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Original article

Recent advances in artificial breeding and larval rearing of silver pomfret *Pampus argenteus* (Euphrasen 1788) in Kuwait

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ABSTRACT

During last several years, Kuwait Institute for Scientific Research (KISR), Kuwait has been trying to develop a sustainable culture technique for silver pomfret (*Pampus argenteus*). This paper reports the recent research advances in the breeding and rearing of silver pomfret fry at KISR. The eggs collected from wild silver pomfret during spawning seasons of 2012 to 2015 were artificially fertilized under laboratory condition. The average hatching rates of artificially fertilized eggs collected from wild silver pomfrets were 25.6%, 44.8%, 76.7%, and 53.5.0% and average survival rates of metamorphosed fry produced from these eggs were 3.7%, 5.7%, 4.4% and 3.8% for the spawning seasons 2012, 2013, 2014, and 2015, respectively. For captive brood stocks, observation on the spawning time at hourly interval by collecting eggs from the nets set at out-flow of brood tanks showed that the spawning time for captive silver pomfret starts at the time of sunset. In 2012, two groups of captive silver pomfret broods spawned a total of $62x10^3$ and $66x10^3$ eggs, but the eggs were unfertilized. No captive spawning occurred during 2013 and 2014 spawning season. In 2015, captive silver pomfret broods in three tanks spawned about $653x10^3$, $673x10^3$ and $270x10^3$ eggs, and in 2016, the broods in four tanks spawned about $669x10^3$, $22x10^3$, $3x10^3$ and $366x10^3$ eggs. However, from these eggs only 1,400 and 1,000 fertilized eggs were collected which produced 300 and 123 hatched larvae in July 2015 and June 2016, respectively. The larvae produced from the 2015 broods did not survive beyond 56 days of metamorphosed stage while larvae produced from 2016 captive broods survived for 9 days only. The mortality of the larvae from captive brood could be related to the poor egg or milt quality. However, efforts are continued to improve the eggs and sperm quality through proper brood management.

1. Introduction

Silver pomfret, *Pampus argenteus* (Euphrasen 1788) locally known as 'zobaidy' in Kuwait, is the most highly priced fish in the local market. It is commercially fished in Kuwait, Iraq, Iran, India and China (FAO, 2003). However, in recent years the commercial catch of zobaidy declined due to overfishing and that the fishery stocks in China Sea are under stress due to both extensive fishing and ecological changes (Wen et al., 2006). A steady sharp decline in the fishable stocks from Kuwait Bay and increasing pressure of demand in Kuwait initiated several research projects towards developing aquaculture technology for this species in Kuwait. Successful mass production of seedlings and commercial farming of silver pomfret to marketable size will narrow the gap between the consumer high demand and depleting supply of fish from the wild.

In spite of the its worldwide importance and market demand, efforts towards developing a commercial culture technology for silver pomfret have been constrained still to-date due to the lack of successful captive breeding of silver pomfret. However, Mariculture and Fisheries Department (MFD) of Kuwait Institute for Scientific Research (KISR) has been successful in breeding and larval rearing of silver pomfret with eggs collected from the wild brood stocks (Almatar et al., 2000; Al-Abdul Elah et al., 2001, Almatar et al., 2004). Studies on the feeding and rearing of fry and juveniles to grow-out and brood stage have been conducted at MFD, KISR using various formulated and commercial feeds (Cruz et al., 2000; Almatar and James, 2007; Almatar et al., 2013). It is well

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known that for formulation of a balanced diet and successful aquaculture, the knowledge of the dietary nutritional requirement of a species is very important. In this regard, the nutritional requirements of juvenile silver pomfrets on optimum dietary protein (Hossain et al., 2010), lipid (Hossain et al., 2011), docosahexaenoic acid, (DHA, Hossain et al., 2012) and vitamin E (Hossain et al., 2016) have also been investigated. However, research on captive breeding of silver pomfret at MFD are still ongoing. In the present paper, we reported the results of the research efforts on the breeding and fry rearing of silver pomfret at KISR during 2012–2016.

2. Materials and methods

2.1. Wild brood stock collection and artificial fertilization

For catching wild silver pomfret brood, a number of fishing trips were made in the Kuwait Bay and the number of fishing days were 43, 25, 20, and 3 in the year 2012, 2013, 2014 and 2015, respectively. Drift gill nets were set in the silver pomfret spawning grounds of Kuwait Bay between Kuwait Tower and Salmiya. Fishing time ranged between 14.00 and 18.00 h. Fishing days were selected to be during the spring tides of around new and full moon. Fish caught by the gill nets were checked by stripping spawners' abdomen for determining maturity stage. Only the fish with ripe and running stage were selected and put inside styrofoam box with ice and transferred to the hatchery at Salmiya Marine Research Station, KISR. In the hatchery, manual artificial fertilization was carried out with ripe females using the dry method. The eggs were cleaned and transferred to a measuring cylinder in order to separate floating and sunken eggs. The floating eggs were transparent and supposed to be fertilized, while the sunken eggs were unfertilized and opaque. The floating eggs were then measured volumetrically to estimate their number and transferred to a incubation net of 30 cm in diameter and 42 cm deep (22.5-L working volume) with continuous supply of fresh seawater and aeration. Eggs started to hatch after 14 h, and hatching was completed by 16 h at about 29 °C water temperature. Defused aeration maintained embryos and larvae in suspension. Average total hatched larvae was estimated by counting three aliquot samples of 100 ml each, and then the larvae were transferred to the larval rearing fiberglass tank of 1 or 2 m^3 capacity for further rearing.

