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**Ecologia trofica dei gattucci definita da isotopi sta-
bili: casi studio dall'Italia e dal Sudafrica**

**Trophic ecology of catsharks elucidated by stable
isotopes: case studies from Italy and South Africa**

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RIASSUNTO

Gli squali sono pesci cartilaginei che occupano i livelli intermedi ed alti della rete trofica marina, caratterizzandosi, a seconda della specie, età e/o taglia sia come mesopredatori che come predatori apicali. In tutto il mondo vengono sovrasfruttati come oggetto di pesca commerciale e sportiva e sono una delle porzioni più consistenti, insieme a mammiferi marini e tartarughe, del *by-catch* (catture accidentali). La rimozione delle specie che fungono da predatori apicali, oltre ad avere effetti a cascata negativi su tutta la rete trofica, può causare un fenomeno chiamato “mesopredator release”, che porta all’aumento dei mesopredatori con un conseguente cambiamento nell’equilibrio dell’ecosistema. Nonostante il loro importante ruolo ecologico, sia come mesopredatori sia come predatori apicali, le informazioni relative alla loro ecologia trofica sono limitate. Informazioni sulla composizione della loro dieta sono essenziali e si possono ottenere tramite l’analisi del contenuto stomacale (Stomach Content Analysis o SCA) e l’analisi del contenuto di isotopi stabili (Stable Isotope Analysis o SIA). Per il progetto descritto in questa tesi, sono stati prelevati campioni di tessuto muscolare, per le successive analisi degli isotopi stabili di azoto e carbonio, da due specie diverse di squali appartenenti alla famiglia degli Scyliorhinidae: *Poroderma africanum* (endemico del Sud Africa) e *Scyliorhinus canicula* (catturato nel Mar Mediterraneo, precisamente nel Mar Tirreno). Per quest’ultima specie sono stati integrati i dati SIA con dati provenienti dall’analisi dei contenuti stomacali di *Scyliorhinus canicula*. Sono state eseguite in seguito analisi statistiche univariate e multivariate utilizzando PRIMER6 a PERMANOVA+ considerando i fattori sito, sesso e lunghezza per *P. africanum* e sesso e lunghezza per *S. canicula*. I risultati ottenuti dalle analisi degli isotopi stabili di N e C sono stati analizzati tramite i pacchetti R SIMMR e SIBER per la stima del contributo delle fonti potenziali di cibo alla dieta delle due specie e la valutazione dell’ampiezza delle loro

nicchie trofiche in base alle significatività ottenute dalle analisi statistiche precedenti. In *Poroderma africanum* si è notato un incremento significativo del $\delta^{13}\text{C}$ con l'aumentare della lunghezza totale. Anche gli output di SIMMR hanno evidenziato che gli adulti selezionano prede maggiormente bentoniche come il mollusco gasteropode *Phalium craticulatum* rispetto ai giovanili, i quali selezionano specie più nectobentoniche e/o planctoniche. Gli output di SIBER hanno mostrato come i giovanili abbiano una dieta più ampia mentre gli adulti hanno una dieta più selettiva, selezionando specie di più alto livello trofico. Anche in *Scyliorhinus canicula* c'è un incremento significativo del $\delta^{13}\text{C}$ con l'aumentare della lunghezza totale. I risultati ottenuti dalle analisi svolte ci confermano come i giovanili siano proiettati a selezionare primariamente prede più nectobentoniche come *Cepola macrophthalma* rispetto agli adulti, che preferiscono prede più bentoniche, come il gamberetto schioccatore *Alpheus glaber*.

Nonostante vivano in luoghi molto diversi la presente tesi ha dimostrato come le due specie abbiano molte caratteristiche in comune. Infatti, entrambe occupano lo stesso livello nella rete trofica, essendo squali mesopredatori. A dimostrazione del fatto che per *P. africanum* (popolazione in aumento, come stimato dalla IUCN), non esistono programmi di monitoraggio ben definiti, i dati sulle catture, accidentali o meno, dovuti alla pesca locale di questa specie in Sudafrica sono pochi rispetto a quelli relativi a *S. canicula*, la cui popolazione è in aumento, come stimato dalla valutazione dello stato della sua popolazione in Mediterraneo da parte dell'IUCN. Approfondire la conoscenza delle diete di questi mesopredatori può aiutare a capire le conseguenze dell'aumento di mesopredatori che potrebbe portare alla diminuzione delle loro prede.

ABSTRACT

Sharks are cartilaginous fishes that may occupy both intermediate and upper levels of marine food webs. Worldwide they are overfished as a commercial and sport fishing target and, together with marine mammal and turtles, they constitute one of the largest portions of the bycatch. In particular, the removal of top-predator species has negative cascading effects on the entire food web, causing the so-called "mesopredator release" phenomenon, which leads to an increase in mesopredators with a consequent change in the ecosystem energetic balance. Despite their important ecological role, information on their trophic ecology is limited. Information on the composition of their diet is essential and can be obtained through the analysis of the stomach contents (Stomach Content Analysis or SCA) and the analysis of the stable isotopes (Stable Isotope Analysis or SIA). For the project described in this thesis, muscle tissue samples were taken from two different species of sharks belonging to the Scyliorhinidae family: *Poroderma africanum* (endemic to South Africa) and *Scyliorhinus canicula* (captured in the Mediterranean Sea, precisely in the Tyrrhenian Sea), for this latter data from SIA have been integrated with those obtained by SCA. Univariate and multivariate statistical analyses were then performed considering site, sex and length factors for *P. africanum* and sex and length for *S. canicula*. The results obtained from the stable isotopes analysis of Nitrogen and Carbon were analyzed using the R SIMMR and SIBER packages to estimate the contribution of potential food sources to the diet of the two species and to evaluate the extent of their trophic niches based on the significance obtained from previous statistical analyzes. In *Poroderma africanum*, a significant increase in $\delta^{13}\text{C}$ with increasing total length was observed. The SIMMR outputs also showed that adults select more benthic prey such as the gastropod *Phalium craticulatum* than juveniles, which select more planktonic species. The SIBER outputs showed that juveniles have a wider diet while adults have a more selective diet in

relation to the prey trophic level. Similar results were obtained for *Scyliorhinus canicula*, where there is a significant increase in $\delta^{13}\text{C}$ with increasing total length. Also for this species, both SIA and SCA confirmed that the juveniles primarily select more nekto-benthic prey such as the red bandfish *Cepola macrophthalma* than adults, which prefer more benthic prey such as the red snapping shrimp *Alpheus glaber*. Although they live in very different places, this thesis has shown that the two species have many characteristics in common. In fact, both act as mesopredators occupying the intermediate level of the food web they belong to. Concerning *P. africanum* (increasing population, as assessed by IUCN), there are no well-defined monitoring programs, as demonstrated by the lack of well-structured catch data for the species, with respect to those for *S. canicula*, whose population is increasing, as assessed by the IUCN evaluation on their population status (European regional assessment). Therefore, given the role mesopredators likely play in determining structure and function of ecosystems, an understanding of the dynamics of mesopredator interactions is crucial to elucidating the potential effects of predation in marine systems.

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1. INTRODUCTION

Sharks are cartilaginous fishes that comprise about half of all contemporary chondrichthyans (492 sharks, 621 batoids, 46 chimeras, catalogueof-life.org). They are a monophyletic group of predatory fishes that originated about 423 million years ago, before any other extant vertebrate predator. Over evolutionary time, natural selection modified these predators which, from small coastal consumers, developed large body sizes, delayed age of first maturity, continuous growth, and the capability to colonize deeper oceanic waters (Grogan & Lund, 2004). This group influenced the distribution and diversification of prey and competitor species, acquiring ecological niches previously occupied by extinct predatory vertebrates like *Otodus megalodon* (Ferretti *et al.*, 2010). Nowadays, sharks are present everywhere in all oceans. Most species are limited to the continental shelves, while a small number of species is fully oceanic, such as the blue shark, the oceanic whitetip, mako sharks, and some migrate between coastal and oceanic waters, such as the hammerhead, the silky, the tiger and the white shark (Compagno, 1990; Ferretti *et al.*, 2010).

The morphological and physiological features of elasmobranchs, sharks in particular, make them particularly adapted to predation. Each species has, in fact, a characteristic series of teeth, which can rapidly change. Teeth grow in parallel rows as on a conveyor belt (Figure 1), since at each attack there is the possibility of losing them. The mouth is ventral, and the teeth are articulated and effective. The jaw is not fused with the skull and varies in shape, size and orientation depending on the type of feeding. The maxillary muscles are particularly strong to ensure the maximum pressure to the bite. The jaw opens several degrees to increase the penetration of the bite, the rotation of the teeth and the removal of the larger parts of the prey (Whitenack & Motta, 2010).



Figure 1. Skull of a mako shark with parallel teeth rows

The integumentary system is made up of placoid scales (dermal denticles), which decrease the friction with water and allow a smoother forward motion, together with the shape of their body. The trunk sustains the pectoral fins, and the propulsion is given by the thrusts of the caudal fin for the large pelagics, while the small ones mainly use a wave motion. The digestive system changes according to the type of nutrition, but in general the intestine is coiled to increase digestive capacity (Bucking, 2015). The enzymes secreted by the pancreas are like those of mammals. The liver is large (about a quarter of the mass of the animal) and it secretes squalene and other oils that have the function to increase buoyancy (Speers-Roesch & Treberg, 2010). They represent an important reserve of energy, in fact, sharks can spend a lot of time fasting. They produce urea, which is also present in high concentration within the blood.

The sensory system is particularly evolved. It is characterized by different sensory organs that include four mechanisms to perceive stimuli from external environment: chemoreception (thanks to the gustatory and olfactory system), photoreception (thanks to the visual system), mechanoreception (thanks to the auditory and lateral line systems) and, finally, electroreception (thanks to the “ampullae of Lorenzini”). The “ampullae of Lorenzini” are tubules innervated by nerve ending, which are an extension of the lateral line system (Figure 2).

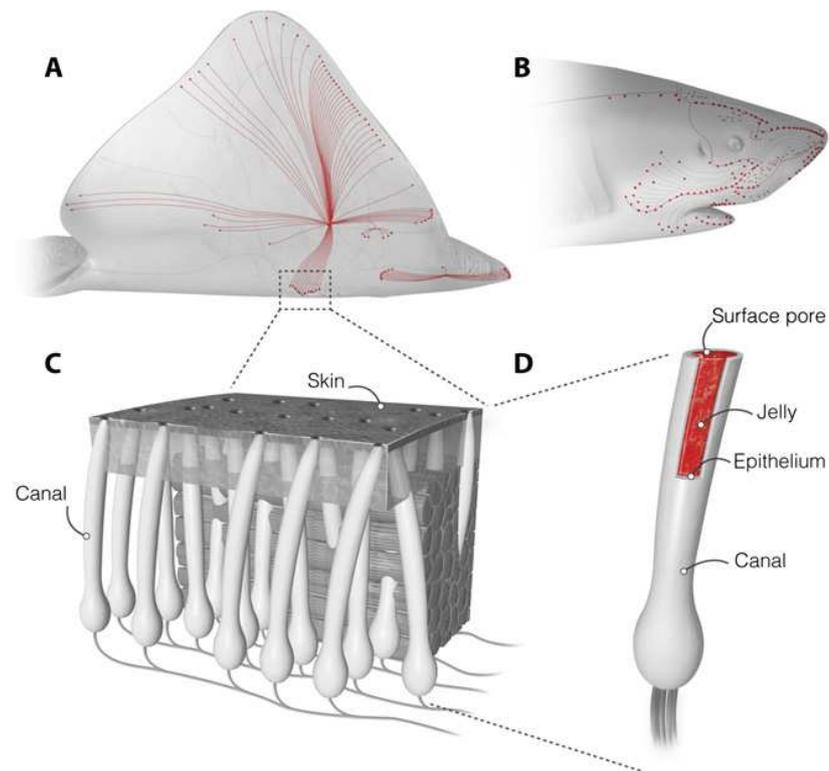


Figure 2. The AoL. (A and B) Skates and sharks locate their prey by detecting the weak electric fields naturally generated by biomechanical activity. (C) A network of electrosensory organs called the AoL is responsible for this sense. (D) An individual ampulla consists of a surface pore connected to a set of electrosensory cells by a long jelly-filled canal. Sharks and skate can sense fields as small as 5 nV/cm despite canals traveling through up to 25 cm of noisy biological tissue (From Josberger *et al.*, 2016).

They are important for the identification of prey, orientation, and movement. The senses of smell and taste are made up of several chemoreceptors. Those of smell are located inside the nostrils and are very sensitive to amino acids, while those of taste are generally present in the oral cavity, in structures called gustatory buttons. The olfactory system allows to detect the presence of food substances in the water even at very low concentrations and at great distances. The gustatory system, on the other hand, allows to recognize food once in contact with it (Meredith & Kajiura, 2010). The visual system consists of eyes disposed laterally on the head, equipped with eyelids or membranes, which are absent in the abyssal species. Species that live in low-light environments have a retina with a cone to rods rate of 1 to 100. This rate allows them to perceive bioluminescence in deep waters. The lateral line is part of the sensory system. It is a set of receptors inserted in a channel and therefore they are not directly in communication with the external environment. They are associated with other sensory organs similar in origin, innervation, and structure (Maruska, 2001). The auditory system is characterized only by the inner ear, very similar to that of mammals and sensitive to low frequency sounds. It is made up of three semicircular canals, which constitute the organs of balance, and three otolithic regions, delegated to perceive sounds: the saccule, the utricle and the lagena. Sharks perceive sounds between 10 and 800 Hz (Popper & Fay, 1977).

Sharks are almost exclusively marine, but some species can go up the rivers such as the bull shark, *Carcharhinus leucas*; very few species live in freshwater.

Regarding the reproductive system, sharks have a sexual dimorphism, that is, males and females show different secondary characters (Kardong, 2012; Awruch, 2015) (Figure 3).

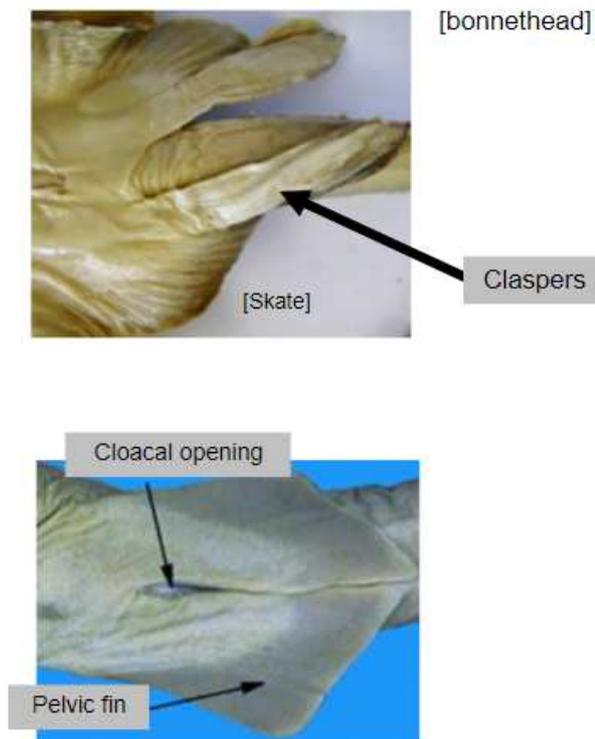


Figure 3. Top: copulatory organs of elasmobranch males (claspers) and bottom: cloacal opening of females.

The copulatory organ of males is made up of modified pelvic fins, called claspers, which are long and flexible structures with calcified ends. The degree of calcification depends on the sexual maturity stage. The sperm is enclosed in protective capsules, called spermatophores, which are pushed into the female's cloaca through claspers. The reproduction of most shark species is little-known. Sharks have three reproductive strategies: oviparity, ovoviviparity and viviparity (Compagno *et al.*, 2005). Viviparity can be placental (eggs are retained in the oviducts until the development of the embryo which feeds thanks to the placenta; it is present in only 10% of known species) and implacental (eggs are retained in the oviducts until the development of embryo which feeds thanks to the yolk sack). In some cases, the embryo feeds on other eggs produced by the mother (oophagy) or on other embryos which are present in the oviduct (adelphophagy).

