Journal of Agricultural Science and Technology B 11 (2021) 73-88
doi: 10.17265/2161-6261/2021.02.003

Impact of Dietary Supplementation on Bioenergetics and Reproductive Performances in *Amphiprion sebae*

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Abstract: Effects of varied types of live feeds and pelleted feeds on the bioenergetics and reproductive performances were studied under laboratory conditions. The highest feeding rate of 279.4 J/g live fish/day was observed in test fishes feed with Moina. Fish fed with pelleted feed exhibited the lowest feeding rate of 104.4 J/g live fish/day. But the absorption efficiency showed the opposite trend. The fish fed with pelleted feed showed the highest absorption efficiency 36% whereas the fish fed with *Brachionus plicatilis* exhibited the lowest absorption efficiency of 28%. The brood stock maintenance and larval growth were closely observed. The larvae grew like adult after 25th day of hatching. The mortality noticed during 2nd and 7th day of hatching was overcome by feeding the larvae with nutrient rich live feed. The sexually mature females and males were injected with 50 µg of GnRHa (gonadotrophin releasing hormone analogue) encapsulated in a biodegradable copolymer of fatty acid dimer and sebacic acid p (FAD-SA) of a 25:75 molar ratio. After fifteen days all treated females commenced spawning. Similar treatment induced a two-fold increase in sperm volume in male. Conclusion of the present study indicated that the laboratory scale production of marine clown fish, *Amphiprion sebae* can be successfully carried out using live feed than the pellet feeds which express better feeding rate, spawning and development.

Key words: *Amphiprion sebae*, larval rearing, live feed, pelleted feed, survival rate.

1. Introduction

The increasing demand for marine ornamental fish due to the recent developments in aquarium keeping has resulted in an over exploitation of the natural stock and consequent destruction of reef areas [1]. The controlled spawning through temperature manipulations to bring about gonadal maturation has been successful with several temperate species. The temperature is one of the most important physical factors influencing fish growth, body composition and energy budget [2]. When temperature is low, growth rates, feeding rates and metabolic rates are suppressed; whereas elevated temperatures correlate with an increase in growth up to an optimum point above which thermal stress occurs [3].

Anemone fish or the more popularly known clownfish is one of the highly preferred tropical marine ornamental fish due to its small size, attractive color, peculiar behaviour patterns, and the symbiotic association with sea anemones. It is an omnivorous fish which attains a standard length of about 100 mm, depending on the species [4]. The several scientists have shown interest to find out the role of specific nutritional components such as essential fatty acid (EFA), phospholipids, vitamins C & E, carotenoids and other dietary components of the feed on the growth of clown fish [5]. The availability of adequate quantities of good quality feed significantly influences the reproductive performance of fish. Brood stock nutrition directly and indirectly influences gonadal development making it one of the essential areas in aquaculture research [6]. Brood stock nutrition studies are limited and relatively expensive. Presently, marine hatcheries operating on a commercial basis depend greatly on wet diets obtained from the sea or oceans. Fresh fish as well as other aquatic animals are used as brood stock feed and have proved to fulfill the nutritional requirements of the fishes ensuring good quality gametes especially eggs [7]. In

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case of fish with a short yolk deposition period, stipulation of enriched diets shortly before or during spawning can manipulate the gonadal development and growth is positively. In this backdrop, an attempt was made to study the impact of various dietary on bioenergetics and reproductive performances in *Amphiprion sebae.*

2. Materials and Methods

2.1 Bio-Energetic Studies

2.1.1 Effects of Varied Types of Live Feed Organisms and Pelleted Feed on Food Utilization of Clown Fish, *Amphiprion sebae*

Juveniles of *Amphiprion sebae* (4.5 ± 0.5 g) were wild collected from Gulf of Mannar and acclimated to as per Table 1. The juveniles were distributed among the tanks at a stocking density of 5 fish/tank with treatments in triplicate (Plate 1). The physico-chemical parameters of the aquarium water were maintained as per the levels shown in Tables 1 and 2 which show the feeding schedule and feeding organism for *Amphiprion sebae* larvae. During acclimation period the fish were fed on pelletized artificial feed prepared by square method of food compounding [8]. Different types of feeds namely pellet feeds, *Daphnia*, *Brachionus* and *Moina* were used during the experimental studies. The flesh feeds are namely Beef, clam, polychaete worm and *Artemia.* The ingredients used for the feed preparation were groundnut oil cake, rice bran, dried fish, dried silkworm pupae and tapioca flour. The test animals were fed twice a day at 6.00 a.m. and 8.00 p.m. at the rate of 15% of their body weight.

The feed acceptance was monitored every day. After the 2 h feeding period (for about 2 h), the food that remains in the trough was collected using pipette without disturbing fish and the actual food consumption was calculated. The culture medium was changed daily to give constant effect of water qualities on fish. At the time of changing the culture medium the entire tank water was filtered for the collection of faeces. The faeces were dried at 95 ℃ and weighed. The rearing experiments were conducted for a period of 60 days. On the final day the test animals were weighed in live condition, killed and dried to constant weight. The dry weight of the test individuals was also estimated. The food utilization scheme was worked out as per the modified form of integration by parts (IBP) formula [9]. The formula adopted in the present study was C = P + M + F where C represented the amount of food consumed; P, production (or) growth; M, energy loss due to metabolism and F, feces.

Table 1 Physico-chemical characters of marine water.

Parameters	Optimum level
pH	7.8-8.2
Salinity	32-36 ppt
Temperature	27-32 ℃
Alkalinity	< 120 ppm
Dissolved oxygen	> 5 mL/L
Ammonia	< 0.01 ppm
Nitrite	< 0.01 ppm
Photo period	12 h/day

Table 2 Feeding schedule for *Amphiprion* larvae.