2.2. Rearing of larvae from eggs collected from wild silver pomfret

Larval rearing commenced after newly hatched larvae were placed in rearing tanks and continued until larvae reached metamorphosis stage. Fiber glass tanks of 1 or 2 m^3 were used for larval rearing with each being supplied with two to three fluorescent units of light hanged on top of each tank providing light intensity of around 2000 to 3000 lx. Temperature and dissolved oxygen were measured daily. Larval stocking density varied depending on the availability of newly hatched larvae and ranged from 1.5 to 20.0 larvae L^{-1}. Stocked larvae were kept in static water until 5 days after hatching (DAH) after which water exchange started at a rate of 50 ml min^{-1}. A mesh screen stand pipe was in the center of the tank. Diffused aeration was supplied to the larval tanks through air stones positioned to create a circular pattern sufficient to maintain larvae in the water column. Water exchange gradually increased to reach 750 ml min^{-1} at 35 DAH. Marine single cell algae, *Nannochloropsis* cultured using the method described by Abu-Rezq et al. (1999) were added to the larval rearing tanks in order to stabilize water condition and as food for rotifers and *Artemia*. Algae were added at a rate of 10 l d^{-1} starting from 1 DAH for 5 days then increased to 20 to 30 l d^{-1} depending on consumption of algae in the tank.

Occasionally *Vibrio* bacteria, a pathogen of marine fish, appeared in the larval rearing tanks, rotifer culture and sometimes in algae tanks. Thus, oxolinic acid, an antibiotic, was regularly added at a rate of 4–8 ppm to the larval rearing tank starting at 1 DAH. Rotifers were supplied from 2 DAH at a density of 5 rotifers ml^{-1} and then increased to 15–20 rotifers ml^{-1} during the end of the first week of the rearing period when the larvae were actively foraging on the rotifers. Since the rotifers multiplied in the larval rearing tanks due to the addition of algae, it was not required to add rotifers daily in the culture system during the first week of the larval rearing period. *Artemia* nauplii were decapsulated and supplied to larvae from 9 DAH. Along with *Artemia* weaning feed was introduced at 18 DAH and was started with 2 g $tank^{-1}$ of paste feed, and the amount was gradually increased with the growth of larvae. The composition of weaning feed was a mixture of 'Otohime B-1′ feed (<0.36 mm, Nissin-Marubeni Ltd. Japan), and high DHA tuna oil. The feed contained a crude protein content of 55–58% and crude lipid 12–16%. The paste feed was smeared over both sides of a specially made feeding net. The feeding net was hung about 20–25 cm deep inside the tank water by a string or rope, and there was a sinker attached underneath the net so that it hanged straight. Larvae could eat the paste feed from either side of the net (Fig. 1). Cleaning of the tank bottom by siphoning was initiated at 5 DAH and was done daily during the rearing period.

2.3. Breeding of captive zobaidy

2.3.1. Maintenance and feeding management of silver pomfret captive brood stock

The silver pomfret brood stocks were developed from the juveniles produced from the wild silver pomfret eggs in the previous years and were maintained in out-door concrete brood tanks marked as C1 and C2 of size 32 m^3 each, and D1, D2, D3 and D4 of size 125 m^3 each. The D tanks were rectangular in shape and C and J tanks were circular in shape. The indoor brood tanks J1, J2, J3 and J4 were fiber glass tanks of size 25 m^3 each. The brood stocks were fed with a special brood feed which was a combination

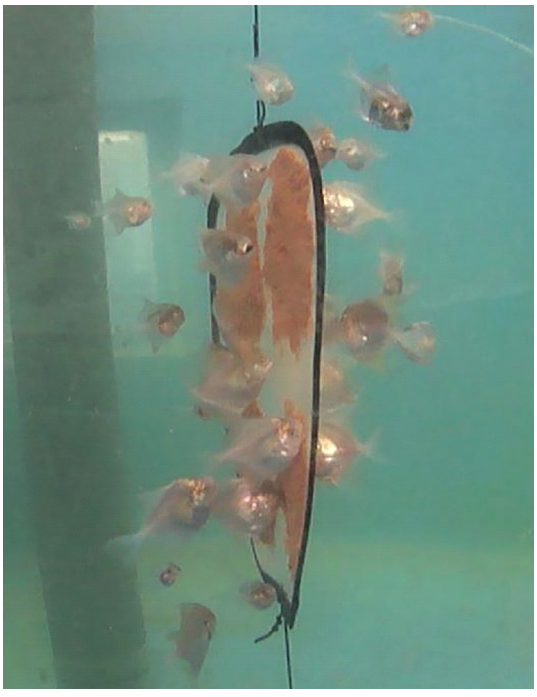

Fig 1. Net feeding of paste feed by metamorphosing silver pomfret larvae.

of different types of commercial feeds, maturation feed, special protein sources, essential n-3 fatty acids (high DHA fish oil), special nutrients and binder (Table 1). The feed was prepared by thoroughly mixing different compound feeds, special nutrients, additives and water together, and was passed through a die of a meat grinder. The resultant feed was in paste form. Since 2014 Japanese eel feed was included to increase the consistency of the paste feed. The combination of different feed ingredients in the brood feed formulation varied in different years on the basis of feed availability and the specialist's suggestions. The feed was offered at 10.00 h and 14.00 h in white plastic bowls suspended about 2 m below water surface or in plastic nets hanged about 30 cm below water surface. The feed supplied in paste/dough form had a soft but sticky consistency which helped to adhere the feed at the bottom of the feeding bowl or on the plastic net resulting in reduced disintegration of feeds. However, complete prevention of disintegration and nutrient leaching could not be ascertained. The tanks were cleaned daily by siphoning out the feces and other deposited solid particles.