Pregnancy is very long; it can last up to 2 years. For this reason, elasmobranchs' populations are very vulnerable, and it is crucial to know their reproduction rates and their longevity to better manage them (Frisk *et al.*, 2005). Vulnerability is related to low resilience and slow recovery and varies from species to species (Dulvy *et al.*, 2003). Fishing could lead to the decline of their population (Norse *et al.*, 2012).

Worldwide, sharks are exploited for their meat, skin, jaws, and fins which especially enrich the tables of Asian countries. Sometimes they are the main target of some types of commercial and / or sport fishing (e.g., *Alopias vulpinus*, *Prionace glauca*) and some species are frequently caught accidentally, i.e., bycatch (Oliver *et al.*, 2015). Bycatch is the unintentional catch of non-target species or sizes and is considered as one of the greatest threats to large marine fish populations. The release of by-catches of threatened species is often required by national and international legislation but often the animals are already dead, so their release does not have any conservation purpose (Gallagher *et al.*, 2014). Altogether such pressures have led to a state of over-exploitation of various species causing their decline (Dulvy *et al.*, 2021). The decline of large predatory animals reduces natural mortality in a range of prey, contributing to changes in behavior, distribution, and abundance of small elasmobranchs, sea turtles and marine mammals which have few other predators (Ferretti *et al.*, 2010).

Elasmobranch fishes are often typified as opportunistic predators, with a wide trophic spectrum that includes plankton to marine mammals. There are different feeding types: filter-feeding as for basking and whale sharks, suction crushing as for carpet sharks and effective raptorial mechanisms as for white and tiger sharks (Compagno, 1990; Ferretti *et al.*, 2010) (Figure 4).

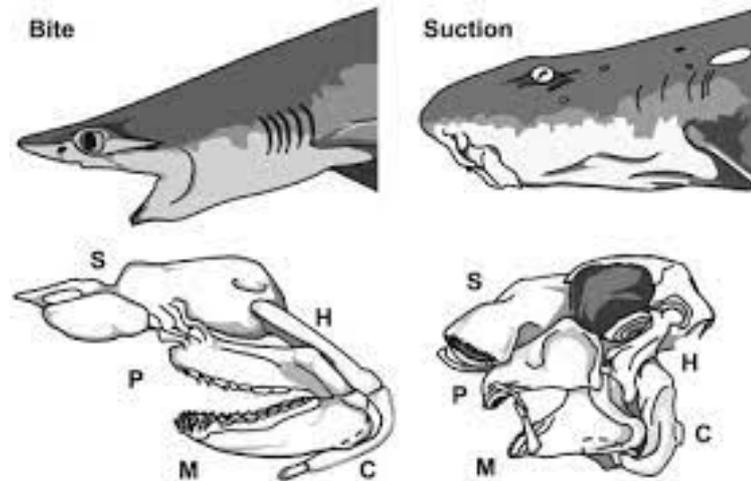


Figure 4. On the left: raptorial mechanism and, on the right, suction crushing mechanism

Most larger species, > 3 m total length, play the role of top predators and can prey on a high diversity of mesopredator elasmobranchs of < 1.5 m total length. Their importance is explained by the likely effects they may have on particular prey species and their high connectivity in the food web (Bascompte & Melián, 2005; Ellis & Musick, 2007; Ferretti *et al.*, 2010).

Information regarding the diet composition of the species in this group can be essential to understand trophic relationships in marine ecosystems (Cortés, 1999; Ferretti *et al.*, 2010). Sharks have the potential to influence the community structure and the habitat use of prey organisms. They are very important for the top-down control that can be a principal determinant of ecosystem structure and function. Top-down control can be exerted on a trophic link or on multiple trophic links, directly or indirectly, respectively (Baum & Worm, 2009). Trophic cascades are among the best-known examples that involve strong predator effects propagating downwards through food webs (Pace *et al.*, 1999). The removal of sharks, especially the apex predator, can cause a trophic cascade that led to an outbreak of

mesopredators, called mesopredator release. This can lead in turn to increased predation on smaller prey (Ritchie & Johnson, 2009). Many shark species, that are at or near the top of a food chain, function as keystone predators keeping the balance with competitor species and supporting high level of ecosystem diversity (Motivarash *et al.*, 2020). Thus, they serve as indicators of ocean health, helping to remove the sick and the weak individuals and ensuring the survival of healthier populations. By eliminating the weak, old and sick fishes, they prevent the spread of diseases and epidemics that could have a devastating effect on the ecosystem.

Despite their important ecological role, the information available on their trophic ecology is limited. Their intrinsic biological characteristics make the study of many species complicated: naturally low population densities, generally highly mobile and elusive behavior, and large distances migrations (Matich & Heithaus, 2014). A deep understanding of the diet, foraging and trophic ecology of sharks is considered fundamental for the management of populations and their successful conservation (Heupel *et al.*, 2014; Gračan *et al.*, 2017; Yemiskén *et al.*, 2019).

Traditionally, the study of the diet and the trophic ecology of fishes is carried out through to the stomach contents analysis (Hyslop, 1980; Cortés, 1999). Stomach contents analysis (SCA) provides instant observation of a consumer's diet, thus a snapshot of the diet of an individual in a certain space and time. Methods used are clearly invasive, such as gastric lavage or stomach eversion, and become destructive when threatened and/ or protected species are considered (Hammerschlag & Sulikowski, 2011; Heupel & Simpfendorfer, 2010). The identification of the species in the stomach is time-consuming and requires extensive taxonomic knowledge. Prey are often digested, making the identification difficult or impossible. Moreover, those items that are slower to digest, such as hard pieces, otoliths, cephalopod beaks, decapod chelae etc., may be overestimated (Baker *et al.*, 2014). For sharks, as well as for many large and highly mobile predatory

fishes, SCA is limited by both insufficient sampling of all size classes and incomplete sampling in the geographic range of the animal from a spatial and temporal point of view (Hussey *et al.*, 2011).

More recently, stable isotopes analysis has become an alternative or complementary method to SCA. Stable isotopes analysis (SIA) can overcome many limitations of SCA for assessing dietary and trophic relationships in marine ecosystems. One of the advantages is that it requires smaller sample size as it reflects assimilated food, but it cannot be used to identify specific prey (Shiffman *et al.*, 2012). Moreover, this method allows to obtain information on long-term diet, since predator stable isotope values reflect those of assimilated nutrients from ingested prey integrated over longer time periods (Domi *et al.*, 2005; MacNeil *et al.*, 2006). Analysis of isotope values of a predator can reveal if stable isotope values are close to equilibrium with its prey. Furthermore, the niche and the dietary breadth estimations allow to show intra- and inter-individual variation in resource use (MacNeil *et al.*, 2005; Hussey *et al.*, 2012; Matich & Heithaus, 2014; Matich *et al.*, 2015). Thus, SIA provides a better understanding of a species' role in the ecosystem that can lead to management and conservation strategies (Hussey *et al.*, 2012). SIA is based on a biological-chemical concept: during normal metabolic functions, the heavier, rare isotope is retained while the lighter, more common isotope is excreted (Fry, 2006). The resource use is indicated by specific ratios of heavy-to-light isotopes, which allow to infer the diet and/or habitat (De Necker, 2017).

The stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are most used and provide powerful tools for estimating carbon flow to consumers ($\delta^{13}\text{C}$) and related trophic positions of species within food webs ($\delta^{15}\text{N}$). However, a consumer's isotopic signature alone, without an appropriate isotopic baseline, is not generally sufficient to infer carbon source or trophic position (Post, 2002). Ratios of heavy-to-light nitrogen and carbon isotopes increase at each trophic transfer, providing a chemical tracer for links in diet

(Logan & Lutcavage, 2010). The analysis of nitrogen isotopes allows to obtain information on trophic position, as $\delta^{15}\text{N}$ increases with increasing trophic level (Figure 5).

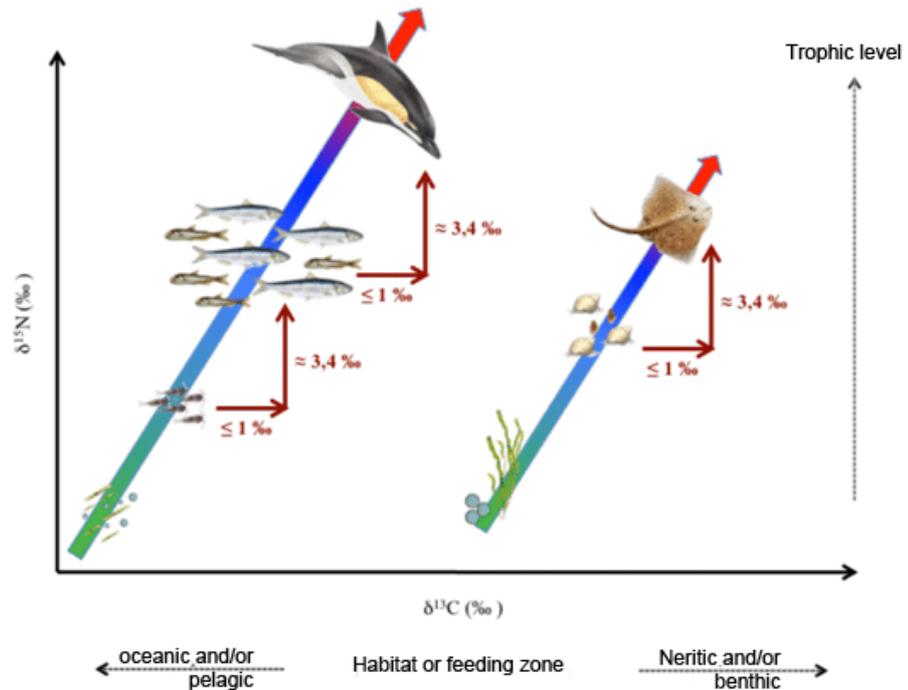


Figure 5. Representation of the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the ecosystem. As $\delta^{15}\text{N}$ increases, the trophic level increases. If $\delta^{13}\text{C}$ increases, there is a preference of neritic and/or benthic habitat or feeding zone, while, if $\delta^{13}\text{C}$ decreases, there is a preference of oceanic and/or pelagic habitat or feeding zone.

This process is known as trophic enrichment or fractionation (Shiffman *et al.*, 2012; Van der Heever *et al.*, 2020). Some analyses have provided convincing results that enrichment decreases with successive trophic levels (e.g., Hussey *et al.*, 2011). However, enrichment is generally considered constant around 3.4‰ for trophic level and is predictable. An estimation of an organisms' trophic position compared to that of others can be obtained through this analysis (Post 2002; Hussey *et al.*, 2011, Van der

Heever *et al.*, 2020). The analysis of carbon isotopes allows to obtain information on the foraging ecology and movement/migration patterns. $\delta^{13}\text{C}$ values vary between primary producers such as pelagic production sources versus benthic ones, C3 plants versus C4 or coastal sources versus offshore ones (Figure 5). Despite this, $\delta^{13}\text{C}$ values undergo minimal enrichment when moving up the food chain, with an average of 0.4 ‰ for trophic level (Post 2002; Shiffman *et al.*, 2012; Van der Heever *et al.*, 2020). If there are two or more species with different $\delta^{13}\text{C}$ values, this may suggest the presence of multiple sources of dietary carbon within an ecosystem (MacNeil *et al.*, 2005).

The isotopic value of an individual can be determined by analyzing a particular tissue. Once compared with other organisms within the relative trophic system, it is possible to estimate the trophic position of a species within the food web. The type of tissue that will be analyzed is recognized as a fundamental aspect of experimental design when applying stable isotopes analysis in ecological studies. Tissue samples used for the SIA of sharks include white muscle, liver, blood (plasma, whole blood), cartilage (usually from vertebrae or spines) and, more recently, fin (Hussey *et al.*, 2011). Each tissue has its own characteristics, different metabolic turnover rates between different species and within the same species. Stable isotope values are correlated with metabolic turnover rates of tissues (De Necker, 2017). Stable isotopes of each metabolically active tissue and with different turnover rates can be used as a representation of a different feeding period and compared to examine temporal dietary shifts (Logan & Lutcavage 2010; Matich & Heithaus, 2014).

The most sampled tissue for stable isotope analysis in sharks is the white muscle. It can provide a long-term integrated measure of a species feeding habits because of the slow growth rate of most species (Hussey *et al.*, 2012). Muscle tissue can be sampled from multiple individuals of varying

size, either sex or different life stages of maturity for an integrated assessment of ontogenetic dietary shifts. Furthermore, the sampling of muscle tissues is not destructive or invasive and this is very important considering the threatened status of many sharks. Stable isotope values of muscle tissue allow to obtain some information on the trophic position, on the role of a species in the trophic webs and in entire ecosystems, on variation in the diet and on the resource use of different species and, also, on movement patterns (Hobson 1999, Estrada *et al.*, 2003, Domi *et al.*, 2005, Hussey *et al.*, 2012). Here, muscle tissue from two different species of sharks belonging to the Scyliorhinidae family were sampled for the project described in this thesis, these are: *Poroderma africanum* and *Scyliorhinus canicula*.

PORODERMA AFRICANUM (Gmelin, 1789)

Order: Carcharhiniformes, Family: Scyliorhinidae, Genus: *Poroderma*,
Species: *africanum*.



Figure 6. *Poroderma africanum*

The Pyjama shark or striped catshark (*Poroderma africanum*) (Figure 6) is endemic of the waters off South Africa coasts, in the Southeast Atlantic Ocean and Western Indian Ocean from Saldanha Bay (Western Cape) to East London (Eastern Cape). It is common off Cape Province, especially the Southern Cape. There are older records of this species from Madagascar and Mauritius, but these are unconfirmed (Figure 7).

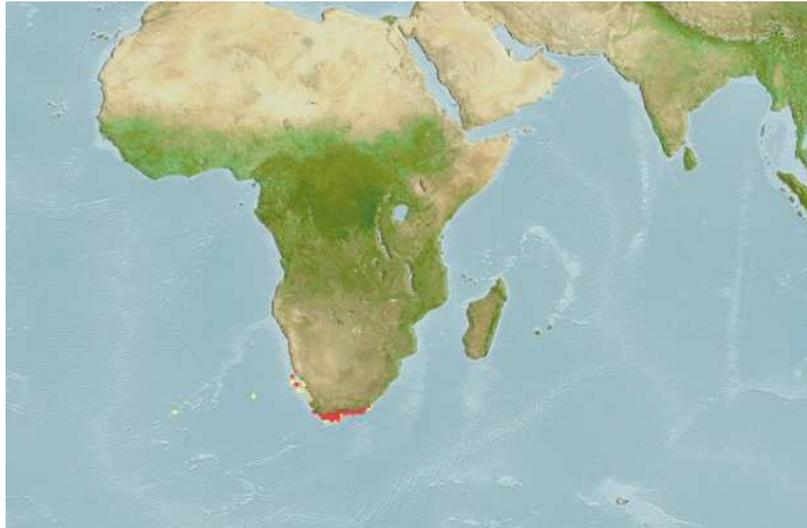


Figure 7. Distribution range of *P. africanum* (from fishbase.de)

There are no estimates of the population size for the Pyjama shark, but it is suspected to be increasing (iucnredlist.org). It has no specific protection, but its retention in commercial line fisheries is prohibited. It is assessed as “Least Concern” by the International Union for Conservation of Nature (IUCN).