Sl. No.	1-6 days	6-10 days	After 10th day
1	*Chlorella*, *Brachionus plicatilis*	*Artemia* nauplii, *Brachionus plicatilis*	Adult *Moina*, *Artemia* nauplii
2	*Isochrysis*, *Brachionus plicatilis*	*Copepod* nauplii, *Brachionus plicatilis*	Adult *Copepod*, adult *Moina*
3	*Chlorella*, *Brachionus plicatilis*	*Moina* nauplii, *Copepod* nauplii	Adult *Moina*, adult *Copepod*
4	*Chlorella*, *Brachionus plicatilis*	*Artemia* nauplii, *Copepod* nauplii	*Artemia* nauplii, adult *Copepod*

The food consumption was determined by subtracting the dry weight of the unfed materials from the dry weight of the food given and expressed in mg, dry food consumed per g, live fish per day. Food absorption was determined by subtracting the faces from the total food consumed by the fish. The feeding rate, absorption rate and growth rate were calculated by using the following formulae:

$$\text{Feeding rate} = \frac{\text{Total dry food consumed}}{\text{Number of days} \times \text{Initial live weight of fish}}$$

$$\text{Absorption rate} = \frac{\text{Total food absorbed}}{\text{Number of days} \times \text{Initial live weight of fish}}$$

$$\text{Growth rate} = \frac{\text{Total gain in body weight}}{\text{Number of days} \times \text{Initial live weight of fish}}$$

Metabolic rate = Absorption rate – Growth rate

The percentages of absorption and conversion efficiencies were calculated as follows:

$$\text{Absorption efficiency} = \frac{\text{Food absorbed}}{\text{Food consumed}} \times 100$$

$$\text{Growth conversion efficiency (K1)} = \frac{\text{Growth rate}}{\text{Feeding rate}} \times 100$$

$$Net\ conversion\ efficiency\ (K2) = \frac{Growth\ rate}{Absorption\ rate} \times 100$$

2.2 Fecundity Studies

2.2.1 Experiments to Assess Impact of Feed Variation on Fecundity in Clown Fishes

We have analyzed the impact of feed variation on fecundity of clown fish, *Amphiprion sebae* under laboratory conditions and experiment set up shown in Plates 2 and 3. Fifteen (15) pairs of anemone fishes of same size and weight were selected and each 3 pairs were fed with five types of feeds like beef, clam, polychaete worm, *Artemia* and pelleted feed. The food utilization scheme was worked out as per the modified form of IBP formula [9].

2.2.2 Microsphere Preparation

To prepare GnRHa (gonadotrophin releasing hormone analogue)-containing microspheres, 22.6 g of GnRHa (82.3% active ingredient) was dissolved in 100 µL of distilled water containing 25 µg of gelatin. This solution was kept at 65 ℃ until addition of 1 mL of methylene chloride containing 452.4 µg of p (FAD-SA) of molecular weight 43 kDa. The mixture was agitated and the coarse emulsion was cooled on ice. It was slowly allowed to reach room temperature, after evaporation of the $MeCl_2$, the microspheres were filtered through a 250 µg sieve in order to remove any amorphous material. The microspheres were then weighed and recovery was estimated by the ratio of the weight of collected microspheres to the combined weight of the polymer, gelatin and GnRHa used. The microspheres were stored at -20 ℃ under a nitrogen atmosphere the amount of GnRHa entrapped in the microspheres was estimated indirectly by measuring the amount of GnRHa in all solutions used for microsphere preparation and rinsing of the microspheres [10]. It was found that 1.6 mg microsphere/kg of body weight contained 50 µg GnRHa/kg of body weight. The shape and size of the microspheres were evaluated using a compound microscope at 40× magnification.

2.3 Ovulation and Spermiation

Studies on *in vitro* release and degradation and *in vivo* GnRHa release were carried out by the techniques [10]. In order to evaluate the efficiency of the GnRHa loaded p (FAD-SA) microspheres in inducing ovulation and spermiation, the experiments with captive reared anemone fish, *Amphiprion sebae* were designed according to the methods suggested [10]. In the first experiment six sexually matured females (15 ± 1 g) were injected with microphere vehicle only. In order to assess the progress of ovulation of various times (multiples of five days) after treatment the ovarian biopsy was collected from test fish. These test fish were reared in rectangular glass tanks (150 L each) connected with flow through water system under ambient temperature ranging from 25-30 ℃.

In the second experiment, six sexually matured males (15 ± 1 g) were injected with GnRHa loaded microspheres at a dose of 1.6 mg microspheres/kg of body weight (50 µg GnRHa/kg of the body weight). The other group of six fishes was injected with microsphere vehicle only and this group served as the control. At every two days after treatment, the total amount of sperm was collected from all tested males by stripping. In the third experiment, six sexually matured females (15 ± 1 g) and six spermiating males (15 ± 1 g) were maintained in aquarium tanks (150 L). The test fish were treated with 1.6 mg microsphere/kg body weight (50 µg GnRHa/kg body weight). Six pairs of control fish were also maintained for comparison. The females were tested for ovulation for every three days after treatment. The sperm was collected from test fish once a week after post-treatment by stripping.

