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Effect of boodstock nutrition on reproductive performance of clownfish *Amphiprion* sp

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Abstract
The marine ornamental fish trade moves nearly 20-25 million live fish worldwide per year. Nearly 90% of these are wild caught from the pristine coral reef environments. The demand for fishes is on the rise and it has led to depletion of natural fish stocks around the globe. Clown fishes (*Amphipirion* sp.) are among the most popular marine ornamental fish, and reliance on the natural reefs has led to depletion of stocks. The efforts to reduce dependence of wild stocks as well as to ensure market sustainability spurred research into captive breeding and several researchers have successfully bred many species of clown fish. Breeding of clown fish is important to reduce the dependence on wild caught. Broodstock nutrition is one of the major hurdles faced by breeders, an improvement in broodstock nutrition improve the brood quality. Traditionally fresh natural diets are used for feed broodstock development. The fresh diets such as squid (38%), cuttlefish (50%), bivalve meat, krill (10%) and other small crustaceans, fish gonads, polychaetes, other worms are popular maturation diets. In this study conducted at Mandapam Centre for Sustainable Aquaculture (TNJFU). With several diets, a combination fresh fish with clam was observed to be the best for gonadal maturation of *A. sebae*.

Keywords: Aquariculture, clownfish, broodstock nutrition, maturation diets

1. Introduction
Marine aquarium keeping has gained momentum among people all over the globe. Recent developments in aquarium technology have made marine ornamental fishes important interior decor in households. Coral reefs are called as rain forest of the sea and it is considered as most diverse productive ecological environment comprising more than 4000 species of fin fish and shell fishes, 800 species of coral rocks including more number of other species of sponges and invertebrate sponges [27]. More than 50 reef fish families with 175 genera and about 400 species are distributed in the Indian seas. The relation between ornamental fishes and corals are complex one.

Anemone fish or the more popularly known clownfish, is one of the most sort tropical marine ornamental fish [28], due to its small size, attractive color, peculiar behaviour patterns, and the symbiotic association with sea anemones. The anemone fish is omnivorous and attains a standard length of about 100 mm, depending on the species. They are highly adaptable species and display interesting behaviour in captivity [13]. Alava and Gomes (1989) [3] first noted the association between a giant sea anemone and a beautiful clown in the South China Sea. Clownfishes belong to the family of Pomacentridae, one of the largest groups of reef fishes, inhabiting tropical and subtropical seas and members of this family incorporate 29 genera and 350 species under four subfamilies [5]. There are 29 species of Clownfishes with two intergeneric hybrids between *Amphiprion* and *Premnas* [4].

Commercial marine fish hatcheries depend heavily on wet diets from marine side. Fresh natural diets are traditionally used for feed brood stock development. This is the most effective way of meeting the nutritional needs of fish and ensuring good quality eggs. Certain hatchers use fresh marine products along with commercial maturation diets for attaining higher success rates. The fresh diets commonly used are squid, cuttlefish, bivalve meat, krill and other small crustaceans, fish gonads, polychaetes, other worms and enriched products.

Though research on marine ornamental fishes had made rapid strides in recent years, a great deal of refinement is still required to make this as viable industry. The development of exclusive and nutritionally complete feeds is of utmost importance in evolving farming and aquarium keeping technology packages for these fishes.

For rapid growth in hatchery side, the availability of suitable artificial feeds and feeding regimes are the key factors. Considering these valid aspects, the present review taken on "Effect of broodstock nutrition on reproductive perfomence of clownfish *Amphiprion sp*.

2. Ornamental Fish Trade

The aquarium fish trade moves more than two billion live fish worldwide per year. For fresh water organisms, more than 90% of them are captive bred, but in case of marine ornamental fishes over 90% are wild-caught (17). Coral reefs and adjacent areas are major source of wild caught organisms. To a great extent, the organization of the ornamental species trade is highly complex and dynamic, involving more than two million people worldwide, from collectors to hobbyists including governments, airlines, associations, etc [17].

According to the Food and Agriculture Organization of the United Nations [19], the ornamental species trade represents only 0.5% of the international fish trade. However, its importance goes beyond its share in the international market. According to the FAO data, the volume of live fish exported increased in value from USD 21.5 millions in 1976 to USD 315 million in 2007 [44]. Specifically, this sector plays an important role in providing income and employment in developing countries. India has been exporting some unique varieties of wild caught ornamental fish to many developed countries of the world since 1969. But, our contribution to global export of ornamental fish remains only 0.32% of a total of US $362 million [20].

