# Development of Hatchery Techniques for the Silver Pomfret *Pampus argenteus* (Euphrasen)

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## Abstract

This is the first report on the successful hatchery larval rearing of the silver pomfret *Pampus argenteus* (Euphrasen) based on eggs stripped from the wild gravid fish during the 1998 and 1999 spawning periods. The result of this investigation shows that males always outnumber the females in the population. The present investigation also confirms that spawning initiates in May and reaches a peak in June and July when the water temperature is between 28 to 30ºC. Gonadal maturity stages in relation to day time have been investigated for the first time and show that gravid female occurs in the early day time period up to 15:00 h and proceeds to attain full ripe ovulated fish in the evening hours. Spawning occurs in the late evening hours. Among the eggs collected, the viable floating eggs varied from 38.6 to 44.0% and egg hatching ranged from 36.5 to 51.8% during the 1998 and 1999 spawning periods. The mean egg diameter was 1.12±0.02 mm and the newly hatched larval size was 2.40±0.10 mm. The egg hatching time was 15 h at 29 to 30ºC. Egg hatching was significantly high (P<0.01) at ambient seawater salinity of 35 to 40‰ compared to that in low water salinity. Larval growth was slow in the early stages and showed a curvilinear pattern. Larval survival did not show any significant difference (P>0.05) between stocking densities of 20,30 and 40 larvae $l^{-1}$. Up to 1.5% larval survival was observed. Larval survival declined during the June to August culture period but was not significantly different (P>0.05) from the June-July culture period. A number of biological and environmental factors have been identified to be associated with low larval survival in the hatchery. The results demonstrate the possibility of producing silver pomfret under hatchery culture conditions. However, further research is required to enhance larval survival towards realizing commercial farming of this species.

## Introduction

The silver pomfret *P. argenteus* (Euphrasen) is one of the most desired food fish worldwide. It has an extensive geographical distribution from the East China Sea to Southeast Asia, Indian Ocean, Arabian Gulf and the North Sea (Kuronuma and Abe 1972, Davis and Wheeler 1985). It is the most sought after marine food fish in Kuwait and the market price is always higher than the other prime fish species such as grouper. Silver pomfret, locally known as

'zobaidy', accounts for about 15% of the commercial fish landings in Kuwait (Morgan 1985). In spite of its worldwide market demand, the development of a commercial farming technology for silver pomfret has not been realized to date due to the difficulty of breeding this species in captivity and of rearing the larvae under hatchery culture conditions. Some attempts were made in Japan by obtaining fertilized eggs from wild broodstock and studying the larval development. However, the hatched out larvae did not survive beyond five days in the first attempt (Mito and Senta 1967) and 28 days in the second attempt (Oda and Namba 1982). Due to the difficulties experienced in breeding this fish by collecting live broodstock as well as in securing their feed, mainly jelly fish, Oda and Namba (1982) concluded that silver pomfret does not seem suitable for aquaculture. The first successful collection of fertilized eggs and rearing of silver pomfret larvae in the laboratory was reported by Almatar and Al-Abdul Elah (1999). The first complete larval developmental stages of laboratory reared silver pomfret *P. argenteus* was reported for the first time by Almatar et al. (2000).

Following the successful larval development of the silver pomfret *P. argenteus* at the Mariculture and Fisheries Department (MFD) of the Kuwait Institute for Scientific Research, much research efforts were directed towards developing a feasible technology for the aquaculture of this species in Kuwait. This is the first report on the larval rearing of silver pomfret under hatchery culture conditions. The major objective of the present study is to evaluate the availability of wild spawners for egg stripping, assessment of egg quality, and larval rearing of silver pomfret using conventional hatchery methods to determine the efficiency of the culture system.

## Materials and Methods

### Gravid fish and egg collection

Several fishing areas in Kuwait waters (29º22.40N, 48º03.76E) were surveyed in 1998 and 1999 for mature and gravid silver pomfret females and males using 14 cm mesh stretch size drifting gill nets. Fishing days were done during spring tides, at new and full moons and most of the fishing was done during day time. A total of 40 fishing trips were made between May and September 1998 and 55 in 1999. The surface water temperature and salinity were recorded in the spawning ground. Fully ripe males and females were caught in the coastal areas with water depth ranging from 5 to 20 m. Fishing trips were made from 10:00 h to 18:00 h. During these trips the gonadal maturity stages were examined to determine the maturity stages in relation to day time. Collections were also done at night to observe the availability of fully ripe females as well as to assess the time of spawning in the wild.

Eggs from fully ripe females were stripped into a plastic bowl and mixed with sperm stripped from fully ripe males. The females provided a good flow of transparent eggs with gentle abdominal pressure. The males, however, produced only a few drops of semen after gentle abdominal pressure due to rather small testes. Seawater was slowly added until it slightly covered the mass of eggs

and sperm, which was kept undisturbed for 5 minutes to facilitate fertilization. The mass of fertilized eggs was then washed gently through a scoop net (300 to 350 mm mesh) to remove body fluids and other debris and transferred to the hatchery facility at MFD in 20 l capacity plastic buckets. The transferred eggs were again washed in the hatchery and placed into one-liter graduated cylinders to measure and separate the floating viable eggs. Viable eggs were then transferred to round hatching nets of 29 cm dia. x 42 cm deep (22.5 l working volume) supplied continuously with fresh gently flowing aerated sea-water of about 29 to 30°C. The eggs were incubated in the hatching nets at a stocking density of 200 to 2,000 eggs·l$^{-1}$, depending on the availability of eggs from wild spawners.

