Experiments on induced breeding of *Liza macrolepis* were conducted by heteroplastic pituitary injection under threshold doses of 3 - 5 mg and 6 - 8 mg/kg body weight of fish for the first and second injections, respectively. Generally, males do not release milt in time and hence stripping is resorted to for better rate of fertilisation. Oozing males did not require hormone treatment. Hatchlings were reared to fry stage over a period of 20 days yielding a survival rate of 36.4%. Salinity of 30% appears to be favourable for incubation and larval rearing. Feeding larvae with cultured *Chlorella* sp. (green water), *Chaetoceros affinis* and *Brachionus plicatilis* from the third day of hatching enhanced the survival rate and growth.

**INTRODUCTION**

Since knowledge of propagation techniques for grey mullets (Lee *et al.*, 1987) and its larval rearing (Kraul, 1983) remains incomplete, Lee and Tamaru (1988) emphasised the importance of continuing the effort to develop a standardised procedure for artificial propagation of mullet. Since the first successful induction of spawning in mullet (Tang, 1964) using injection of mullet pituitary homogenate, Liao *et al.* (1971) achieved success in ovulating *Mugil cephalus* using pituitary glands combined with Synahorin and vitamin E. Subsequently, many studies were conducted to improve spawning techniques using wild caught animals (Liao, 1975).

In India, successful ovulation in *M. cephalus* was achieved by Chaudhuri *et al.* (1977) by using homoplastic pituitary extract. Successful ovulation in *Liza macrolepis* has been reported by Alikunhi *et al.* (1971), Sebastian and Nair (1975), Radhakrishnan *et al.* (1976) and Kowtal and Gupta (1986) by using homoplastic pituitary glands, while James *et al.* (1983) induced ovulation using carp pituitary and chorionic gonadotropin. Though success has been achieved on an experimental scale in India, it has not so far been possible to rear the induced-bred hatchlings in mass scale (Nammalwar and Mohanraj, 1990). The results of experiments conducted for induced breeding of *L. macrolepis* using crude human chorionic gonadotropin (HCG), chorionic gonadotropin and carp pituitary extract, and larval rearing are reported in this paper.
MATERIAL AND METHODS

The experiments were conducted during July 1989 - February 1990 at Ennore Field Centre of Central Institute of Brackishwater Aquaculture. In the wild population of _L. macrolepis_ in the Ennore Backwaters, two peaks in the occurrence of brooders (migration of spawning stock) during February to April and September to November were noticed. Matured females and males were obtained from the commercial catches of _siruvalai_ (bag net) operated during receding spring tides. Oozing males and females with bulged belly and round vent were selected, placed in plastic basins with sufficient water and transported to the hatchery with frequent change of water. The fish were treated with potassium permanganate or acriflavin at 1 ppm for 3 min under constant aeration. The ovarian eggs were collected using a cannula for measuring oocyte diameter. Females with oocyte diameter exceeding 0.56 mm and oozing males were further selected for hypophysation, and the length and weight of the fish were recorded.

The females were injected twice with the respective hormone (Table 1) at an interval of 6 h. The crude HCG in powder form was dissolved in distilled water and injected. In respect of pituitary, the technique of hypophysation was similar to the one adopted by Chaudhuri et al. (1977). After treatment, the females were released in 500-l plastic pools, 3-4 fish for each pool and the water was aerated. The untreated males were maintained separately in similar pools. Water with different salinities ranging from 30-35% was used to find out the suitable salinity for ovulation.

Sea water used for egg incubation and larval rearing was stored in black fibreglass reinforced plastic (FRP) tanks under dark condition and treated with calcium hypochlorite at 10-25% depending on the silt load and thoroughly aerated for 3 days to expel the excess chlorine. The water was filtered using 50-μm mesh fabric filter bags. Incubation of fertilised eggs was done in 500-l plastic pools. Larval rearing was carried out in 500-l round FRP tanks for the first 20 days and in 1.75-t rectangular FRP tanks thereafter. Aeration was controlled from the third day after hatching onwards to avoid the possible damage to the larvae. During incubation and early larval rearing period, slow but continuous running water was provided at 0.5 l/min. From the tenth day, 20-50% water exchange was done daily. The faecal matter accumulated at the bottom was siphoned out every day. The water was treated with chloromycetin at 1% thrice a week. Salinity, pH and temperature were recorded once daily at 10.00 a.m.

