Mediterranean Aquaculture Journal 2010 1(1); 28-35

Original Article

Out of Season Artificial Propagation of The Black Scorpionfish (*Scorpaena Porcus L.*) in Captivity

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Abstract

Successful artificial propagation of black scorpionfish (*Scorpaena porcus*), in captivity is reported for the first time. Broodfish were collected from the Adriatic Sea after the natural spawning season. Maturation stage of the gonads was determined using histological analysis. Broodfish were kept in two 700-L aquaria within a recirculation system and held under controlled conditions. Fish were stimulated to reach pre-ovulatory stage by weekly IP injections of common carp pituitary at $3mg.kg^{-1}$ and the speciments showing soft and swollen abdomen were induced to ovulate by $6mg.kg^{-1}$ CP. Frequency of ovulation and pGSI of the females were recorded. The mean pseudo gonado-somatic index (pGSI) reached 29% and the average egg number per stripping ranged 5-6000. The dry fertilisation method proved to be more efficient than the wet method. Fertilised eggs, which floated on the water surface in gelatinous mass, were incubated in a fine net cage. Fry hatched out 53-59 hours post-fertilisation and began exogenous feeding three days after hatching at 20°C. This method could be adapted to the largescaled scorpionfish (*Scorpaena scrofa L.*), an endangered close relative, which is a commercially valuable candidate species for marine aquaculture.

Key Words: Scorpionfish, *Scorpaena porcus*, Sexual maturation, Artificial propagation.

Received: 20 March 2009
Accepted: 12 August 2009

Introduction

Black scorpionfish (*Scorpaena porcus L.*) and largescaled scorpionfish (*Scorpaena scrofa*) are valuable catches in commercial fisheries of the middle Mediterranean Sea. The stock of the latter species is decreasing due to overfishing but it is not yet included in the CITES list (Matic-Skoko et al. 2008). According to a 2007 FAO report, the price of

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largescaled scorpionfish was 18€.kg^{-1}. Although the largescaled scorpionfish could be a promising future candidate for marine aquaculture, relatively little is known about its reproductive cycle and natural reproduction, and virtually nothing about how to artificial breed this species in captivity. Laboratory studies on artificial propagation of the black scorpionfish could serve as a model for its bigger and commercially important relative, the largescaled scorpionfish.

The black scorpionfish is distributed in the Eastern Atlantic from the British Isles to the Azores and the Canary Islands, including Morocco, the Mediterranean, and the Adriatic and Black Seas. It is a solitary and sedentary species, commonly found among rocks and algae from the surface down to 800m, in temperate water; 55°N-25°S, 32°W-42°E. It feeds mainly on crustaceans and small fish: gobies, blennies, wrasses; (Hureau and Litvinenko, 1986). The maximum length of this species is 37.0cm TL and its maximum published weight is 870g (IGFA, 2001). The females grow faster and reach larger size than males but other sexual dimorphism cannot be distinguished (Bilgin and Çelik, 2009), except close to ovulation, when females display a soft and swollen abdomen.

The embryonic development of the largescaled scorpionfish was described by Segvic et al. (2007). The natural reproduction of black scorpionfish was first described by Sparta (1941). The morphological characteristics of fertilised eggs from spontaneous reproduction in aquaria, as well as embryos and newly hatched fry were described by Jug-Dujakovic et al. (1995). They found that eggs ranged 1.09-1.14mm in diameter and had a slightly oval shape, contained no oil drops and were embedded in a gelatinous matrix. Hatching started 1.91 days (45.84 hours) post fertilisation and the newly hatched fry had a total length of 2.21-2.33mm. In contrast, Celik and Bircan (2004) observed egg diameters between 0.60 and 0.87mm, which is a significant deviation (25-45%) from the data of Jug-Dujakovic et al. (1995). Since the eggs were not produced by artificial propagation in either case, it can be hypothesized that the eggs were examined at different times after fertilisation, resulting in different degrees of swelling and thus different diameters.