To evaluate the effect of salinity on egg hatching, studies were carried out in 2 l volume beakers with aerated water salinity of 0, 10,20,30, 35 and 40‰. The experiment was carried out using three replicates for each treatment. Water temperature was kept constant at 29.5°C. Each beaker was stocked with 100 fertilized eggs in a liter of water and aerated to keep dissolved oxygen above 5 mg·l$^{-1}$ as well as to keep the eggs suspended in the water column. The hatched larvae were individually counted to determine the egg-hatching rate (%) in relation to water salinity regimes.

### Live food production

Live food for larval rearing consisted of the microalgae *Nannochloropsis* sp., *Isochrysis* sp., *Chlorella* sp., and *Tetraselmis* sp., produced using the methods described by James and Al-Khars (1990), Abu-Rezq et al. (1999). The S-type rotifer *Brachionus plicatilis* was produced in rotifer chemostats by using batch cultures following the methods described by James and Abu-Rezq (1989a) and Abu-Rezq et al. (1997). Marine *Chlorella* sp., and bakers' yeast were used to culture the rotifers in the production system. The harvested rotifers from the production tanks were further treated for 24 h with nutritional enrichment media to enrich the rotifers with the essential ω-3 highly unsaturated fatty acids (ω-3 HUFA). The following enrichment media were tested in two replicates for each treatment: 1) marine *Chlorella* sp.; 2) *Nannochloropsis* sp.; 3) *Tetraselmis* sp.; 4) *Isochrysis* sp.; 5) mixture of algae consisting 25% each of *Chlorella, Tetraselmis, Nannochloropsis* and *Isochrysis*; 6) mixture of algae plus commercial products Super Selco and DHA Protein Selco; and 7) unenriched rotifers. For fatty acid analysis, the rotifers from different treatments were lyophilized at −73°C and the lipids extracted from lyophilized samples, using a 2:1 chloroform:methanol mixture and ultrasonicated for 1 h, were esterified and analyzed by gas chromatography (Perkin-Elmer sigma 3B) using high-resolution FFAP fused silica capillary columns (OV 225, length 25 m; internal diameter 0.25 mm). Flow rate was maintained at 2 ml·min$^{-1}$ with nitrogen as the carrier gas. Temperature was programmed from 140 to 220°C and maintained at 2°C min$^{-1}$. A flame ionization detector was used, the area percent was calculated and quantified using the standards and a Schimadzu C-R 1A Chromatopac recorder and integrator. Based on the results obtained (Table 1), the rotifers treated with a mixture of algae plus Super Selco and

DHA Protein Selco were used to feed the silver pomfret fish larvae in the hatchery. These enriched rotifers were washed in 20‰ water for 20 min to remove any possible bacterial load in the culture media prior to being fed to the silver pomfret larvae.

## Larval rearing

The newly hatched larvae (1 to 2 h old) were stocked in 1 m$^3$ capacity round fiberglass indoor hatchery tanks (130 cm dia. x 89 cm deep). The tanks were painted black and the bottom had a 1$^o$ slope towards a 6.5 cm diameter central drainage standpipe with provision for water drainage either from the top or from the bottom. The tank bottom had a smooth surface and was not covered with any substrate. Fluorescent lights were used 24 h·day$^{-1}$ to provide a light intensity of 400 to 480 lux above the tank water surface. A single air stone was placed in each rearing tank to supply about 100 to 200 ml of air·min$^{-1}$. The ambient seawater used in the hatchery first passed through a skimmer to remove floating debris and sediments, then the seawater passed through a storage tank of 2 m$^3$ capacity. Two 2HP self-priming pumps were used to pump the seawater further through two rapid sand filters (Triton-II, Model 140, AREA Inc., U.S.A.). Each sand filter had a filtration area of 0.65 m$^2$ and a filtration flow rate of 401 l·min$^{-1}$. The sand filtered seawater first passed through two 5 mm cartridge filters and then through three 1 mm cartridge filters (model PL 2805-2-316-G7, AREA Inc., U.S.A.) to filter off most of the debris. Two units of UV-sterilizers (model WG-6-L-B, AREA Inc., U.S.A.) were used to sterilize the seawater before it was stored in a header tank of 2 m$^3$ capacity. Water salinity was 40‰ and water temperature was 28.0 to 29.5$^o$C during the observation period.

