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Research Article

Evidence of Sperm Storage in Nursehound (*Scyliorhinus stellaris*, Linnaeus 1758): Juveniles Husbandry and Tagging Program

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Nursehound, *Scyliorhinus stellaris* (Linnaeus 1758), is a shark of the Scyliorhinidae family, close to the *Scyliorhinus canicula* (Linnaeus 1758), frequently hosted in public aquaria. Information on biology and ecology is deficiently available regarding this species of sharks. In the Mediterranean basin, they are occasional rare and vulnerable species (Serena, 2005). In 2003 a female specimen of *Scyliorhinus stellaris*, 90 cm long, fished in the Tyrrhenian Sea was transferred to Tuscany Argentario Mediterranean Aquarium and placed in a 20.000 L tank. The female laid 42 eggs and juveniles were born on 2004 and 2005. They were transferred to the aquarium laboratory in order to get standard protocol for correct juveniles husbandry. After a total of 18-month observations, some of them were tagged and let free on 2006. To collect data about nursehound shark needs in terms of feeding and growing in captivity, especially during the first life years, is a necessary and fundamental step in order to develop a Mediterranean program of tagging and study in the field of conservation policy proposal. Husbandry protocol for this species' juveniles was developed in this study. This is the first reported case of a nursehound storing sperm for 2 years, in captivity (Pratt, 1993; Hamlett et al., 2002; Awruch, 2007).

1. Introduction

The *Scyliorhinus stellaris* (Linnaeus 1758) is a benthonic shark. It is distributed in the Mediterranean Sea, being absent from the Black Sea, and it also occurs in the Atlantic Ocean, from Shetlands and southern Scandinavia to Senegal being rare in the North Sea. Greater-spotted dogfish (*S. stellaris*) is a rare species. At the southern and western coasts of the British Isles, the species occurs over shallow rough inshore grounds (e.g., Gower Peninsula, Pembrokeshire, and Lleyn Peninsula) at depths from 13 to 100 m deep, where maximum catch rate is 18 ind.hr.$^{-1}$ [1, Fig. 4b]. In the Mediterranean Sea, data from MEDITS surveys indicated that the species could be caught down to 500 m deep, extending its previous bathymetric range [2]. The species feeds on crustaceans, molluscs, and small fishes, which may include small specimens of the species [3, 4]. Although the maximum size of the species is set as 162 cm, length of larger fished specimens varies from 50 to 60 cm. This species leaves on rocky bottom and it is bigger than small-spotted catshark *Scyliorhinus stellaris* (Linnaeus 1758); female is mature at about 79 cm and male at 77 cm [5]. The conservation and exploitation status is: FAO, B1; Mediterranean, rare and vulnerable species [5]. Egg-cases of *S. stellaris* were never caught in large numbers, and it is likely that they are laid in shallow water, attached to macroalgae [6, 7]. The *Scyliorhinus stellaris* can be easily maintained in captivity [8], which facilitates the development of physiological studies on the species observations [9–15]. In December 2003, a mature female fished in the Tyrrhenian Sea, was transferred to the Argentario Aquarium, in a large tank with 3 other young specimens, 2 females and 1 male. During the two years of captivity, the mature female laid 42 eggs. Some of the fertilised eggs completed embryonic development and hatched in the aquarium. Juveniles were kept in the laboratory to observe growing rate and to obtain information on a correct captivity husbandry protocol. This was the first case in which a female laid fertilised eggs during a 2-year period in the absent of males [16–18]. This implies that sperm from previous insemination could be stored in a shell gland and be viable after 2 years. Metten [19] and Richards et al. [20] have reviewed earlier evidence on sperm survival [21]. Some of the juveniles, 11 and 18 months old, were tagged in the aquarium for the National Elasmobranch tagging program from Italian ARPAT (Tuscany Agency for Environmental Protection) and let free on July 2006, but they were not fished again until 2014. The aim of this study was to verify a correct husbandry protocol for juveniles. Their behaviour can be observed during their growth and when they are 2 years old, they can be used for tagging programs to study sea nursery and movements.

