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A non-invasive manual method for the assessment of egg cases from oviparous sharks

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Abstract

Enhanced survival of oviparous shark populations can rely on only one easily applicable method. We tested a powerful method to retrieve egg cases from dead oviparous females at the fish market which is non-invasive. We approved this method for *Scyliorhinus canicula* and *Scyliorhinus stellaris* (smallspotted catshark and Nursehound) in Valletta, Malta in 2014. Here, catsharks are landed in whole, whereas in many other Mediterranean countries sharks are already eviscerated when brought ashore. Saving egg cases bears the grateful opportunity to rescue shark embryos and this then helps again to restore fish stocks. The common work flow of fishermen is to throw away the egg cases when eviscerating the shark and so the embryos were left in the bin to die. To apply a non-invasive method is very important at fish markets where sharks are landed in one piece as fish brokers do not tolerate many dissections. So, our goal was to detect pregnant females with maximum accuracy and dissect only females with egg cases inside. Formerly, the common method used by members of Sharklab-Malta was to dissect females more or less randomly based on only two parameters (i) a total length above 40cm and (ii) a relatively corpulent body shape. This was not effective as many females were dissected without containing egg cases. This led to the more problematic fact that pregnant individuals were not found and so their offspring died in the end. The basic concept in our newly tested method is based on using a fixed touching pattern which allows to screen the belly of all females available simply by hand. To screen one female only 2 seconds are needed, and this works surprisingly well. We called this method "gotIt" simply because we got all available egg cases and could save all embryos. A validation underpinned the success and showed that gotIt works with 100% accuracy. After rescuing the egg cases they were transported to an aquarium. Egg cases were attached to a lanyard by their tendrils and left for development in the tank for 3-6 months (depending on their initial developmental stage at the fish market) at a water temperature of 15-19°C. After hatching, sharks were fed with squid and released into the sea. The number of rescued embryos arose significantly from five to above hundreds of shark pups which were saved and released by applying gotIt. Additionally, fishermen can be encouraged to use this method, as awareness programs can be promoted to encourage consumption of this sustainable fish, which can therefore increase the marketable prices. Widely applied, gotIt can help to preserve oviparous shark populations at fish markets where sharks are landed whole.

Keywords: *Scyliorhinus stellaris, Scyliorhinus canicula*, shark conservation, sustainable fishery, smallspotted catshark.