**Table 1. Fatty acid composition relevant to ϖ3 HUFA (%) content in rotifers treated with different species of algae and nutritional enrichment media and without any treatment from the rotifer production tank.**

| Rotifer Treatment | % Fatty acids (ϖ3 HUFA)* | | | |
|---|---|---|---|---|
| | 18:3ϖ3 | 20:5ϖ3 | 22:6ϖ3 | Σϖ3 HUFA |
| Rotifer from production tank (Control | 1.03±0.01[e] | 8.63±1.15[e] | 0.37±0.11[e] | 10.61±1.03[f] |
| Chlorella | 13.89±0.41[b] | 6.29±0.42[f] | 1.41±0.16[c] | 24.67±1.08[e] |
| Mixture of algae | 11.03±0.01[c] | 16.93±0.42[c] | 0.93±0.05[d] | 30.94±0.47[bc] |
| Mixture of algae + SS & DHA P.S.** | 6.49±0.04[d] | 19.35±0.33[b] | 9.12±0.29[a] | 36.32±0.68[a] |
| Nannochloropsis | 0.67±0.12[e] | 29.18±1.67[a] | 0.18±0.08[e] | 30.25±1.47[c] |
| Tetraselmis | 18.16±0.30[a] | 12.56±0.38[d] | 0.84±0.03[d] | 32.56±0.09[b] |
| Isochrysis | 6.60±1.77[d] | 18.58±1.28[bc] | 2.03±0.28[b] | 27.69±0.23[d] |

In each column,values with different superscript are significantly different, ANOVA(P<0.05)
*Values are means of two replicates
** SS = Super Selco
DHA P.S.= DHA Protein Selco

Larval rearing was carried out in the same tank throughout the larval rearing period (45 days) in all treatments. Larval survival in the hatchery was evaluated in relation to different stocking densities of 20,30 and 40 larvae·l$^{-1}$, using randomized block design to achieve a minimum replication of five per treatment. Studies were also carried out to assess larval survival in relation to spawning period from June to August during the 1998 and 1999 culture period. During this period, larval rearing initiated in the respective months were considered for evaluating the survival of larvae in relation to spawning time. To assess larval survival from June to August, data obtained from the 19 production trials made in 1998 and 28 production trials made in the 1999 culture period were used. During this period the larval stocking density was 20 to 30 larvae·l$^{-1}$. All tanks received the same treatment for feeding and tank management during the spawning season (June to August).

Table 2 shows the feed regime and tank management used during the larval rearing period. The microalga *Chlorella* sp. was introduced in the larval rearing tanks after 24 h of stocking the larvae (day 1). Algal cell density was maintained at 250 to 300 x 10$^3$ cells·ml$^{-1}$ from day 1 to day 11. The *Chlorella* sp. cell density was increased to 500 to 800 x 10$^3$ cells·ml$^{-1}$ from day 12 to day 28. The S-type rotifers were introduced on day 2 when the larvae had partly absorbed their yolk. The rotifer density in the larval rearing tanks was maintained at 5 individuals·ml$^{-1}$ till day 7 and then maintained at 10 rotifers·ml$^{-1}$ till day 28. Maintenance of *Chlorella* sp. as well as S-type rotifers in the larval rearing tanks was terminated on day 28. From day 12 onwards newly hatched *Artemia* nauplii were introduced in the larval rearing tanks. The number of *Artemia* nauplii was increased from 0.1 individuals·ml$^{-1}$ on day 11

Table 2. Feed regime and tank management used during the larval rearing of *P. argenteus*.

| Days after hatching | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 |
|---|---|---|---|---|---|---|---|---|
| **1. Feed regime** | | | | | | | | |
| Marine *Chlorella* sp. | [ 250-300x10$^3$cells ] | | | | | | | |
| | | | [ 500-800 x 10$^3$cells ml$^{-1}$ ] | | | | | |
| S-type rotifers | [5 ind. ml$^{-1}$ ] | | | | | | | |
| | | [ | 10 ind. ml$^{-1}$ | | | ] | | |
| *Artemia* nauplii | | [0.1 ml$^{-1}$] | | | | | | |
| | | | [ 0.2 ml$^{-1}$ ] | | | | | |
| | | | | [ 0.4 ml$^{-1}$ ] | | | | |
| | | | | | [ 0.6 ml$^{-1}$ ] | | | |
| | | | | | | [ 0.4 ml$^{-1}$ ] | | |
| Inert feed (fed ad libitum) | | | | | | | | |
| Shrimp paste | | | | [ 5 times day$^{-1}$ ] | | | | |
| | | | | | [ 3 times day$^{-1}$ ] | | | |
| **2. Tank Management** | | | | | | | | |
| Water exchange | | | | | | | | |
| Flowthrough day$^{-1}$ | [stagnant] | | | | | | | |
| | | [ 10 % ] | | | | | | |
| | | [ 20 % ] | | | | | | |
| | | | [ 50 % ] | | | | | |
| | | | | [ 100 % ] | | | | |
| | | | | | [ 150 % ] | | | |
| | | | | | [ 200 % ] | | | |
| Siphoning of bottom | | [ | | | | | | ] |

to 0.6 individuals·ml$^{-1}$ on day 25. From day 30 onwards the *Artemia* nauplii supplied was reduced to 0.4 individuals·ml$^{-1}$, since the larvae actively started feeding on inert feed. Weaning of larvae to inert feed was initiated on day 18 by suspending 10 x 10 cm plastic meshes (3 mm mesh size) spread with shrimp paste in one or two locations in the tank. The shrimp paste consisted of one part of minced shrimp mixed with one part of Lansy A-2 (INV Aquaculture NV, Belgium). From day 18 to day 25 the paste feed was fed five times a day and from day 25 onwards, feeding frequency was reduced to three times a day. During this period the number of feeding stations (plastic mesh) was increased to 4 tanks$^{-1}$. The silver pomfret larvae were fed ad libitum when they were fully weaned to inert feed by day 30. Dispensing excess feed was avoided.