The striped catshark lives both inshore and offshore over the continental shelf. It prefers kelp beds and rock reefs, from intertidal zone to 100 m depth (Compagno *et al.*, 1989). It is a nocturnal species, living in caves and crevices during the day and being active in the night, when they search for prey (Compagno *et al.*, 1989). This catshark has a snout bluntly



rounded. It has short nasal barbels that measure less than half the length of the nostrils and do not reach the mouth. The mouth is arched with the lower symphysis slightly behind the upper exposing the upper teeth. Each jaw has several rows of sharp, thin tricuspid teeth (Figure 8).

Figure 8. A particular of the mouth of a specimen of *P. africanum*.

This shark has two dorsal fins: the first is located in front of the middle base of the pelvic fins, while the second, much smaller, terminates at the origin of the caudal fin. The pectoral fins are broad and rounded in shape. The skin is thick and is made up of well-calcified dermal denticles (Compagno *et al.*, 1989). The striped catshark is greyish. It is characterized by seven distinct dark longitudinal broad stripes extending the entire length of the body, on the dorsal and lateral sides. They terminate towards the tail and the lower sides. Some individuals possess one or two (rarely more) large black or white spots scattered across the dorsal surface (Figure 9).



Figure 9. An example of white spots across the dorsal surface of a specimen of *P. africanum*

Ventral coloration, on the other hand, ranges from pale grey to white and, usually, when white is present, there is a rough margin between grey and white. When the ventral surface is white, dark grey spots may be present. The color pattern of the embryos generally mirrors that of the adults. Some individuals may be lighter than adults and, sometimes, the stripes can be very dark brown (Compagno *et al.*, 1989).

This species may reach a maximum total length (TL) of 109 cm. The average size of the adult striped catshark is 60-80 cm TL. Males mature at 72 cm TL, and females at 78 cm TL (Compagno, 1984). In this species little sexual dimorphism is present, with both sexes growing up to approximately 100 cm, although the males mature at a smaller size than the females. The striped catshark is an oviparous species and lays a single egg per oviduct. The embryos feed exclusively on yolk. Each season the female

releases two purse-shaped brownish eggs, which are enclosed in a case (Figure 10).

The egg case measures 5 x 10 cm and has a sticky surface that adheres to kelp and other marine vegetation. It is unknown how long it takes before hatching in the wild. However, in an aquarium test, an egg hatched after about five and a half months. Hatchlings measure around 14-15 cm in length and look like the adult striped catshark (Dulvy & Reynolds, 1997).



Figure 10. An egg case of *P. africanum*

The Pyjama catshark feeds primarily on teleosts. Other prey items include crustaceans and mollusks. It also feeds on annelids, cnidarians, agnathans and echinoderms (Compagno, 1984; Dainty, 2002). Larger sharks, such as the sevengill shark (*Notorynchus cepedianus*), are common predators of the striped catshark.

The striped catshark is well-adapted to life in captivity. It is often caught as bycatch in bottom trawling and recreational fishing. It is not used for human consumption, and usually discarded (ufl.edu).

SCYLIORHINUS CANICULA (Linnaeus, 1758)

Order: Carcharhiniformes, Family: Scyliorhinidae, Genus: *Scyliorhinus*,
Species: *canicula*



Figure 11. *Scyliorhinus canicula*

The Small-spotted Catshark (*Scyliorhinus canicula*) (Figure 11) is found in the Northeast and Eastern Central Atlantic Ocean (from Norway and the Shetland Islands to Senegal) and in the Mediterranean and Black Seas. This species is one of the most abundant elasmobranchs in the Northeast Atlantic and Mediterranean Sea (Figure 12).

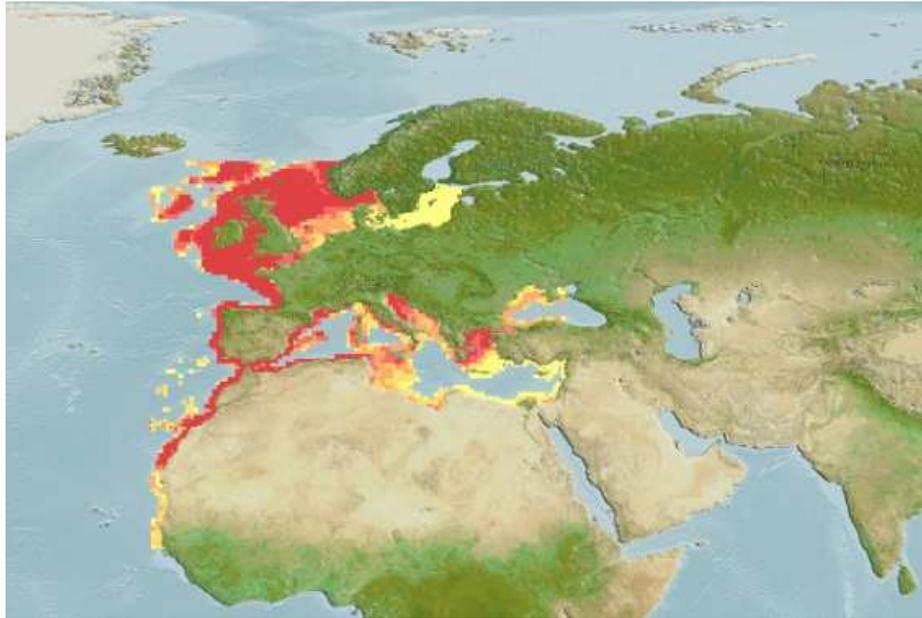


Figure 12. Distribution range of *S. canicula* (from fishbase.de)

Scientific surveys suggest that the population is stable or even increasing in some areas (confirmed by global IUCN assessment and European regional IUCN assessment, respectively). There is no evidence of population decline. Global and European IUCN assessment indicated *Scyliorhinus canicula* as “Least Concern” (iucnredlist.org).

The small-spotted catshark lives along continental shelves and higher slopes. It prefers sandy, coral, algal, gravel and muddy bottoms at depths between 10 and 400 m. This species is nocturnal, in fact, during the day it remains motionless. During the night, it increases its activity searching for prey (Sims *et al.*, 2001; Koehler *et al.*, 2018).

This catshark has a blunt head, elongated eyes and a rounded and short snout. The expanded anterior nasal flaps reach the mouth and cover the shallow nasal furrows while the labial furrows are only found on the lower jaw. The teeth are numerous and very small, consisting of a large cusp and small cusplets on either side, near the central portion of the mouth. The rear teeth, on the other hand, have a comb-like shape. This catshark has five pairs of gill slits; the last two are overlapped on the pectoral fins. It

has two dorsal fins and the first is larger than the second. The first dorsal fin is located posterior to the pelvic fin insertions, while the second is located above the anal fin insertion. The anal fin is long and low, in fact, it measures less than the space between the two dorsal fins. The skin of this shark is not extremely rough to the touch, in fact, it is made up of small denticles (Soares & De Carvalho, 2019).

The dorsal surface is greyish-brown and is usually characterized by many small dark spots. The small-spotted catshark also has 8-10 dark patches that form dark saddles along the dorsum. Ventral coloration ranges from light grey to white.

This species generally reaches a maximum of 80 cm TL. In the Mediterranean Sea, the maximum length is less than 60 cm, while, in the Atlantic Ocean, it can reach 100 cm TL. In the Mediterranean Sea, males mature at 39 cm TL and females at 44 cm TL, while, in the Atlantic Ocean, males mature at 53.5 cm TL and females at 57 cm TL (Koehler *et al.*, 2018; Soares & De Carvalho, 2019). In the Atlantic Ocean, the age at first maturity of females is estimated at 9 years, with a maximum age of 17 (Rodríguez-Cabello *et al.*, 2005). Mating occurs all year round in deep waters, from late summer to autumn, when females return to their spawning areas and release their eggs until June or July (Ellis & Shackley, 1997; Koehler *et al.*, 2018). Similarly, to *P. africanum*, the small-spotted catshark is an oviparous species and lays a single egg per oviduct. The embryos feed exclusively on the yolk. The large egg cases are laid in pairs and snagged, thanks to the tendrils, on suitable substrate such as macroalgae. In deeper habitats, egg cases are deposited on sessile invertebrates such as sponges or hydroids. The size of egg cases is around 4.9 and 7 cm in length and 1.5-3 cm in width and depends on the location and the size of the female. Up to 18-20 eggs can be released during each reproductive season. Embryos remain in the egg cases for 4-6 months before hatching (Ellis & Shackley, 1997; Koehler *et al.*, 2018).

This small shark feeds on marine invertebrates including mollusks, crustaceans, cephalopods, and polychaeta worms. It also feeds on small bony fish such as gobies, pilchards, juvenile mullet, damselfish and dabs, and occasionally larger fish, such as gurnards or whiting (Compagno, 1984). The small-spotted catshark can be a potential prey of larger fish, including sharks.

This catshark has a relevant importance for commercial fishing, being a target species of bottom trawling (both in Mediterranean Sea and Atlantic Ocean). Along the British Isles, it is mainly caught by bottom trawls. Once landed by fishermen, it is used fresh and dried-salted for human consumption or for fishmeal and oil. The small-spotted catshark is considered harmless to humans due to its small size, feeding habits and habitat preference (ufl.edu). It is also used as ornamental species in public aquaria.

2. AIMS OF THE STUDY

The main objectives of this study were to investigate the trophic ecology and the diet of two catsharks, the spotted catshark *Scyliorhinus canicula* and the Pyjama catshark *Poroderma africanum*. Specifically, we aimed to evaluate:

- spatial differences in the trophic ecology of *P. africanum*, also related to sex;
- ontogenetic shift in the feeding ecology of *S. canicula*;
- similarity between the diets of these mesopredators and their positions within the local food webs they belong to.

Trends in their population status were also discussed in the light of the “mesopredator release hypothesis” and insights on their trophic ecology.

3. MATERIALS AND METHODS

3.1. Study area for *Poroderma africanum*

The southernmost tip of Africa is characterized by the meeting of two major water masses: the Indian Ocean in the east and the Atlantic Ocean in the west, creating a hydrographically complex area. The east coast is influenced by the warm Agulhas current, which drives rapidly down the south-east coast under the influence of the tropical waters of the Indian Ocean. The west coast is influenced by the upwelled cold Benguela current that slowly drifts northwards (Bustamante & Branch, 1996). Biogeographically, the southern African region has been divided into three main provinces: the subtropical east coast, the warm-temperate south coast and the cold-temperate west coast (Bustamante & Branch, 1996). This region is characterized by a high variety of habitats, including both soft sediments and large areas of rocky reefs. This feature, together with the oceanographic conditions, favors high productivity, which, in turn, supports rich and diverse fauna (Smale *et al.*, 1994). The study area is located in Mossel Bay, in the western Cape province of South Africa, exactly halfway between the provincial capitals of Cape Town and Port Elizabeth (each 400 kilometers away) (Figure 13). The main feature of this site is the occurrence of a rocky reef.

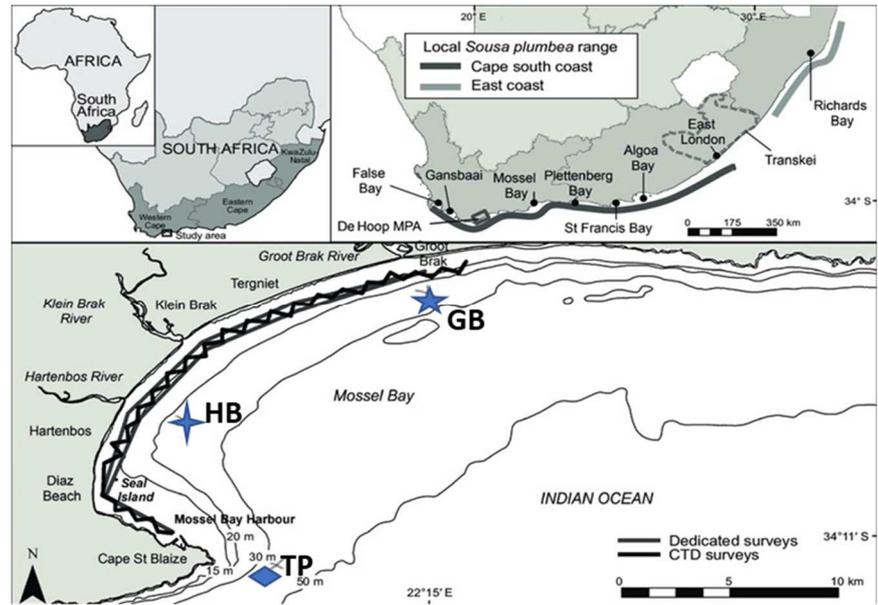


Figure 13. Study area in Mossel Bay, in the Western Cape province of South Africa, with the indication of the sampling sites (light blue symbols) of *P. africanum*. HB = Hartenbos, GB = Groot Brakrivier, TP = The Point (Downloaded on 24-09-2021 and modified from James *et al.*, 2015)

One of the most important abiotic factors in structuring South African rocky intertidal communities is the role of wave action (McQuaid, 1981; McQuaid & Branch, 1985; Emanuel *et al.*, 1992). Rocky reefs are characterized by inshore (< 40 m) fish communities that are species-rich and have complex trophic relationships. In shallow waters (< 30 m), endemic sparids represent the larger teleosts of the reef and cover a wide range of trophic niches from herbivores to piscivores. There are numerous benthic fishes, such as sandsharks, catsharks, stingrays, and skates, which feed on both fish and invertebrates (Smale *et al.*, 1994). Different large marine mammals also inhabit these waters, such as humpback whales (*Megaptera novaeangliae*), humpback dolphins (*Sousa plumbea*), southern right whales (*Eubalaena australis*), and southern right whale dolphins (*Lissodelphis peronii*). A colony of Cape fur seals (*Arctocephalus pusillus*) is present at Seal Island, which is an important hunting ground for great white sharks (*Carcharodon carcharias*).

For this project, three sampling sites were chosen inside the bay. These were, from the closest to the furthest from Mossel Bay harbour: The Point ($34^{\circ}10'983''$ $22^{\circ}09'774''$, hereafter TP), Hartenbos ($34^{\circ}07'775''$ $22^{\circ}07'392''$, hereafter HB) and Groot Brakrivier ($34^{\circ}05'469''$ $22^{\circ}14'514''$, hereafter GB) (Figure 13). Hartenbos and Groot Brakrivier are within the bay and therefore likely to be more sheltered, whereas The Point is more exposed to the wind and waves. Along the bay there is an important freshwater contribution deriving from three main rivers which take their name from the respective cities that pass through: Hartenbos, Little Brakrivier and Groot Brakrivier. All three rivers, crossing agricultural areas and raised beaches, likely funnel great amounts of nutrients into the sea, enhancing primary production. Changes in the basal food source may influence the isotopic signatures of sharks, which are at the apex of the food chain (Watling & Watling, 1982).

3.2. Collection of specimens of *Poroderma africanum*

The collection of muscle samples took place over 6 days during August 2021. Sharks were captured using a handline or a fishing pole with a small hook in order not to damage animals. Squid and fish-like mackerel were used as baits. Once caught and pulled into the boat, they were placed in a container half filled with salt water to reduce the stress during subsequent processes, like removing the hook from the mouth (Figure 14a). Then, total length (TL), from the tip of the snout to the longest length of the tail, and pre-caudal length (PCL), from the tip of the snout to the pre-caudal dimple were measured (Figure 14b).



Figure 14. a) On the left, the removal of the hook from the mouth, b) on the right, the measurements of TL and PCL in a specimen that is rolled up on itself.