3. Results and Discussion

3.1 Bioenergetic Studies

The results of 60-day feeding trial using live feed and flesh feed in the bioenergetics of fishes were tabulated in Tables 3-5. Effects of different types of live feed on feed utilization in *Amphiprion* are shown in Table 3. The highest feeding rate of 279 J/g live fish/day was observed while the test fish were fed with *Moina*. Similarly the fish fed with pelleted feed exhibited the lowest feeding rate of 104 J/g live fish/day. The absorption efficiency showed the opposite trend. For example, the fish fed with pelleted diets had the highest absorption efficiency (36%) whereas the fishes fed with the *Brachionus* showed the lowest efficiency in absorption (28%). The analysis for crude protein in the chosen experimental feeds showed that *Moina* contained 45%, *Brachionus* contained 40%, *Artemia* 40%, *Daphnia* 35% and the pelleted feed also contained 35% of protein. The highest level of protein content in *Moina* may be one of the reasons for the better growth performance of *Amphiprion* fed with *Moina*. Even though *Brachionus* and *Artemia* were found to contain same level of protein there was significant variation in the feeding rate of *Amphiprion* fed on *Brachionus* (332 J/g live fish/day) and *Artemia* (277 J/g live fish/day). Similarly dietary protein level of *Daphnia* and pelleted feed were same (65%). But intake of experimental feeds differed in the fish tested with *Daphnia* (252 J/g live fish/day) and pelleted feed (168 J/g live fish/day). These observations suggest that *Amphiprion* is more sensitive to the type of feed used at the time of captive rearing. Similar, the maturity level in *A. sebae* fed with a diet of clam meat, supplemented with polychaete worms for 3 months [11, 12]. Among the other treatments, fresh fish and a combination of fresh fish and squid on alternate days, showed lower weight gain, which was similar to the findings, which reported that *A. sebae* fed with trash fish and commercial feed had lower maturation efficiency.

Effect of different types of flesh feed on feed utilization in *Amphiprion sebae* are showm in Table 4. The highest feeding rate of 1,644.7 J/g live fish/day was observed while the test fishes were fed with

Table 3 Effects of different types of live feed on feed utilization in *Amphiprion sebae*.

Feed utilization parameters	*Moina*	*Brachionus*	*Daphnia*	Pelleted feed
Feeding rate	279 ± 4	221 ± 7	168 ± 4	104 ± 4
Absorption rate	202 ± 9	180 ± 5	140 ± 5	91 ± 2
Conversion rate	99 ± 9	96 ± 4	63 ± 3	46 ± 4
Absorption efficiency	32 ± 2	28 ±2	32 ± 3	36 ± 2
Conversion efficiency	11 ± 1	10 ± 2	8 ± 2	7 ± 1

Table 4 Effects of different types of flesh feeds on feed utilization in *Amphiprion sebae*.

Feed utilization parameters	Types of feed			
	Beef	Clam	Poly chaete worm	*Artemia*
Feeding rate	1,428 ±5	1,503.3 ±7	1,644.7 ±6	1,608 ±4
Absorption rate	1,400 ±9	1,468.8 ±5	1,645.9 ±3	1,581 ±5
Conversion rate	67.2 ±6	44.92 ±4	66.52 ±5	108 ±3
Metabolic rate	1,332.8 ±2	1,423.8 ±2	1,578.2 ±3	1,473 ±3
Absorption efficiency	98.04 ±2	97.7 ±3	97.3 ±2	98.32 ±3
Growth conversion efficiency	4.71 ±1	2.99 ±2	4.04 ±1	6.72 ±2
Net conversion efficiency	4.8 ±1	3.06 ±3	4.05 ±3	6.83 ±3

Table 5 Impact of feed variation on growth in clown fish, *Amphiprion sebae* under laboratory conditions.

Feed utilization parameters	Beef	Clam	Polychaete worm	*Artemia*	Pelleted feed
Feeding rate	1,123.2	1,260.0	1,398.4	1,372.8	1,257.9
Absorption rate	1,094.4	1,224.0	1,349.7	1,335.3	1,221.3
Growth rate	68.0	72.0	80.88	38.68	28.8
Metabolic rate	1,026.4	1,152.0	1,268.8	1,296.6	1,192.5
Absorption efficiency	97.4	97.14	96.52	97.27	97.08
Growth conversion efficiency	6.05	5.88	5.78	2.82	2.28
Net conversion efficiency	6.21	5.88	5.99	2.89	2.36

polychaete worms. The feeding rate for beef was the lowest as 1,428.0 J/g live fish/day. Clam was 1,503.3 J/g live fish/day and *Artemia* was 1,608.0 J/g live fish/day. Similar trend was observed in other food parameters like absorption rate and metabolic rate. But, the absorption efficiency showed the opposite trend. The absorption efficiency for *Artemia* was high as 98.32% and for poly chaete worm it was very low as 97.3%. Beef has the absorption efficiency of 98.04% and for clam it was 97.7%.

From the above results, we came to knowing that when the fish were fed with polychaete worm it showed the highest feeding rate and the fish fed with beef showed the lowest feeding rate. The fish fed with *Artemia* and beef had the highest absorption efficiency (98.32% and 98.04% respectively) whereas the fish fed with the clam and polychaete worm showed the lowest efficiency in absorption (97.77% and 97.3% respectively). The highest level of EFA and protein content in polychaete worms may be one of the reasons for the better growth performance of *A. sebae* fed with polychaete worm. Even though *Artemia* and clam were found to contain some level of protein there was significant variation in the feeding rate of *A. sebae* fed on clam (1,503.3 J/g live fish/day) and Artemia (1,608 J/g live fish/day). These observations suggest that *Amphiprion* is more sensitive to the type of feed used at the time of captive rearing. There is limited work in the field of bioenergetics for *A. sebae*. Polychaete worm fed fishes had the highest feeding rate and conversion efficiency. So, we have to conclude that polychaete worm is best fed for *A. sebae*.