Water quality in the larval rearing tanks was monitored regularly to maintain the larval tanks in good condition and to determine the water exchange required during the larval rearing period. Point aeration was provided to maintain the dissolved oxygen level above 5 mg·l$^{-1}$. During the larval rearing period from day 0 to day 5, water in the rearing tanks was kept stagnant (Table 2). From day 6 onwards water was dripped through the tanks to achieve an exchange of 10%·day$^{-1}$. The water exchange rate was increased to 20% on day 10, 50% on day 15, 100% on day 21, 150% on day 26 and 200% from day 30 onwards till the end of the larval rearing period. Siphoning of the tank bottom to clean the sediments was initiated on day 10 and continued till the end of the larval rearing period.

Aliquot samples of 20 to 30 larvae from the larval rearing tanks were made daily for morphometric studies as well as to monitor the growth of larvae in the culture system. Larval rearing was terminated on day 45 and the juveniles were transferred to nursery tanks.

### Statistical analyses

Statistical analyses were performed by one-way ANOVA using CSS:Statistica (version 5.0, Statsoft Inc.). Differences were compared using the least significant difference (LSD) test and were considered significant at $P<0.05$. Values are given as means ± standard deviation.

# Results

### Availability of gravid fish

During the May to September 1998 spawning period, a total of 2,743 fish were caught. Of these, males constituted 68.6% and females 31.4%. The catches of both males and females were highest in June (703 and 262) and July (558 and 181) and least in September (107 and 23). Fully mature running males outnumbered the females with an average ratio of 5:1. Mature gravid females were at the maximum (Fig. 1) in June (38%) followed by July (30%) and August (23%). Mature gravid females were minimum in May (8%) and September (1%) in the catch. Mean size of gravid females spawning in June

(FL 26.6±3.0 cm, wt 630.3±221.4 g) was significantly higher (P<0.01) than the fish caught in July (FL 25.1±2.7 cm, wt 539.1±203.6 g) or in August (FL 24.8±2.7 cm, wt 470.2±169.2 g). The mean size of males was always smaller than the females and it averaged FL 21.6±1.6 cm compared to that of female FL 26.6±3.0 cm during the peak spawning period in June. The surface water temperature averaged 26.7°C in May, 29.5°C in June, 29.7°C in July and 33.2°C in August. Water salinity was 35 to 38‰.

During the May to September 1999 spawning period, a total of 1,786 fish were caught. Of these, males constituted 64.6% and females 35.4%. Unlike in the 1998 spawning period, the catch of both males and females were highest in July (392 and 301) and August (381 and 154) and was at the minimum in May indicating the delayed initiation of the spawning period. Mature gravid females were at the maximum (Fig. 1) in July (55%) followed by August (26%). Mature gravid females were at a minimum in May (0.05%) and September (2%). Similar to the 1998 spawning period, the mean size of mature gravid females was high in June (FL 26.9±2.5 cm, wt 677.4±180.6 g) compared to that of the fish in July (FL 26.2±2.6 cm, wt 594.2±174.4 g), although it was not statistically significant (P>0.05). The mean size of male was FL 23.0±1.7 cm compared to that of female FL 26.9±2.5 cm in June. In May 1999, water temperature averaged 25.9±1.32°C and increased to 29.0±0.72°C in June and 29.9±1.19°C in July. The water temperature increased to above 32°C in August and decreased to 30.9±1.5°C in September. Water salinity ranged from 36.9 to 41.5‰.

### Maturity stages in relation to day time

Mature gravid females with very much enlarged yellowish to red ovary, defined as 'stage 5 females', normally occurred between 12:00 and 15:00 h. At this stage the eggs were not released with slight pressure on the abdomen. Whereas fully ripe gravid females with hydrated and oozing ovulated eggs, released with slight pressure on the abdomen, defined as 'stage 6 females' were obtained between 15:01-18:00 h (Figs. 2 and 3) suggesting that most spawning activities increase towards the latter part of the day during ebb tides. However, only spent females were observed during night hours. Maturity stages in relation to day time in 1998 showed the occurrence of 87 to 100% 'stage 6' females in the evening hours of May-June period (Fig. 2). This was significantly
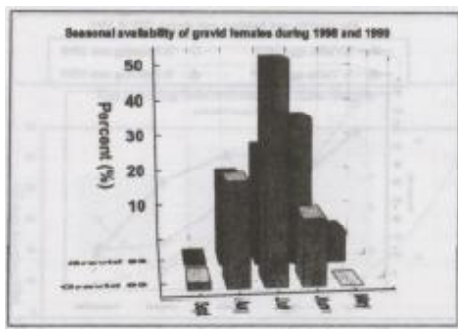


Fig. 1. Seasonal availability of gravid *P. argenteus* females during 1998 and 1999.
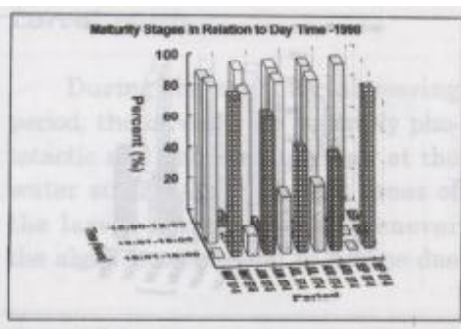
Fig. 2. Gonadal maturity stages of *P. argenteus* in relation to day time during 1998

higher (P<0.05) than the occurrence of 'stage 6' females in the population in July (64%) and August (59%). After a decline in July and August, 100% 'stage 6' females were observed in September 1998.