2. Material and Methods

2.1. Sexually Mature Female. A female, 90 cm long, fished along the Argentario coasts, was transferred on December 2003 in the exposition pelagic tank (20.000 L) of Argentario Mediterranean Aquarium, Tuscany. The pelagic aquarium is equipped with external filter, controlled temperature, iodure lamp 250 w, 100% per week seawater changing, and a pipeline directly keeping seawater in front of the aquarium at 8 m depth. Later the female laid eggs (Figure 1); a total of 20 eggs were laid in 2004 (group A) and 22 eggs were laid in 2005 (group B). All the eggs were laid between January and March and hatched between August and September.

The laid eggs were transferred to the aquarium laboratory. On late August of 2004, 6 eggs from group A completed the embryonic development and hatched. Between August and September 2005, 11 eggs from group B completed the embryonic development but only 9 survived.

2.2. First Group A, 2004–2006/Second Group B, 2005-2006. Every hatched egg was transferred to an aquarium in the laboratory. Each aquarium was equipped with internal filter for nanoreef aquarium, illuminated from the laboratory fluorescent tubes 50 watts from 9:00 to 18:00 in October–May and from 9:00 to 20:00 in June–September. Twice a month the 20% of aquarium water was changed by natural and filtered seawater. During the first 10 months, juveniles were weekly feed with food representing 10% of its body weight. After that, the food increased to 15% of the body weight. Initially, at the first 3 months, food consists in prawns and after that it was composed of anchovy. The total lengths (TL) were measured on neonates and specimens to the nearest millimeter, and the weight was measured with Hanna pce-pm 6 t precision balance. The same husbandry protocol was followed and the data were collected for 19 months for group A (2004–2006) and along 11 months for group B (2005-2006). The analysis instruments include mercury thermometer, electronic pH-meter, refractometer for salinity, field Spectrophotometer Hanna C-200, for nitrates and nitrites, and Hanna pce-pm 6 t precision balance.

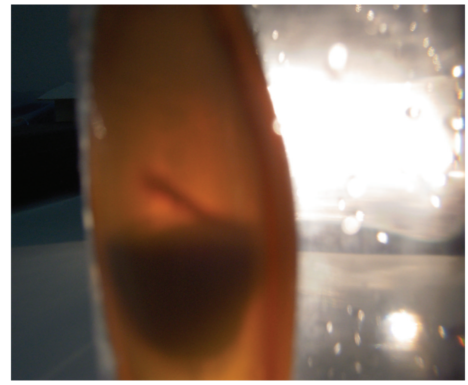

Figure 1: *Scyliorhinus stellaris* egg.

Table 1: Tagging information.

Identification code	Tagging Sex	TL
01899	Female	20,5 cm
01898	Female	20,5 cm
01897	Male	20,5 cm
01896	Male	21 cm
01895	Female	21,5 cm
01894	Male	21,2 cm
01893	Male	20 cm

2.3. Tagging and Free. In June 2006, 4 juveniles belonging to group A and 5 to group B were tagged (Figure 2) following the protocol for the National Elasmobranch Tagging Program from ARPAT Tuscany Agency for Environmental Protection, managed by Dr. Fabrizio SERENA, and seven were released and let free on July (Figure 3). The remaining juveniles were maintained in the aquarium as control cases. Yellow short strings tags with progressive number were placed in the dorsal fin (Table 1).

3. Results

Between 2004 and 2006, 42 eggs were laid and then grouped as A: first 2004–2006, B: second (2005-2006): 17 juveniles hatched in the laboratory where one specimen was hosted per aquarium which were daily controlled and monitored. Of those 15 survived. Data on weight, total length, and weight of food in gr weekly distributed were collected along 18 months. Water parameters such as T°, pH, salinity, nitrates, and nitrites were also collected. In group A, the total length of specimens at birth varied between 145 and 150 mm; after 11 months, specimens have a total length of 213.3 mm in average and after 19 months they measured 267.5 mm (Figure 4). In group B, the total length at birth varied between 145 and 150 mm; after 11 months, specimens have, in average, a total length of 207 mm, group B (Figure 5). Length at birth was similar in the two groups but the aquarium conditions were slightly different.