Ornamental fish keeping has increased in popularity over the past 50 years or so with a resulting increase in demand for knowledge on the feeding and care of fish [12]. But even today the dietary requirements of ornamental fish remain one of the least explored areas of animal nutrition. Most of the data currently available are based on research carried out by the aquaculture industry on a relatively small number of commercially formed food fish species, which is of limited relevance to ornamental fishes, because they are kept under totally different husbandry regimes [13]. Now-a-days pomacentrids are popular among marine aquarists all over the world because they are generally small and hardy. They have attractive colours. They are highly adaptable to life in captivity and display interesting behavior [1]. But very little work has been done on the acclimation of bioenergetics of commercially important marine ornamental fishes like the clown fish, *A. sebae*. Therefore, the present work intends to study the effect of various types of feed on food utilization in clown fish, *A. sebae*.

The fishes were distributed among the tanks at a stocking density of 6 fish/tank with treatments in triplicate. Four types of feeds—beef, clam, artemia and polychaete worm were used during the experimental studies. The test animals were fed twice a day at 6.00 a.m. and 8.00 p.m. at the rate of 15% of their body weight. The maintenance of fish and feeding was as same as in the previous experiment [9].

3.2 Fecundity Studies

3.2.1 Impact of Feed Variation on Fecundity in Clown Fishes

In the beef fed fishes, the feeding rate was 1,123.3 J/g live fish/day and their average number of eggs were 335. The hatching rate was 85.97% and spawning interval days were 18. The clam fed fishes showed the feeding rate of 1,260.0 J/g live fish/day. These fish laid around 900 eggs/spawning. The hatching rate was 88.88% and spawning interval days were less than beef fed fishes. The produced eggs are subjected to fanning and mouthing by parent fish. The infected eggs were also removed by the mouthing process as shown in Plates 3-8. The polychaete worm fed fishes showed the highest feeding rate of 1,398.40 J/g live fish/day and their total number of eggs per spawning is very high as 1,126 eggs when compared to other fishes. The hatching rate is 100% and spawning interval days were 9 in polychaete worm fed fishes while in other fishes the spawning interval days were above 12 days. When the fishes were fed with *Artemia*, their feeding rate was 1,372.8 J/g live fish/day. The fish laid 1,002 eggs/spawning and the hatching rate was 95.21%. The spawning interval days were reduced to 13 (Table 6). The ammonia level was found to be within the permissible range even though it showed slightly higher level in trash fish and clam meat experiments [14]. The pelleted feed fed fishes showed the lowest feeding rate of 1,257.9 J/g live fish/day and they laid less number of eggs 328. Their hatching rate was 84.75% and the spawning interval days were 18. From this we came to knowing that the clown fishes cannot acclimatize to the pellet feed and they only preferred live feed. From the above experiment, it is understood that feed also plays a main role in fecundity of the fishes. The polychaete worm fed fishes spawned earlier than any other feed fed fishes. The hatching rate is also higher and the spawning interval days are also very much reduced (Table 7, Fig. 1 & Plates 9 and 10).

3.2.2 Induced Breeding

The 35-day treatment of matured female fish with GnRHa-loaded p (FAD-SA) microspheres induced ovulation in 65% of the tested sexes and when the treatment was prolonged to 50 days, 80% of the experimental fishes ovulated (Fig. 2). The ovulation started 15 days after hormonal treatment, 50% of ovulation was achieved after 30 days of treatment and 80% ovulation was obtained after 50 days of treatment. Total failure of ovulation in non-treated fish underlines the fact that in captivity, the ovaries in the females do not undergo the maturation process of the

Table 6 Impact of feed variations on fecundity in clown fish, *Amphiprion sebae* under laboratory conditions.

Feed	Total number of eggs	Un hatched eggs	Hatching rate (%)	Spawning interval days
Beef	335	47	85.97	18
Clams	900	100	88.88	16
Pelleted feed	328	50	84.75	18
Polychaete worm	1,126	-	100	09
Artemia	1,002	48	95.21	13

Table 7 Spawning, hatching and survival data of *Amphiprion* spp.

No. of spawning	Date of spawning	Date of hatching	No. of eggs	Survival rate (days)					
				1	5	7	14	21	30
I	January 17 6-6.45 p.m.	January 24 8.30-10 p.m.	300	95% 285	88% 264	81% 243	79% 237	74% 222	71% 213
II	January 24 5-6 p.m.	January 31 7.30-9 p.m.	800	98% 784	90% 720	82% 656	78% 624	73% 584	68% 544
III	January 27 3-4 p.m.	February 3 10-12 p.m.	450	97% 436	93% 418	90% 405	84% 420	78% 351	72% 324
IV	February 5 7-8 a.m.	February 12 11.30-1 a.m.	1,000	100% 1,000	91% 910	87% 870	81% 810	76% 760	71% 710

