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Reproduction of Persian Gulf anemone fish (*Amphiprion clarkii*) in captive system

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Abstract. The present study was carried out to assess the reproduction of Persian Gulf anemone fish, *Amphiprion clarkii* (Bennett, 1830), in captive conditions with artificial features. Persian Gulf, having good relation with Indian Ocean, is one of the important niches of fishes and the specific position of this Gulf makes its fishes popular. The yellow tail clown fish which originates to this gulf has the best survival rate and health than the other areas. Live food is the most important factor in production of this species and enrichment of their live prey with probionts improve the nutritional value of fish's diet.
Key words: clown fish, jar, spawn, rotifer, artemia, Persian Gulf, Iran.

Introduction. Although Persian Gulf has a vast potential of marine ornamental fish production, an organized marine ornamental fish trade has not yet been developed in Iran. It is well known that the marine ornamental fishes are mostly associated with coral seas. The coral reefs provide a large variety of ecological niche which are the abode of extremely rich and complicated animal communities consisting of an enormous diversity of taxa. Because of the global water quality change (Gomoiu 2004; Szabo et al 2010) and due to the tropical climate of southern Iran, Persian Gulf water quality was changed respectively and these changes caused an improvement of Persian Gulf fishes tolerance (Sahandi, unpubl. data).

As a serious branch of fish trade, the marine ornamental fish trade is continuously expanding and marine aquarium fishes are still in great demand all over the world. About 15% of the world aquarium fish industry is based on marine aquarium fishes and more than 90% of these fishes are contributed from countries like Singapore, Sri Lanka, Hong Kong and Maldives (as presented in 1999 and 2001 by Gopakumar et al; however the situation seems to be similar at present).

The breeding and rearing of clown fish is promising due to their ability to produce large eggs and larvae, frequent spawn in captivity and due to their hardy nature (Gopakumar et al 1999, 2001). The breeding and rearing of two species of clownfish, *A. clarkii* and *A. percula* was reported long time ago by Alva & Gomes (1989). Since then, several fish hobbyists were able to breed different species and varieties of clown fish. As far as we know, the clown fish was the first marine species that breed for ornamental fish industry target. Rotifers and *Artemia* nauplii are usually used as live food for their fries, but also calanoid copepods and other invertebrates administration improves yellow tail clown fish larviculture (Olivotto et al 2009). While the large-scale culturing of clown fish has not always been successful, technically the breeding of the clown fish is a real success considering the difficulty in obtaining good survival of coral fishes which usually go through one or many larval stages and are very sensitive to environmental changes.

The clown fishes belonging to the family Pomacentridae are among the most popular tropical marine ornamental fishes due to their generally small and hardy nature, attractive colors, high adaptability to life in captivity and the interesting display of behavior due to their association with sea anemones. The present research was embedded in a paper in order to enrich the literature with data on reproduction of Persian Gulf anemone fish (*A. clarkii*) in captive conditions.

Persian Gulf Anemone Fish. The black clown fish, *A. clarkii*, is also known as yellow tail clown fish or Clarkii clownfish. It can be found in many different color variations, but it is usually yellow and black with broad, white stripes that run vertically over the body (Sandford 2007). The black clown fish is a so called anemone fish and in the wild it will always try to find an anemone that can protect it from predators. This fish is one of the most commonly kept anemone fishes among saltwater aquarists. If you want to obtain an anemone, you can choose any of the ten anemone species that the fish is known to interact with: *Cryptodendrum adhaesivum, Entacmaea quadricolor, Heteractis aurora, H. crispa, H. magnifica, H. malu, Macrodactyla doreensis, Stichodactyla gigantea, S. haddoni* and *S. mertensii*.

Brood Stock. In the entire bloodstock tank one pair grew ahead of others and became the dominant and spawning pair. This evolutionary advantageous behavior is also often observed in most cichlid species. The size of the mature fish ranged between 8 and 9 cm. Sexual dichromatism was more visible in the spawning pair as compared to other individuals. The snout of the female was dusky yellow whereas that of the male was conspicuous of an intense yellow color. Brood stock usually choose form group of brood stocks which show the reproduction behavior.

Brood Stock Tank. The minimum volume for breeding tank was 120 liters tank with good filtration. There are two breeding methods available: natural breeding and desert breeding (Sahandi & Sahandi 2011). Natural breeding was carried out with preparation of natural environment with anemone and rocks; desert breeding was done in tank without any thing just jar. The jar can be replaced with natural stones in cases of commercial breeders. This method is same as cichlids family reproduction. After spawning the jar was changed with a new jar and eggs were transferred to the breeding tank which was at least 60 liter size. A percent of 10% water in the breeding tank was siphoned and replaced every day.

Spawning. This species is territorial fish which protect their eggs and fry. Usually, in the wild, eggs are laid beside anemone and this is because of preparation of safe location for fry. Our fish spawned several times in the bloodstock tanks. The spawning pair drove out other fishes intruding into their territory. The spawning started with the cleaning of the jar at which the eggs were to be laid. Then the egg laying started and lasted for about one hour. Jar would be used for egg laying and this is because of fish behavior for finding cave beside anemones to spawn. Before spawning the sexual dichromatism of fishes would be emerged and it means that spawning will be occurred soon. Before spawning, male starts to clean the surface of its nest. With acceptance of female, pairs start to clean the nest with each other for the second time. After that, spawning occurs and eggs are laid in the nest.