Introduction

Sharks evolved around 400 million years ago and hence appeared on the planet before the dinosaurs (Bowmaker, 2008). During this time, they had the chance to adapt perfectly to their environment and were able to find their niche (Lingham-Soliar, 2016). They became apex predators at the top of the marine food chain and are one of the most important group of species needed for a healthy marine ecosystem (Friedlander & DeMartini, 2002). The fact that they have not changed significantly in the last 100 million years implies an early development of features to adapt perfectly to their environment (Carey & Teal, 1969). Sharks and all elasmobranchs have a K-selected life strategy which is more focused on conservation, including features like slow growth rate, late maturing, long life span and a small amount of offspring (Daves & Nammack, 1998). In contrast, a R-selected strategy would be set more on replicating and less on conservation. All these attributes typical in K-strategists are disadvantageous when facing anthropogenic impact (Ward- Paige *et al.,* 2010), and therefore the design and implementation of specific concepts for shark conservation is a very important and challenging task (Daves & Nammack, 1998). Currently, many marine ecosystems are undergoing a drastic change and unmanaged fishing exploitation may lead to the collapse of several shark populations within a few decades (Baum *et al.,* 2003). If sharks as apex predators are removed from the food chain it will lead to an increase of its prey population, leading to a severe imbalance of the ecosystem (Stevens *et al.,* 2008). Although the implementation of shark conservation measures is hampered by the lack of information about their status and population trends (Baum *et al.,* 2003), positive results are shown in areas where strong fishing regulations are introduced. For example, sharks occurring in densely populated areas, like coral reefs, have benefited from the implementation of conservation measures (Ward-Paige *et al.,* 2010). Furthermore, the minimum landing size (MLS) is an important tool for stock management of exploited fish and is being used in shark conservation all around the world (Tsikliras & Stergiou, 2014). The MLS defines the smallest size of fish of a certain species in a certain area, which can be kept and/ or sold, to ensure that individuals can reproduce at least once before caught. If they are smaller than the MLS they must be released back into the sea. Prominent and successful conservation actions revealed through experiments where catsharks were tagged and discarded by trawlers demonstrated that the survival rate is over 90% (Rodriguez-Cabello et al. 2001). The main challenge for this tool is that the MLS should be defined on a regional basis as most species mature at different sizes depending on the geographic area. For instance, the same species can mature later in areas with lower water temperature (Angilletta & Dunham, 2003). In the context of fish conservation, there are two main differences between the reproduction of Chondrichthyes (sharks) and Osteichthyes (bony fish). These differences make the conservation much more difficult for sharks than for bony fish. Firstly, bony fish release eggs and sperm into the sea, i.e. exhibit external reproduction, while sharks reproduce internally and must search for mating partners. They have a long gestation period and pregnant females are vulnerable while migrating into shallow waters. When female bony fishes get caught after releasing their eggs there is less harm to the actual offspring. When pregnant sharks get caught it causes damage to the stock as the female is caught including the offspring. While bony fish are losing one individual, sharks can lose depending on the species, up to hundred individuals (*Prionace glauca*). Some species (*Squalus acanthias*) even carry the offspring for up to 24 months (Owen, 2009). So, conservation measures to protect vulnerable pregnant or egg-laying females should be implemented for all shark species to improve the chance of reproduction before getting caught. The MLS overcomes this problem because it ensures that immature sharks get released based on their size. According to the tagging study mentioned above, these released sharks have a high percentage of survival (over 90%). It is well known that the size of sharks in the Mediterranean Sea differs from other areas such as the Atlantic Ocean, and that they reach sexual maturity at a smaller length (Filiz & Taşkavak, 2006). Therefore, to propose adequate conservation measures, knowledge should exist on the biological parameter of the species in question for that region (Ellis & Shackley, 1997; Ivory *et al.,* 2004). To investigate and provide area specific data for the Maltese waters, the locally occurring smallspotted catshark (*Scyliorhinus canicula*) were chosen for the present study. Another reason for this selection was because this species is oviparous, where the pregnancy assessment and rescuing offspring is much easier in comparison to viviparous species. During the investigation, a new method for egg case detection for this species was introduced in Malta. The aim of this study was (i) to investigate the size range of egg-laying female sharks of the species smallspotted catshark (*Scyliorhinus canicula)* in the Maltese waters and (ii) to improve the method of egg case detection from dead females of these oviparous shark species. The methodology proposed ("gotIt") is a simple, fast and effective way that can ensure that only pregnant females get investigated and therefore avoid needless random dissection of non pregnant females which is time consuming.

Material and Methods

Data collection

All data was collected from the wholesale fish market in Valletta (Malta) from February to June 2014. Data collections always started one hour prior to opening to avoid interactions with the selling activities of fish brokers and customers.

Catsharks of both sexes were landed in boxes. By checking the presence of claspers, males and females were identified and sorted in different piles. To investigate pregnancy two methods were applied. The first one, named "Previous Method" relied on basic visual inspection regarding body size and shape. Females with TL above 40cm and a bulky body shape were assumed to be pregnant and were dissected. The second method, gotIt, works differently by eliminating the parameters. Instead of visual inspection females were investigated mechanically with a touching pattern at their belly to detect the egg-cases immediately without opening as it is assumed that size and shape may mislead investigators. To record the size range of pregnant females, which were carrying egg cases, size records were made with measurements to the nearest cm (see Fig. 4(B)), including recording of body shape (slim and corpulent which was used in the Previous Method).