Seawater was continuously flowed through the tanks to maintain exchange rate of about 8–10 times a day. Ambient seawater mixed with ground well water of equivalent salinity was used to control the water temperature just as not to reach above 30 °C during summer and not below 20 °C during winter since the ambient seawater temperature rises to over 33 °C during summer and declines to less than 15 °C during winter.

2.3.1.1. Brood stocks for 2012. These broods were produced from eggs collected from the wild silver pomfrets during 2010 spawning season. The brood fish were grouped into two and marked as group B10A and B10B. A total of 48 broods (B10A) were stocked in D2 (125 m^3) tank on 5 June 2012. Average standard length and body weight of the broods were 18.8 cm and 252.9 g, respectively. Six broods were over 400 g in body weight. The biggest brood was recorded as 25 cm in standard length and 627 g in body weight. Another group of 55 captive broods (B10B) were stocked in tank D4 (125 m^3) on 24 May 2012. Average standard length and body weight of fish were 19.2 cm and 275.6 g, respectively. Among the broods, 7 fishes were over 400 g in body weight which were expected to be female (Table 3). It may be mentioned that no spawning occurred for captive broods for 2013 and 2014.

2.3.1.2. Brood stocks for 2015. Three groups of brood stock such as B12, B13A and B13B of silver pomfret which were produced from the wild eggs in year 2012 and 2013 were used for 2015 captive spawning. Total number of brood in group B12, B13A and B13B were 48, 43, and 53, respectively. In May 2015, the average standard length and body weights of brood stocks of B12, B13A, B13 were 20.2 cm and 270 g; 20.1 cm and 273 g; 17.4 and 178 g; respectively (Table 3).

2.3.1.3. Brood for 2016. Four groups of brood stock B12, B13, B14A and B14B which were produced from the eggs collected from the wild silver pomfrets in 2012, 2013 and 2014 were used in captive spawning for 2016. The total number of broods in group B12, B13, B14A and B14B were 57, 63, 80 and 40 respectively. In May 2016, average standard length and body weight of broods of B12, B13, B14A and B14B were 18.9 cm and 242 g; 18.1 cm and 220 g; 16.4 cm and 155 g; 18.6 cm and 266 g; respectively (Table 3).

2.3.2. Collection of eggs from captive silver pomfret brood stocks

Starting from late May, a conical net with 400-μm fine mesh was fixed at the brood tank water overflow channel in a submerged trough at 15.00 h to collect the eggs during the natural spawning. Daily inspection for eggs was carried out between 19.00 and 22.00 h as well as morning at 08.00 h. When eggs were present, the collected eggs were washed to separate from the suspended algae and other debris by using 2 mm mesh net. The washed eggs were placed in 1000 ml capacity graduated measuring cylinders to quantify the floating and sunken eggs. Floating eggs are transparent which include fertilized eggs and the sunken eggs are whitish and unfertilized. The eggs were also examined under

Table 1
Formulation of brood stock feeds used for captive silver pomfret during different years.

Ingredients	2011 (g/kg)*	2012 (g/kg)	2013 (g/kg)	2014 (g/kg)	2015 (g/kg)	2016 (g/kg)
Maturation feed (USA)	–	–	–	100	100	150
Eel feed (Japan)	–	–	–	200	200	200
GEMA/BIOMAR feed (Greece)	250	250	150	–	–	–
Lansy Breed Maturation (INVE)	150	150	150	100	100	100
Lansy Breed Recovery (INVE)	150	150	–	–	–	–
FISH Breed-M (INVE)	150	150	150	100	50	50
Optibream (Skretting, Italy)	–	–	150	–	–	–
Gema diamond (Skretting, Italy)	–	–	100	–	–	–
Raw squid muscle	50	50	50	200	150	100
Raw shrimp muscle	50	50	50	200	300	300
Blood worm (Frozen)	50	50	50	50	50	50
Krill (Freeze dried)	40	40	20	50	50	50
Frozen Artemia	50	50	50	50	50	50
Squid oil	50	50	50	–	–	–
DHA oil (Cultech, UK)	50	50	50	10	10	10
Bio-max	4	4	4	4	4	4
Carpmin forte (mineral)	10	10	10	10	10	10
Astaxanthin (Naturose)	2	2	2	2	2	2
Cyclopeeze	1	1	1	2	2	2
Gut conditioner (probiotics and yeast)	2	2	–	–	–	–
Stay-C (Vit-C)	5	5	5	5	5	5
Nutra-Brood Enhance	4 ml	4 ml	4 ml	4 ml	4 ml	4 ml
Vitamin E (α-tocopherol)	250 mg	250 mg	250 mg	250 mg	250 mg	250 mg
Betaine (attractant)	5	5	5	5	5	5
Eco-Plus Feeding Mixing	–	3	3	3	3	3
Special Live Germ	–	1	1	1	1	1
Omega Enzyme Powder	–	0.5	0.5	0.5	0.5	0.5
Sodium Alginate (binder)	–	3	3	3	3	3

* Feed was fed as paste form and all ingredients were mixed as fed basis. Total amount could exceed 1 kg as liquid nutrients and raw shrimp muscles, squid, frozen artemia, blood worm etc. were added.

the microscope to determine whether the egg were fertilized or not. The spawned eggs were separated as floating and sunken. Usually if there are any fertilized eggs among the floating eggs, the eggs were stocked in the egg hatching nets which were suspended in a hatching tank in the hatchery. Next morning newly hatched larvae were collected and numbers estimated and transferred to larval rearing tanks. The larval rearing procedure were similar to those followed for rearing of larvae from wild silver pomfret eggs.