During the 1999 spawning period, a maximum number of 'stage 6' females occurred in July (75%) coinciding with the peak spawning period (Fig. 3). The occurrence of 'stage 6' females was less in August (70%) and September (57%). Fishing trips made in June and July showed the occurrence of 100% 'stage 5' females in the morning hours.

## Egg quality and hatching

The fertilized eggs were spherical, transparent and pelagic, similar to the description of Mito and Senta (1967). Egg diameter ranged from 1.08 to 1.19 mm with a mean of 1.12±0.02 mm. The floating viable eggs ranged from 11.0 to 44.0% with a mean of 24.18±13.37% in 1998 and ranged from 0.0 to 38.6% with a mean of 12.73±15.27% in 1999. The egg hatching rate ranged from 5.0 to 51.8% with a mean of 23.6±17.53% in 1998 and ranged from 0.0 to 36.5% with a mean of 12.7±15.27% in 1999. The availability of viable eggs declined in relation to spawning season. Maximum viable eggs were obtained at the beginning of the spawning season in May 1998 (44.0%) and the minimum (11.0%) in September (Fig. 4). Egg hatching rate was also maximum (51.8%) in May 1998 and declined steadily till August (5.0%) and again increased at the end of the spawning season in September (19.1%). No viable eggs were obtained in May 1999. A maximum of 38.6% viable eggs was obtained in June 1999 and onwards; egg viability declined to 37.5% in July to a minimum of 10.2% in September 1999. The egg hatching rates also showed a similar trend of decline from June 1999 (36.5%) to a minimum of 1.3% in September 1999 (Fig. 4).

Egg development until hatching lasted for 15 h at 29 to 30ºC. At this temperature, egg hatching initiated at 15 h after fertilization and all the eggs hatched within 30 to 40 min. The mean size of newly hatched larvae was 2.40±0.10 mm in total length. The larvae had a large ellipsoid yolk, length ranging from 0.64 to 0.73 with a mean of 0.69±0.04 mm and width ranging from 0.28 to 0.35 mm with a mean of 0.30±0.02 mm, with a posteroventral oil globule.
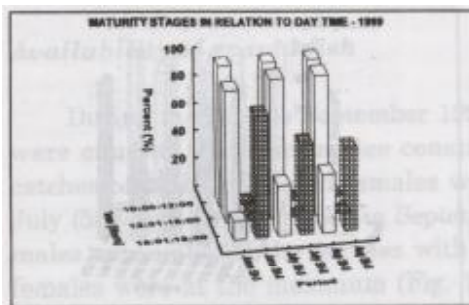


Fig. 3. Gonadal maturity stages of *P. argenteus* in relation to day time during 1999
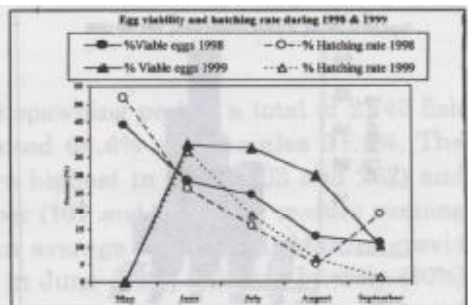
Fig. 4. Egg viability and hatching rate of *P. argenteus* during 1998 and 1999

Studies carried out on the hatching of eggs in relation to different salinity regimes showed a curvilinear relationship between different salinities tested and egg hatching rates (Fig. 5). The egg hatching rates were significantly low (P<0.01) in salinity less than 35‰ and the eggs did not hatch at 0‰. No significant difference (P>0.05) in egg hatching was observed between 35 and 40‰ salinity. At 35‰ salinity, egg hatching ranged from 15 to 27% with a mean of 21.33±6.03%, and in 40‰, egg hatching ranged from 14 to 33% with a mean of 20.33±10.97%. During this investigation, water pH varied from 8.14 to 8.63 in different treatments and the dissolved oxygen ranged from 6.1 to 6.9 mg·l$^{-1}$. Water temperature was 29.5ºC.

### Live food

The algal culture cell densities varied for different species of algae produced in the indoor culture system. During this investigation, *Chlorella* sp. cell density varied from 52 to 70 x 10$^6$ cells·ml$^{-1}$, *Nannochloropsis* sp., 40 to 50 x 10$^6$ cells·ml$^{-1}$, *Isochrysis* sp., 5 to 8 x 10$^6$ cells·ml$^{-1}$ and *Tetraselmis* sp., 4 to 7 x 10$^6$ cells·ml$^{-1}$. The algal culture temperature varied from 21 to 23ºC in the culture system.