RESULTS AND DISCUSSION

Fifty-six experiments on induced breeding of _L. macrolepis_ were conducted during 8 months (13 in July-November 1989 and 43 in January-February 1990) with a view to standardise the hormone dosage and the optimum salinity for ovulation. The details of experiments are presented in Table 1.
<table>
<thead>
<tr>
<th>Length (mm)</th>
<th>Weight (g)</th>
<th>Month and year</th>
<th>No. of fish</th>
<th>Oocyte diameter range (mm)</th>
<th>Hormone</th>
<th>Dosage/kg fish</th>
<th>Salinity range (%)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>110-215</td>
<td>50-140</td>
<td>Jul 1989 Aug 1989</td>
<td>2</td>
<td>0.560-0.577</td>
<td>Crude HCG (activity: 30 IU/mg)</td>
<td>5 mg 10 mg</td>
<td>31-32</td>
<td>One plugged after 2nd injection; one died after 1st injection; one did not respond</td>
</tr>
<tr>
<td>130-230</td>
<td>60-145</td>
<td>Sep 1989 Oct Nov 1989</td>
<td>3</td>
<td>0.560-0.577</td>
<td>Chorionic gonadotropin</td>
<td>1500 IU 3000 IU</td>
<td>30-32</td>
<td>Four did not respond; one died after 1st injection; two died after 2nd injection; one showed good response stripped, 20% fertilisation, development continued only up to 5th cleavage</td>
</tr>
<tr>
<td>136-261</td>
<td>60-225</td>
<td>Jan Feb 1990</td>
<td>10</td>
<td>0.560-0.577</td>
<td>Carp pituitary extract</td>
<td>3-5 mg 6-8 mg</td>
<td>30-35</td>
<td>Did not respond</td>
</tr>
<tr>
<td>140-272</td>
<td>60-220</td>
<td>Jan Feb 1990</td>
<td>3</td>
<td>0.560-0.586</td>
<td>Carp pituitary extract</td>
<td>3-5 mg 6-8 mg</td>
<td>31-34</td>
<td>All spawned after 2nd injection, poor rate of fertilisation, development continued only up to 5th cleavage or gastrula stage</td>
</tr>
<tr>
<td>140-278</td>
<td>60-220</td>
<td>Feb 1990</td>
<td>3</td>
<td>0.560-0.586</td>
<td>Carp pituitary extract</td>
<td>3-5 mg 6-8 mg</td>
<td>30-34</td>
<td>Responded well, stripped 6 h after 2nd injection, 50-70% fertilisation, development continued only up to 5th cleavage or gastrula stage</td>
</tr>
<tr>
<td>225</td>
<td>110</td>
<td>Feb 1990</td>
<td>1</td>
<td>0.577</td>
<td>Carp pituitary extract</td>
<td>3 mg 6 mg</td>
<td>30</td>
<td>Stripped after 6 h of 2nd injection, 75% fertilisation, hatching completed after 20 h, 50% hatching rate, larvae reared up to fingerlings</td>
</tr>
</tbody>
</table>
Only one female (225 mm/110 g) having an average oocyte diameter of 0.577 mm which was administered with the pituitary extract at 3 mg/kg body weight at 12.30 p.m. showed good response. By 6.30 p.m. the belly became softer and enlarged and the second dose of 6 mg/kg body weight was administered. Water salinity was maintained at 30% and temperature and pH were 27.5-29.0°C and 8.2, respectively. By midnight, after 6 h of the second injection, the ovarian eggs became loose, turned light brown and were flowing freely on application of gentle pressure on the sides of the abdomen. Dry stripping was resorted to at 12.15 a.m. Milt from two oozing males (140, 150 mm/45, 55 g) was used for fertilising the eggs. The eggs and milt were well mixed for 3-4 min using a clean quill.