Before proceeding with the extraction of the muscle tissue, information on sex was also annotated. If the specimen was a female, an additional effort to understand, if it was pregnant was made, by sliding the hand under its belly. The males were easy to recognize thanks to the presence of claspers. For sample extraction, a tool usually employed for larger sharks, like great white sharks, was used, which is not the most suitable tool for this kind of procedure on small animals, because of the lack of a biopsy punch. This tool (Figure 15a) was inserted behind the first dorsal fin and a tiny piece of muscle tissue was taken. Such process was hard as the shark's skin was tough. To obtain the necessary amount (ca 1 g of wet weight), the same process was repeated two/three times. Subsequently the samples were placed in a numbered test tube (figure 15b). To take the samples from the extraction tool, tweezers and a piece of fishing line are used to help us pushing it into the test tube. At the end, the sharks were immediately released (Figure 15c). This process, from capture to release, had to last as little as possible because the sharks had to return to their environment with normal physiological functioning and behavioral attitude (Enrico Gennari, personal communication). Finally, all the samples were placed in a dryer at 60°C for 48 hours and then stored in a freezer at -20°C until the day of

departure to Italy. During the 18 hour-flight a rigid bag with ice was used for better storage. Once arrived in the laboratory, the samples were put in the oven at 60°C until the weighing process began.



Figure 15. a) On the left, the tool used for sample extraction, b) in the middle, the collection of muscle tissue sample, c) on the right, the release of the shark.

3.3. Study area for *Scyliorhinus canicula*

The north-western part of the Mediterranean Sea is included in the geographical sub-area (GSA) 9 of the GFCM sub-division (General Fisheries Commission for the Mediterranean) (Figure 16). This area extends for 1245 km of coastline from Ventimiglia, in Liguria, to the mouth of the Garigliano river, in the Gulf of Gaeta. Furthermore, it includes the seas in front of Liguria, Tuscany and Lazio, reaching depths well over 1000 m (Mannini *et al.*, 2015). GSA 9 is a heterogeneous area both from a morphological and ecological point of view (Mannini *et al.*, 2015; MiPAAFT, 2011). There are two distinct basins, the Ligurian Sea and the Tyrrhenian Sea, geographically separated from the Elba Island. The Ligurian Sea is characterized by the presence of Corsica to the North and by numerous small islands belonging to the northern Tuscan archipelago to the South. The continental shelf varies from east to west: it goes from being very

wide, constituting over 60% of the seabed of this area, to being quite narrow with an extension of about 2000 km². In this area there are two main currents: the Eastern Corsican current, characterized by calm waters, and the Western Corsican current, characterized by colder waters, both flowing northward (Mannini *et al.*, 2015). One of the most important features of the Ligurian coast is the presence of numerous canyons that plow the continental slope (MiPAAFT, 2011). Their presence induces the transport of nutrients to the euphotic zone, on the continental shelf, increasing primary production.

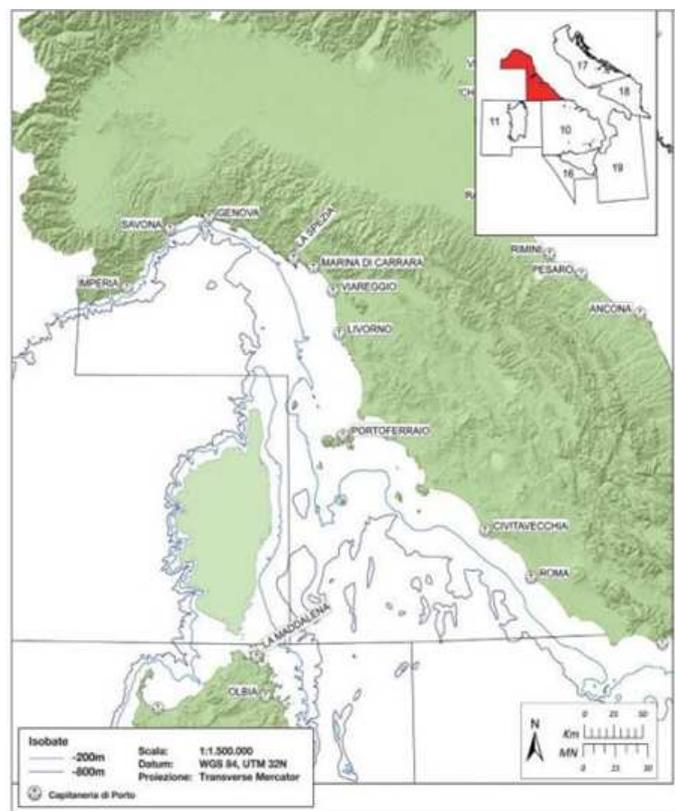


Figure 16. Study area in the Southern Tuscan archipelago (Downloaded 22-12-2021 from Mannini *et al.*, 2015)

The Tyrrhenian Sea is surrounded by islands and is separated from the rest of the basin by a channel at a depth of 450 m. Both the Central and Northern parts are characterized by a large continental shelf, which represents about 50% of the total area (Mannini *et al.*, 2015). Here, the circulation of

water is characterized by a series of eddies originating from the wind. The 3 main ones, 2 cyclonic and 1 anticyclonic, are characterized by the presence of cold water and undergo important seasonal changes. During the winter, the current in the frontal region of the eddies increases and the associated upwelling moves westward. In this season a direct connection arises between the Ligurian Sea and the Tyrrhenian Sea through the Corsican channel (MiPAAFT, 2011). As for the dynamics of phytoplankton there is a peak in the cold seasons, between October and April, and a minimum in summer.

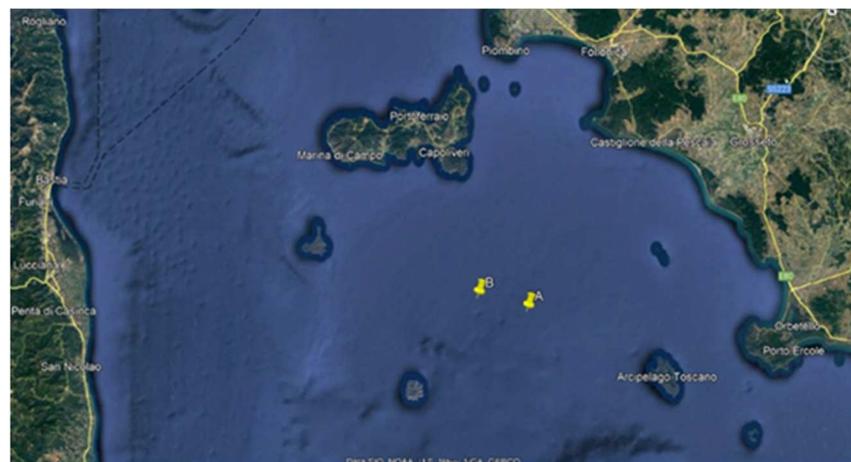


Figure 17. Sampling location in the South of Elba Island (Downloaded 23-12-2021 from Google Earth Pro).

The study area of this thesis is located in the Southern Tuscan archipelago, South of Elba Island, exactly. Here, the continental shelf is slightly narrower. The benthic populations show a high heterogeneity; they are characterized by both soft bottom and hard bottom biocenosis, typical of the oligotrophic waters of the Mediterranean Sea. Furthermore, the seagrass beds are particularly luxuriant (Mannini *et al.*, 2015). For this project, sharks were caught along a stretch A-B with coordinates, respectively, 4228.03 LAT, 1034.32 LON and 4229.48 LAT, 1027.36 LON (Figure 17). Depth of collection ranges from 330 to 255 m.

3.5. Stomach content analysis (SCA)

SCA was used only for *Scyliorhinus canicula*.

Each stomach was removed from the defrosted animals and weighed with the contents (stomach weight or SW, expressed in g). Then, each stomach was carefully emptied, and the contents were collected in Petri dishes. Each stomach wall was weighed, and the wet weight of the stomach contents (expressed in g) was obtained by subtracting the weight of the stomach wall from SW (Fanelli & Cartes, 2010). Stomach contents were analyzed under a light microscope (Leica ICC50) and the ingested items were identified to the lowest taxonomic level, as possible. The volumetric contribution of each item was obtained using the points' method (Swynnerton & Worthington, 1940): each food category ('very common', 'common', 'frequent', 'rare', etc.) on the basis of rough counts and judgement by eye, is awarded with some points that are proportional to its estimated contribution to stomach volume (expressed as a percentage).

Then, the fullness percentage was calculated for each specimen (weight of stomach contents/shark weight \times 100) as it is considered a proxy of feeding intensity. Traditional trophic indices were also estimated: percentage of frequency of occurrence (%F) and percentage of weight (%W) (Hyslop, 1980). An Index of Relative Importance ($IRI = \%F (\%N + \% V)$) was used to determine the importance of prey items in catsharks' diets (Dainty, 2002).

3.6. Stable isotope analysis (SIA)

First, the laboratory workbench and the analytical scale were carefully cleaned to avoid the contamination of samples. Sample tissue of defrosted *S. canicula* and of *P. africanum* were oven-dried at 60° for 24 h (Fanelli *et*

al., 2015). Each dried sample was ground using a mortar and a small pestle, and ca. 1-1.2 mg was placed into tin capsules (Elemental Microanalysis Tin Capsules Pressed, Standard Weight 5 × 3.5 mm) using a rectangular-tipped spatula (Figure 19).

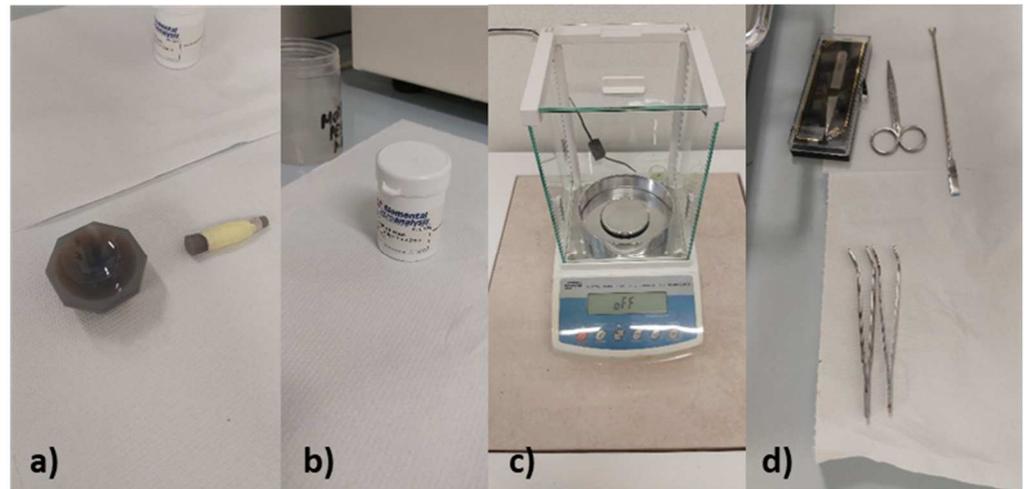


Figure 19. a) Mortar and pestle, b) tin capsules (5 × 3.5 mm), c) analytical scale, d) rectangular-tipped spatula and tweezers.

After weighing, each capsule containing the sample was closed using a rectangular-tipped spatula and tweezers and ordered in a plastic rack. Then, the rack was stored in a freezer at -20° until the delivery to the specialized laboratory for subsequent analysis. Samples were analysed through an elemental analyser (Thermo Flash EA 1112) for the determination of total carbon and nitrogen, coupled through a continuous-flow with an isotope-ratio mass spectrometer (Thermo Delta Plus XP), for the determination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, at the Laboratory of Stable Isotope Ecology of the University of Palermo (Italy).

Usually, for large sharks, urea should be extracted. It has high N content and, being a waste product, could modify the result of N isotope analysis. For these shark species, urea extraction was not necessary because they are not large sharks. Furthermore, lipid extraction is also important as sharks have a high concentration of lipids because of the presence of large

amounts of squalene. This could affect the $\delta^{13}\text{C}$ values and thus the results of the analysis. Here, lipids were not extracted from samples because the amount of samples, at least for *P. africanum*, was not enough. Thus, a correction equation was applied to $\delta^{13}\text{C}$ values, by using the relationship between C:N ratios and the $\delta^{13}\text{C}$ signatures according to Post *et al.* (2007): $\delta^{13}\text{C}_{\text{corrected}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 * \text{C:N}_{\text{bulk}}$.

3.7. DATA PROCESSING

3.7.1. Statistical analysis

The statistical design used to test for differences in the obtained data varied according to the two species. For *Poroderma africanum* a three fixed factors design was used: 'TL', 'Sex' and 'Site'. The first two factors have two levels ('AD'/'JUV' for TL, and 'Male'/'Female' for Sex, respectively). The median of the length frequency distribution of *P. africanum* allowed to separate juveniles (< 82 cm) from adult (\geq 82 cm). The third factor (Site) has three levels ('HB'/'GB'/'TP'), according to the three sampling sites. Differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, among considered factors, were tested by univariate and multivariate PERMANOVA (Permutational Multivariate Analysis of Variance) carried out on the resemblance Euclidean matrix of untransformed $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, respectively.

For *Scyliorhinus canicula* the design was based on two fixed factors 'TL' and 'Sex' with two levels 'AD'/'JUV' for TL, and 'Male'/'Female' for Sex, respectively. The median of the length frequency distribution allowed to discriminate between juveniles (< 39.6 cm) and adult (\geq 39.6 cm). Differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, among considered factors, were tested by univariate and multivariate PERMANOVA carried out on the resemblance Euclidean matrix of untransformed $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, respectively.

Then, a multivariate PERMANOVA test was also performed on a modified Gower resemblance matrix of fourth root transformed prey biomass

data to test the significance of any dietary changes based on sex and total length. Stomach contents data are generally distorted and contain many zeros, so this measure of resemblance was considered the most suitable for this kind of data (Clarke & Warwick, 2001, Anderson *et al.*, 2008). SIMPER analysis was applied to stomach contents data to determine the most important food sources (Clarke & Warwick, 2001).

For all PERMANOVA tests, the significance value was set at $p < 0.05$. Both univariate and multivariate PERMANOVA tests were carried out under an unrestricted permutation of raw data, with 9999 permutations. The Monte Carlo test was applied because of low sample size. In addition, if the main test of PERMANOVA highlighted significant differences, a pairwise test was performed to identify the source of variation.

Multivariate and univariate statistical analyses on the obtained results were conducted using PRIMER6 and PERMANOVA+ (Clarke & Warwick, 2001, Anderson *et al.*, 2008).

PAST (version 4.0.9), which is a free software for scientific data analysis, was used to calculate the correlation between $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ and the total length of *P. africanum* and *S. canicula* (Hammer *et al.*, 2001).

3.7.2. Mixing models

A Bayesian model SIMMR (Stable Isotope Mixing Models in R, Parnell, 2021) was run to estimate the contribution of the different food sources to the diet of *P. africanum* and *S. canicula* with the software R 4.0.5 (R Development Core Team 2009). This model allowed the inclusion of sources of uncertainty. In particular, the variability in the isotope values (mean and standard deviation) of prey species can be incorporated into the model (Parnell *et al.*, 2013). SIMMR is designed as an upgrade to the SIAR (Stable Isotope Analysis in R) package and contains many of the same features. This new version contains a slightly more sophisticated mixing model, a simpler user interface, and more advanced plotting features. SIMMR, as

SIAR, uses Markov-chain Monte Carlo modelling, takes data on consumer stable values and fits a Bayesian model of the diet habits based on a Gaussian likelihood function with a Dirichlet prior mixture distribution for the mean. The model also assumes that each target value (i.e., the stable isotope data for each individual) comes from a Gaussian distribution with an unknown mean and standard deviation (Fanelli *et al.*, 2015). The structure of the mean is a weighted combination of the food sources' isotopic values. The key differences between SIAR and SIMMR are that: i) SIMMR has a slightly richer mixing model based on code from the Parnell *et al.* 2013; ii) SIMMR does not have a menu system; all commands must be run through the console or script windows; iii) SIMMR uses ggplot2 to create graphs and JAGS to run the mixing model.

Before running the model, the isotopic values of the sources and sharks were plotted, applying the correct trophic enrichment factors (TEFs) to potential sources to build a mixing polygon (Phillips *et al.*, 2014). TEFs used for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are 2.29 and 0.9, respectively (Tilley *et al.*, 2013). The list of potential prey of *P. africanum* is shown in Table 1 while that of potential prey of *S. canicula* is shown in Table 2.