The annual reproductive cycle of a third scorpionfish species, the small red scorpionfish (*Scorpaena notata*), was studied by Muñoz et al. (2005). The authors reported that sexual dimorphism did not occur in the studied population, which was clearly dominated by males. According to that study, multiple spawning events took place between July and October, with 6000 to 33000 eggs per female, each measuring about 500µm in diameter.

Propagation methods of fish reared in captivity are based on various hormone treatment regimes (Zohar and Mylonas, 2001). The optimal type, dose and manner of application of hormones for gonad maturation and for induced ovulation are known for the most important cultured marine fish species, such as sea bass, *Dicentrarchus labrax* (Forniés, et al. 2000), sea bream, Sparus aurata (Zohar, et al. 1995), sole, *Solea solea* L. (Ramos, 1986 a,b) and European eel, *Anguilla anguilla* (Muller, et al. 2004). Repeated injections of pituitary extract proved to be efficient for inducing sexual maturation of European eel (Pedersen, 2003). Due to the lack of knowledge on artificial propagation of the black scorpionfish, we examined the reproductive stages of mature fish, and attempted to reproduce these fish in captivity using hormone injections, applying wet and dry fertilization methods. We further characterized the early larval behavior of this fish.

Materials and Methods

Origin of the study fish

The study fish were caught in the northern part of the Adriatic Sea, near Pula, Croatia in September 2007, after the natural spawning season. Sixteen specimens were collected by local fishermen using gillnets. Fish that showed signs of injuries were sacrificed and used for external morphological examinations and microscopic gonad studies. Twenty-three

specimens were caught with a fine texture dip net by snorkeling and diving at 1-25m depth and transported to the Budapest Tropicarium. These fish were used for the hormonal maturation and artificial propagation study. Their weight, length and sex data are listed in (Table 1).

Table 1: Weight, length and sex data of fish used for the maturation and propagation study:

		females	males	unidentified	total
	number	16	3	4	23
	%	70%	13%	17%	100%
length (cm)	min.	14.0	17.0	11.0	11.0
	max.	26.0	22.0	21.0	26.0
	mean	19.1	19.7	15.1	18.5
	variance	3.8	2.5	4.3	3.9
weight (gram)	min.	57.2	107.5	30.2	30.2
	max.	397.0	193.7	187.0	397.0
	mean	159.3	151.4	86.7	145.7
	variance	104.8	43.1	69.9	95.3

Circumstances of fish keeping

The fish were kept in two 700-L aquaria (12 and 11 specimens per aquarium) which was part of a recirculation system that was equipped with a protein skimmer. The water temperature was regulated by an electric cooling device and maintained at 20±0.5°C. It was slowly decreased to 12°C (Winter temperature) by 2°C. week^{-1} increments. Fish were maintained at this temperature for 4 weeks. This was followed by increasing the temperature to 20±0.5°C where the induced maturation and ovulation studies were carried out (Figure 1). The water had the following chemical parameters: dissolved oxygen at saturation, NO_3- below 25 ppm, PO_4^{3-} less than 2 ppm, pH 7.7 and salinity 38g.L^{-1} (Specific weight 1.028g.cm$^{3-1}$). Illumination, cca.500 lx at the front side of the aquaria, was applied continuously. The fish were fed 4-5% of their body weight with live prey fish (*Carassius a. gibelio*), or frozen squid (*Todarodes pacificus*), shellfish (*Mytilus edulis*) and shrimp (*Pandalus borealis*) twice a week.