Filtered water of 30% was gently added and the fertilised eggs were transferred to incubation tanks. About 80,000 eggs had been obtained of which about 75% were fertilised. They were transparent and floating, and sometimes formed clusters on the surface. After 10-12 h of fertilisation, when the embryonic streak was formed, the developing eggs exhibited a tendency to float in the water column. The average diameter of the eggs was 0.735 mm and that of the oil globule was 0.294 mm.

Divergent views have been expressed by various workers on the threshold dosage of hormone for inducing breeding of mullet. Pioneering experiments done on induced breeding of *M. cephalus* using homoplasmic pituitary extract by Chaudhuri et al. (1977) showed that the effective dosages varied from 8-16 mg/kg weight of fish and 2-8 pituitary glands per fish. Tang (1964) has reported that 2 mullet pituitary glands and 40 rabbit units (RU) of Synahorin might be the threshold dosage for initiating ovulation in *M. cephalus*. Ling (1970) has noticed the effective dose for fully ripe *M. cephalus* females for ovulation as 2-3 homoplasmic pituitary glands with 20 RU Synahorin per fish. Liao et al. (1971) have noticed successful ovulation when 2.5-6.0 pituitary glands combined with 10-16 RU Synahorin and 0 to 300 mg vitamin E per fish were administered. Mohanty (1971) has used 6-10 homoplasmic pituitary glands per fish in induced breeding of *M. cephalus*.

In respect of *L. macrolepis*, Alikunhi et al. (1971) suggested a dosage of 2.5-3.0 and 4 homoplasmic glands per fish for the first and second injections, respectively, for ovulation. Sebastian and Nair (1975) reported that this fish could be induced to spawn by administration of homoplasmic pituitary alone and the threshold dosage was found to be 3 and 4 glands per fish for the first and second injections, respectively, at an interval of 6 h. Radhakrishnan et al. (1976) have suggested a dosage of 15-20 mg carp pituitary per kilogramme body weight. James et al. (1983) have reported that the effective dose varied between 600 and 1200 mg, and 110,000 and 340,000 IU/kg body weight of the fish in the case of major carp pituitary gland and chorionic gonadotropin, respectively. In combination, the effective dosage was 1200 mg and 12,000 to 15,000 IU of pituitary gland and chorionic gonadotropin, respectively. In the present series of experiments where the major carp pituitary glands were used, the threshold dose for initiating ovulation was found to be 3-5 mg and 6-8 mg/kg for the first and second injections, respectively, at an interval of 6 h depending on the condition of ripeness of the females. In about 25% of females, ovulation was noticed when the above dosages were administered. The wide range of dosages reported by various workers indicate that it is difficult to arrive at a precise dose
uniformly applicable for mullet, especially when the brooders are collected from the wild where the external visual assessment of the correct ripeness of females is difficult. The effective dose is bound to vary from fish to fish as their maturity condition will be varying at least slightly. Again, the potency of the pituitary gland used also varies depending upon the maturity condition of the donor fish, mode of preservation and also on the freshness of the gland. Prevailing environmental parameters like salinity and temperature also have an effective role to play in the process of ovulation (Walsh et al., 1991).

About 30% of the fertilised eggs hatched out 18 h after fertilisation and hatching was completed 2 h later with an overall 50% hatching rate. The newly hatched larvae measured an average total length of 1.656 mm. During the incubation period, water temperature ranged between 27.5 and 29.0°C. The hatchlings were transferred to larval rearing tanks and reared at a density of 38/l. The 6-h old larvae registered an average total length of 2.16 mm. During the first 30 days of larval rearing, salinity was maintained at 29.8-31.0‰, water temperature 27.2-30.40°C and pH 7.8-8.3.