3. Results

The summary of the eggs collected, larvae and fry produced from the wild silver pomfret during 2012 to 2015 spawning seasons is shown in Table 2. Maximum number of female brood used was 19 in 2012 while it was only 2–3 females in other years under study. Although only two females were used in year 2014, it produced the highest number of larvae (104×10^{-3}) followed by year 2012 which produced $93x10^{-3}$ larvae using 19 females. The average hatching rates of eggs collected from wild silver pomfret were 25.6%, 44.8%, 76.7%, and 53.5% for the spawning seasons 2012, 2013, 2014, and 2015, respectively. The total number of metamorphosed fry produced were 2,378, 1,042, 4,524 and 872 for the spawning season 2012, 2013, 2014, and 2015, respectively. The average survival rates of fry produced were 3.7%, 5.7%, 4.4%, and 3.8% for spawning seasons 2012, 2013, 2014, and 2015 respectively.

3.1. Captive spawning of silver pomfret

3.1.1. Spawning in 2012

In 2012, two-year-old captive silver pomfret broods were fully matured and started spawning at the end of June. These broods were produced from eggs collected from the wild silver pomfrets during 2010 spawning season. Broods in D4 tank started spawning on 30 June and ended on 16 August (Fig. 2). Brood stock was spawning intermittently until 24 July but they were spawning consecutively from 26 July to 5 August. They stopped spawning for 8 days and started again from 13 to 16 August (Fig. 2) The spawning occurred for 20 days. A total of about $66x10^3$ eggs were collected from tank D4 but unfortunately eggs were unfertilized.

Brood stocks in tank D2 started spawning on 11 July and consecutively spawned until 18 July 2012. The spawning was stopped for 9 days and restarted on 27 July which continued until 30 July (Fig. 2). Broods spawned for a total of 16 days in July and August. A total of about $62x10^3$ eggs were collected from tank D2 and the eggs were not fertilized too. The spawning pattern of the captive silver pomfret broods in tanks D2 and D4 are shown in Fg.2. The X- axis of the graph indicating two kind of date. The upper one is Moon Age (Arabic calendar) and lower is Gregorian calendar. This graph was plotted to see the correlation between occurrence of spawning and moon phases. It seemed that spawning was not exactly correlated with moon phase. A summary of the size of brood stocks, spawning days and total number of eggs produced by the captive broods in 2012 is shown in Table 3.

To determine the precise time of spawning, routine inspections of the egg collection was carried out from 13 to 16 July 2012 at 17.00, 20.00, 22.00, 24.00, 03.00, 06.00 and 08.00 h. Data on egg collection during this time was plotted in Fig. 3. From the result of eggs collection, it showed that the fish spawned around 20.00 h on 13 and 14 July (Fig. 3). In case of July 15 and 16, fish started spawning before 20.00 h. However, roughly it can be estimated that the spawning time of silver pomfret is between 17.00 and 22.00 h.

The floating eggs were examined microscopically see the development stage and found that they were transparent (Fig. 4A) and the sunken eggs were opaque (Fig. 4B). Unfortunately, both eggs were unfertilized. It seems that the floating eggs were in good condition because they were transparent with perivitelline space and blastodisc (Fig. 4A), and they seemed to be in good condition to accept spermatozoa. Reason for eggs not being fertilized is not yet clear, but this could be due to males not producing enough milt or that the males were not involved in spawning activity.

3.1.2. Spawning in 2015

As mentioned earlier, no spawning occurred for captive broods in 2013 and 2014. However, in 2015, the group B12 started spawning on 4 June which ended on 24 October 2015 and produced a total of $653x10^3$ eggs. Group B13A started spawning on 3 May which ended on 3 September 2015 and produced a total of $673x10^3$ eggs. Group B13B started spawning on 18 July which ended on 4 August 2015 produced a total of $270x10^3$ eggs. A summary of the average size of brood stocks, spawning days and total number of eggs produced by the captive broods in 2015 is shown in Table 3. Fig. 5 shows the spawning intensity of the captive silver pomfrets in 2015.

The B13B spawned about 1,400 floating eggs on 29 July 2015 which included fertilized eggs. Although B13B was the smallest sized brood stock (average weight 178 g) among the groups, there was only one fish which weighed more than 400 g. We predict that this biggest fish could be a female which might have spawned quality eggs which were ultimately fertilized. The fertilized eggs were incubated and the larvae were reared following the standard procedure used for rearing wild silver pomfret larvae at KISR. The summary of the results of the fertilized eggs collected, hatching rates and larvae produced from the captive silver pomfret in 2015 are shown in Table 4.

