at 4.5 ppm and above DO concentration. The recovery extent for adult after 48 hrs shows variations in the mortality rate with different DO levels.

**Experimental studies**

The repeated injections of brain and thoracic ganglion extract in the prawn *M. lamarrei lamarrei* shows stimulation by increased GSI, acinar diameter and oocyte diameter. A gonadal development and maturation was observed histologically. Whereas the repeated injections of eyestalk extract shows a fall in the GSI values as well as in acinar diameter and oocyte diameter values. But the values observed from control prawns are not significant. And resulted the inhibition of ovarian and testicular development. Histologically almost similar pictures were observed as in control prawns. From the experiments it can be concluded that the brain and thoracic ganglion produced the gonad stimulating hormone whereas the eyestalks contains the gonad inhibiting hormone.

In the eyestalk ablation (bilateral) experiment in both male and female prawns shows a precocious gonadal development with increase in GSI as well as acinar diameter and oocyte diameter. Histologically after 40 days experiment in the testes, induced maturation was observed up to secondary spermatocytes, few spermatids and sperms whereas in ovaries, almost ripe ova are observed which converted from early primary vitellogenic oocyte and previtellogenic oocyte.
The influence of synthetic hormone i.e. ovaprim and ovatide were also studied in the prawn *M. lamarrei lamarrei*. Ovaprim is manufactured on the principle of M/s Syndel Laboratories Limited, Canada. It is ready to use product in a liquid form for both male and female prawns. Ovaprim comprises Salmon gonadotropin releasing hormone (sGnPHa) (20 ug) and a Dopamine antagonist domperidone (10 mg/ml) and it proves as convenient and effective ovulating agent. Similarly ovatide is cost effective new hormone used for induced breeding. Ovatide contains gonadotropin releasing hormone (GnRH). The observation of present experiment shows increase in GSI value with increase in acinar diameter and oocyte diameter in both the male and female prawns. Histologically, proper development in testes and ovaries of prawn was shown.


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