Previous Method

The Previous Method was generally used by Sharklab-Malta members from 2007 until March 2014, with an overlap of one month with the gotIt method introduced in the present study. The Previous Method was determined through fixed parameters. Firstly, every single female was measured to the nearest cm. Possible pregnant females were classified by (i) total length (TL), above 40 cm and (ii) corpulent body shape. The threshold of 40 cm was the length of first maturity estimated in previous investigation done by the team members, but there was no methodically produced evidence or reference in support of that value, so that this parameter was removed in 2014. All females which were smaller and/ or had a slim body shape were not considered to be pregnant, though no empirical evidence for the reliability of this method was available. After this, among the pre-selected females a smaller number of individuals was chosen randomly according to personal preference by the investigator. From around 50 individuals with the selected features, 10 females were chosen and dissected to investigate if they were pregnant.

gotIt

gotIt was used from March to June 2014. To approach an optimization of the method the parameter TL and body shape were removed. For a survey, many females were opened without any preference and compared to find possible correlations. Comparisons were made and similarities between pregnant and not pregnant females were investigated. With anatomical studies a touching pattern was devised (see below) to detect egg cases with the aim of optimized accuracy. After this step, it was possible to detect pregnant females non-invasively within seconds, via the systematic touching pattern.

Development of the systematic touching pattern

For rapid and accurate identification of pregnant females, the anatomy was studied. It was possible to identify the location of egg cases inside a female (Fig. 1A). To assure the shark´s pregnancy it has to be turned around a few times and investigated very softly to avoid damaging the embryo. Possible detection errors were identified. The most difficult point was to distinguish between stomach and egg case (Fig. 1B) since the content of the stomach is sometimes quite solid (depending on ingestion).

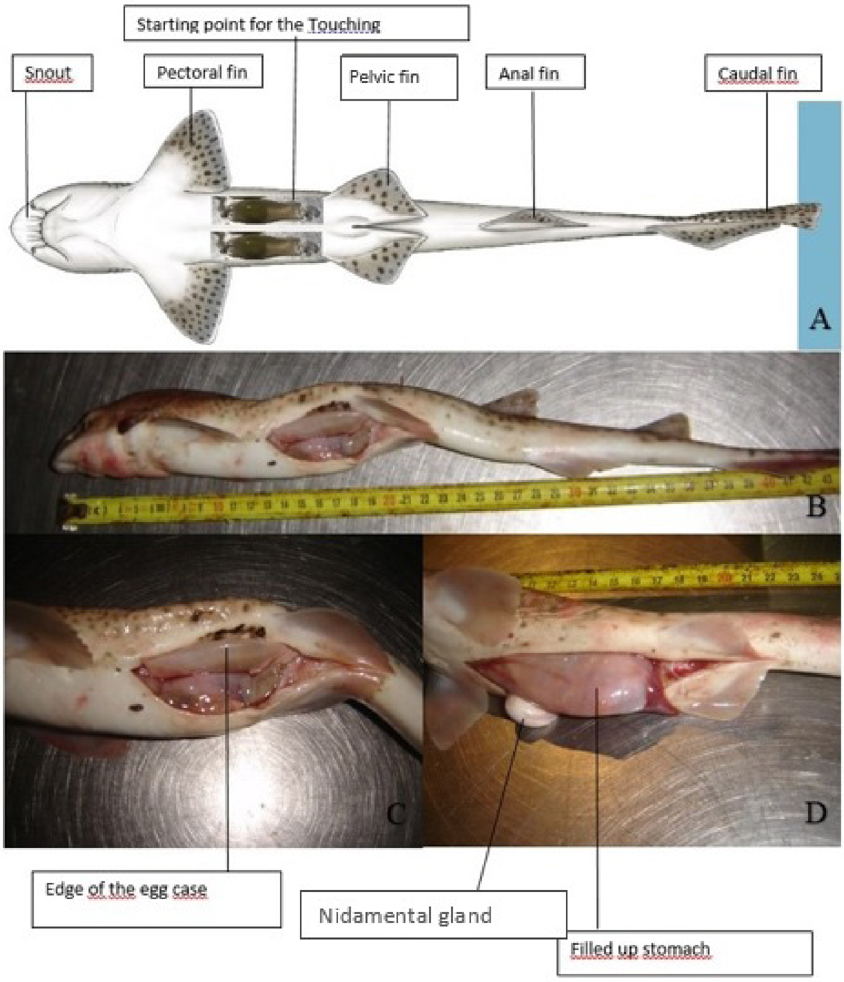

Fig. 1: This illustration shows the anatomical features of a female smallspotted catshark (Scyliorhinus canicula). A) Schematic location of egg cases (adapted from www.sharktrust.com). B) Overview of the position of egg cases inside a pregnant female. C) Identification of the edge of an egg case. D) Female with full stomach covering the egg cases.