The fatty acid composition of rotifers in different nutritional enrichment treatments shows (Table 1) that significantly high (P<0.05) ω3 HUFA was present in rotifers treated with mixed algae plus Super Selco and DHA Protein Selco compared to that of other treatments. A maximum of 36.32±0.68% total ω3 HUFA was present in the above treated rotifers that was significantly higher (P<0.05) than in other treatments. The most essential long chain docosahexaenoic acid (22:6ω3) was also significantly high (P<0.05) in the above treated rotifers (9.12±0.29%) compared to other treatments. Significantly high (P<0.05) quantities of eicosapentaenoic acid (20:5ω3) was present in rotifers treated with *Nannochloropsis* sp. (29.18±1.67%) compared to other treatments. However, the most essential docosahexaenoic acid (22:6ω3) was significantly high (P<0.05) in rotifers treated with *Isochrysis* sp., (2.03±0.28%) compared to that of other algal species, except mixed algae with Super Selco and DHA Protein Selco. Results showed that the ω3 HUFA content in untreated rotifers from the production tank was significantly lower (P<0.05) than in other treatments.

### Larval rearing

During the early larval rearing period, the larvae were positively phototactic and gathered together at the water surface in the bright areas of the larval rearing tanks whenever the algal densities tend to decline due
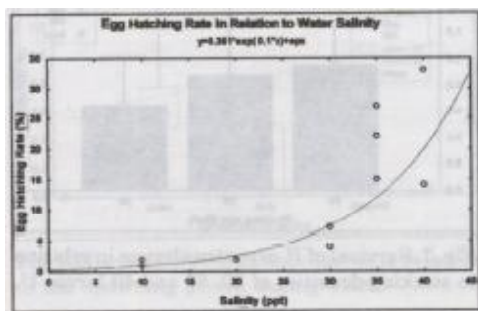


Fig. 5. Egg hatching rate of *P. argenteus* in relation to different water salinity regimes

to active feeding of rotifers on *Chlorella*. Maintenance of algal cell densities of 250 to 300 x $10^3$ cells·ml$^{-1}$ in the initial stages (Table 2) was effective to reduce the phototactic behaviour and to disperse the larvae in the culture system. However, with increasing water exchange rates after day 11, the *Chlorella* cell density had to be increased from 500 to 800 x $10^3$ cells·ml$^{-1}$ to avoid phototactic behaviour of larvae in the culture system. Yolk absorption was rapid and some yolk still remained on day 2. The larvae initiated ingesting rotifers from day 2 onwards. Active feeding of larvae on rotifers increased from day 4 onwards. Swimbladder inflation initiated from day 3 onwards and almost 100% of the larvae had their swimbladder developed by day 8. From day 12 onwards the larvae started feeding on newly hatched *Artemia* nauplii. By day 18, the larvae started feeding on inert paste feed.

Larval morphology in relation to its growth was as described by Almatar et al. (2000). Larval growth was slow in the early stages of development and was curvilinear during the larval rearing period (Fig. 6). During the present investigation the size of day 1 larvae ranged from 2.95 to 3.22 mm with a mean of 3.12±0.11 mm. The larval size variation increased with the growing period. On day 4 the larval size ranged from 3.24 to 3.95 mm with a mean of 3.42±0.17 mm. At the time of initiating feeding on *Artemia* nauplii on day 12, larval size ranged from 4.70 to 5.00 mm with a mean of 4.85±0.11 mm. Larval size ranged from 6.80 to 7.45 mm with a mean of 7.11±0.27 mm on day 18 when the larvae started weaning to inert paste feed. Larval growth steadily increased to 9.10 to 12.20 mm with a mean of 10.50±1.17 mm on day 25 and 13.80 to 17.60 mm with a mean of 15.70±1.63 mm on day 30. On day 35 most of the larvae were transformed to juveniles and the size ranged from 22.90 to 28.10 mm with a mean of 25.82±2.36 mm. By day 40, larval transformation to juvenile was complete and the size ranged from 32.93 to 38.90 mm with a mean of 35.78±2.83 mm. From then onwards, larval size variation increased and on day 45 the size ranged from 36.00 to 59.00 mm with a mean of 45.18±6.53 mm.

The investigations carried out on the survival of larvae at different stocking densities of 20,30 and 40 larvae·l$^{-1}$ showed no significant difference (P>0.05) in larval survival between different treatments (Fig. 7). However, a declining trend in larval survival was observed with increasing stocking densities. Larval survival ranged from 0.44 to 1.24% with a mean of 0.95±0.32% at a stocking density of 20 larvae·l$^{-1}$. Although larval survival was up to 1.5% at 30 larvae·l$^{-1}$, mean survival was 0.87±0.33% which was less than that at a
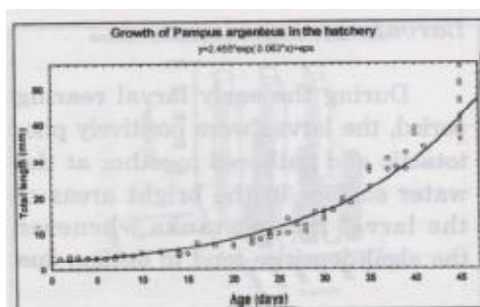


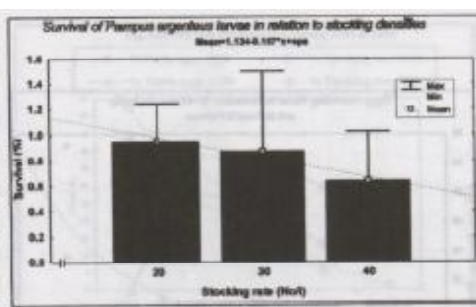Fig. 6. Growth of *P. argenteus* larvae in the hatchery



Fig. 7. Survival of *P. argenteus* larvae in relation to stocking densities of 20, 30 and 40 larvae l$^{-1}$.

stocking density of 20 larvae·l⁻¹. Larval survival at 40 larvae·l⁻¹ ranged from 0.31 to 1.02% with a mean of 0.64±0.26%.