The estimated annual global trade of marine organisms involves between 20 and 25 million live fishes, 12 million pieces of coral and 10 million other invertebrates (44). The ornamental trade is dominated by freshwater species; however, the increasing popularity of coral reef aquaria has become a leading trend since the late 1980s. Prices have become increasingly affordable for European and American markets [47].

The UNEP World Conservation Monitoring Centre Report on the Global Trade In Marine Ornamental Species (2003) reported that 69 fish species associated with captive breeding, which was in sharp contrast with the Marine Fish Breeding Records (MFBR), which reported 575 species, of which 211 were recorded as being bred and grown to the juvenile stage and beyond [37]. The main marine fish groups traded are Pomacentridae, Acanthuridae, Balistidae, Labridae, Pomacanthidae, Chaetontidae and Syngnathidae primarily due to their appearance, colour, robustness and long life (47). Marine fish species constitute more than 15% of the market by value, with about 98% collected from the wild while the rest are captive-bred. Inspite of difficulty in maintenance and expensive, still marine aquarium keeping emerging as a greater hoppy.

The value of ornamental fish trade has grown significantly over the past decades. Between 2000 and 2011, global exports of ornamental fish increased from US$181 million to US$372 million. Total trade in live marine ornamentals is estimated at around US$44 million annually. Most of the market supplies originate from Asia, with Singapore dominating as the top exporting country in the world. In 2013, Singapore exported around US$56 million worth of ornamental fish to over 80 countries [21].

In India, about 90 percent of ornamental fish is traded from Kolkata port followed by 8 percent from Mumbai and 2 percent from Chennai [24]. Global fish market records were established in 1976 with just 28 countries exporting ornamental fishes, later increased to 105 in 2004; presently more than 125 countries are involved in this trade. The global import of ornamental fishes began with only 32 countries in 1976, later their numbers increased significantly and reached 130 in 2001; presently more than 150 countries are involved in this trade [44].

The global export market rose steadily from 2000 valued atUS$177.7 million and reached a peak value of US$364.9 million in 2011 then declining slightly to US$347.5 million in 2014 [14]. The ornamental fish exports from India showed an increasing trend and exponential growth over the years. The share of India in world ornamental fish exports fluctuated and remained less than one percent for most of the years. India's share in the world market ranged from 0.12% to 1.16% during 1991-2009 and India gained the highest market share of 1.16% during the year 2007 US$ 1.06 million. Export value for the Indian ornamental fish industry in 2016 stood at US$ 1.06 million and contributed to 0.3% of the total export. India ranks 31stposition among world exporting countries. During the fifteen-year period from 2000 to 2014, the import value for ornamental fish rose from US$ 247.9 million in 2000 to an all-time high of US$402.1 million in 2008. Thereafter, there was a declining trend until 2013 (US$287.2), and then a slight rise (+4.1%) to US$299 million in 2014 [14].

3. Nutritional Requirements of Ornamental Fishes

Protein is the most significant and expensive ingredient in fish feed, representing about 60% of fish feeds. Therefore, best use of dietary protein is necessary for economical production [6]. Kruger *et al*., [35] reported that a diet should have at least 45% crude protein and 6% lipid level for better growth performance in *Xiphophorous helleri*. Lovell [39] reported that there are some factors that differentiate the nutritional requirement in fish, they can absorb minerals through the gills and some fish require more dietary unsaturated fatty acids and vitamin C (ascorbic acids), ornamental fish are known worldwide, despite the nutritional requirement has not been studied deeply and so far it has been formulated based on the information from other species used in aquaculture.

According to Elangovan and Shim [18], the comparison of protein requirements between fish species is complex since this can vary according to the size, life stage, diet formulation and farming condition. In Red tailed tinfoil (*Barbodes altus*), the optimal dietary protein has been reported to be 41.7% with positive effect on weight gain. According to Sales and Janssens [49], the lipids are important sources of energy and fatty acids are essential for normal growth and survival. Ling *et al*. [38] suggested that the muscle lipid content acts as a source of lipid in the ovary making it as a useful indicator of reproductive performance. Increase in dietary lipid from 8 to 16% with the same protein level, improved the growth performance in Swordtail. The muscle lipid content had the same trend as the protein level, with the highest accumulation observed with the highest dietary lipid. Afzal khan *et al*. [1] observed that fish fed with low dietary protein had lower egg protein content. Similarly, decrease in the ovarian mass was observed in fish fed with 20% of dietary protein as a result of poor oocyte development; in contrast, the Hepato Somatic Index was higher in fish fed with 30% of dietary protein level, probably as a mechanism to improve the vitellogenesis.