3.1.3. Spawning on 2016

Four groups of brood stock B12, B13, B14A and B14B which were produced from the eggs collected from the wild silver pomfrets in 2012, 2013 and 2014 were used in captive spawning in 2016. In May 2016, average standard length and body weight of broods of B12, B13, B14A and B14B were 18.9 cm and 242 g, 18.1 cm and 220 g, 16.4 cm and 155 g, 18.6 cm and 266 g, respectively. Group B12 started spawning on 21 May which ended on 7 November 2016 and produced a total of $669x10^3$ eggs. Group B13 started spawning from on 14 April which ended 29 October 2016 and produced a total of $22x10^3$ eggs. Group B14A started spawning on 1 July which ended on 27 July 2016 and produced a total of $3x10^3$ eggs. Group B14B started spawning from 18 June which ended on 23 October 2016 and produced $366x10^3$ eggs. Spawning frequency and number of eggs collected from different

Table 2
Eggs collected, larvae and fry produced from wild silver pomfret eggs during 2012 to 2015.

Year	No. of female used	Amount of eggs (ml) collected	Hatching rate (%)	No. of larvae produced	No. fry produced	Survival rate (%)
2012	19	729.5	25.6	93,000	2378	3.7
2013	3	75.0	44.8	18,370	1042	5.7
2014	2	205.0	76.7	104,000	4524	4.4
2015	2	169.5	53.5	23,000	872	3.8

Table 3
Summary of natural spawning and sizes of the captive silver pomfret brood stock during 2012, 2015 and 2016*.

Spawning Year	Spawning Group	Spawning period	Spawning days	Total No. of eggs	No. of fish >250 g	No. of fish <250 g	Total No. of fish	AverageFork length (cm)	Average Body weight (g)	AverageCondition factor (K)
2012	B10A	11/6–7/8	13	62,267	15	33	48	18.8	253	3.62
	B10B	30/6–16/8	18	66,470	33	22	55	19.2	276	3.69
2015	B12	4/6–24/10	50	653,000	21	27	48	20.2	270	3.12
	B13A	30/5–3/9	50	673,950	27	16	43	20.1	273	3.29
	**B13B	18/7–4/8	11	270,550	5	48	53	17.4	178	3.26
2016	**B12	21/5–7/11	26	667,203	19	38	57	18.9	242	3.24
	B13A	14/4–29/10	15	21,548	16	47	63	18	207	3.26
	B14A	1/7–27/7	4	2,717	9	71	80	16.5	156	3.31
	B14B	20/6–23/10	40	366,268	18	30	48	18.6	266	3.86

* Brood fish during year 2013 and 2014 did not spawn.
** Group which produced fertilized eggs.

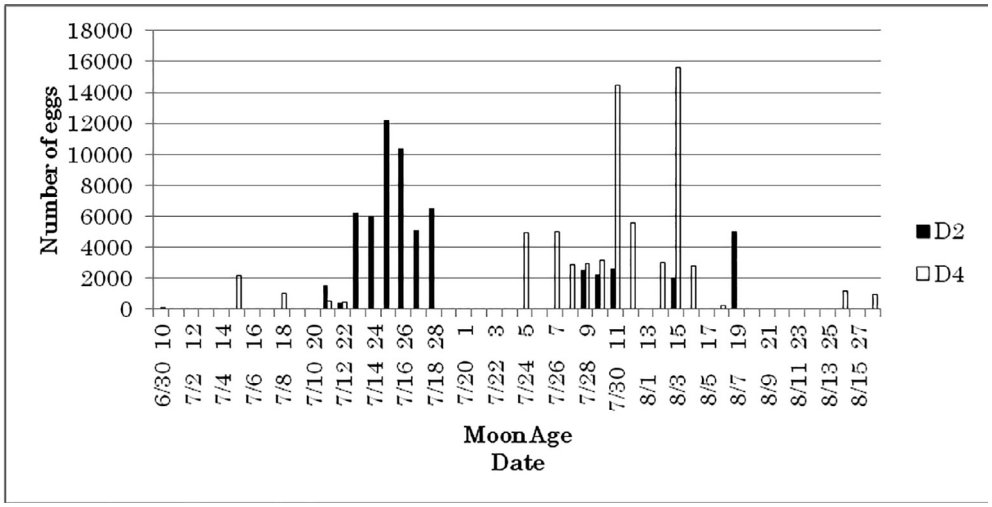

Fig. 2. Spawning frequency and number of eggs collected from different brood groups during 30 June to 16 August in 2012.

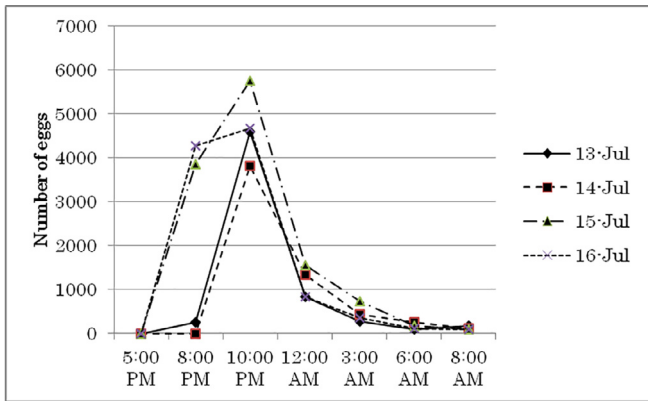

Fig.3. Number of eggs collected with respect to time during 13 to 16 July in 2012.

brood groups during 14 April to 7 November in 2016 is shown in Fig. 6.

Group B12 spawned about 1,000 floating eggs on 12 June 2016 which included fertilized eggs. The eggs were transferred to hatching trough and hatched larvae were collected and reared as per the procedure followed for rearing of eggs collected from wild silver pomfret. A summary of the results of the fertilized eggs collected, the hatching rates and larvae produced from the captive silver pomfret in 2016 are shown in Table 4.

