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Recovered and released - A novel approach to oviparous shark conservation

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ABSTRACT

The small spotted catshark *Scyliorhinus canicula* and the greater spotted catshark *Scyliorhinus stellaris* are benthic elasmobranchs frequently caught as bycatch in commercial fishing gears and landed at local fish markets for consumption. In recent years landings have begun to decline raising concerns for their population numbers and conservation status. In this study we present a novel, direct approach to shark conservation: removal of eggcases from dead *Scyliorhinus* specimens. Any viable embryos were observed during development and hatching. Post-hatching, pups were reared for 6 months and then released back into the wild. Eggcases were collected throughout the year, indicating the absence of a discreet breeding season in these species. Since January 2012, 689 eggcases were collected from females landed at the wholesale fish market in Malta, 548 *S. canicula* and 141 *S. stellaris*. From these a total of 186 shark pups were released back into the Maltese waters between January 2014 and March 2016. *S. canicula* carrying eggcases were found within a range of 36–52 cm total body length (TL), with most eggcases found in females of 41–47 cm TL. In *S. stellaris* eggcases were present in females ranging from 64 to 94 cm TL, with the majority of eggcases recovered from females of 77–88 cm TL.

The recovery and release program is on-going with eggcase collection continuing for both species. This is to the best of our knowledge, the first report of the successful hatching and release of viable eggcases recovered from dead elasmobranchs. The program provides a practical methodology which can be optimised for other oviparous elasmobranch species landed by commercial fisheries globally; especially for unprotected species facing extensive local fishing pressure.

1. Introduction

Benthic sharks such as catsharks, like all elasmobranch populations, are subjected to a variety of anthropogenic mortalities, including incidental capture (bycatch) in commercial fisheries. In the Mediterranean catsharks make a substantial contribution to trawl fishery bycatch and are landed for consumption at local markets (Colloca et al., 2003; Soykan et al., 2016). Recently, an analysis of a long-term series of landings data and market surveys has shown a dramatic decline in elasmobranch landings, particularly in catsharks of the genus *Scyliorhinus* (Tudela, 2004; Barausse et al., 2014).

The small spotted catshark (*Scyliorhinus canicula)* is a bottom-dwelling species, found along continental shelves and upper slopes at depths ranging from 10–400 m (Ragonese et al., 2013), where they feed on a range of small invertebrates (Šantić et al., 2012). Juveniles can be found in deeper water, often segregated by sex (Rodríguez-Cabello et al., 2007). Size of females at sexual maturity differs depending on location and has been recorded as between 43.0 and 44.0 cm TL in the Mediterranean (Capapé et al., 2008), and 36.4–46.7 cm in the Aegean Sea (Kousteni et al., 2010). Mating occurs all year round in deeper waters (Wearmouth et al., 2013).

The greater spotted catshark (*Scyliorhinus stellaris)* is a larger species of catshark, reaching a total body length of up to 170 cm (Reiner, 1996). This species occurs in coastal waters and continental shelves, most commonly in depths of 20–150 m (Bauchot, 1987), but has also been recorded at greater depths > 400 m (Baino et al., 2001; Gulyugin et al., 2006). Juvenile *S. stellaris* feed on a variety of crustaceans and molluscs (Ford, 1921; Saldanha et al., 1995), with cephalopods and smaller teleosts constituting the majority of the adults diet (Ellis et al., 2009a).

Both *S. canicula* and *S. stellaris* are oviparous species which like all elasmobranchs utilise internal fertilization. They produce one eggcase per oviduct and usually lay them simultaneously, whereby the female twists her body to secure the eggcases with their tendrils on sea grass or macro algae for further development. In *S. canicula* the embryo remains in the eggcase for 4–6 months until hatching, feeding on the yolk inside (Ellis and Shackley, 1997). The size of the eggcases depends on the locality and size of the female (Compagno, 1984) and ranges between 4.9 and 7.0 cm length and 1.5–3.0 cm width (Bor, 2002). In *S. stellaris* embryos remain in the eggcases for around 9 months, the size of

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eggcases again varies due to the size of the female and their origin, to between 10.0 and 13.0 cm in length and 3.5–4.5 cm width (Bor, 2002).

S. canicula and *S. stellaris* are commonly found and captured in commercial fishing gears throughout the Mediterranean Sea (Tudela, 2004; Gulyugin et al., 2006). Despite on-going fishing pressures *S. canicula* are still considered one of the most abundant elasmobranchs in the Mediterranean Sea (Navarro et al., 2016) and are currently listed as a species of least concern by the International Union for the Conservation of Nature (IUCN) (Ellis et al., 2009b). In comparison *S. stellaris* show scattered patches of occurrence (Ellis et al., 2009a; Ragonese et al., 2013) and are markedly less abundant than *S. canicula* (Gulyugin et al., 2006). They are therefore more vulnerable to exploitation (Gibson et al., 2008), which is reflected by their IUCN listing as near threatened (Ellis et al., 2009a).