4. Broodstock Nutrition

Broodstock nutrition plays a major role in the development of gonads and is one of the most important research areas in aquaculture [57]. The dietary requirement of broodstock is different from those of rapidly growing juvenile fishes. Nutrient requirements vary depending on species, size, developmental status, sex, gonad, and egg maturation process. There are many reports on the influence of nutrition on growth, gonad maturation and reproduction in ornamental fishes. The role of nutrients and supplementary feeds on regulating the reproductive physiology of broodstock is well documented. It has been reported that reduction in feeding rate can cause an inhibition of gonadal maturation in several fish species including goldfish. Since, the diets play a major role in the maturation and further breeding of ornamental fishes [25].

In marine fishes' reproductive performance is unpredictable and is the major limiting factor hindering seed production. Broodstock performance is determined by biological factors such as age and size of the fish, diet, physiological status, genetic makeup, health etc. and abiotic factors such as water quality, photoperiod, season, rearing system *etc.* The diet provided to the broods considerably influences the success of hatchery operations directly by affecting the fecundity, fertilization rate, egg quality, embryo development and larval quality [10, 23, 32].

In fishes with a short vitellogenic period, the gonadal development and fecundity can be manipulated by providing proper enriched diets shortly before or during spawning [32]. In fishes like sea breams, brooders continue to feed during spawning and the nutrient composition of egg and the larvae are greatly influenced by the diet within short duration [59, 55]. Clownfishes hold several vital characteristics viz., continuous spawning nature, (it spawns two to three times a month), early maturity, easy to maintain and manage fairly large groups of individuals in a small area, acceptance of formulated diets, etc. These fishes expressed the influence of changed dietary carotenoids within a short span of 48 h [58].

Several methods have been developed to assess the egg quality of fish [34, 22]. Fecundity, is used to determine egg output, which is primarily affected by a nutritional deficiency in broodstock diets. Fecundity is the total number of eggs produced by each fish expressed either in terms of eggs/spawn body weight. Reduced fecundity, reported in several marine fish species, could be due to influence of a nutrient imbalance on the brain-pituitary–gonad endocrine system or by the restriction in the availability of a biochemical component for egg formation.

Enrichment of broodstock diet is important as it has a considerable influence on the success of hatchery operations directly by affecting the fecundity, fertilization rate, egg quality, embryo development, and larval quality. Nutrition of brood fishes critically influences breeding success and is vital during early larval stages, especially during the yolk nourishment stage. Despite the remarkable advances in the field of fish larval and juvenile nutrition, new frontiers need to be explored regarding the nutritional requirements of brood fish. Dearth in information on broodstock enrichment diet is affecting the diversification and development of potential mariculture technologies [15].

5. Maturation Diet in Marine Aquariculture Practices

In an aquaculture system, feed contributes 60-80% of the total operational cost, which is a major challenge for the industry. The availability of an optimal diet is a crucial factor for the maturation and reproduction of shrimps. It has been reported that an unbalanced or incomplete diet causes poor reproductive performance or may even stop animals from reproduction [8].

Feed contributes 60-80% of the total operational cost in aquaculture system which is a major challenge for aquaculture industry. The availability of an optimal diet is a crucial factor for the maturation and reproduction of shrimps. It has been reported that an unbalanced or incomplete diet causes poor reproductive performance or may even stop animals from reproduction [8].

6. Broodstock Dietary Sources

In general, commercial marine fish hatcheries are heavily dependent on fresh diets of marine origin. Fresh marine products along with commercial maturation diets are also used in certain hatcheries. The fresh diets commonly used are squid, cuttlefish, bivalve meat, krill and other small crustaceans, fish gonads, polychaete and other worms, and enriched products. Formulated commercial maturation diets are available only for a few species. Most of these diets are prepared by using high quality nutrient sources to meet the requirements of essential fatty acids and amino acids and the additives which are expected to boost/enhance the brood stock performance. The major constraints with these fresh diets are disease transmission, nutrient imbalance, higher cost and the difficulties in production, maintenance and storage [58].

The ultimate goal of the brood stock nutrition studies is to optimize dietary nutrients to maximize egg production and quality. To achieve this, it is essential to understand the factors influencing initial recruitment of oocytes into the pool of maturing eggs. A properly formulated broodstock diet must satisfy requirements for higher fecundity as well as egg quality for an optimum spawning performance. The better understanding of nutritional factors involved in maturation and spawning processes and their interactions with other factors are critical to meet the future aquaculture demand [52].