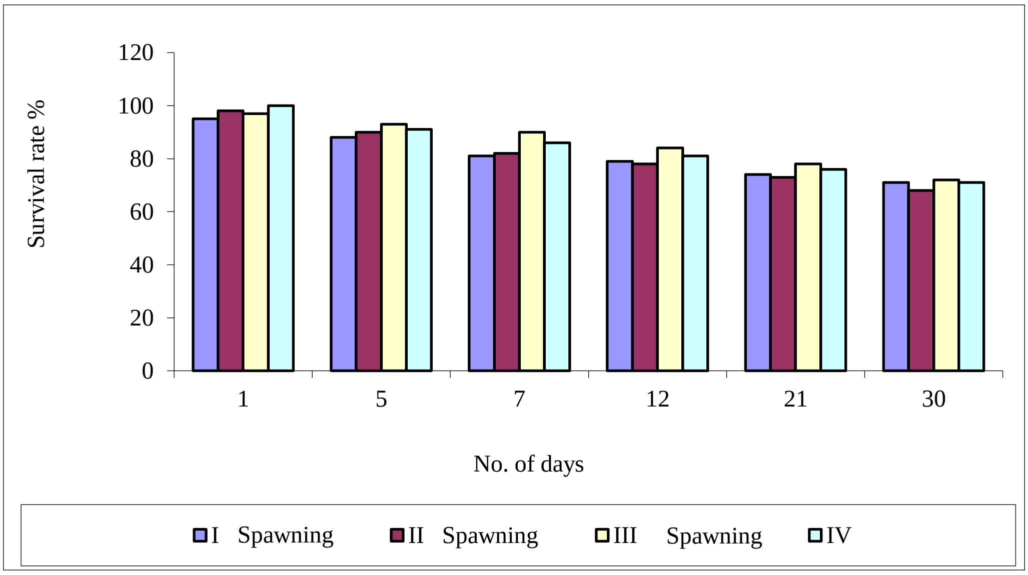

Fig. 1 Survival rate of eggs laid by *Amphiprion* spp. at varied spawning periods.

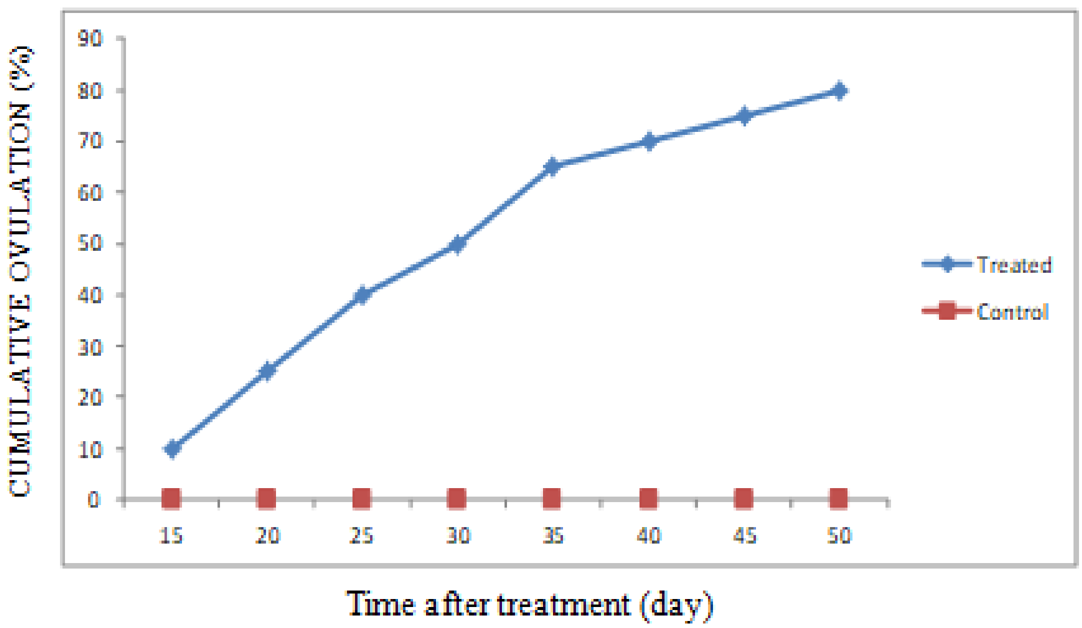

Fig. 2 Cumulative percentage ovulation in *Amphiprion sebae*. Brood stock versus time (days) in response to treatment with GnRHa (gonadotrophin releasing hormone analogue) loaded p (FAD-SA) microspheres (50 µg GnRHa/1.6 mg microsphere).

ovary (Table 8). In the second group of experiment, the GnRHa loaded p (FAD-SA) microspheres were effective in enhancing sperm production (Fig. 3). The mean total sperm volume of GnRHa microsphere treated fish was higher, when compared to control at every sample time within the duration of two weeks of experimental period. Two days after treatment, sperm production was doubled when compared to sperm in control, but in males treated for 14 days the sperm production was 4 times higher than that of the control (Table 9). This increased sperm volume was the clear indication of higher sperm cell production.

In the third experiment, all the females and males treated with GnRHa loaded microspheres ovulated and spermiated within fourteen days of treatment (Fig. 4). This higher enhancement of ovulation and spermiation in a shorter period of treatment may be attributed to the injection of GnRHa-loaded microspheres (1.6 mg microspheres having 50 µg GnRHa/kg of body weight). The first fish spawned after 6 days of treatment and the percent of ovulation was obtained within 21 days. At the same time, in the control fishes

Table 8 Cumulative percentage of ovulation in *Amphiprion sebae* treated with GnRHa (gonadotrophin releasing hormone analogue) loaded p (FAD-SA) microspheres (50 µg GnRHa/1.6 mg microsphere).

Days	Cumulative of ovulation (%)	
	Treated	Control
15	10	0
20	25	0
25	40	0
30	50	0
35	65	0
40	70	0
45	75	0
50	80	0

Table 9 Variation in sperm volume mL/g in relation to treatment period in GnRHa microsphere treated *Amphiprion sebae* males.