Hatching the Eggs. Generally, the eggs started darkening at two days and the developing larvae were clearly visible through the egg capsule at 3-4 days. The larval hatching period was between 6 and 7 days. In the hatching day the egg capsule became very thin and pellucid. Shining of the larval eyes in this period was very prominent. The last larvae broke the capsules and came out in the 7^{th} day. Darkness or diffuse light accelerated the hatching process. The eggs hatched mainly in the 6^{th} day night, but in a few cases a part of the eggs hatched on the 7^{th} day night (see also rather similar observations on hatching in Gopakumar et al 1999, 2001). Usually, under good environmental condition, 85-95% of eggs were hatched (better percentage compared to that of Gopakumar et al 1999, 2001).

Larval Rearing. The length of the newly hatched larvae ranged from 2.5 to 3.0 mm. The larvae metamorphosed into juveniles 12-15 days post-hatching. The average length of the early metamorphosed young was of 8 mm.

Larval Nutrition. After eggs hatching the fry used their rest yellow sack and after starting the swimming they were fed with rotifer *Brachionus plicatilis* (Sahandi & Sahandi

2011). Rotifer feed would be used for a period 12-20 days. From the 5th day *Artemia* nauplii were used besides rotifer feed. Whenever possible, enrichment of rotifer or *Artemia* nauplii with Omega 3 and probiotics would be effective (see preparation in Jafaryan et al 2010, Sahandi & Jafaryan 2011, Olivotto et al 2011 and Divya et al 2011). This enrichment would improve the digestive tract bioprocesses and consequently the larvae survival and health.

Results and Discussion. Several studies have been made on the biology, more exactly on the ethology and physiology of clown fishes (Hattori & Yanagisawa 1991ab; Moyer & Nakazono 1978; Moyer & Bell 1976; Moyer 1980; Ochi 1986; Olivotto et al 2009; Nelson et al 1996). They are known as benthic egg layers and protandrous hermaphrodites (many clownfish males become females at a certain point in their lifespan). Social hierarchies in clownfish populations were also deeply investigated (Moyer & Nakazono 1978; Moyer 1976). The largest individual in an anemone is usually a female with a smaller male, having a variable number of juveniles among them. Hirose (1995) studied the patterns of pair formation in *A. clarkii, A. frenatus* and *A. perideraion* on coral reefs of Okinawa, Japan which indicated that re-pairing occurred in these species. Hattori & Yamamura (1995) described the coexistence of sub adult males and females as alternative tactics for breeding post acquisition in *A. clarkii*. From all the ethological studies on clownfish (this and close related species) inventoried in our survey results the species' good perspective on large scale reproduction and culture in captivity.

Today, saltwater results from a large number of types of industrial activity (Oboh et al 2009). However, not all these water resources are suitable for fisheries (Viman et al 2010), and the permanent access to natural marine water or minerals is a great advantage for the marine aquarium industry.

There are opinions that marine resources were not exploited enough (Doroftei et al 2010). Even they are right in part (algae, minerals, water etc), the exploitation of marine ornamental fish from the wild would lead to the over exploitation of the coral seas (Harsan & Petrescu-Mag 2008). Uncontrolled fishing for trade or even for scientific purposes may have unpredictable effects for the aquatic ecosystems (Battes et al 2004; Pricope et al 2004). The indiscriminate methods of capture or collection can damage the coral reef ecosystem of Persian Gulf, which provides the microhabitat requirement for the recruitment of the different species of coral reef fishes. By scientific cooperation and international experience exchange, under the most recent educational concepts (Darabaneanu 2004), using the present technologies of natural feeds production and water quality control (Raja et al 2009; Sugumar 2010; Dragos et al 2010), clownfish species (but also many other marine species) can be breed in captive conditions for commercial purposes and it can be helpful for wild life conservation (Rhyne 2010; Olivotto et al 2011b).

The breeding and rearing of Persian Gulf clown fish is promising due to the production of large eggs and larvae, frequent spawning in captivity and the hardy nature of the fish. It was reported in the past century by Allen (1998) on the clownfish hatchery production by two companies connected with marine aquarium. The major technological aspects of clownfish rearing program are the successful development of bloodstock, methods of hatching the eggs, development of a biological detoxifying filtration system for larval rearing and appropriate larval feeding schedule. This technology can be considered as a milestone towards the development of a sustainable marine ornamental fish trade in Iran, which has immense potential for foreign exchange earnings. Dufour (1998) showed that many millions of fish are 'caught' worldwide and that the export of 100,000 ornamental fish would bring approximately 200,000 U.S. dollars in business. This species can be breed in vast number and exported to all over the word. The tolerance of this species to environmental factors change makes this fish the best between other clown fish and the variation color either improved their attrition. Persian Gulf with stone beach and unlimited saltwater resources can be good place for this species breeding in natural or semi-natural conditions.

Conclusion. Persian Gulf, having good relation with Indian Ocean, is one of the important niches of fishes and the specific position of this Gulf makes its fishes popular.

The Clarkii clown fish which originates to this gulf has the best survival rate and health than the other areas. Live food is the most important factor in production of this species and enrichment of their live prey with probionts improve the nutritional value of fish's diet.

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