In case it is very full it can cover the egg case, so the stomach must be moved around through gentle pressure until a detection of the edge of the capsule was possible. The shark got turned on its back and were systematically touched on the sides of the stomach, were the egg cases are located. After spotting the stomach, which is usually located in the middle, the edge of the egg case and the bowing between the two edges (Fig. 1C) was identified. After finding the edge, it was necessary to follow the edge until the tendrils were detected. If the same could be repeated on the other side of the shark it was indicative that the female was carrying egg cases, since females usually carry two egg cases at the same time. After some training, the detection through gotIt took about 3 seconds per shark with a maximum of 8 seconds for sharks with a filled stomach with highest accuracy.

Validation of gotIt

To validate the developed gotIt method all females were selected and placed in a pregnant and not pregnant pile. Females from both piles were dissected to validate pregnancy. To validate this method, it was necessary to ensure that all females from the ´pregnant´ pile carried egg cases, whereas from the ´not-pregnant´ pile all females did not carry egg cases. This validation was done with 124 females.

Maintenance of egg capsules and pups

Egg-laying females identified by gotIt were dissected and after removal, egg cases were taken in a sealed container filled with fresh sea water to be relocated into a private aquarium for development at 17 to 19°C in fresh sea water tanks for approximately 3 weeks (see Fig. 4(C)). Afterwards they were transported in a sealed and cooled container to the Malta Aquaculture Research Center (MARC), where they were kept for approximately 3 to 6 months (depending on the developmental state at removal at the fish market) until the main location, the Malta National Aquarium (MNA) was opened (see Fig. 4 (D)). After hatching (see Fig. 4 (E, F)) they got fed with squid and released into the sea by members of Sharklab-Malta and the MNA crew into the sea (see Fig. 4 (G)). The release location of the shark pups was Cirkewwa, in the North of Malta.

Results

In total 678 females of *S. canicula* (smallspotted catshark) were tested, of which 93 (13.7%) were pregnant (see Table 1), including the total from both methods and the validation during the whole time of data collection.

Previous Method

With the Previous Method only 5 of 121 (4.1%) of the randomly chosen females considered pregnant were pregnant. A total of 9 egg cases were recovered in 4 weeks (see Table 1). It could not be confirmed that the parameter "body shape" used in the Previous Method is related to pregnancy. Our results indicate that slim females as well as more corpulent females were pregnant.

gotIt

With gotIt it was possible to retrieve 176 egg cases in 12 weeks (22.03.2014 until 22.06.2014) from 557 sharks, with 88 pregnant (15.8%) individuals (see Table 1).

Validation of gotIt

For gotIt validation 124 individuals were opened from both the pregnant and not pregnant pile. 103 were declared to be not pregnant and sorted to the ´non-pregnant´ pile, 21 were declared to be pregnant and sorted to the ´pregnant´ pile. The validation occurred randomly between April and June 2014. The validation showed that the new method works with 100% precision. All pregnant sharks were identified, and the work was performed quickly.

Size at maternity

Measurement of pregnant females showed that the smallest size of pregnant females was 37 cm. One of two females sampled with that size was taken as a sample and stored in alcohol as evidence. The suggestion that only females above 40 cm can be pregnant was quite close. But it was possible to confirm the sizes now with clear evidence. Our data shows the highest number of pregnant females at a TL of 44cm.