3.1.4. Fertilized eggs from natural spawning of captive silver pomfret brood in 20,015 and 2016

As mentioned earlier, about 1,400 floating eggs that included fertilized eggs obtained from B13B spawning group on 29 July 2015 were carefully scooped by fine meshed net and stocked in a hatching trough. Larvae were hatched out next morning around 10:00 am. Fig. 7 shows the fertilized egg developmental stage of early gastrula.

A total of about 300 newly hatched larvae were divided two groups and stocked in two 100 l conical shape fiber glass tanks with water volume of about 60 l. The hatching rate of larvae was 21.4%. A standard larval rearing method used at KISR for rearing of larvae from wild silver pomfret eggs was followed. Frequent mortality were observed during the larval rearing process and only few larvae reached metamorphosed stage and only one reached to full fry stage attaining a length of 5.4 cm and body weight of 3.6 g on 56 days of rearing (Fig. 8).

About 1,000 fertilized eggs collected from B12 (4 year old) on 12 June 2016 were put into hatching trough in a similar way as mentioned earlier. A total of only 123 newly hatched larvae were collected next day and stocked in a larval rearing tank. The hatching rate of larvae was 12.3%. Unfortunately, all the larvae died after 9 days of rearing. Usually normal larvae swim straight but larvae produced were swimming in circle near the surface. This early mortality of the larvae could be related to the poor quality of the eggs.

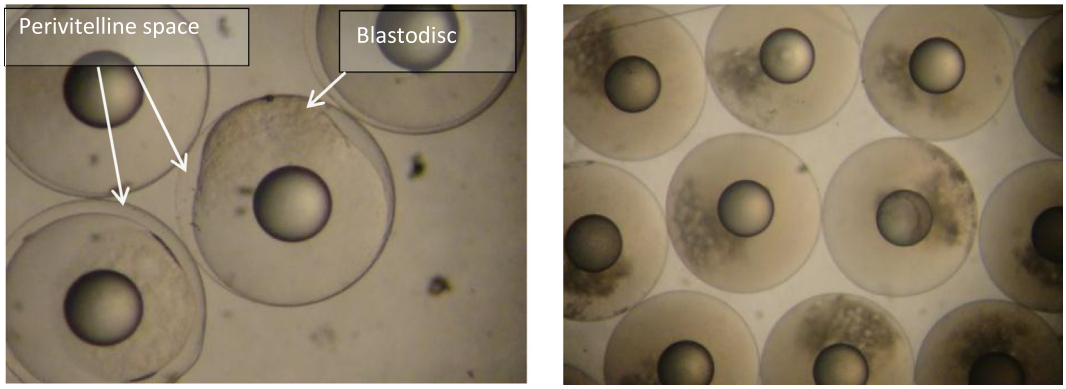

Fig. 4. (A) Floating transparent eggs showing perivitelline space and blastodisc (B) Sunken but opaque eggs.