7. Effect of Feed Quality

The availability of adequate quantity of feed significantly influences the reproductive performance of fishes [51, 40, 36]. Feed availability reportedly affects the process of maturation in rainbow trout [50], goldfish [50], European seabass [11], and Atlantic salmon [9]. Restricted feeding has been shown to affect the maturation and fecundity in fish [51, 54]. Food shortage resulted in lowered fecundity through follicular atresia in the rainbow trout, *Salmo gairdneri* [51]. Cerda *et al.* [11] observed that feeding the European seabass broodstock with half ration decreased the growth and spawning, and also produced smaller eggs and larvae compared to those fed full ration.

7.1 Clam as Feed Ingredient

Muthu and Laxminarayana [45] reported the maturation and spawning in ablated *P. indicus* fed with fresh clam and live mysids. Akiyama [2] showed clam powder based diet for *P. indicus*had better conversion. Clam meal possess various essential nutrients and chemo-attractant which acts on the maturation process of shrimp. Normally, clam meal consisted of 56.5% protein and 8.8% lipid, which meets the protein and lipid requirement of broodstock [30]. Maheswarudu *et al.*, [43] reported repetitive spawning of *P. indicus* for prolonged period when fed with clam meal in combination with oligochaete and squid meal

7.2 Squid as Feed Ingredient
Squid mantle, head and tentacles are often used for human consumption. After processing, the squid by-products viz., viscera, fins, skin and pen, typically amounts to about 52% of the whole weight. These by-products can be made into a meal which can be used as a high-quality feed ingredient for fish, shrimp and other animals [30]. Fresh or frozen squids are generally used as feed and are usually chopped before being fed to shrimp broodstock [12]. Squid has the highest level of protein but contains low lipid levels, however, the portion of HUFA was found to be high [30]**.** Of the total lipid present in squid mantle, 18.0% is cholesterol, which is higher than any other animal feed ingredient [46].
Squid is rich in protein (84.5%) and low in lipid content (3.1%) when compared to other fresh feeds like polychaete, clams and oysters but the portion of highly unsaturated fatty acids (HUFA)of the total lipid is relatively high (56). Cholesterol plays a vital role in animal growth and reproductive performance [2]. Squid meal is rich in DHA(>12%) and EPA(> 9%) [52]. These qualities make squid a good source of maturation diet.

8. Influence of Dietary Protein And Amino Acids
The quantity and quality of dietary protein in broodstock diets have been reported to affect the reproductive output. Varghese *et al*. [57] observed egg production to be significantly influenced by diet and cuttlefish meat gave the highest number of eggs per clutch (*Amphiprion sebae*). Broodstock diets formulated with cuttlefish meal and squid meal were found to be superior to fishmeal as a protein source in seabreams [60]. The superior performance of squid protein due to its essential amino acids (EM) composition, which resembles that of sea bream egg protein [28, 55].
In rainbow trout, broodstock fed with high protein diets (48-49% protein and 16-17% lipid) produced more eggs with a larger size than those fed diets with lower levels (36 or 42% protein, and 6 or 9% lipids) of protein [53]. An optimum protein level of 45% in the diet has been suggested for red seabream broodstock [61]. In European sea bass, a reduction of dietary protein from 51 % to 34% resulted in a significant reduction in the broodstock performance [11]; a similar trend was also observed in red seabream [60]. Dietary essential fatty acids (EFA) affect egg quality mainly through changing the egg EFA composition without any apparent effect on Vitellogen synthesis.

9. Influence of Dietary Lipid and Fatty Acids
One of the nutritional factors that have been found to greatly affect spawning quality in fish in essential fatty acids content [60, 61]. Lipids are the main constituents of the broodstock diet that directly influence the composition of eggs and larvae [59]. Lipids form an important membrane constituent and energy reserve in fish eggs. The fatty acid composition of eggs is affected by the fatty acid composition of the diet.
Harel *et al*. [29] fed gilthead sea bream broodstock with diets containing up to 1 % n-3 HUFA (highly unsaturated fatty acids) and observed that the composition of the female organs which are associated with reproduction were modified by the essential fatty acid levels of the diet and could affect egg quality over a short time period. Fernandez-Palacios *et al*., [22] reported that excessive levels of n-3 HUFA in the broodstock diet (31.5 g kg1 DW) resulted in lower fecundity and yolk-sac hypertrophy in newly hatched *Sparusaurata* larvae and a level of 16 g n-3 HUFA kg1 diet was recommended for improved spawning performance.
Dhert *et al*. [16] did not observe any significant difference in the reproductive output of *Scophthalmus maximus* when fed diets supplemented with n-3 HUFA. Spawning quality in gilthead sea bream has been shown to be improved by the elevation of the dietary n-3 HUFA up to 1.6% [22]. The egg composition and spawning quality of gilthead sea bream broodstock were affected by dietary essential fatty acid levels only three weeks after feeding [22]. In continuous spawners with short vitellogenetic periods such as sparids spawning quality seems to be affected by dietary lipids during the verge of the spawn or even during spawning [61].