Larval survival during the June to August 1998 spawning period (Fig. 8) showed wide variation and had a declining trend in larval survival from June to August. Larval survival was up to 1.1% in June with a mean of 0.62±0.51%. Larval survival in July averaged 0.25%. No significant difference (P>0.05) in larval survival was observed between the June and July culture period. The larvae crashed during the August larval rearing period resulting in 0 survival till day 40. Wide fluctuation in larval survival was also observed during the June to August 1999 culture period (Fig. 9). Although mean survival tend to decline during the June to August 1999 culture period, no significant difference (P>0.05) in larval survival was observed. Larval survival varied from 0.00 to 1.21% with a mean of 0.71±0.67% in June. In July, larval survival varied from 0.00 to 1.24% with a mean of 0.86±0.29%. In August, survival varied from 0.00 to 0.88% with a mean of 0.18±0.39%

## Discussion

Based on the description of morphometric and meristic characters of the silver pomfret *P. argenteus* larvae reared at MFD (Almatar et al., 2000), this is the first report on the availability of eggs and hatchery larval rearing of this species. The results obtained during the present investigation on the availability of mature fish show that males always outnumber the females in the population. This is contrary to the observations of Oda and Namba (1982) who observed more females in the catch in Okayama Prefecture in Japan. Dadzie et al. (1998) observed that there were less males in the samples collected from the fish market landings in Kuwait, especially in June and July, and opined that the males could be less vulnerable to the fishing gear used during this period or they might have migrated to the feeding grounds after spawning. The above observations were made based on either small catches or fewer fish sampled from the fishermen. During the present investigation, several fishing trips were made over two years to investigate the availability of silver pomfret mature fish. The data obtained during this investigation confirms the occurrence of more males and less females in the population during the breeding season in Kuwait waters.
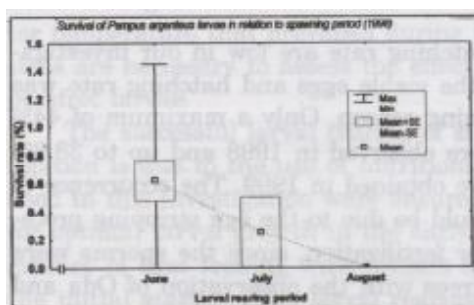


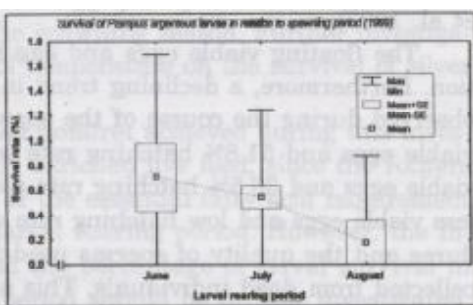Fig. 8. Survival of *P. argenteus* larvae in relation to the spawning period during 1998.

Fig. 9. Survival of *P. argenteus* larvae in relation to the spawning period during 1999.

Seasonal availability of mature gravid females observed during the May to September 1998 and 1999 spawning periods is in agreement with the observations of Abu-Hakima et al. (1983) and Dadzie et al. (1998) in Kuwait waters. Although Abu-Hakima (1984) observed two peaks of gonad maturation, one in March to May and another in August to September, the present investigation confirms that spawning initiates in May and reaches a peak in June and July when the water temperature range between 28 and 30ºC. Therefore, peak availability of gravid females in June 1998 and July 1999 was associated with the optimal water temperature of 28 to 30ºC as observed in this investigation. Furthermore, spawning of silver pomfret occurs in different periods (Mito and Senta 1967; Gopalan 1969; Oda and Namba 1982; Pati 1981, 1984) depending on the geographical area and environment.

Gonadal maturity stages of silver pomfret females in relation to day time have been observed for the first time during this investigation. The results show that stage 5 females occur in the early day time period up to 15:00 h and proceed to attain full ripe maturity stage 6 in the evening hours. Since spawning occurs in the late evening, only spent females could be observed in the night collections. The data obtained during the present investigation confirms the spawning of silver pomfret in the evening hours in the Kuwait in-shore waters.

Although the egg diameter of 1.08 to 1.19 mm (mean 1.12±0.02 mm) obtained during the present investigation in Kuwait waters is larger than that of Almatar et al. (2000), 1.05-1.12 mm (mean 1.08±0.02 mm), it is smaller than that of Mito and Senta (1967), 1.20 to 1.35 mm; Oda and Namba (1982), 1.31±0.35 mm; in Japanese seas and 1.26 to 1.32 mm as observed by Gopalan (1969) in the Indian waters. Kim and Han (1989) observed egg diameter ranging from 0.83 to 1.27 mm for *P. argenteus* in the Korean waters. The newly hatched larval size of 2.40±0.10 mm observed in the present investigation concurs with that of Almatar et al. (2000) in Kuwait waters. However, it is smaller than that of Mito and Senta (1967), 2.75 to 3.10 mm and Oda and Namba (1982), 3.75±0.07 mm, observed in the Japanese waters at temperatures ranging from 25.2 to 26.7ºC. The smaller egg and newly hatched larval size observed in our investigation may be attributed to the high salinity and temperature conditions of Kuwait waters compared to that in Japan, Korea and in the Indian waters. The present observation also concurs with the egg diameter and newly hatched larval size of other fish species recorded in the hypersaline waters in the Gulf and in the Red Sea (Hussain et al. 1975; James et al. 1997).