Regarding weight, group A (Figure 6) specimen varied at birth between 10 and 12 gr, they attained a mean of 25,66 g

Figure 2: Juvenile tagged with yellow short string.

Figure 3: Juveniles let free in Monte Argentario island on 5 July 2006.

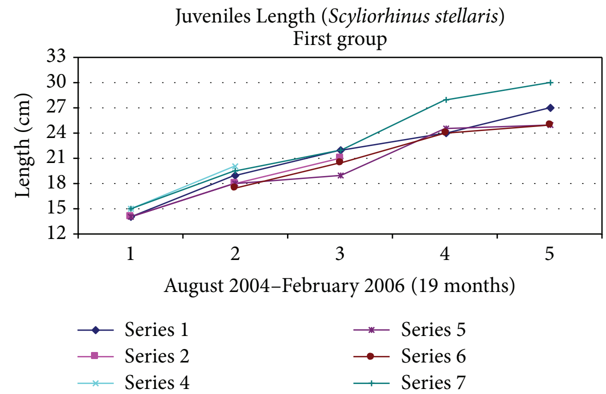

Figure 4: Total length group A.

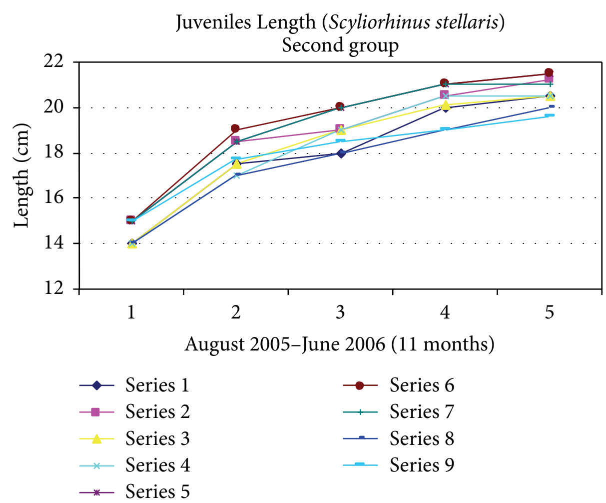

Figure 5: Total length group B.

after 9 months and 63 g after 18 months in these captivity and feeding conditions.

Also group B (Figure 7) varied between 10 and 12 gr when born to be attaint in average 26 g, after 11 months in the same captivity and feeding protocol.

In group A, water temperature varied between 14°C and 20°C, pH between 8 and 8,5, salinity between 36 and 42‰, and nitrites between 0,00 and 0,34 ppm and nitrates maximum was 146 ppm, and in group B, water varied between 18°C and 21°C, pH between 7,4 and 8,6, salinity between 36 and 42‰, and nitrites between 0,00 and 0,38 ppm and nitrates maximum was 83,72 ppm.

4. Discussion

The female caught in 2003 laid eggs in the absence of mature males. This derives from the capacity of Scyliorhinidae and elasmobranchs in general to store sperm for at least 2-3 years and fertilise the egg later. The tank where the female was hosted contained one male specimen with 30 cm length but not sexually mature. In 2004 the female laid 20 and in 2005 22. This total fecundity is lower that what is referred by Capapé [22], which indicated 77–109 per year in natural conditions [23]. A total of 17 of 42 eggs were able to complete the embryonic development in individual aquarium. This means that 40% of fertilised eggs survived which is also lower than what is estimated by Capapé et al. in natural conditions (74%) [23]. The surviving percentage of neonates was 88% (15 of 17 neonates survived), which is higher than 30% determined by Capapé et al. [23]. Both eggs laid in 2004 and in 2005 hatched after 7 and 8 months; this time interval is slightly lower than the estimate by Serena [5] for the Mediterranean Sea (9 months) and more than the 12 months estimated by Moreau [24] and Ehrehbaum [25] for *S. stellaris* specimens in the Atlantic Ocean and North Sea. These faster rates are likely to be related to captivity conditions. Length of birth was also larger than the estimate of Skaramuca and Prtenjaca [26] for Adriatic nursehounds in natural conditions (in average 145 mm). Such difference contradicts Capapé et al.'s hypothesis [23] according to which in captivity the neonates grow less than in natural conditions.