10. Clownfish Breeding
Studies have been done on the captive maturation of Clownfish *Amphiprion* spp with different feed combinations; and revealed the influence of the feed combinations on the maturation, survival and metamorphosis of marine ornamentals. Combinations of various fresh feed like clam, mussel and fish meat were also used for maturation. However, the feed combinations of the indigenous marine ornamental in various space and time have not been done. ICAR - Central Island Agricultural Research Institute has successfully bred marine ornamental fishes like *Amphiprion percula* and *Premnas biaculeatus* in past and in similar attempt Sebae anemone fish, *Amphiprion sebae* was successfully bred in captivity for the first time in Andaman & Nicobar Islands at the Marine Research Laboratory [7].
First time in India, successful breeding and larval rearing of tropical clown fish *A. sebae* was accomplished at Regional Centre of Central Marine Fisheries Research Institute, Mandapam Camp. Adult pairs of clown fishes along with sea anemones collected from the in-shore waters of Gulf of Mannar were maintained in one tonne glass aquarium fitted with bio-filters. The fishes were fed with polychaete worms, clam and fish meat. After three months of maintenance natural spawning took place and the fish deposited its eggs on an asbestos substratum placed in the tank [31].
Fresh natural diets are traditionally used to feed broodstock and are the most effective way of meeting the nutritional needs of fish and ensuring good quality eggs [57]. According to Dhaneesh *et al.* [15], the maximum number of spawning in *Amphiprion sebae* was observed in the summer and spring months and the brooder fishes showed higher reproductive efficiency when they fed with live *Acetes* sp. The energy requirements of anemone fishes are higher during spawning, because of their protracted spawning nature, continuous growth even after sexual maturity, and extensive parental care behaviours. All these energy-demanding processes need to be catered through adequate diet to maintain consistent spawn quality [15].
In the case of protracted spawners like anemonefish, the diet forms a major source of amino acids required for vitellogenesis than the muscle proteins. Further studies on the process of vitellogenesis in protracted spawners are essential to establish the role of dietary nutrients and the regulatory factors. The significant variations observed in egg production between the treatments may be due to the difference in the rate of vitellogenin synthesis and uptake [57].
Captive breeding and larval rearing of tropical clown anemonefish *Amphiprion percula* was accomplished for the first time in India [42]. According to them in all the pairs significantly higher percentage of spawning was obtained 1 to 5 days after and before the full moon and new moon. 95-98%

hatchability was obtained under complete darkness on 7th day of incubation after sun set and peak hatching took place between 19.00 to 20.30 hrs.

First time in India, successful breeding and larval rearing of maroon Clown *Premnas biaculeatus* was accomplished at *Marine Hatchery of Central Marine* Fisheries *Research Institute, Kochi*. The fish bred successfully under captive conditions, within a period of 4 to 6 months of rearing. In one breeding event, they laid 115 numbers of capsule shaped eggs in the first spawning at 15.00 hrs during day lime and the spawning lasted for one to one and a half hour [41].

In general, the ornamental fish producers do not have the same restrictions as that of the producers of food fish with respect to body composition. The nutritional needs of multi-species aquarium systems are very difficult to satisfy as the feeding patterns and dietary preferences differ greatly among the fish species. Therefore, the critical requirements of a satisfactory diet for aquarium fish are different, and actually more demanding, than those for commercial food fish.

11. Conclusion

The review of literature revealed that information on nutrition of marine ornamental fishes is meager and that on their nutrient requirements are still lacking. In marine finfish hatcheries the production depends heavily on the proper (qualitative and quantitative) nutrition of broodstock and larvae. However, studies are mostly focused on captive maturation and spawning of clown fishes. The role played by different feed combinations on the growth, maturation, and spawning of clown fishes are yet to be properly acknowledged. In order to have a better understanding on the influence of different feed combinations on the maturation and spawning of sebae clownfish the present investigation was designed. More studies in similar line using clownfish as a model for broodstock studies can yield valuable information which can be applied to other marine species.

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