Management plans and applied action are required to combat the observed decline of elasmobranchs in the Mediterranean Sea (Abdulla, 2004). However, they are often difficult to put into practice and/or are implemented too late for population numbers to adequately recover (Rosenberg, 2003). This is largely as a result of the sharks' k-selected life history characteristics, such as late maturity, slow growth rates and long gestation periods (Stevens et al., 2000). New conservation approaches are required to increase public engagement in marine policy in support of shark conservation (Friedrich et al., 2014). Education and public awareness campaigns can be effective tools used to drive policy actions and market demands in regard to elasmobranch conservation (Ward-Paige et al., 2012).

The aim of the study was to determine whether or not eggcases from dead females would develop successfully. If so, could a recovery and release program be developed for oviparous shark species, which could engage stakeholders and increase general shark awareness and conservation?

2. Materials and methods

Visits to the wholesale fish market in Valetta, Malta (Fig. 1) were conducted on average three times per week over four years (January 2012 to December 2015) at 3 a.m. for approximately 1 h prior to the official sale starting.

2.1. Shark sampling

Samples of landed catsharks were investigated to determine species composition, sex ratio and size distributions. Sample sizes of *S. canicula* varied depending on the man power, catch size and composition available and ranged from 1 to 10 individuals per day up to 180 per day. In the case of *S. stellaris* all specimens landed were investigated, as these were lower in number and detection of eggcases is simpler and faster due to their size.

2.2. Determination of sexual maturity

Sexual maturity of females was determined by the presence of eggcases in accordance with Musick and Bonfil (2005), who stated that maturity can be assessed by absence or presence of "reproductive products within the reproductive tract"; and a report published by the International Council for the Exploration of the Sea on sexual maturity of elasmobranches (ICES, 2010), which determined that the presence of eggcases can serve as the determination of sexual maturity on a macroscopic scale. Total length of eggcase carrying females was measured to record length at maturity for both species.

2.3. Eggcase removal

Females were separated from male specimens and examined for the presence of eggcases in the ovaries by touching the female's abdomen. The shell gland is located just below the pectoral fin and this was the starting point for the physical examination. The method was to carefully feel for the edges of the eggcase where the dorsal muscle transitions into the thinner ventral muscle covering the body cavity. To validate this method a number of females, where eggcases could not be felt, were cut open and as expected encapsulated eggs were not found. This comparison was undertaken on 10–15 occasions and has proved to be greater than 90% accurate in identifying the presence of eggcases. It is a non-invasive examination method, which is beneficial for two reasons; less interference prior to sale by the brokers resulted in their continued support and it saved processing time allowing for a much faster and efficient check when many specimens were landed.

Females, which were suspected of carrying eggcases, were measured (total length) and opened by cutting carefully through the skin and muscle layer from the cloaca upwards until the presence of eggcases was confirmed. They were then removed by opening the ovarian tubes and pulling the tendrils slowly. Occasionally tendrils from eggcases were found at the opening of the cloaca and eggcases were carefully pulled out. It was essential to handle the eggcases with care throughout the process to ensure no damage was incurred.

2.4. Post removal observations

After removal eggcases were immediately placed into a sea-water filled container, chilled to < 18 °C, in accordance with the average sea surface temperatures (Skliris et al., 2012). Within 24 h the eggcases were placed in a holding tank and tied onto a piece of string, to enable close observation. Water was taken directly from the coast of Malta with an average salinity of 38 psμ (Vidal-Vijande et al., 2010). An observation period of 2–4 weeks was conducted to determine embryonic development. The transparency of the majority of eggcases allows for such observation. When eggcases were not transparent enough to determine development, a torch light was used to illuminate the contents of the eggcase. Embryonic development was confirmed by signs of yolk shape changes in accordance with observations by Ballard et al. (1993) and by the presence of an embryo on the upper side of the yolk. Unfertilised or damaged eggcases showed signs of deterioration of the main yolk structure and were removed immediately. Developing eggcases were transferred to the Malta National Aquarium.

2.5. Aquarium set-up and husbandry

The holding tank where the eggcases were initially placed for the first 2–4 weeks for close observation was a 500 L aquarium with three Eheim professional filters for mechanical filtration, a wet dry bio-tower comprising of bio-balls and a protein skimmer. The aquarium also had a chiller unit installed to maintain a constant temperature range of 17.5–18.0 °C. Testing of water quality was done twice a week for nitrates, nitrites, ammonia (SERA aquarium test kit), and salinity (hand held refractometer). 100 L water changes were carried out twice per week as part of general maintenance. Water was collected from two different locations around Malta where water quality tests had been carried out to check zero levels (0 mg/L) for nitrate, nitrite and ammonia; as well as salinity levels.

The tank at the Malta National Aquarium was a native marine tank kept at 18 °C with an average dissolved oxygen content of 9.19 mg/L and 30 psμ salinity (Hach HQ 30d Multimeter). The salinity was kept lower than normal sea water (38 psμ) and is standard procedure by the Malta National Aquarium in order to reduce the proliferation of external parasites. Levels of ammonia, nitrite, nitrate (Hanna instruments HI 83200 Multiparameter Photometer) and pH SERA digital pH Meter), as well as oxygen level were monitored weekly as part of the aquariums normal operating procedures. Salinity and temperature were measured on a daily basis.

The Malta National Aquarium tank was 1750 L in total volume and has mechanical, chemical and biological filtration, it was supplied with seawater from a reservoir which had already passed through high