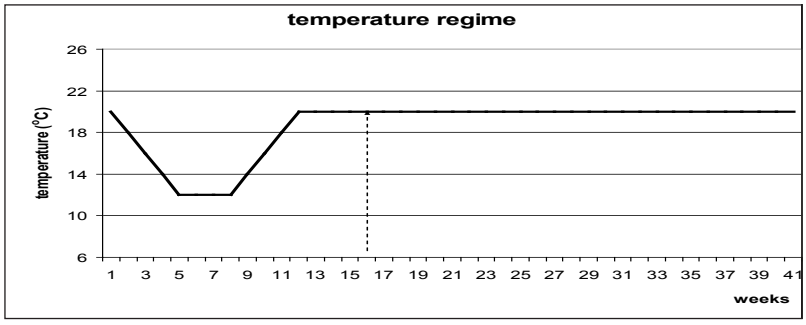

Fig. 1: Water temperature regime and start of hormone treatment.

Condition of the gonads

In order to study the maturation stage, 16 specimens were sacrificed at the beginning of the study. The gonads were removed, weighed, and slices were cut for microscopic analysis. Gonadosomatic index (GSI) was calculated as a ratio of the weights of the gonad and the body using the formula: GSI= (gonad weight/body weight)×100.

Gonads were fixed in 5% formaldehyde and sliced into 5-µm sections that were stained with haematoxylin and eosin. Maturational stages of the testes and ovaries were determined using a microscope.

Pseudo-gonadosomatic index (pGSI) was calculated for the experimental fish used in the artificial propagation experiment as a ratio of the weights of ovulated eggs with gelatinous mass included and the body: pGSI= egg weight with gelatinous mass/body weight)×100.

Hormone treatment and fertilisation

Hormone treatment aimed at inducing maturation in the black scorpionfish was initiated after a 4-week adaptation period at 20°C. The sex of the fish could not be identified through external examination at this stage. After the adaptation period, 23 specimens were selected for hormone treatment. Weekly intraperitoneal (IP) injections of carp pituitary extract were applied to induce maturation of the gonads. The injections (3mg.kg^{-1} BW, dry carp pituitary) were administered IP for 12 weeks. In order to provide the same hormone/body weight ratios to fish of various sizes, the weight of each fish was recorded weekly. The fish were first weighed 4 weeks before starting the hormone injections, and the last weighing was carried out 10 weeks after the last injections. The pre-ovulation phase was characterized by a sharp increase (15-20%) in body weight. For specimens showing such a sharp weight increase–and swollen abdomen-a double dose (6mg.kg^{-1} BW) of carp pituitary was injected in order to induce ovulation. Males were injected only by 3mg.kg^{-1} CP. Fertilisation was carried out with both "wet" and "dry" methods. In the case of the wet method 10-15µL of sperm was mixed to 15-20mL of seawater and immediately added to an equal quantity (15-20mL) of eggs. In the case of the dry method the sperm was first distributed uniformly on the surface of the egg mass using a pipette, and then seawater was added to activate the sperm. The fertilised eggs were kept in an incubation cage.

Results

From the 16 sacrificed fish only three were male and the rest female. The GSI values of these females ranged between 2-3% and that of the males between 1-2%. In all of the ovaries early oogonia as well as oocytes starting vacuolisation were present, indicating that the black scorpionfish has asynchronous ovaries (Figure 2). In testes spermatogonia were dominating. Majority of the tubules were empty and only a few spermatids were observed. Both the ovaries and the testes showed post-spawning season condition.

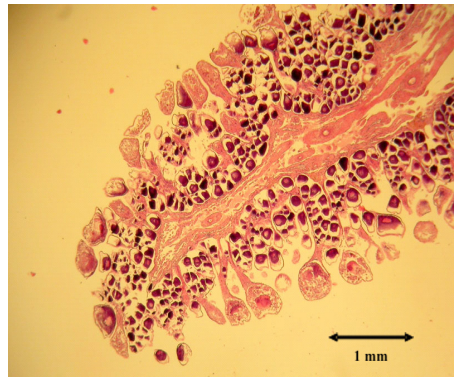

Fig. 2: Longitudinal section of the ovary of black scorpionfish (*Scorpaena porcus*).