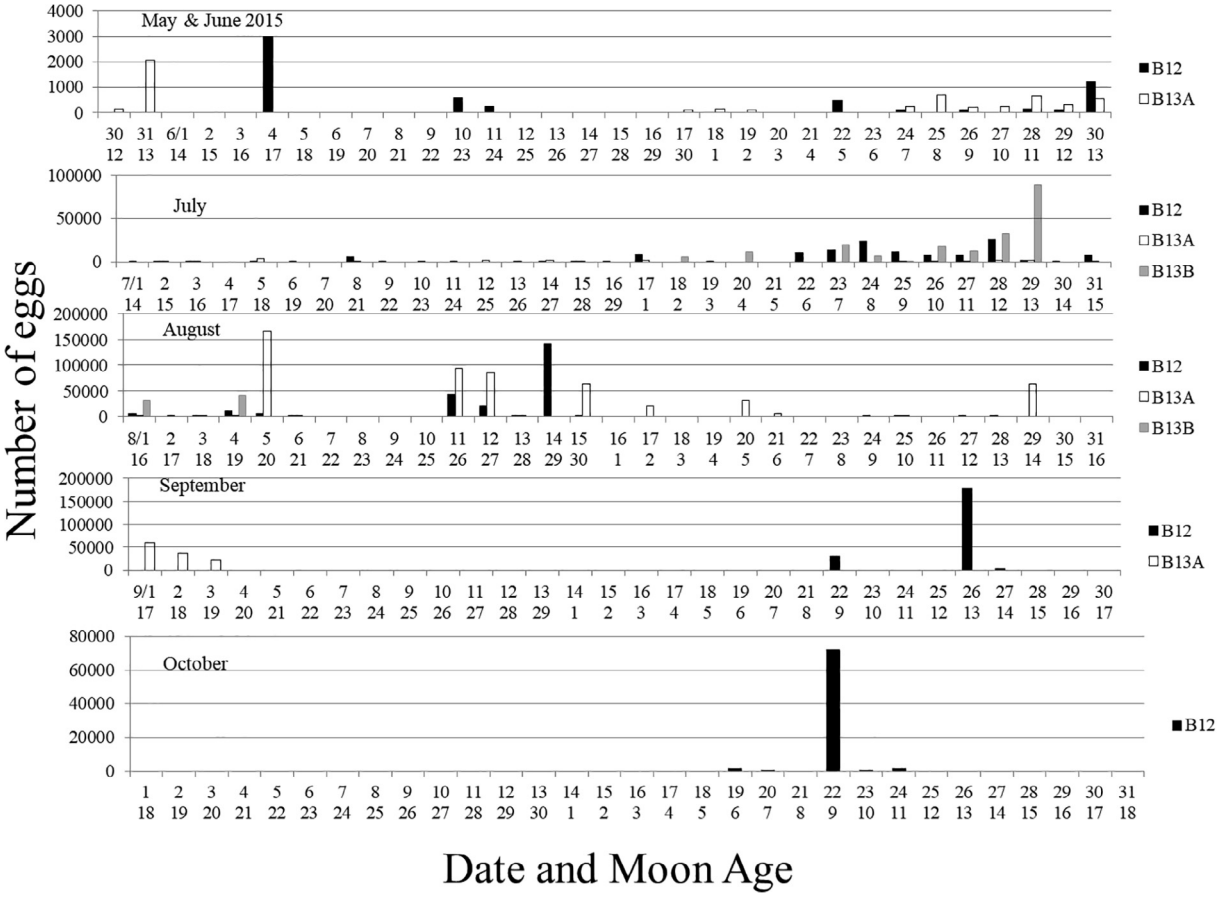

Fig. 5. Spawning frequency and number of eggs collected from different captive brood groups during 30 May to 24 October in 2015.

Table 4
Summary of brood stock weight, eggs collected, larvae produced and hatching rate of captive silver pomfret in 2015 and 2016.

Date of hatching	Origin of brood group	Average brood weight (g)	No. of floating eggs	No. of sunken eggs	No. of hatched larvae	Hatching rate (%)
29/07/2015	Year 2013	178	1,400	89,400	300	21.4
13/06/2016	Year 2012	242	1,000	53,720	123	12.3

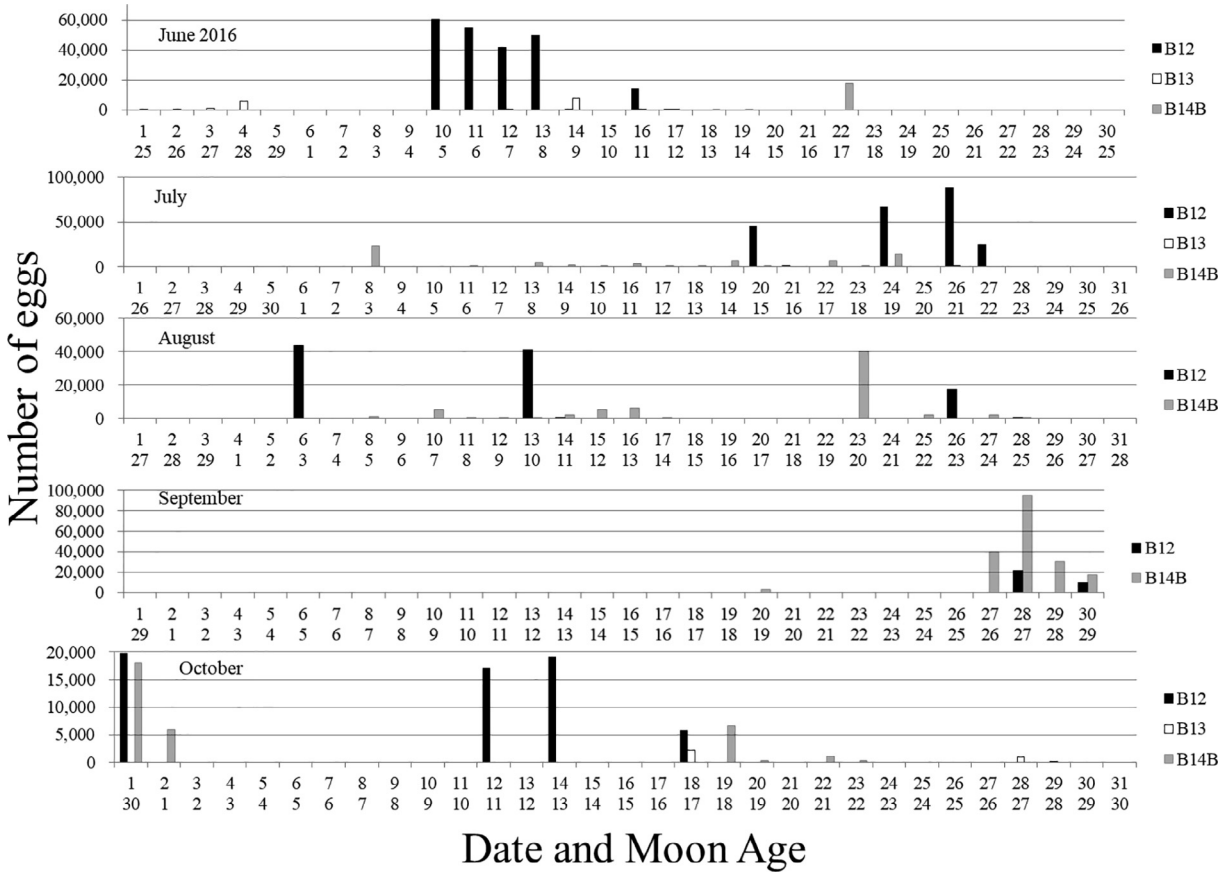

Fig. 6. Spawning frequency and number of eggs collected from different captive brood groups during 14 April to 7 November in 2016.