We decided to feed sharks with a quantity of weight food per day, corresponding weekly to the total of food weight included between 10 and 20% of their body weight and change it in function of juveniles length, daily needs, and ability to search for food, in order to get a standard husbandry protocol to be used. After 150 days, the mean length was, in first group, 188,5 mm and, in second group, 179,1 mm; these lengths in Capapé et al. [23] and Skaramuca and Prtenjaca [26] were reached in 60 days. After 19 month of captivity, young specimens from group A have a growth rate in weight of 0,226 gr per day while specimens from group B had a growth of 0,205 gr per day after 11 months. Both values are about half

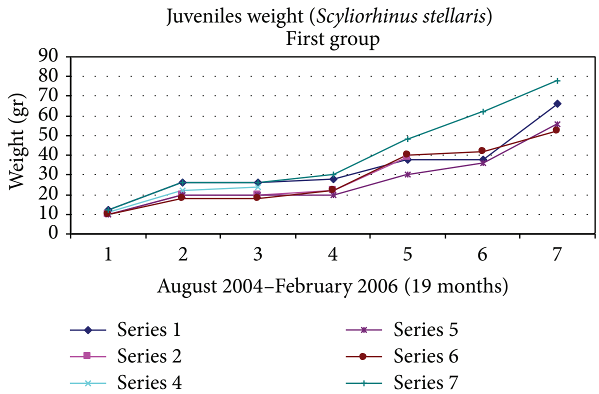

Figure 6: Weight group A.

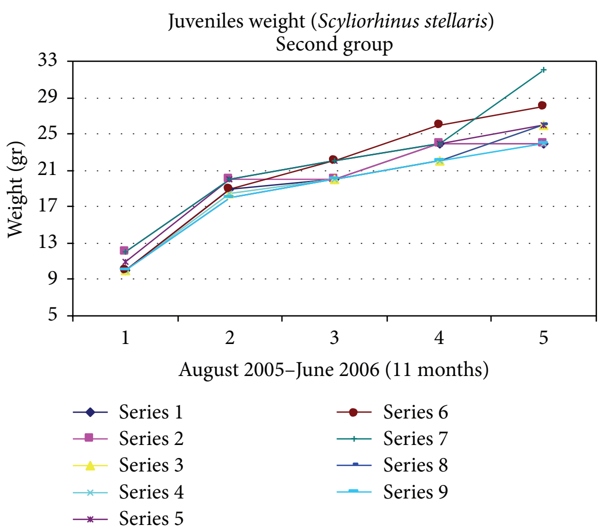

Figure 7: Weight group B.

of the one observed by Capapé et al. [23]. All the juveniles were in good health state and none died during 11- and 19-month observations. In captivity embryos can hatch at the same length as in natural conditions but during development the weekly food ratio in captivity can be increased to more than 10% and 15%. Seven specimens were tagged on June 2006 with a yellow string with a code number useful to identify them if fished. Other specimens were hosted in the aquarium to be able to compare data with those let free on July 2006 in the Argentario Tyrrhenian coast close to Porto Santo Stefano village (Tuscany). In the program, the coast guard and professional fishermen were involved; no information about the free juveniles was collected since 2006 until 2014.

5. Conclusions

The captivity hatching and juveniles husbandry of nursehound seem to be not particularly difficult in public aquaria equipped with large tanks and laboratory; this husbandry protocol permitted us to obtain a regular growing rate along the observed period, and more information is needed to establish at which age Tyrrhenian nursehound is sexually mature in captivity. To lay and hatch each year fertilised eggs, it could be useful to research about reproductive physiology of benthic sharks and to develop programs of tagging in the Mediterranean Sea, where conservation problems seem to attend the nursehound population.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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