Discussion

The main aspect of the Previous Method was that in *S. canicula* (smallspotted catshark) no egg cases could be detected without opening the female due the small size of egg cases. Nevertheless, after studying the anatomy of females and the egg cases, it was possible to show with gotIt that the egg case is quite easily detectable via touching, even when small in comparison to *S. stellaris* (Nursehound). The results show that improvements have been made in the methodology to recover egg cases from dead pregnant *S. canicula* (smallspotted catshark) females at the fish market. The observation and documentation of the various sizes are showing that the species is having a relatively wide variety of size at pregnancy and it is expected that females above 37cm can be pregnant. Data was recorded from the smallest to the largest pregnant females as a basis to calculate a MLS more adequate for Maltese waters. The assumption of previous Sharklab-members that pregnancy starts at a TL of 40 cm was close, but we could show with the two pregnant individuals with a TL of 37 cm that females can get pregnant below 40 cm as well. Also, the

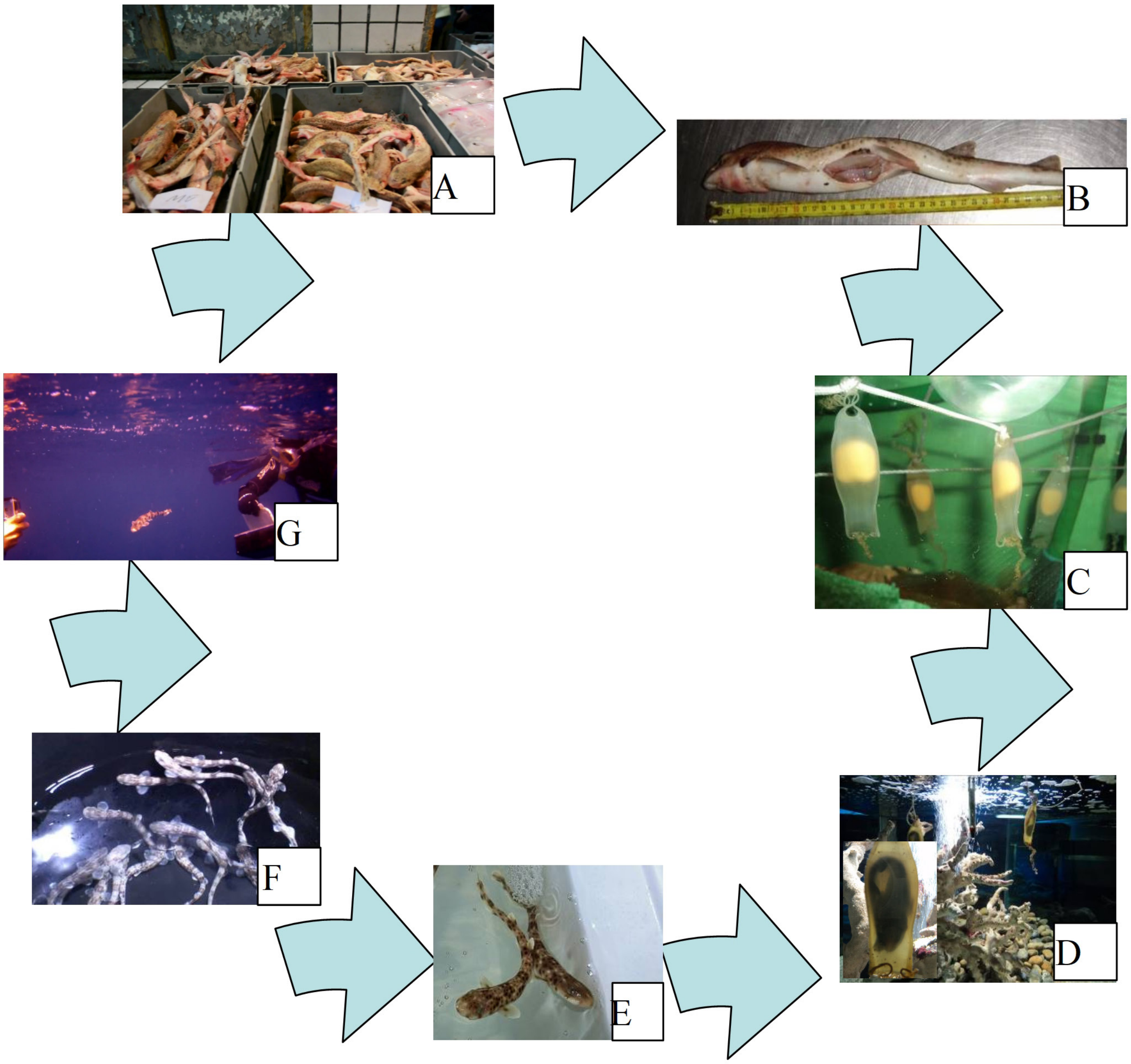

Fig. 4: Protocol used to retrieve catshark´s from the fish market. It can be used for smallspotted catsharks and for Nursehounds (Scyliorhinus canicula and Scyliorhinus stellaris), basically to every oviparous elasmobranch. (A) Identification of female catsharks from boxes of mixed gender at the fish market using gotIt, (B) Retrieval of egg cases by dissection, (C) Placing of egg cases in an aquarium for the development of embryos during 3 weeks, (D) Maintenance of egg cases in the aquarium under controlled conditions, (E) Hatch of shark pups after 24 weeks (depending on the initial stadium), (F) Feeding of catshark pups with squid, (G) Pups released into the sea.