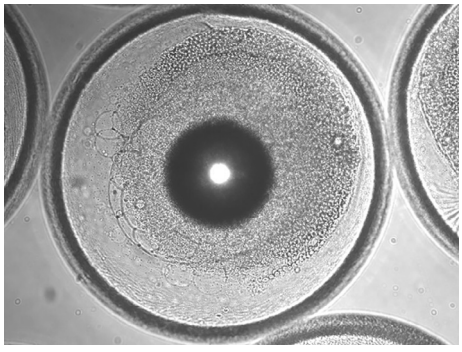

Fig. 7. Gastrula stage of silver pomfret egg collected from brood Group B13B on 29 July 2015.

Fig. 8. A 56 days old dead metamorphosed silver pomfret fry produced from natural spawning of captive brood (group B13B) in 2015.

4. Discussion

The results of the larval rearing of silver pomfret produced from wild brood during the years from 2012 to 2015 showed an improvement in the survival rate of metamorphosed fry produced. The survival rate ranging between 3.7 and 5.7% in the present study, is slightly higher than those of 3.6–4.2% achieved by Al-Abdul-Elah et al. (2001). This improvement could be related to better feeding strategy and the use of 1.5 in. PVC air-lift pipes inside the rearing tanks to avoid air bubbles in the system. As the tiny air bubbles may mimic jelly fish medusa in appearance, the larvae consume them and tend to float, losing their balance in the water column and eventually die (Al-Abdul-Elah et al., 2001). Because of this behavior of consuming air bubbles, Oda and Namba (1982) suggested that silver pomfret is not suitable species for aquaculture. Another strategy was introduction of a net feeding method for weaning paste diets to the larvae in rearing tanks which might have contributed to the better survival of larvae. The paste feed was smeared over both sides of a specially made feeding net which allowed the larvae to eat the paste feed from either side of the feeding net (Fig. 1).

Spawning of cultured silver pomfret under captive conditions is challenging and remained elusive over the years (James and Almatar, 2008). However, during period from 1998 to 2006 attempts have been made at KISR to bring a success to the captive spawning but with no success. In the following years, attempts

have been made to study the nutritional requirements (protein, lipid, Vitamin E) for juvenile and grow-out of silver pomfret (Hossain et al., 2010; 2011; 2012; 2016). Use of optimum water temperature for rearing and improvement of brood stock diet led to captive spawning of silver pomfret in 2012 but unfortunately eggs were not fertilized (Table 3). Since then efforts have been made to modify the brood stock diet formulation to contain higher level of DHA (9.34% of total fatty acids) by using high DHA tuna oil, raw shrimp muscle, and raw squid muscle based on the information available for marine brood fishes in the literature and suggestions from the experts working with marine fish breeding elsewhere. Further, inclusion of Japanese eel feed and maturation feed (Aquafauma Biomarine, USA) in the formulation from 2014 onward resulted in production of higher number of eggs during 2015 and 2016 compared to 2012. The total number of eggs produced in 2015 and 2016 from captive broods were $1596x10^3$ and $1060x10^3$, respectively, while it was only $128x10^3$ in 2012. But the most important achievement was that in 2015 and 2016 the captive broods produced 1400 and 1000 fertilized eggs which resulted in 300 and 123 hatched larvae. The hatching rate was 21.4 and 12.3% in 2015 and 2016, respectively.

Many species of fish are known to have a lunar spawning interval where spawning occurs in synchrony with spring tides of new and full moon (Johannes 1978; Taylor and Dimichele, 1980). Almatar et al. (2004) observed semi lunar spawning frequency rather than a continuous daily spawning occurred in wild silver pomfret in Kuwait waters. In the present study, correlation between the frequency of spawning and moon phase also indicated that the captive spawning of silver pomfret was not exactly correlated with moon phase (Figs. 2, 5 and 6). In the wild, silver pomfret is reported to spawn between May–June when water temperature range between 28 and 30 °C. Again this fish may spawn in late summer in September and October when water temperatures decrease to 28–30 °C (Almatar et al., 2004). Almatar et al. (2004) also reported that the mean percentage of females spawning daily was very high in June, July and August compared to May and September indicating the peak spawning season is June to August. Present study also showed that the spawning season for the captive silver pomfret is mainly between June to September (Figs. 2, 5, and 6).

The natural captive spawning was improved in quantity and quality in 2015 and 2016 (Table 3) and such improvement could be related to the inclusion of more fresh shrimp muscle and special types of compound feeds, maturation feeds, high DHA fish oil and other special nutrients in brood stock diet or due to better water temperature profile used. However, although using the standard larval rearing procedure for the captively produced larvae, high mortalities were observed during the larval rearing process and only few larvae reached metamorphosed stage of 45 days. Only one larvae reached to a size of a full fry attaining a length of 5.4 cm and body weight of 3.6 g, which died at age of 56 day (Fig. 8). The mortality of the larvae could be related to the poor quality of the eggs and milt. Further improvement of the brood stock diet or the maintenance of desired water temperature during winter and summer is extremely important for better spawning success of the captive stock. It was not possible to maintain the water temperature in the brood rearing tanks at desired optimum level of 28 °C. The temperature falls down to below 20 °C during winter (December–February) leading to less or no feed intake by the brood stocks resulting in poor growth and condition factor (K). Again during summer, the rearing water temperature rises above 30 °C hampering the feed intake and resulting in poor growth and condition factor of fish. These problems might hinder the proper gonadal developments of the captive broods. Thus, to maintain the desired temperature (25–27 °C) during winter and summer for the brood stock tanks, a recirculatory aquaculture system (RAS) should be used to control the environmental parameters as desired. Further research requirements to enhance the egg quality and spawning of domesticated brood stocks needs improvement in the brood stock diet which may help to enhance egg and sperm quality of the captive silver pomfret in the coming years.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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