Table 1. List of potential prey of *P. africanum*.

Sources	Mean $\delta^{13}\text{C}$ (‰)	Mean $\delta^{15}\text{N}$ (‰)	SD $\delta^{13}\text{C}$ (‰)	SD $\delta^{15}\text{N}$ (‰)
<i>Perinereis nuntia vallata</i> (Richoux, 2007)	-12.7	11.8	0.3	0.1
<i>Sesarma catenata</i> (Richoux, 2007)	-17.0	10.1	1	0.2
<i>Upogebia africana</i> (Richoux, 2007)	-15.0	9.3	1	0.4
<i>Hyporhamphus capensis</i> (Bergamino <i>et al.</i> , 2014)	-22.3	13.6	0.01	0.01
<i>Phalium craticulatum</i> (De Lecea <i>et al.</i> , 2013)	-14.8	15.0	0.01	0.01

Table 2. List of potential prey of *S. canicula*.

Sources	Mean $\delta^{13}\text{C}(\text{‰})$	Mean $\delta^{15}\text{N}(\text{‰})$	SD $\delta^{13}\text{C}(\text{‰})$	SD $\delta^{15}\text{N}(\text{‰})$
<i>Sepiola affinis</i> (Barria <i>et al.</i> , 2018)	-19.43	8.82	0.26	1
<i>Alpheus glaber</i> (Barria <i>et al.</i> , 2018)	-17.97	7.82	0.84	0.24
<i>Solenocera membranacea</i> (Barria <i>et al.</i> , 2018)	-18.35	8.46	0.64	0.74
<i>Cepola macrophthalma</i> (Barria <i>et al.</i> , 2018)	-20.42	8.14	0.05	0.04
<i>Heteromastus filiformis</i> (Bongiorni <i>et al.</i> , 2018)	-20.27	4.79	0.01	0.01

The SIBER package (Stable Isotope Bayesian Ellipses in R 3.5.3) (Jackson *et al.*, 2011) was used to compare isotopic niche widths among and within communities. It was also used to calculate TA (Total Convex Hull Area) and SEAc (Standard Ellipse Areas corrected for small sample size) (Layman *et al.*, 2007) and SEA (p interval = 0.95 in order to encompass the 95% of our data) for the different communities. Three models with different combinations were run for *P. africanum*. In the first two, the three sites were used as groups. Value 1 was used as community, in the first, and the two sexes were used as communities, in the second. In the third, TLs was used as groups and value 1 as community. For *S. canicula*, only one model was run with TLs as groups and value 1 as community.

4. RESULTS

4.1 *Poroderma africanum*

4.1.1. Length frequency distribution

The length frequency distribution, considering all individuals, shows a different trend for males and females (Figure 20). The 18% of 25 sampled males of *P. africanum* has a TL comprised between 81 and 90.1 cm. Most of the collected specimens have a TL between 91 and 100 cm. Females are 30 and predominate in the three size classes below 90.1 cm; the 5% of them has a TL above 91 cm.

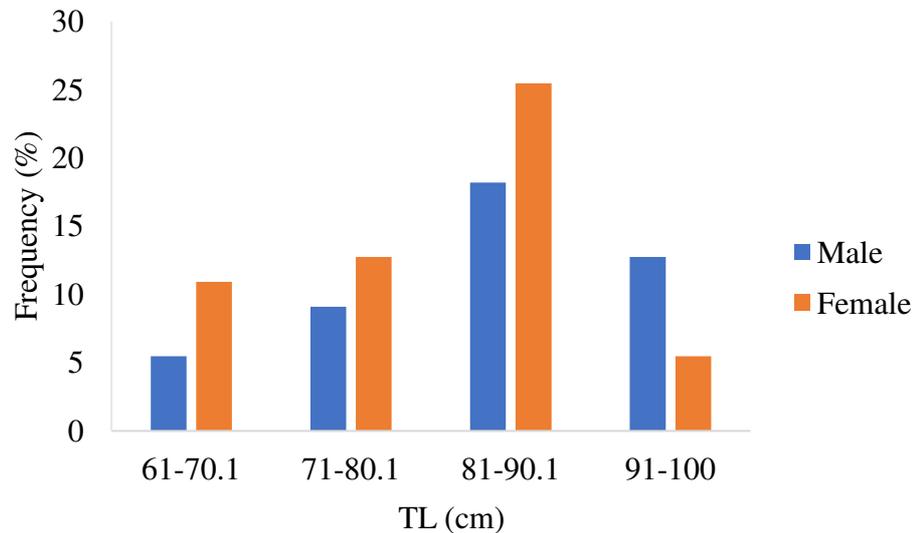


Figure 20. Length frequency distribution of *P. africanum*, N = 55.

Males and females occurred in the three study areas, with a higher number of specimens collected at TP (Figure 21). All size classes are represented at TP, from 61 to 100 cm. At GB there are no individuals between 71 and 80.1 cm, while at HB no individuals between 61 and 70.1 cm occurred (Figure 22).

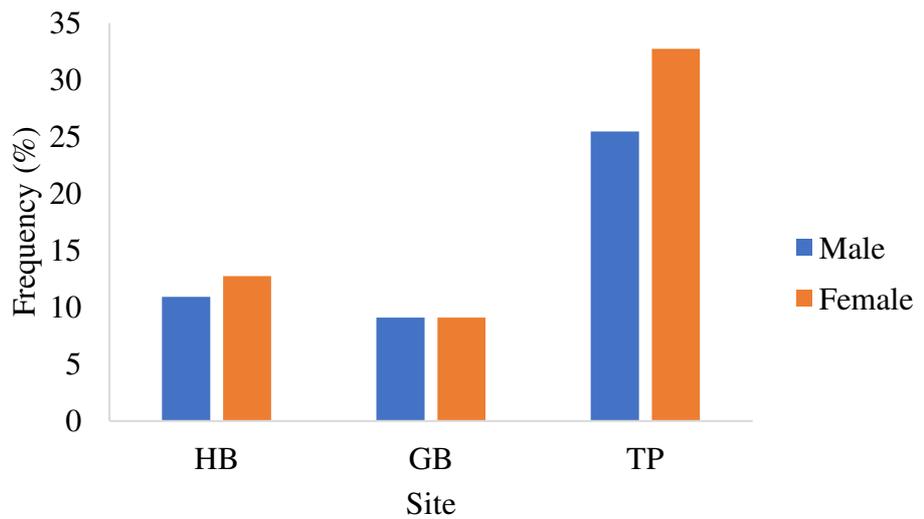


Figure 21. % of the presence of *P. africanum* in the different sites; N = 55

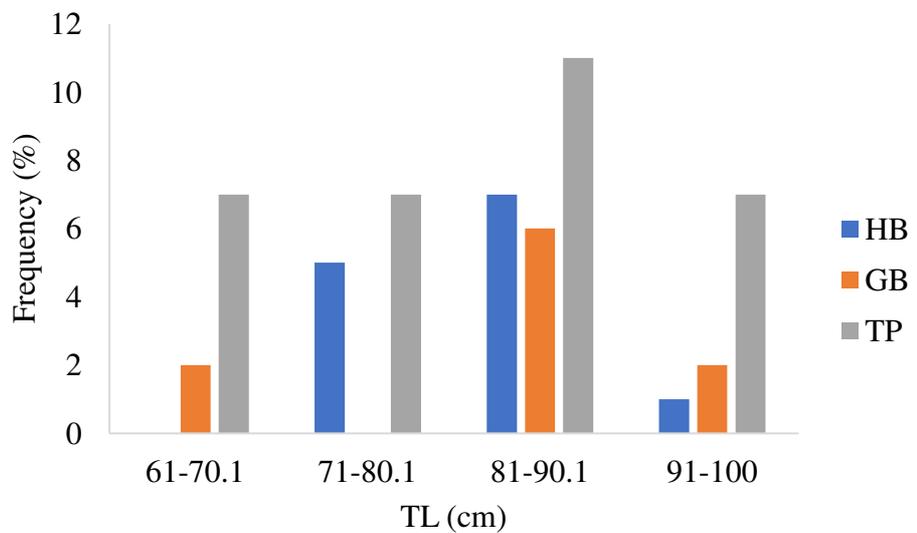


Figure 22. Length frequency distribution according to the site and the TL; N = 55

4.1.2. Changes in stable isotope composition in *P. africanum*

$\delta^{13}\text{C}$ was on average $-14.97 \pm 0.33\text{‰}$ while $\delta^{15}\text{N}$ was $15.46 \pm 0.52\text{‰}$. $\delta^{13}\text{C}$ values vary from females to males (from $-15 \pm 0.31\text{‰}$ to $-14.9 \pm 0.33\text{‰}$).

$\delta^{15}\text{N}$ values vary from females to males (from $15.5 \pm 0.41\text{‰}$ to $15.4 \pm 0.63\text{‰}$). $\delta^{13}\text{C}$ values vary from juveniles to adults (from $-15.1 \pm 0.34\text{‰}$ to $-14.9 \pm 0.27\text{‰}$). $\delta^{15}\text{N}$ values vary from juveniles to adults ($15.4 \pm 0.41\text{‰}$ and $15.5 \pm 0.60\text{‰}$). $\delta^{13}\text{C}$ values are very similar in all three sites around -15 ± 0.30 . $\delta^{15}\text{N}$ values are very similar in all three sites around 15.7 ± 0.30 (Table 3).

Table 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean and sd) for F (females), M (males), JUV (juveniles), AD (adults), HB (Hartenbos), GB (Groot Brakrivier) and TP (The Point)

	Mean $\delta^{13}\text{C}(\text{‰})$	sd Mean $\delta^{13}\text{C}(\text{‰})$	Mean $\delta^{15}\text{N}(\text{‰})$	sd Mean $\delta^{15}\text{N}(\text{‰})$
F	-15	0.31	15.5	0.41
M	-14.9	0.33	15.4	0.63
JUV	-15.1	0.34	15.4	0.41
AD	-14.9	0.27	15.5	0.60
HB	-15	0.24	16.0	0.28
GB	-14.9	0.32	15.7	0.30
TP	-15	0.36	15.2	0.43

$\delta^{15}\text{N}$ values show a slight, not significant, positive correlation with length ($R = 0.08$, $p > 0.05$, Figure 23).

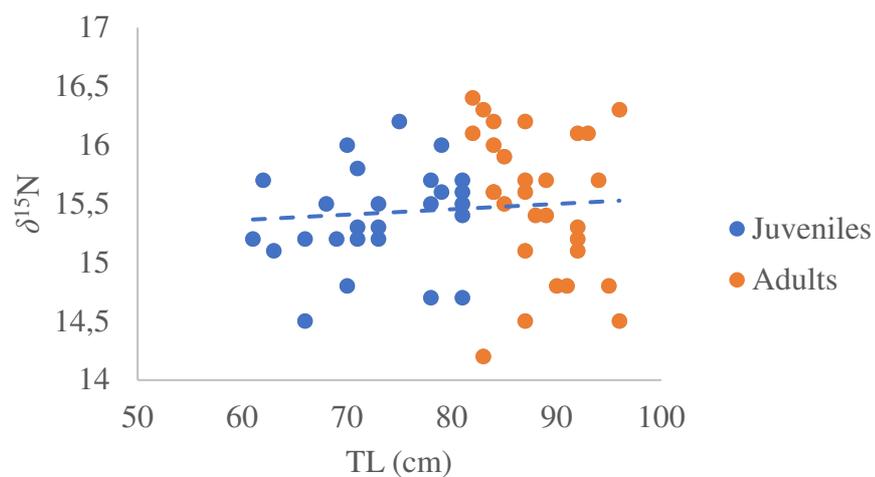


Figure 23. Scatterplot of $\delta^{15}\text{N}$ values vs. total length (cm)

Conversely, $\delta^{13}\text{C}$ values increased significantly with size ($R = 0.5$, $p < 0.001$; Figure 24).

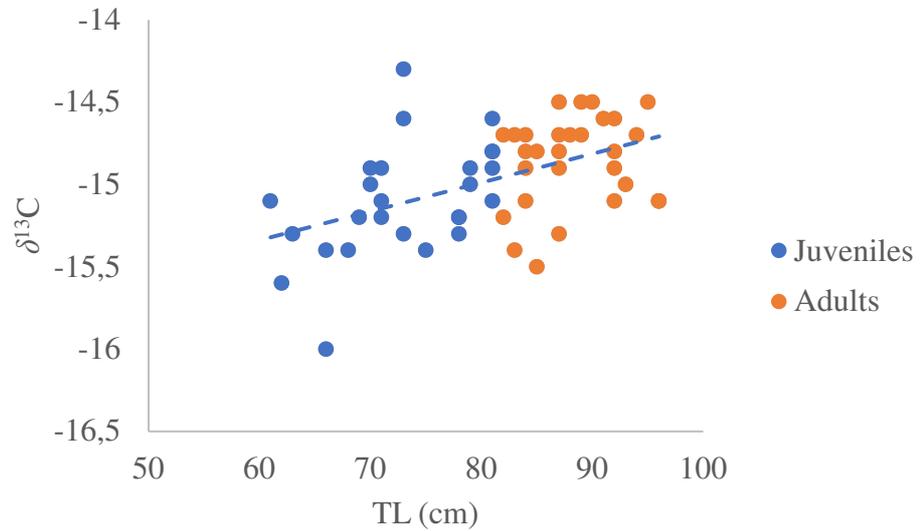


Figure 24. Scatterplot of $\delta^{13}\text{C}$ values vs. total length (cm)

The PERMANOVA test carried on $\delta^{15}\text{N}$ values showed significant differences for the factor “Site” (Table 4a). Pairwise tests showed variations between the levels of the factors “Site” (between TP and the other two sites) and “Sex” (between males and females) (Table 4b). The PERMANOVA main test on $\delta^{13}\text{C}$ values showed significant differences for factor “TL” (Table 5).

Table 4. Results of the PERMANOVA main test (a) and of the pairwise comparisons (b) for $\delta^{15}\text{N}$. df = degrees of freedom; MS = mean square; Pseudo-F = statistic F; t = statistic t for pairwise comparisons; p(MC) = probability level with Monte Carlo test; TL = total length; Se = Sex; Si = Site; HB = Hartenbos; GB = Groot Brakrivier; TP = The Point; F = female; M = male.

a)

Source	df	MS	Pseudo-F	P(MC)
TL	1	0.22	1.60	0.21
Se	1	0.00	0.03	0.85
Si	2	3.18	23.38	0.0001
TLxSe	1	0.00	0.00	0.98
TLxSi	2	0.19	1.39	0.26
SexSi	2	0.40	2.95	0.07
TLxSexSi	2	0.19	1.38	0.26
Res	43	0.14		
Total	54			

b)

Groups	t	P(MC)
HB, GB	0.94	0.38
HB, TP	4.94	0.0007
GB, TP	4.16	0.002

Groups	t	P(MC)
F, M	2.19	0.047

Table 5. Results of the PERMANOVA main test for $\delta^{13}\text{C}$. df = degrees of freedom; MS = mean square; Pseudo-F = statistic F; t = statistic t for pairwise comparisons; p(MC) = probability level with Monte Carlo test; TL = total length; Se = Sex; Si = Site

Source	df	MS	Pseudo-F	P(MC)
TL	1	0.71	7.12	0.01
Se	1	0.00	0.04	0.84
Si	2	0.04	0.35	0.70
TLxSe	1	0.01	0.11	0.74
TLxSi	2	0.11	1.15	0.33
SexSi	2	0.14	1.46	0.25
TLxSexSi	2	0.06	0.57	0.58
Res	43	0.10		
Total	54			

We have chosen species that fall into the mixing polygon (Figure 25).