Maturation, induced ovulation and spermiation

The length of the maturation time of females showed significant individual variation. A sharp increase (>10%) in body weight as compared to the previous week occurred in one female during the first week after the start of the hormone treatment. At the same time, a swollen and soft abdomen indicated the pre-ovulatory stage. During the following time, 0-6 specimens showed to reach the pre-ovulatory stage per week. From 23 fish injected 3 proved to be male, 16 females and 4 unidentified. Six females spawned ones, four females spawned twice and 6 females spawned 3 times during the 22 week long observation period. The stripping technique used in this study differed from that previously reported in other species. In our

study "Stripping" refers to "Pulling" rather than pressing. The egg matrix could be pulled out from the genital pore when it started gushing out (Figure 3).

The weight of the stripped eggs mass-including the gelatinous matrix-is given as a function of body weight in (Figure 4). It shows that the mean pGSI of the black scorpionfish in these experimental circumstances was 29%. The average egg number per stripping was estimated between 5-6000.

Fig. 3: Gelatinous egg mass of ovulating black scorpionfish.

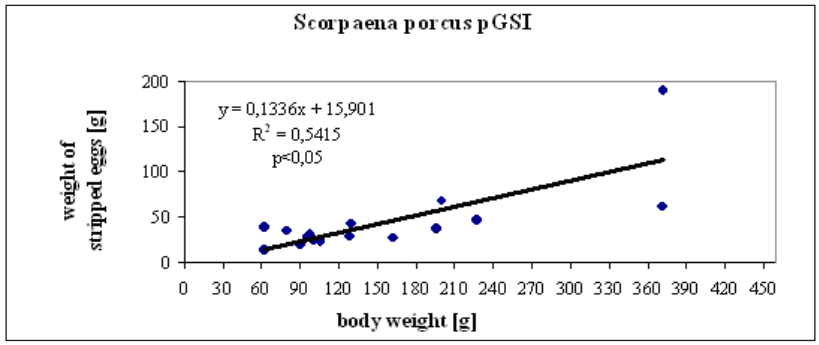

Fig. 4: Weight of the stripped eggs as a function of body weight.

There was not much difference between the 3 males regarding the start of spermiation. One male began to spermiate on the 3rd week of the hormone treatment and the two other males one week later. The quantity of sperm given by each fish was very little, only 15-20µL. Spermiation lasted for about 3 weeks, followed by a 3 to 4-week pause and spermiation.

Fertilisation, hatching, larval rearing

The wet method was less efficient than the dry method. Surprisingly, when adding seawater to the sperm part of the sperm seemed to coagulate. Fertilisation rates using the wet method were below 15%. With the dry method, the best fertilisation rates exceeded 30%. The spawn floated on the surface of water. Hatching began 56 hours after fertilisation (Range 53 hours to 59 hours) at 20°C water temperature. By the time hatching began, the gelatinous matrix around the eggs had dissolved and disappeared. The estimated average egg number per spawning using the dry method was 5-6000 and the number of hatched fry per female when using the dry method was around 1500. The newly hatched larvae were 2mm long and had a large yolk sack. They were transparent and even their eyes had no pigmentation (Figure 5). On the first day after hatching the larvae swam at the water surface and reacted to mechanic stimuli by diving rapidly and then returning to the surface after a few seconds. On the second day the fry moved to the middle of the water body but did not form groups. By the third day their body length reached 2.5mm but they were still not pigmented. On the 4th day the eyes of the fry started pigmentation and small black spots appeared on the fins. Their length ranged between 2.5-2.8mm, the head was significantly enlarged and the size of the gaping mouth was about 150µm. At the same time the fry moved to the bottom and still did not form groups. They were uniformly distributed on the bottom and appeared to be searching for food. By day 8 the eyes of the fry became dark black and the content of their intestines (Probably

detritus) showed a yellowish coloration (Figure 6). Brine shrimp (*Artemia salina*) nauplii and live *Brachionus plicatilis* were too large as first feed. Unfortunately, SS size *B. plicatilis* (90-150µm) (Stephanou and Georgiou, 2000) was not available and the fry died by the 9th day after hatching.