Natarajan and Patnaik (1972) have collected oozing eggs of L. macrolepis and the diameter ranged from 0.658 to 0.732 mm. Sebastian and Nair (1975) have reported that the unfertilised eggs immediately after stripping measured 0.633 mm in diameter. The fertilised eggs, after 20 min, measured an average of 0.665 mm in diameter. James et al. (1983) have stated that fully matured eggs measure 0.70 to 0.74 mm and the fertilised eggs 0.74 to 0.78 mm in diameter. In the present series of experiments, in the specimens where ovulation was noticed, the average diameter of mature ova was 0.577 mm and that of fertilised eggs after 2 h was 0.735 mm. Similar variations in the size of mature ova in respect of M. cephalus have also been reported (Ling, 1970; Chaudhuri et al., 1977). In the present study, the average size of larvae at hatching was 1.656 mm in total length. The length of newly hatched larvae has been reported as 1.519 to 1.556 mm (Natarajan and Patnaik, 1972), less than 2.0 mm (Sebastian and Nair, 1975) and 1.43 mm (James et al., 1983).

Initially, the larvae floated on the surface at an angle to the water surface, later they sank to the bottom head down and moved vertically up with jerking movements. Mouth formation was noticed on the second day of hatching. The larvae were fed with live feed (Chlorella sp., Chaetoceros affinis, Brachionus plicatilis) from the third day of hatching. Observation of the larvae under microscope on the fifth day of hatching revealed the dominance of B. plicatilis over Chlorella sp. and C. affinis in the gut. In respect of feeding of the hatchlings, Sebastian and Nair (1975) have adopted the technique of releasing the hatchlings into 'seasoned greenwater' in small tanks which had a population of Chlorella sp., blue green algae, diatoms, vorticellids, dinoflagellates, Euglenoids and copepods. They have noticed that when the larvae reached about 1 cm in length, they fed exclusively on copepods. The pigmentation that has started developing on the dorsal side had spread and covered the whole body when the larvae were one week old.
The larvae measured an average total length of 2.565 mm on day 5, 3.475 mm on day 10 and 6.25 mm on day 20 (fry stage). The survival from hatchlings to fry stage was about 36%.

The fry were transferred to 2 rectangular FRP tanks of 1.75 t capacity. Along with B. plicatilis, powdered artificial feed of groundnut oil cake and rice bran in the ratio 1:1 was also supplied, initially in powder form and later in dough form for weaning the fry from live feeds. Artificial feed was supplied approximately at 10% of body weight of the fry in two instalments, morning and evening. Water exchange to the tune of 50% daily was carried out from day 20. Within a week, they had acclimatised to artificial feed and thereupon supply of live feed was stopped. The fry developed characteristic grey colour on the dorsal side, and silvery white on the lateral and ventral sides as they attained a size of about 9 mm in length. The change in colour is related more to size than to age. A few fry which did not attain the above size even after a month remained black and developed normal colouration only after attaining the above size. At the end of 90 days' rearing, the fry had attained fingerling stage and had an average size of 34.7 mm in total length and 1.0 g in weight.

Results of experiments conducted in India and in other countries demonstrate that though induced ovulation in mullet can be achieved by hypophysation, the crux of the problem lies in larval rearing. The few experiments on the rearing of L. macrolepis larvae done earlier have shown that mass mortality of hatchlings occurred during the first 10 days of rearing. Natarajan and Patnaik (1972) have stated that they could rear the larvae only for 6 days. Though Alikunhi et al. (1971) and Sebastian and Nair (1975) have reported that they could rear the larvae to juvenile stage, they have not mentioned the survival rate of the larvae to fry/fingerling stage. James et al. (1983) could breed this fish but the hatchlings survived only for 166 h. Fish held in captivity have been bred through acclimatisation and stripping (Kowtal and Gupta, 1986). Rajyalakshmi et al. (1991) could obtain 400,000 hatchlings from one female and they survived for 14 days. Krishnamurthy et al. (personal communication) obtained 150,000 larvae which suffered heavy mortality on day 4, 8 and 12 with only a few survivors to the fry stage. It is significant to note that mass mortality of the hatchlings or fry as has been reported by earlier workers was not noticed during the present experiment. The improved water quality management in the larval rearing system, the appropriate feed and feeding scheme used and successful weaning of larvae from live food have been largely responsible for the higher survival rates achieved in the present study.

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