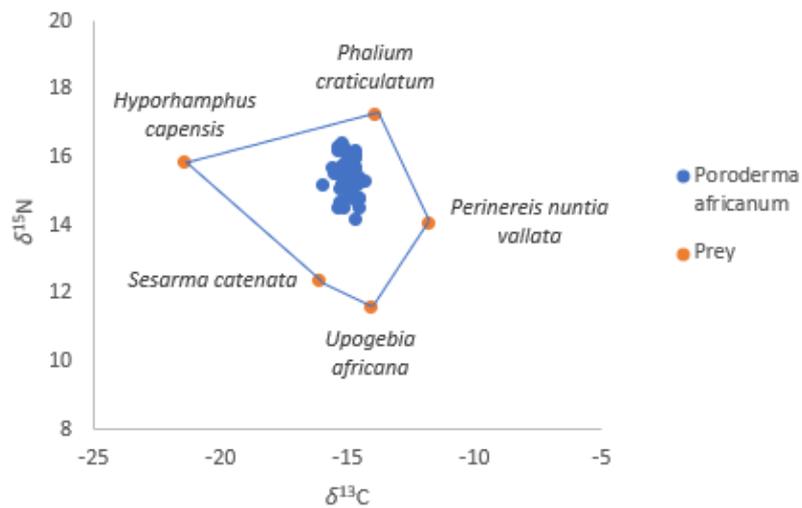


Figure 25. Mixing plot of the potential food sources for *P. africanum*: orange dots indicated mean isotopic values of the food sources, corrected with the TEF. Blue dots represent the isotopic values of *P. africanum* samples.

Three Bayesian models SIMMR were used to estimate the potential food sources for *P. africanum* considering the significance obtained with the univariate analysis. The first two isospace plots showed a difference between TP and the other two sites, but with no significant differences between males and females (Figures 26a and 26b). The third isospace plot did not show important differences between adult and juveniles related to diet (Figure 26c). *Phalium craticulatum* contributed the most to *P. africanum* diet (Figures 27; 28; 29a-b; 30a-c; 31d-f; 32; 33). The mean percentage contribution of each source to *P. africanum* diet was shown in Tables 6-8.

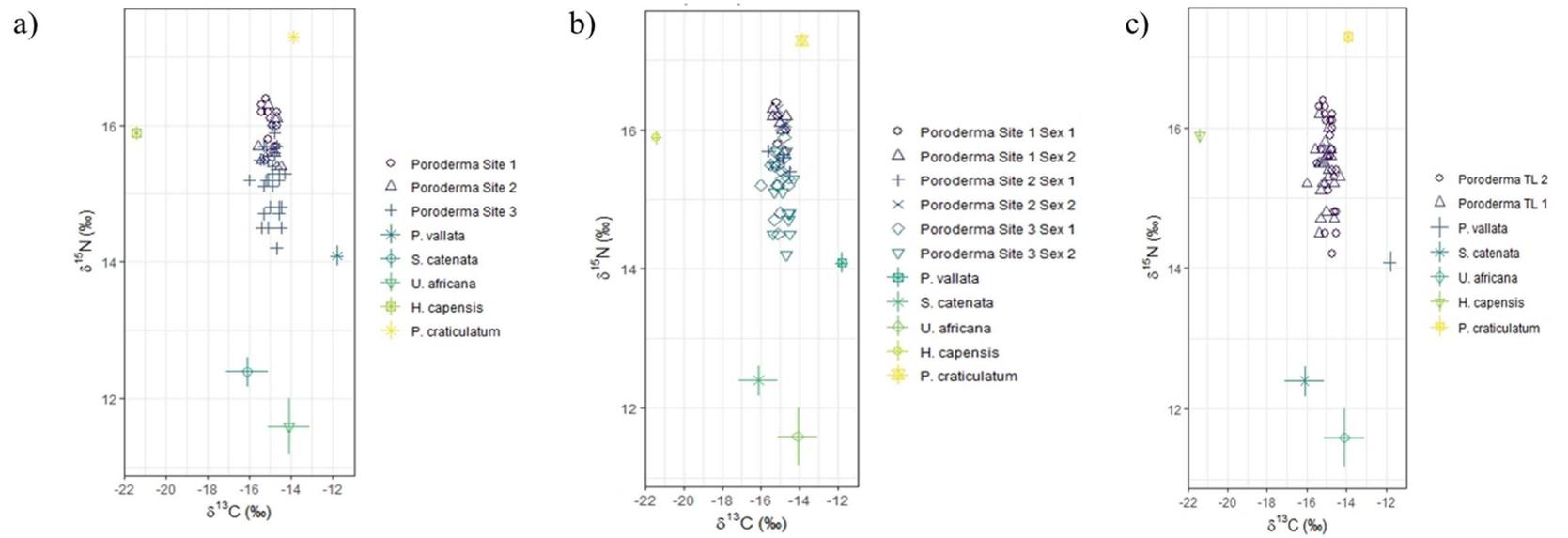


Figure 26: a) Isospace plot of *Poroderma africanum*, factor = “Site”; Site 1 = HB, Site 2 = GB, Site 3 = TP, b) isospace plot of *Poroderma africanum*, factor = “Site”, “Sex”; Site 1 = HB, Site 2 = GB, Site 3 = TP, Sex 1 = Female, Sex 2 = Male, c) isospace plot of *Poroderma africanum*, factor = “TL”, TL 1 = juvenile, TL 2 = adult.

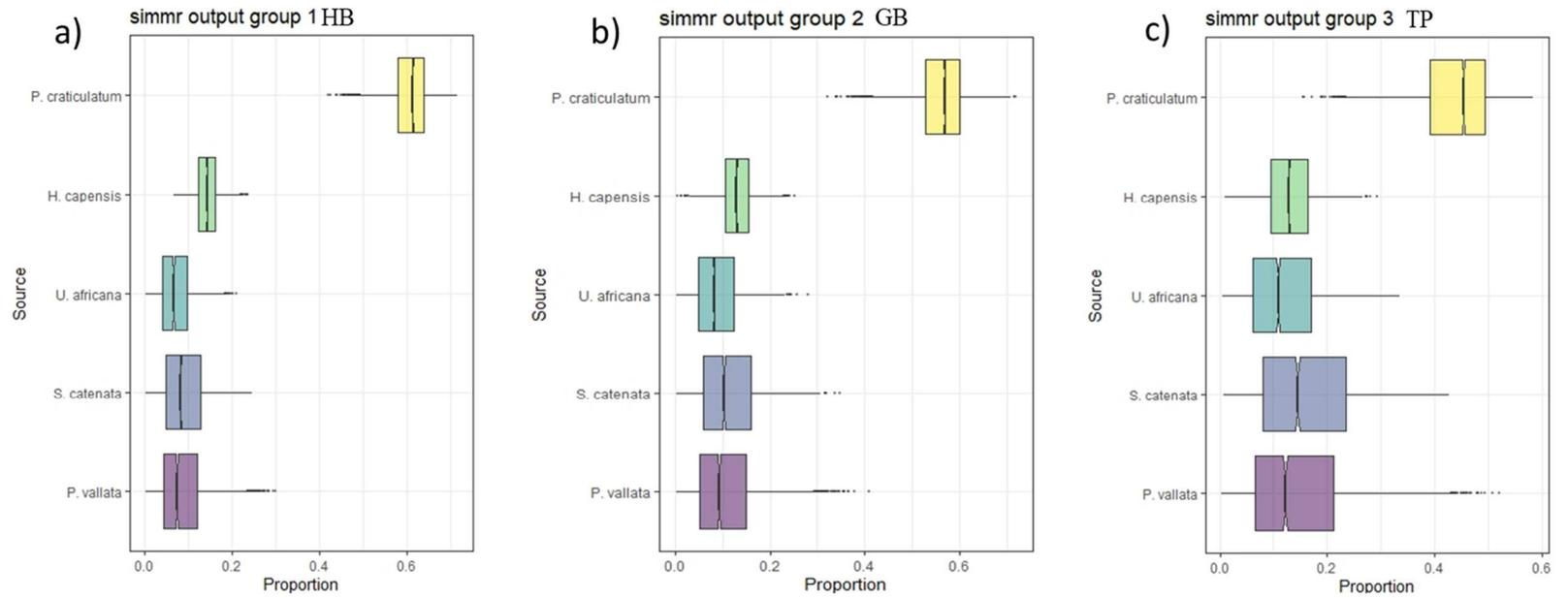


Figure 27. Proportions of each food source in the diet of the catshark collected in a) Site 1 = HB, b) Site 2 = GB, c) Site 3 = TP. Boxplots were obtained with stable isotope analysis mixing models. Each plot shows proportions for each food source in the specific site. Boxes indicate 50%, 75% and 95% Bayesian confidence intervals.

Table 6. Summary percentage contribute for each site.

	HB		GB		TP	
	mean	sd	mean	sd	mean	sd
deviance	7.94	3.38	15.24	3.43	66.24	3.08
<i>P. vallata</i>	0.09	0.06	0.11	0.07	0.15	0.10
<i>S. catenata</i>	0.09	0.05	0.11	0.07	0.16	0.10
<i>U. africana</i>	0.07	0.04	0.09	0.05	0.12	0.07
<i>H. capensis</i>	0.14	0.03	0.13	0.04	0.13	0.05
<i>P. craticulatum</i>	0.61	0.04	0.56	0.06	0.44	0.07
sd[d13C]	0.22	0.09	0.34	0.13	0.27	0.09
sd[d15N]	0.32	0.08	0.37	0.11	0.45	0.06

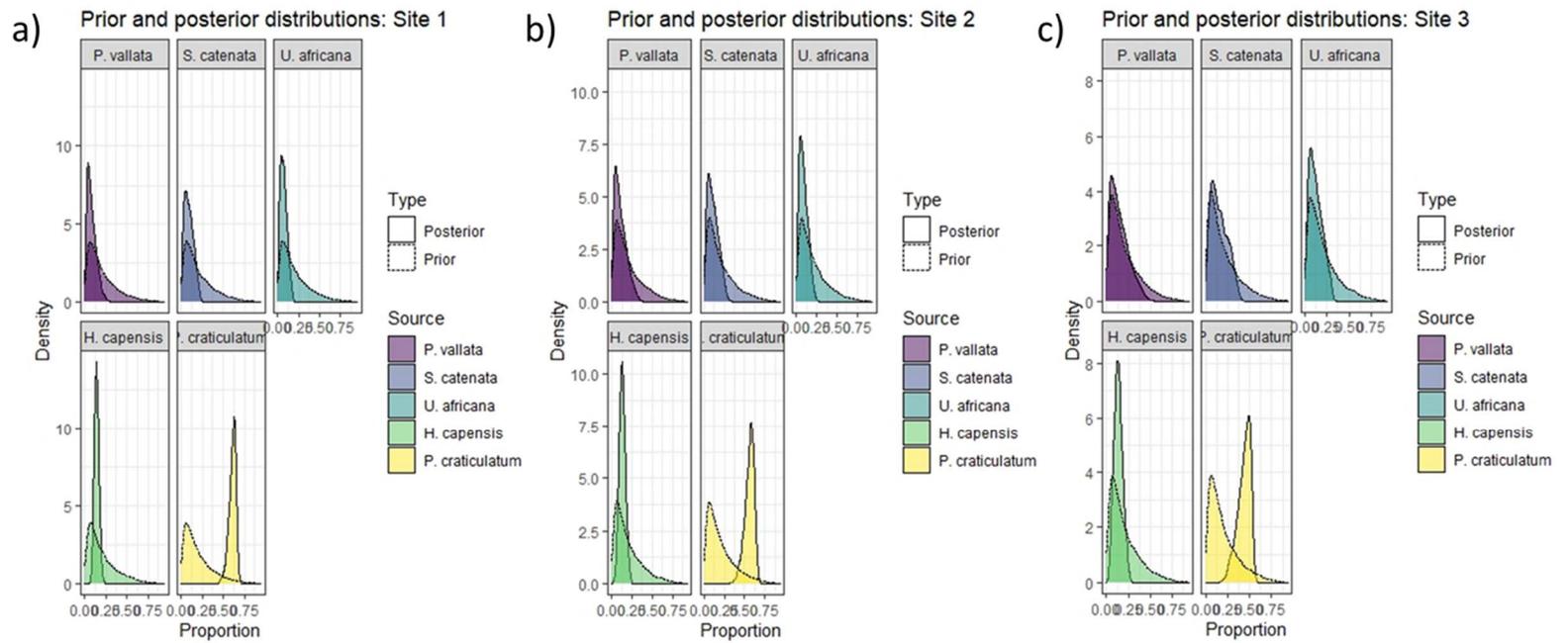
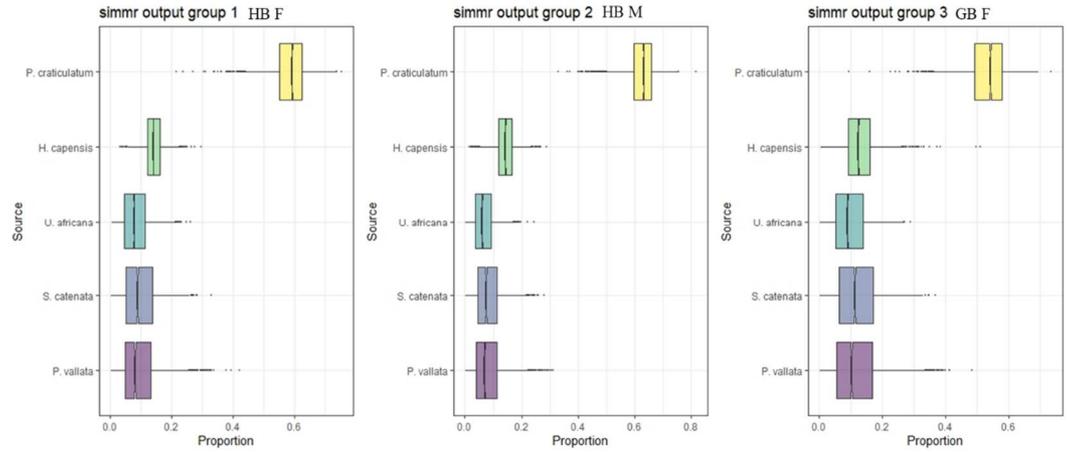


Figure 28. Prior and posterior distribution of the proportional contribute of each source to the diet of *Poroderma africanum* between sites. a) Site 1 = HB, b) Site 2 = GB, c) Site 3 = TP.

a)



b)

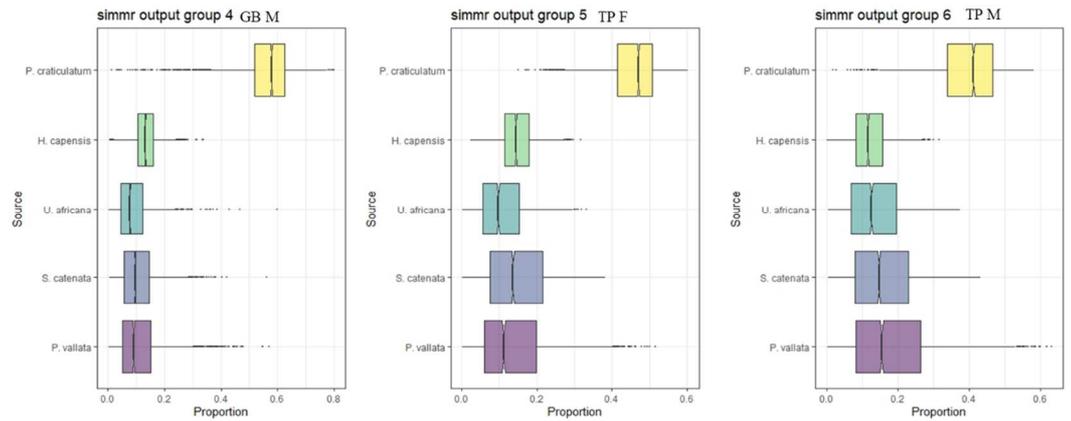


Figure 29a-b. Proportions of each food source in the diet of the catshark collected in Site 1 = HB, Site 2 = GB, Site 3 = TP, related to F = females and M = males. Boxplots were obtained with stable isotope analysis mixing models. Each plot shows proportions for each food source in the specific site-sex. Boxes indicate 50%, 75% and 95% Bayesian confidence.