source of the previous estimated size in 2007 is not clear. It is important to consider that market data can always give just a very small insight into the real fish stocks. So even if the smallest pregnant female we found with a TL of 37 cm at the market it is not advisable to conclude to the stock. Our data gives a small insight to the size range at this time of the year. More studies should be done over the winter time as well and then again over several years. In general, the successful detection of egg cases depends on the person who is investigating the sharks. Every person, regardless of their aim or research, should undergo training and validation to ensure accuracy of the data. The results show that the Previous Method revealed some egg cases but in comparison to gotIt significant improvements could have been made. It is not known how many pregnant females were not spotted before but it is clear the with gotIt all available egg cases were identified. Reasons for the misidentification in the Previous Method could have occurred due to differences between members who investigated and chose the sharks and additionally the wrong parameters set for preselection, like the shape of the body or length of the female. We are sure that other labs and fishermen can use our gotIt easily with the same goal, to preserve the stock

Table 1: Total Results of sharks examined with different methods.[1]

Method	Status	Frequency	[%]
All together	Total	678	
	Pregnant	93	13.7
	Not pregnant	585	86.3
Previous method	Total	121	
	Pregnant	5	4.1
	Not pregnant	116	95.9
gotIt	Total	557	
	Pregnant	88	15.8
	Not pregnant	469	84.2
Validation gotIt	Total	124	
	Pregnant	21	16.9
	Not pregnant	103	83.1

[1]During the whole study 678 females were investigated. Considering both methods 93 sharks were pregnant. In comparison of the Previous method and gotIt the percentage of identified pregnant individuals rose from 4.1% to 15.8%.

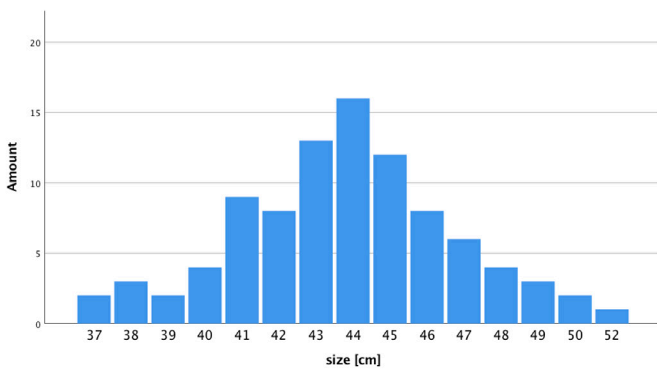

Fig. 2: Size- range of 93 pregnant smallspotted catshark females (Scyliorhinus canicula) in Total (Previous method + gotIt and Validation of gotIt. The two smallest pregnant females had a TL of 37 cm and the largest pregnant female had a TL of 52 cm. But the highest amount of pregnancy were found in sharks with TL of 43 to 45 cm.