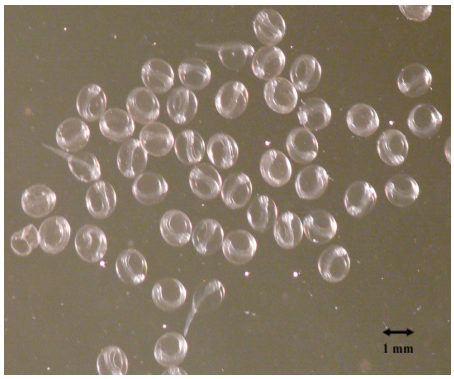

Fig. 5: Hatching of black scorpionfish 56 hours PF at 20°C.

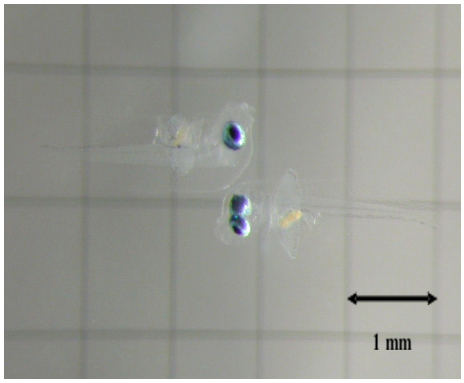

Fig. 6: 9-day-old black scorpionfish with visible gut content.

Discussion

Natural reproduction of black scorpionfish was first reported by Sparta (1941) while its embryonal development and newly hatched fry was described by Jug-Dujakovic et al. (1995). Similar description of embryonal development of the largescaled scorpionfish is given by Segvic et al. (2007). Though both black and largescaled scorpionfish are valuable cathes of the middle Mediterranean Sea, no report has been published on their artificial propagation until now. Artificial propagation of black scorpionfish is described for the first time in the present paper. Out-of-season spawning was induced by repeated injections of carp pituitary extract and a specific temperature regime. Bilgin and Çelik (2009) found that females of the black scorpionfish grow to larger size than males. In a close species, madeira scorpionfish, *Scorpaena maderensis*, La Mesa et al. (2005) found that males grow larger than females. In our study we did not find sexual dimorphism except close to ovulation, when females displayed soft and swollen abdomen.

In accordance with the results of Koya and Muñoz (2007) the females showed asynchronous ovaries. The joint application of a temperature and hormone regime led to out of season maturation and ovulation. Six fish from the 23 ones ovulated once, 4 fish twice and 6 of them ovulated 3 times during the 22-week long period. This forecasts the chance of multiple use of breeders for propagation purposes in one reproduction season. The stripping of the eggs was different from that of the species having freely ovulating eggs. The dry fertilisation method proved to be more efficient than the wet method. I the present experiment the fry hatched out 53-59 hours PF at 20°C. This is significantly longer what Jug-Dujakovic et al. (1995) found at the same temperature. Since their eggs were collected from spontaneous spawning supposedly the exact determination of the time of fertilization was not easy in that case. In a previous account of fry development of the *Scorpaenidae* Nagasawa and Domon (1997) stated that the fry shifted from planctonic to benthic feeding at the size of 25-40mm. According to our observation, when the yolk sack is absorbed and external feeding starts, the black scorpionfish fry immediately and undoubtedly search for benthic food and stays on the bottom.

Conclusion

The method described in this paper could be adapted to the largescaled scorpionfish and other close relative species. Further research is

needed to find suitable starter feeding of larvae and to develop the artificial propagation of the *Scorpaena* species to suit into pilot or farm conditions.

Acknowledgements

The authors wish to thank the staff at Aquarium Pula, Neptun Dive Center (Hungary) and Tropicarium Budapest for their help in catching and keeping the fish.

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