Days	Cumulative of ovulation (%)	
	Treated	Control
0	0.2	0.1
2	0.2	0.1
4	0.35	0.15
6	0.42	0.15
8	0.56	0.20
10	0.64	0.25
12	0.76	0.20
14	0.8	0.20

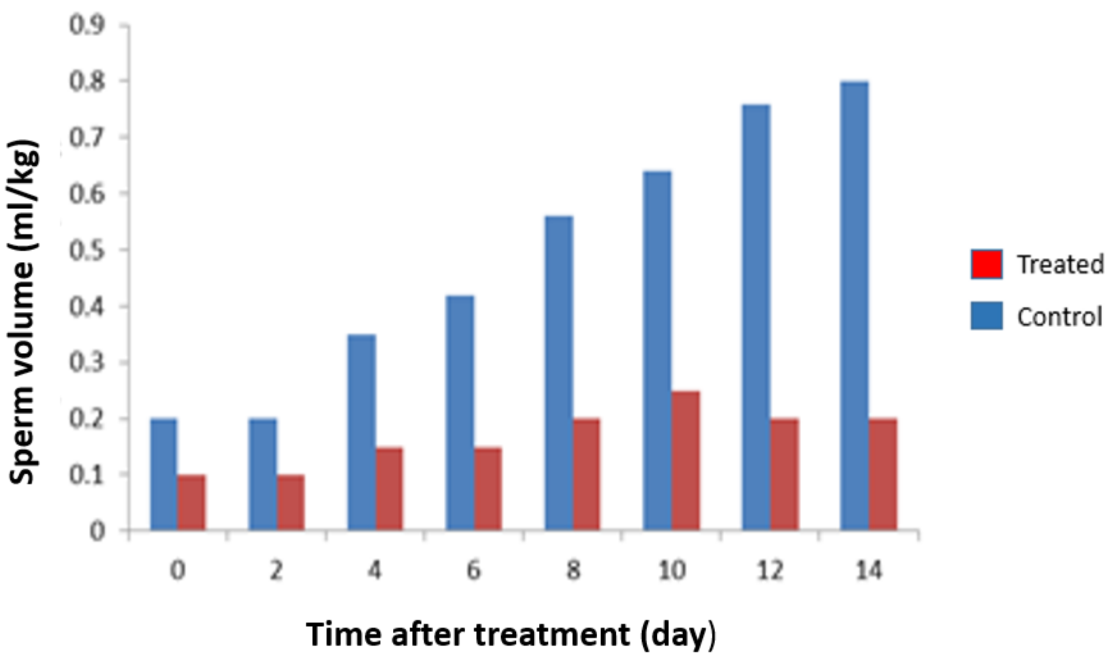

Fig. 3 Total sperm from six *Amphiprion sebae*. Brood stock in response to treatment with GnRHa loaded p (FAD-SA) microspheres (50 µg GnRHa/1.6 mg microsphere).

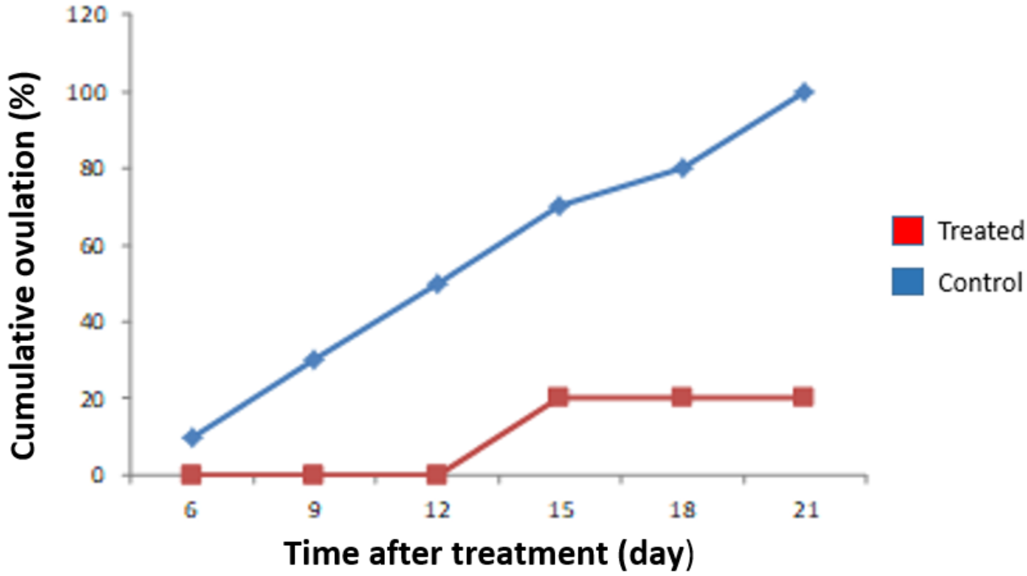

Fig. 4 Cumulative percentage ovulation of GnRHa microsphere treated with six *Amphiprion sebae*.

Table 10 Variation in cumulative percentage of ovulation in relation to treatment period in matured *Amphiprion sebae*.

Days	Cumulative of ovulation (%)	
	Treated	Control
6	10	0
9	30	0
12	50	0
15	70	20
18	80	20
21	100	20

Table 11 Variation in sperm volume (mL/kg) in relation to treatment period in GnRHa microsphere treated *Amphiprion sebae*.

Days	Cumulative of ovulation (%)	
	Treated	Control
7	0.6	0.2
14	0.8	0.2
21	1.0	0.2

the ovulation commenced only after 15th day (Table 10). After two days of injection, the sperm production was increased three times more than control. After 21 days, microsphere treated fishes produced five times more sperm than control (Table 11). The presence of both male and female fish in one anemone may be another favorable factor for the higher inducement of ovulation and spermiation in all the test fishes. The presence of opposite sex may cause the natural inducement of hormonal secretion in both sexes [15].