of any oviparous elasmobranch. In the research team of 2014, all members introduced to gotIt through the author were quickly able to identify pregnant females after about two days. Many sharks were not investigated in the Previous Method whereas with gotIt every female got checked itself and investigated simply because it worked quite fast. So even when all females sampled at the market got investigated, time was still saved in comparison. The reason is basically that in the Previous Method every female got measured anyway, and when above 40 cm, rated by body shape and then dissected mostly unsuccessfully. This is time consuming when considering the output of retrieved egg cases. With gotIt the sharks were measured and checked in one quick step with the highest accuracy within seconds. Only during validation, where all females from both piles were dissected, it took some seconds more. GotIt was proven to be 100% reliable due to validation. The next step now is to introduce gotIt to other Sharklab-members and people in general working at the fish market. Only females with complete egg cases were spotted as pregnant. We also found 3 females in the non-pregnant pile which started to produce egg-case tendrils which is a very early stage. It was not possible to detect this stage. Now future studies need to be done for introducing the method for fisher men.

The following task for sustainable fishing of catsharks is providing fishermen with solutions for how to handle pregnant sharks or egg cases. Option a) would be throwing sharks directly back into the sea after detecting with the gotIt egg cases. It must be investigated if it is possible for the embryo to develop in dead females and if they can hatch when not removed from the uterus. This can be easily investigated with some, not dissected pregnant female which can be attached to a structure (stone, reef, parts of a ship wreck etc.) in the sea, so that the shark cannot drift away. A dissection after the maximum time of 6 months should be done to check if the embryos have developed and hatched within the female's body, to prove that this works, as this option would require a minimum of efforts. Predation should be considered as well. Option b) would be throwing retrieved egg cases back into the sea. For this freshly removed egg cases can be attached to a structure in the sea (stone, reef, parts of a ship wreck etc.) and checked weekly if hatched. If that works fishermen can simply throw removed egg cases into the sea and thereby save the shark pups. This is the option with the second minimum effort. We also need to check if locations play an important role in development. If it does, it is important to give fishermen information where they should throw them back into the sea to save the offspring. Until transport to that location they can put them in a sealed container of cooled fresh sea water. Option c) would be to relocate them into the aquarium. This was done in our work here, and is also very easy, but especially in Malta quite risky as Malta suffers from frequent power cuts throughout the year, during which the cooling system of the aquarium is not maintained and it becomes unbearably hot during the

A B

Fig. 3: These two illustrations of pregnant smallspotted catshark (*Scyliorhinus canicula*) females indicate that the body shape is not interfering with pregnancy and that it is a poor indicator to spot pregnant females. Fig A shows a very slim pregnant *Scyliorhinus canicula* individual and Fig B shows a more corpulent pregnant female.

summer months. The fast-rising temperature will certainly lead to the death of the embryos. To avoid this, a location like the MNA with emergency generators is preferable. Development of embryos works very well in the aquarium (Fig.4).

Conclusion

Sharks are killed daily for food products, luxury goods (furniture covered with shark skin), cosmetics, medicinal products or as by-catch. In terms of numbers this means 63 up to 273 million dead sharks per year (Worm *et al.,* 2012). Conservation strategies against this global destructive fishing pressure on sharks are heavily relying on methods and legislations to save these apex predators in every way possible. Already up to 90% of the global shark population has vanished and this is extremely dramatic (Graham *et al.,* 2010). In the Mediterranean Sea, the situation is critical for large sharks, as shown by abundance and biomass calculations over time (Ferretti *et al.,* 2008). This work is contributing to save embryos of smallspotted catsharks (*Scyliorhinus canicula*) and can easily be applied on Nursehounds (*Scyliorhinus stellaris*) and other oviparous elasmobranches in general. We managed to rescue dozens of shark pups with the method explained in this study and released them back into the wild. At the fish market in Malta the egg cases including the embryos are thrown away in the trash and left there to die. It is important to raise awareness that there is a simple possibility to rescue these pups so they can still survive and be released when grown up, even when the mother is caught. This study gave embryos inside the egg case the chance to develop and to get released back into the sea. Due to this study, already hundreds of shark pups got released in Cirkewwa, Malta. Furthermore, there are possibilities to teach fishermen and brokers this new method so they can sell sustainable fish in the future as they contribute to restock the shark population, of course for higher prices. This would not only be advantageous for the shark population but it would also ensure that this species will be in future fishermen's haul.

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