Table 7. Summary percentage contribute for each site according to the sex (F = females, M = males).

	HB F		HB M		GB F	
	mean	sd	mean	sd	mean	sd
deviance	5.92	3.83	5.09	4.31	1.20	4.47
<i>P. vallata</i>	0.10	0.06	0.08	0.05	0.12	0.08
<i>S. catenata</i>	0.10	0.06	0.08	0.05	0.12	0.07
<i>U. africana</i>	0.08	0.05	0.07	0.04	0.10	0.06
<i>H. capensis</i>	0.14	0.03	0.14	0.03	0.13	0.05
<i>P. craticulatum</i>	0.58	0.06	0.63	0.05	0.53	0.06
sd[$\delta^{13}\text{C}$]	0.20	0.13	0.38	0.21	0.56	0.39
sd[$\delta^{15}\text{N}$]	0.42	0.18	0.29	0.17	0.13	0.14
	GB M		TP F		TP M	
	mean	sd	mean	sd	mean	sd
deviance	11.91	4.78	30.32	3.17	31.09	3.29
<i>P. vallata</i>	0.11	0.08	0.14	0.10	0.18	0.12
<i>S. catenata</i>	0.11	0.07	0.15	0.09	0.16	0.09
<i>U. africana</i>	0.09	0.06	0.11	0.07	0.14	0.08
<i>H. capensis</i>	0.13	0.04	0.15	0.05	0.12	0.05
<i>P. craticulatum</i>	0.56	0.10	0.46	0.07	0.40	0.09
sd[$\delta^{13}\text{C}$]	0.40	0.32	0.26	0.11	0.27	0.13
sd[$\delta^{15}\text{N}$]	0.65	0.48	0.41	0.08	0.50	0.12

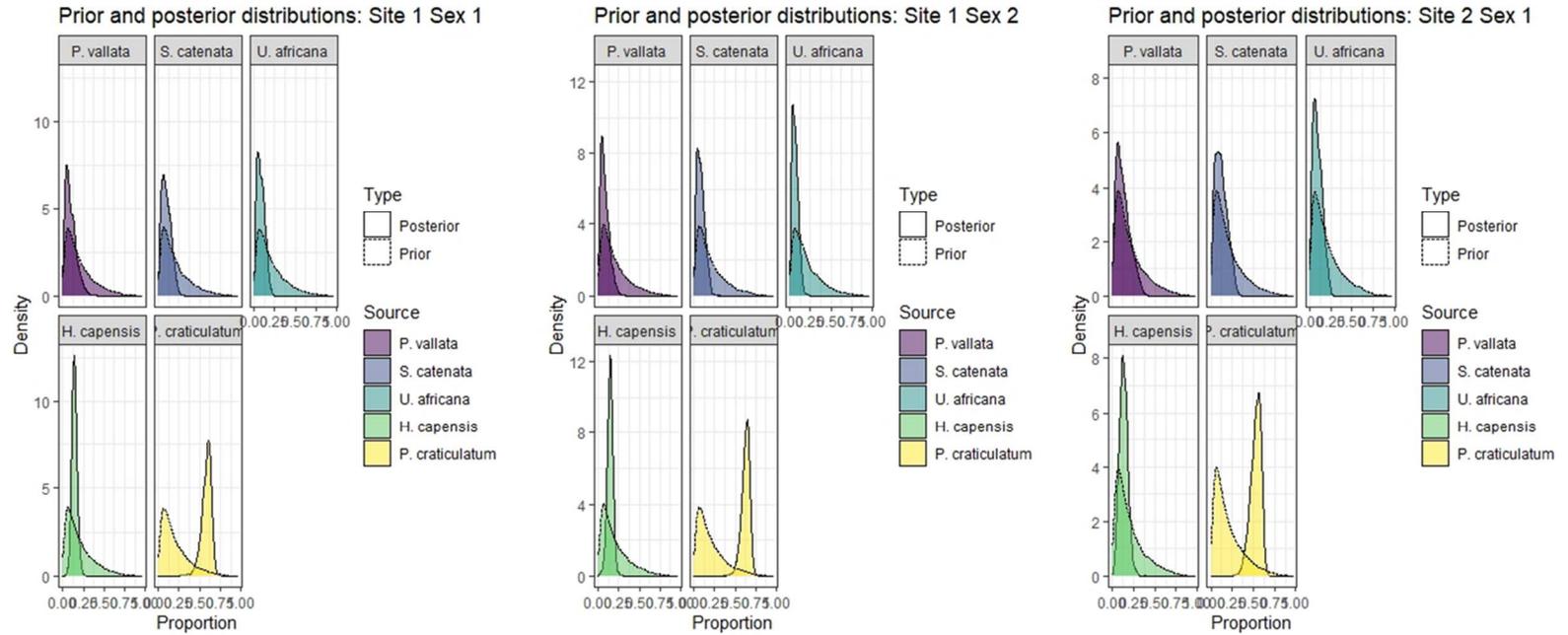


Figure 30a-c. Prior and posterior distribution of the proportional contribute of each source to the diet of *Poroderma africanum* between site and sex. a) Site 1 Sex 1= females in HB, b) Site 1 Sex 2 = males in HB, c) Site 2 Sex 1 = females in GB, d) Site 2 Sex 2 = males in GB, e) Site 3 Sex 1 = females in TP, f) Site 3 Sex 2 = males in TP.

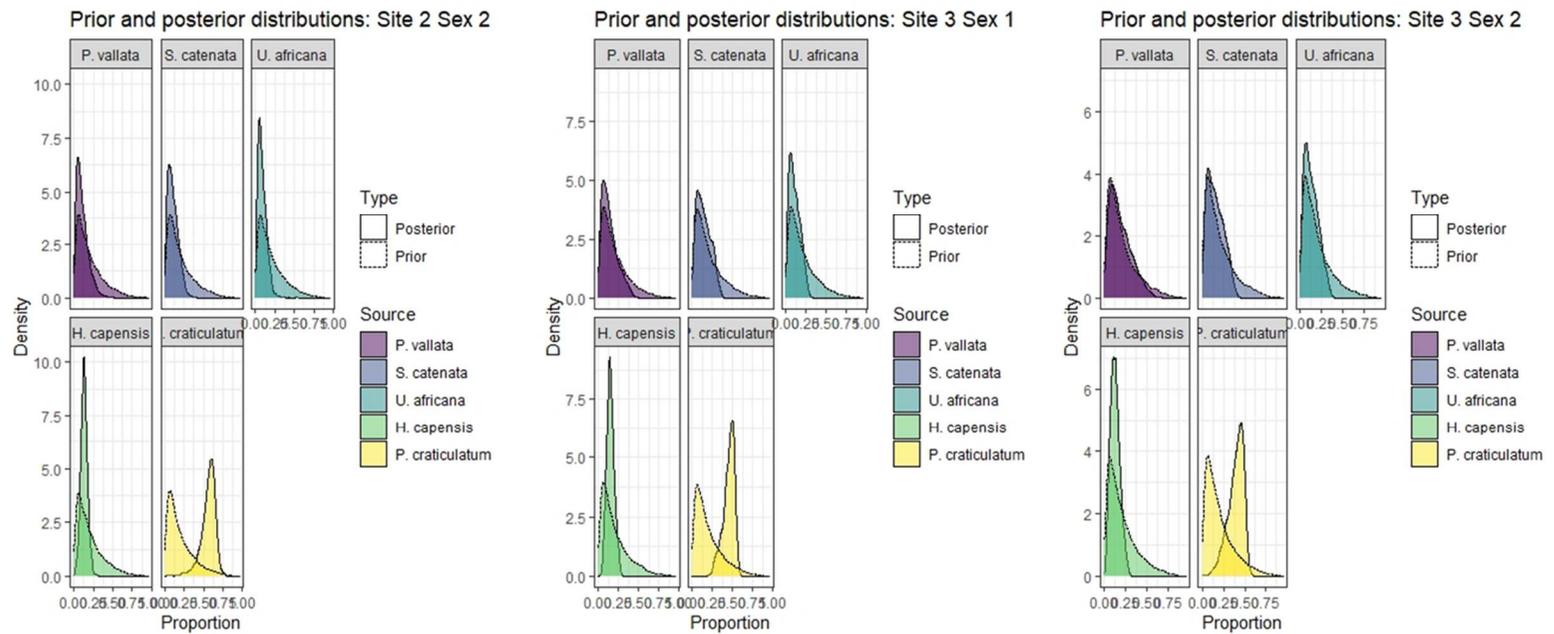


Figure 31d-f. Prior and posterior distribution of the proportional contribute of each source to the diet of *Poroderma africanum* between site and sex. Site 1 Sex 1= females in HB, Site 1 Sex 2 = males in HB, Site 2 Sex 1 = females in GB, Site 2 Sex 2 = males in GB, Site 3 Sex 1 = females in TP, Site 3 Sex 2 = males in TP.

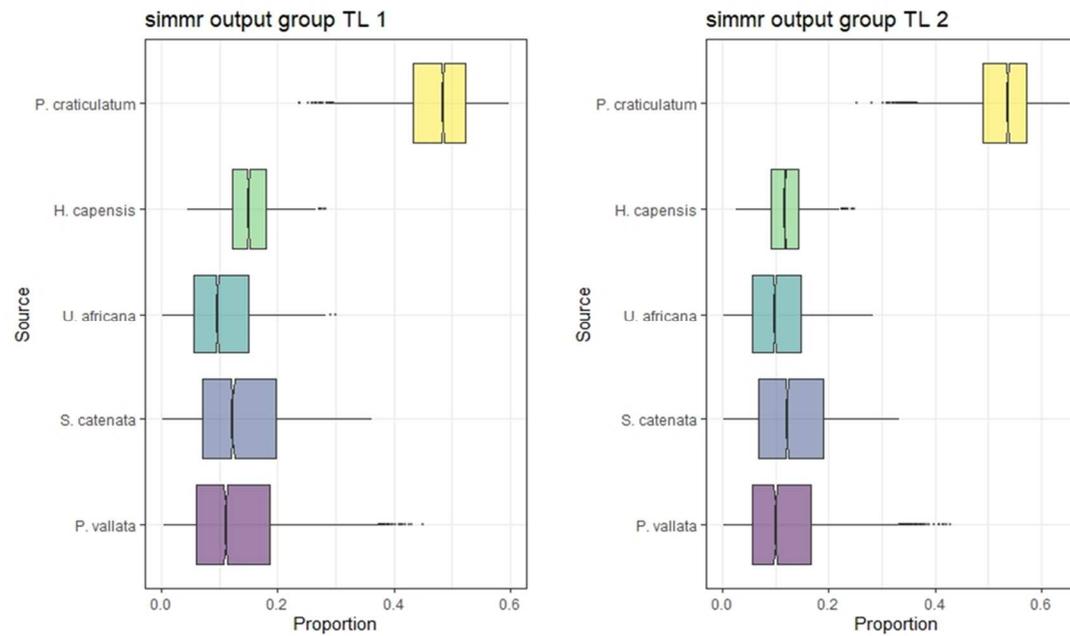


Figure 32. Proportions of each food source in the diet of the catshark collected for TL 1 = Juveniles, TL 2 = Adults. Boxplots were obtained with stable isotope analysis mixing models. Each plot shows proportions for each food source in the specific TL. Boxes indicate 50%, 75% and 95% Bayesian confidence intervals.

Table 8. Summary percentage contribute for juveniles and adults.

	Juveniles		Adults	
	mean	sd	mean	sd
deviance	49.20	2.98	62.91	3.02
<i>P. vallata</i>	0.13	0.09	0.12	0.08
<i>S. catenata</i>	0.14	0.08	0.13	0.07
<i>U. africana</i>	0.11	0.06	0.11	0.06
<i>H. capensis</i>	0.15	0.04	0.12	0.04
<i>P. craticulatum</i>	0.47	0.06	0.53	0.06
sd[d13C]	0.28	0.09	0.18	0.08
sd[d15N]	0.43	0.07	0.63	0.09

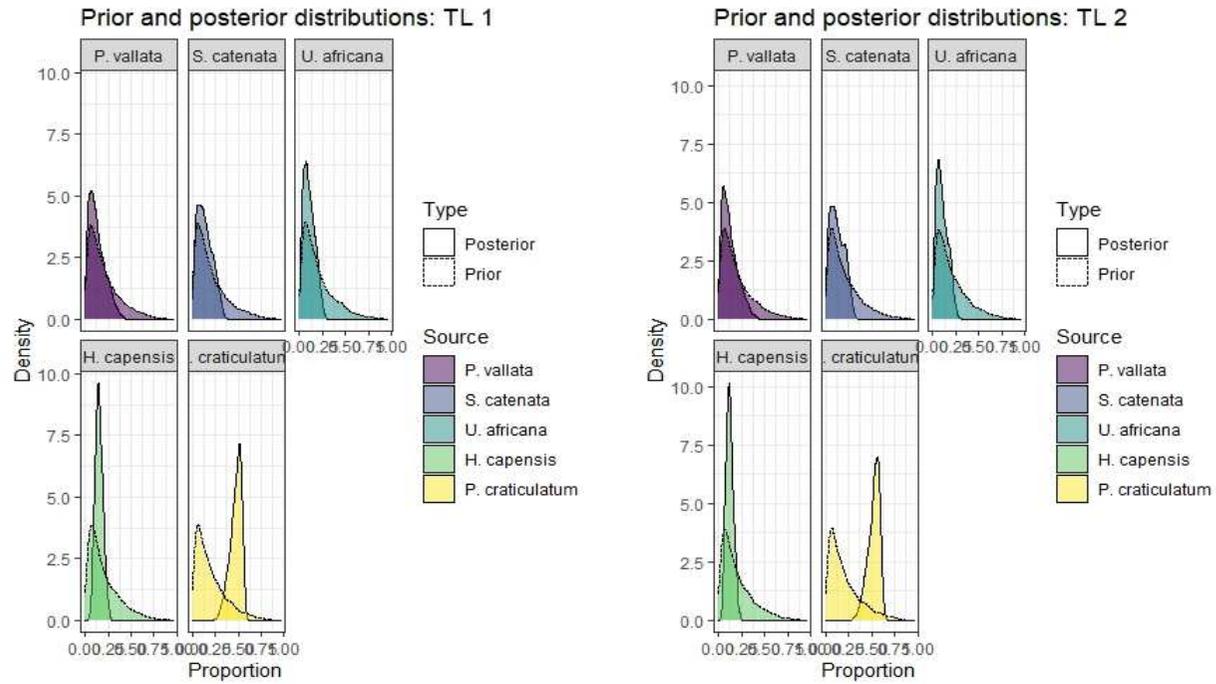


Figure 33. Prior and posterior distribution of the proportional contribute of each source to the diet of *Poroderma africanum* between juveniles and adults. TL 1 = juveniles, TL 2 = adult

Standard ellipses obtained by SIBER show the isotopic niche widths of the groups. The niche width of *P. africanum* in TP is greater than in the other two sites (Figure 34). The SEAc of TP is bigger than that of the other sites (Table 9). The color of the column indicates the order of groups, from the top to the bottom.