The floating viable eggs and egg hatching rate are low in our investigation. Furthermore, a declining trend in the viable eggs and hatching rate was observed during the course of the spawning season. Only a maximum of 44% viable eggs and 51.8% hatching rate were observed in 1998 and up to 38.6% viable eggs and 36.5% hatching rate were obtained in 1999. The occurrence of less viable eggs and low hatching rate could be due to the egg stripping procedures and the quality of sperms used for fertilization, since the sperms were collected from dead individuals. This agrees with the observation of Oda and Namba (1982) who obtained only 6.3% egg fertilization for the individuals collected from the fishing boats. Further investigations are required towards en-

hancing the quality of eggs collected from the wild by stripping procedures. Further research is also required towards developing a domesticated silver pomfret broodstock that could solve this problem.

The egg hatching time of 15 h at 29 to 30ºC observed in this investigation is similar to that of Almatar et al. (2000) and shorter than that of Mito and Senta (1967) who observed 24 h hatching time at 25.2 to 26.4ºC. The short egg hatching time is due to the higher water temperature during our investigation. Egg hatching under various salinity regimes show that ambient seawater salinity ranging from 35 to 40‰ is optimum for hatching the eggs since it gives significantly high (P<0.01) hatching rate compared to that at low salinity.

The live food algal culture cell densities observed during this investigation is within the range of cell densities observed in the conventional culture systems at MFD (James et al. 1988; James and Al-Khars 1990; Abu-Rezq et al. 1997, 1999). The total ω3 HUFA content of 36.32±0.68% in rotifers treated with mixed algae plus Super Selco and DHA Protein Selco during the present investigation is considerably higher than that of the previous records at MFD (James and Abu-Rezq 1989a,b). Significantly high (P<0.05) quantities of the most essential fatty acid 22:6ω3 in the above treated rotifers ensure the nutritional requirement for marine fish larval growth and survival as discussed by Al-Abdul-Elah (1984), Watanabe (1991) and Sargent et al. (1993).

During the initial stages of larval rearing, addition of *Chlorella* enabled the larvae to disperse in the water column and helped in efficient feeding as observed in the Atlantic halibut *Hippoglossus hippoglossus* L., larvae by Naas et al. (1992). Addition of algae also helped to avoid the phototactic behaviour of larvae in the culture system. In clear water devoid of algae, the larvae tend to float on the water surface and resulted in considerable mortality in the culture system. Further investigations are required to determine the optimal algal densities in the culture system towards enhancing larval survival. Larval growth was slow in the early stages and the growth curve during the larval rearing period showed a curvilinear pattern.

Although larval survival at different stocking densities do not show any significant difference (P>0.05) between stocking densities of 20,30 and 40 larvae·l$^{-1}$, survival is very low (maximum 1.5%) till the transformation of larvae to juveniles under the present hatchery culture conditions. The declining trend in larval survival during the course of the larval rearing period from June to August could have been due to the egg quality as well as the seawater temperature that prevailed during the spawning season. Further investigations are necessary to assess the effect of temperature on the survival of silver pomfret larvae.

The successful larval rearing of silver pomfret achieved during this investigation is due to the use of nutritionally enriched live feed, since the rotifers used in this investigation were ensured of the essential fatty acid requirement for optimal larval growth in the early larval rearing period. However, the incidence of first feeding with rotifers and the percentage of larval survival in the initial stages of the larval rearing period should be further investigated. Larval mortality due to cannibalism was low during the larval rearing period compared to that in groupers (James et al. 1997).

In the present study period, one of the reasons for larval mortality was larval rearing tank water management constraints and larval behaviour, since the larvae tend to engulf tiny air bubbles on the water surface. This is due to the innate feeding habit of silver pomfret that consume small jelly fishes and medusae (Pati 1977, 1980a,b; Higashikawa et al. 1981). Since tiny air bubbles mimic jellyfish in appearance, the larvae consume these air bubbles and tend to float on the water surface and loose their balance in the water column. This tendency of consuming air bubble increased at the latter part of the larval rearing period from day 25 onwards. Based on the eating habits of silver pomfret, Oda and Namba (1982) opined that silver pomfret is not a suitable species for aquaculture. Further improvements in the culture system to avoid the occurrence of air bubbles at the water surface will enhance larval survival in the hatchery.

One of the constraints in the present hatchery system is the difficulty in obtaining good quality eggs from the wild. Research on developing domesticated broodstock could solve this problem. Further improvement in egg collection and fertilization procedures could also help improve the egg quality in the present culture system. The successful larval rearing of silver pomfret achieved during the present investigation shows the possibility of producing the fingerlings under hatchery culture conditions. However, larval survival should be enhanced considerably towards realizing commercial farming of this species.

## Acknowledgments

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