The above observations corroborate the results obtained in the studies made by Mylonas *et al.* [10] in striped bass and Atlantic salmon. In the third experiment, treatment of both male and female fish with GnRHa-loaded microspheres induced spermiation and ovulation in all the test fish within 21 days (Fig. 5). Captive-reared marine fishes can undergo final oocyte maturation and spermatid formation leading to optimum ovulation and spermiation without any hormonal manipulation [15]. Unfortunately, females do not spawn in captivity and the eggs have to be stripped manually and fertilized artificially [10]. Moreover, ovulation in many of marine fishes is asynchronous within brood stocks and it may prolong from 1 to 3 months for all the fish to ovulate. During this period, the test fish have to be verified for ovulation frequently (2 to 3 times a week). This may cause handling stress leading to very high mortalities. GnRHa has been successfully used in the past to synchronize ovulation in various cultured Salmonids [15]. A GnRHa delivery system can alleviate the need for repetitive handling then by preventing mortality

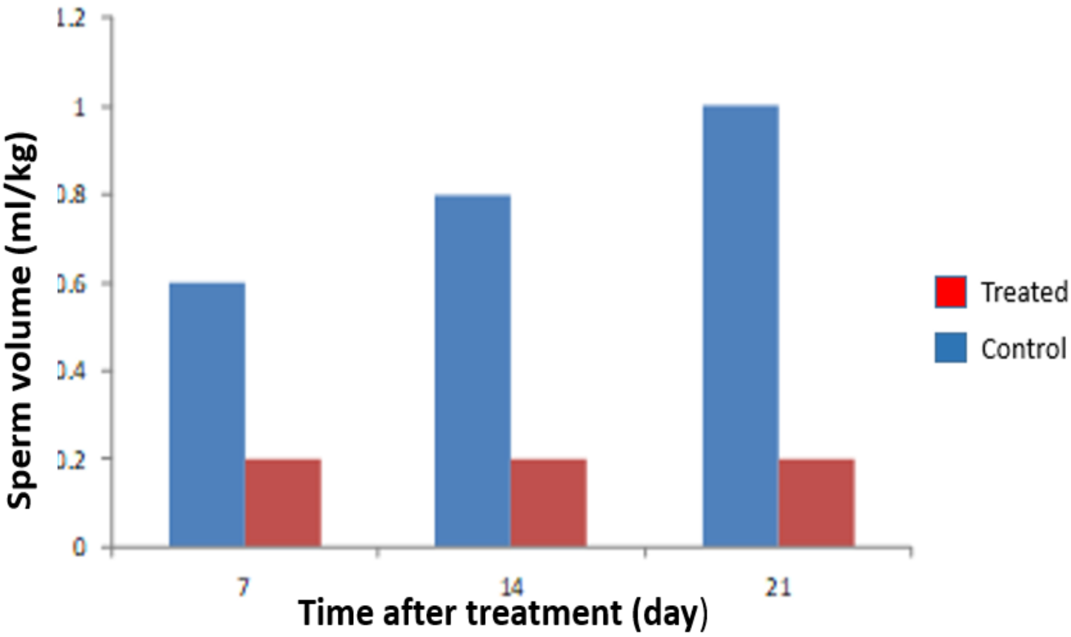

Fig. 5 Mean total sperm from GnRHa microsphere treated six male *Amphiprion sebae* reared in cement tanks having both E and T fish in one anemone.

due to handling stress, furthermore, due to the long-acting properties of p (FAD-SA) microspheres, a single treatment can successfully induce ovulation up to 6 weeks prior to the natural onset of ovulation [10]. The growth and survival of clown fish *A. chrysogaster* fed with various feeds. Among the feed combinations, fishes fed on polychaetes and live acetes grew faster and showed better colour quality [16]. The sperm availability is a general factor for consideration in aquaculture, and in some situations it may be a limiting factor [17]. In hatcheries where the eggs are mainly stripped from the females and fertilization is achieved *in vitro* with an excess of sperm there is often shortage of sperm. Hence, relatively large amounts of sperm are collected from a selected group of males every time a female is found to have ovulated. Sperm collection may occur many times over the course of a week, and often over the course of a day, and as a result the males can run out of sperm (Fig. 4). Hormonal manipulations can be used to increase sperm production but the effects are short-lives, or require multiple treatments ranging from once weekly to once daily [18-21].

4. Conclusion

From the various results obtained during the present study it suggested that polycheate worm fed fishes exhibited the highest number of egg laying, highest hatching rate, less number of spawning interval and highest feeding rate. The conclusion of the study revealed the GnRHa delivery system can be better used to increase sperm production many times and also for a prolonged period of time in clown fish under laboratory condition.

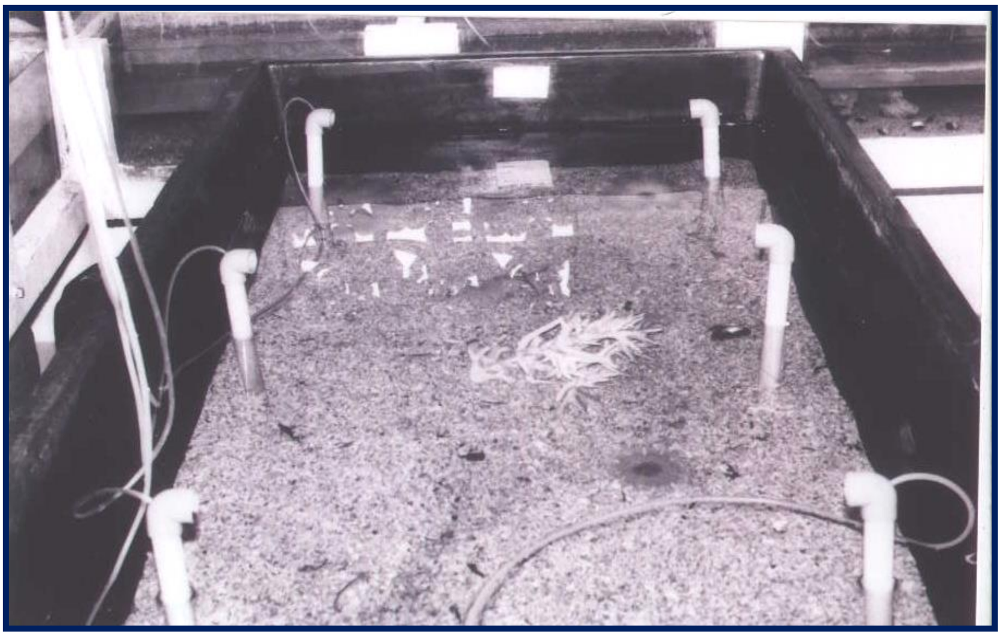

Plate 1 Mother aquarium.

Plate 2 Breeding tank.

Plate 3 Spawning process.

Plate 4 Eggs in circular patch.

Plate 5 A single egg.

Plate 6 Fanning by male.

Plate 7 Mouthing by the male.

Plate 8 Infected egg.

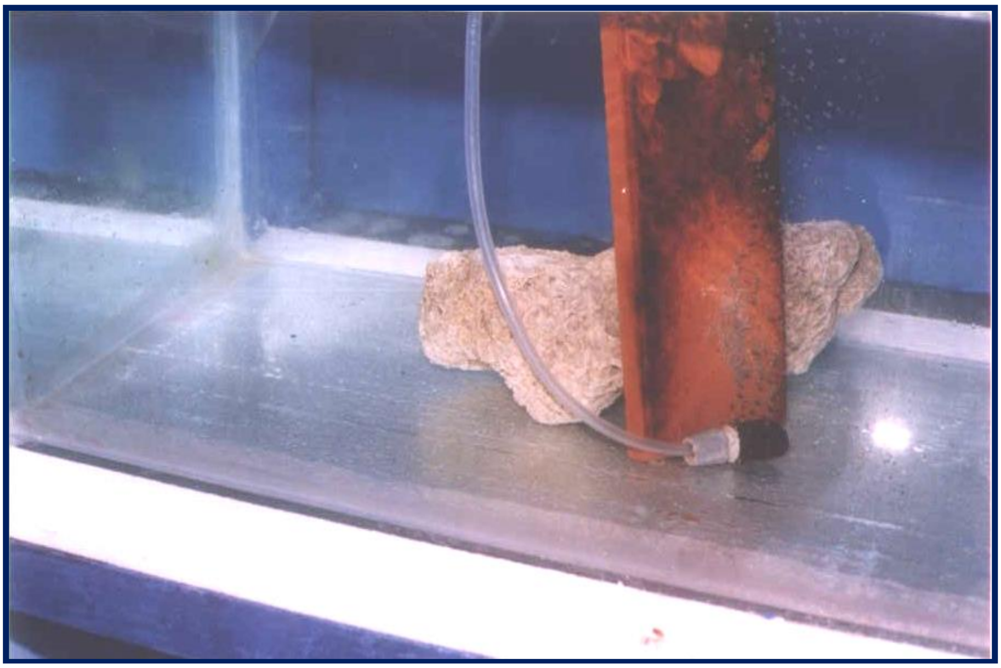

Plate 9 Eggs in hatching tank.

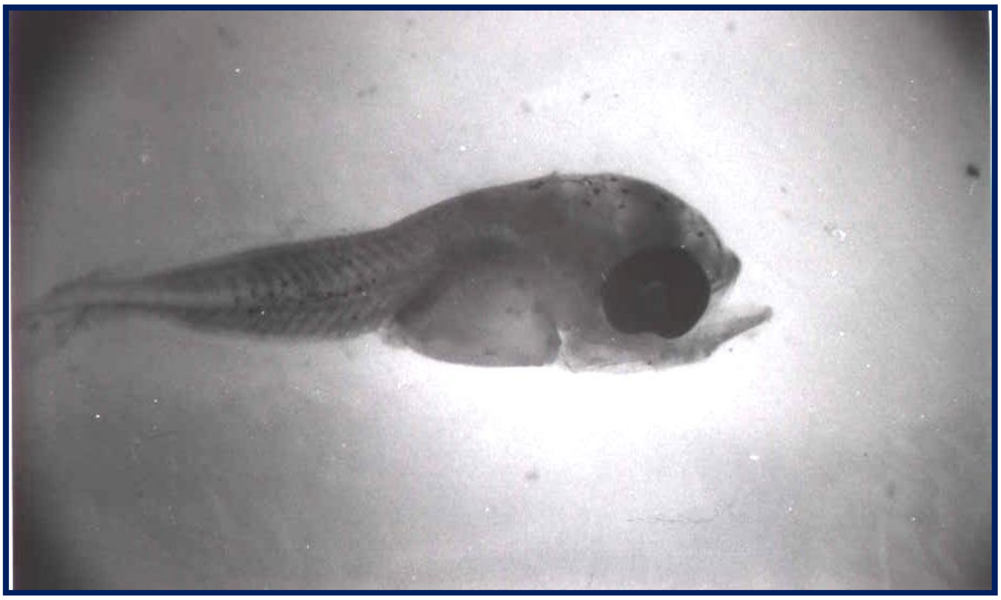

Plate 10 Newly hatched larva.

Acknowledgement

The authors are grateful to the secretary, principal, of Kamaraj College, Tuticorin, for providing their support to carry out this research work. The authors also would like to express sincere gratitude to the funding agency, OSTC (Ocean Science and Technology), New Delhi Project Sanctioned No: 30.3.2000/ Ref: DOT/11-MRVF/4/1/UN/97/P-5.

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