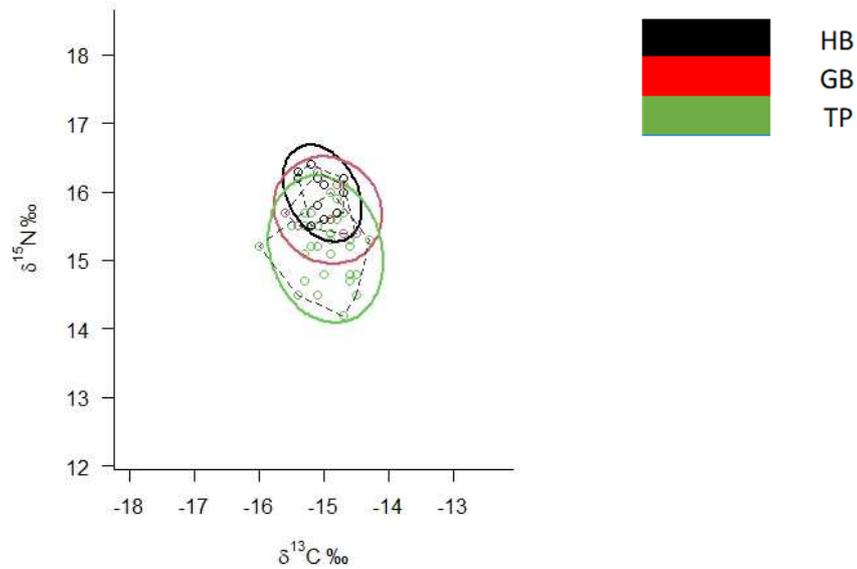


Figure 34. $\delta^{13}\text{C} - \delta^{15}\text{N}$ scatterplot with standard ellipses (p interval = 95%) corrected for small sample size (SEAc) for *P. africanum* collected in Mossel Bay, by site.

Table 9. Total Convex Hull Area (TA) and Standard Ellipse Areas corrected for small sample size (SEAc) values of *P. africanum* between sites. 1.1 = HB; 1.2 = GB; 1.3 = TP.

	1.1	1.2	1.3
TA	0.45	0.62	1.75
SEA	0.21	0.34	0.49
SEAc	0.23	0.38	0.51

The SEAc of TP, for both sexes, is bigger than that of the other sites. The SEAc of GB, for females, is very low, compared to the others (Figure 35 and Table 10). The SEAc value of adults is stretched along $\delta^{15}\text{N}$ -axis (Figure 36 and Table 11). The color of the column indicates the order of groups, from the top to the bottom.

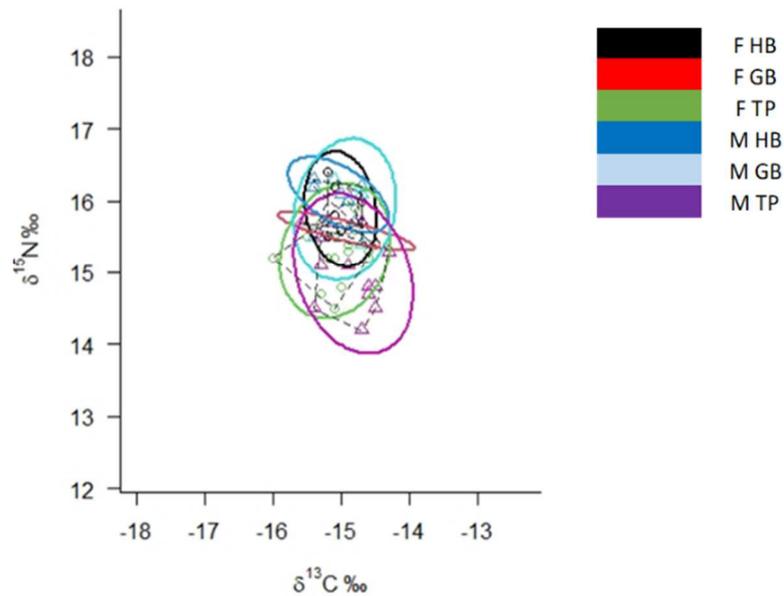


Figure 35. $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ scatterplot with standard ellipses (p interval = 95%) corrected for small sample size (SEAc) for *P. africanum* collected in Mossel Bay, by site and sex. F HB = females in HB; F GB = females in GB; F TP = females in TP; M HB = males in HB; M GB = males in GB; M TP = males in TP.

Table 10. Total Convex Hull Area (TA) and Standard Ellipse Areas corrected for small sample size (SEAc) values of *P. africanum* between sites and sexes. 1.1 = females in HB; 1.2 = females in GB; 1.3 = females in TP; 2.4 = males in HB; 2.5 = males in GB; 2.6 = males in TP.

	1.1	1.2	1.3	2.4	2.5	2.6
TA	0.30	0.07	1.09	0.21	0.44	1.22
SEA	0.22	0.07	0.39	0.17	0.38	0.49
SEAc	0.26	0.10	0.42	0.21	0.51	0.54

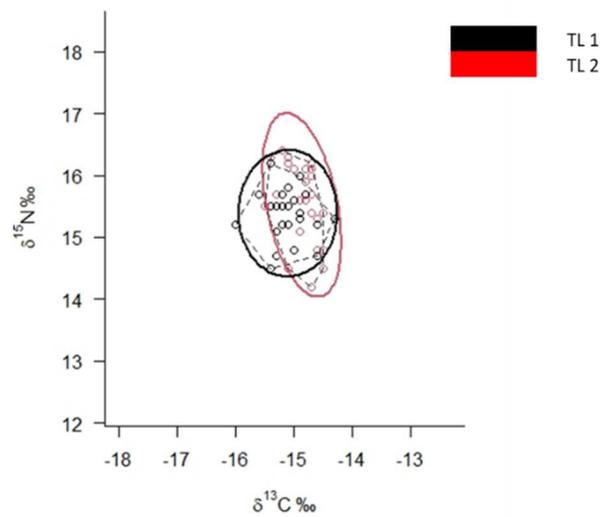


Figure 36. $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ scatterplot with standard ellipses (p interval = 95%) corrected for small sample size (SEAc) for *P. africanum* collected in Mossel Bay, by TL. TL 1 = juveniles; TL 2 = adults.

Table 11. Total Convex Hull Area (TA) and Standard Ellipse Areas corrected for small sample size (SEAc) values of *P. africanum* between TLs. 1.1 = juveniles; 1.2 = adult

	1.2	1.1
TA	1.56	1.77
SEA	0.48	0.45
SEAc	0.50	0.47

4.2 *Scyliorhinus canicula*

The length frequency distribution, considering all individuals, shows a different trend for males and females (Figure 37). There are 5 males, and none have a TL comprised between 20.01 and 30 cm. Females are 14 and belong to all size classes considered, with a 47% of them between 30.01-40 cm.

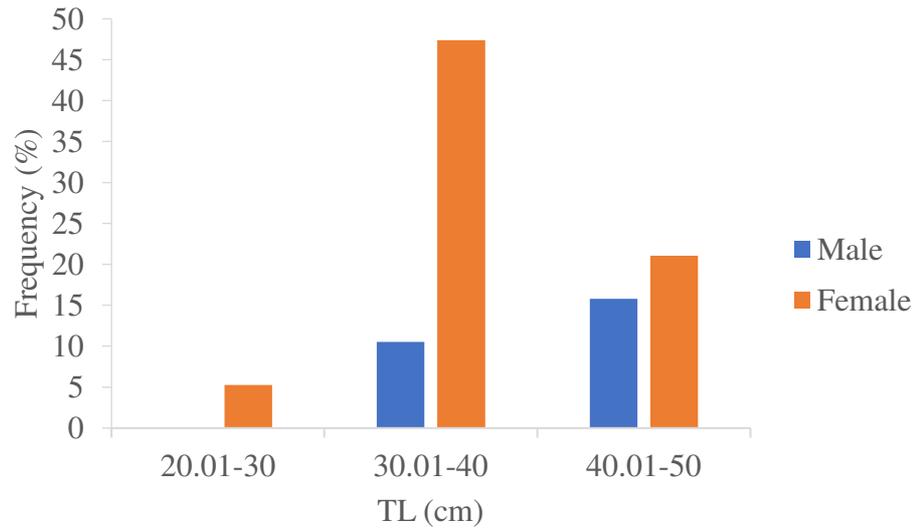


Figure 37. Length frequency distribution of *S. canicula*; N = 19

19 full stomachs of *Scyliorhinus canicula* were analyzed. The organisms found are part of several Phyla: Annelida, Arthropoda, Mollusca, and Vertebrata. Within Annelida, the Polychaete family of Aphroditidae was found, within Arthropoda, the crustacean order of Decapoda, and for Mollusca the Sepidae family and the order Teuthida of Cephalopoda. Decapoda has the largest number of recognized species. Finally, many remains of Osteichthyes were found. Most of the stomach contents were not recognizable and were classified as digested material. %W and %F values are shown in Table 12. Diet composition of these specimens of *Scyliorhinus canicula* is represented in Figure 38.

Table 12. %W and %F values of stomach contents. %W = percentage by weight, %F = frequency of occurrence.

Subphylum	Order	Genus/Species	%W	%F
Polychaeta			0.07	0.04
	Aphroditidae		0.07	0.04
Crustacea	Decapoda		0.40	0.04
		<i>Parapenaeus longirostris</i>	0.18	0.04
		Peneidae n.d.	0.06	0.04
		Natantia n.d.	0.01	0.04
Cephalopoda			0.22	0.23
	Saepidae			
	Sepidae	Sepidae n.d.	0.14	0.04
	Teuthida			
		Teuthida n.d.	0.03	0.04
Osteichthyes			0.29	0.31
Nematoda			0.02	0.04
Digested material			0.5	0.42

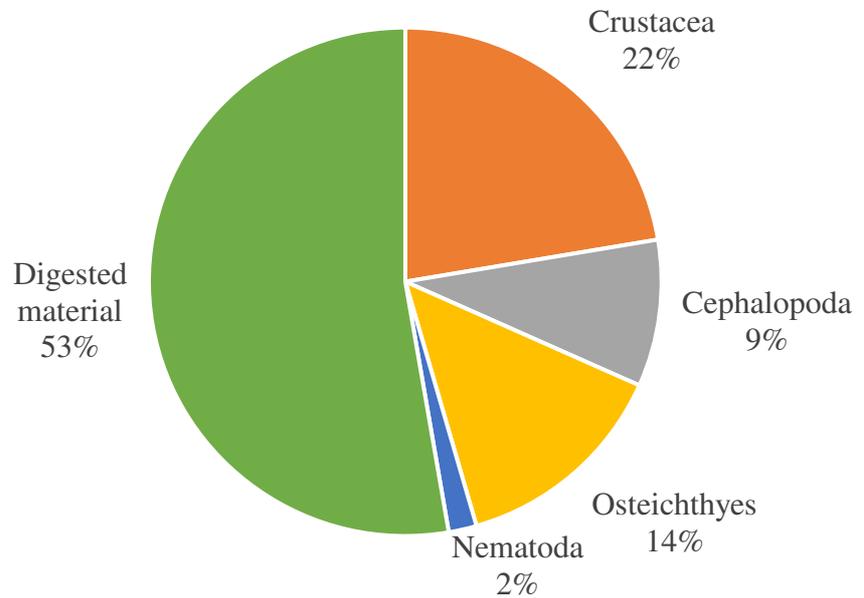


Figure 38. Diet composition of these 19 specimens of *S. canicula*

The SIMPER analysis shows a high contribution of Osteichthyes for juveniles and a high contribution of Crustacea for adults. Despite this, the digested and unrecognized material represents about 80% of the contribution in both cases (Table 13).

Table 13. Percentage of similarity between prey between juveniles and adults. Low contribution cut-off at 100%. JUV = juveniles; AD = adults.

Group JUV		Average similarity: 56.13		
Species	Av.Abund	Av.Sim	Contrib%	Cum.%
Digested material	1.08	42.81	76.27	76.27
Osteichthyes	0.55	6.33	11.28	87.55
Crustacea	0.46	3.82	6.8	94.35
Cephalopoda	0.41	3.17	5.65	100
Nematoda (<i>Anisakis</i>)	0.1	0	0	100
Polychaeta	0.13	0	0	100
Group AD		Average similarity: 66.96		
Species	Av.Abund	Av.Sim	Contrib%	Cum.%
Digested material	1.13	55.5	82.87	82.87
Crustacea	0.52	9.66	14.43	97.3
Osteichthyes	0.24	1.39	2.08	99.38
Cephalopoda	0.17	0.42	0.62	100

$\delta^{13}\text{C}$ was on average $-18.29 \pm 0.62\text{‰}$ while $\delta^{15}\text{N}$ was $9.51 \pm 0.71\text{‰}$.

Mean $\delta^{13}\text{C}$ content of juveniles and adults are, respectively, $-18.5 \pm 0.64\text{‰}$ and $-18.1 \pm 0.56\text{‰}$. Mean $\delta^{15}\text{N}$ content of juveniles and adults are, respectively, $9.6 \pm 0.75\text{‰}$ and $9.5 \pm 0.66\text{‰}$.

$\delta^{15}\text{N}$ values show a slight, not significant, positive correlation with length ($R = 0.09$, $p > 0.05$, Figure 39).

Table 14. Results of the PERMANOVA main tests for $\delta^{15}\text{N}$ (a) and $\delta^{13}\text{C}$ (b).

a)

Source	df	MS	Pseudo-F	P(MC)
TL	1	0.03	0.05	0.82
Se	1	0.46	0.78	0.38
TLxSe	1	0.12	0.20	0.65
Res	15	0.59		
Total	18			

b)

Source	df	MS	Pseudo-F	P(MC)
TL	1	1.12	3.05	0.10
Se	1	1.25	3.41	0.09
TLxSe	1	0.15	0.42	0.52
Res	15	0.37		
Total	18			

We have chosen species that fall into the mixing polygon (Figure 41).

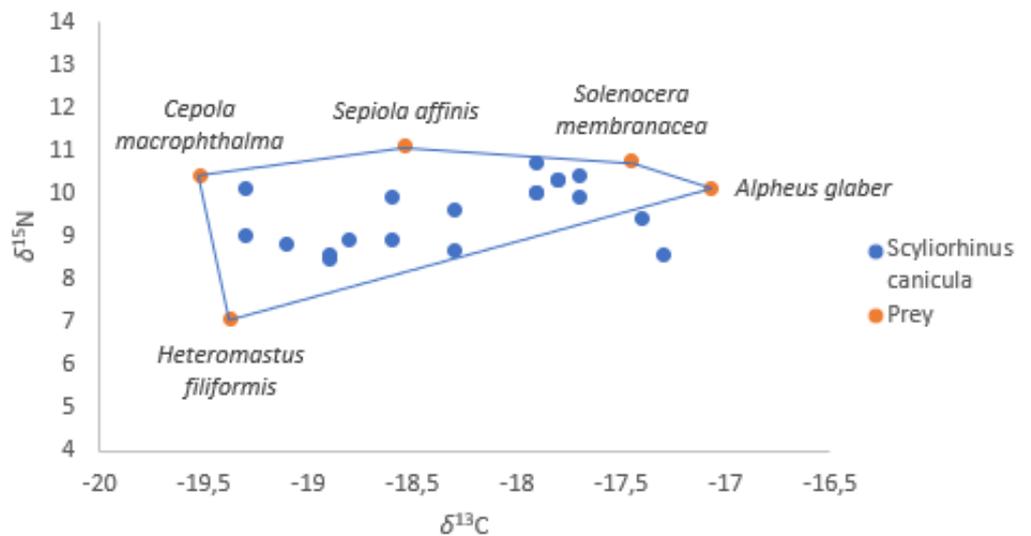


Figure 41. Mixing plot of the potential food sources for *S. canicula*: orange dots indicated mean isotopic values of the food sources, corrected with the TEF. Blue dots represent the isotopic values of *S. canicula* samples.

A Bayesian model SIMMR was used to estimate the potential food sources for *S. canicula*. Because of the absence of significance demonstrated with univariate analysis, it was considered TL for the correlation with $\delta^{13}\text{C}$ (Figure 40). The two groups considered are homogeneously distributed (Figure 42), confirming the absence of significance reported by PERMANOVA analysis (Table 14). *Heteromastus filiformis* and *Alpheus glaber* contributed the most to juveniles' and adults' diet, respectively (Figures 43 and 44). The mean percentage contribution of each source to the isotopic signals of the analyzed specimens of *S. canicula* is shown in Table 15.

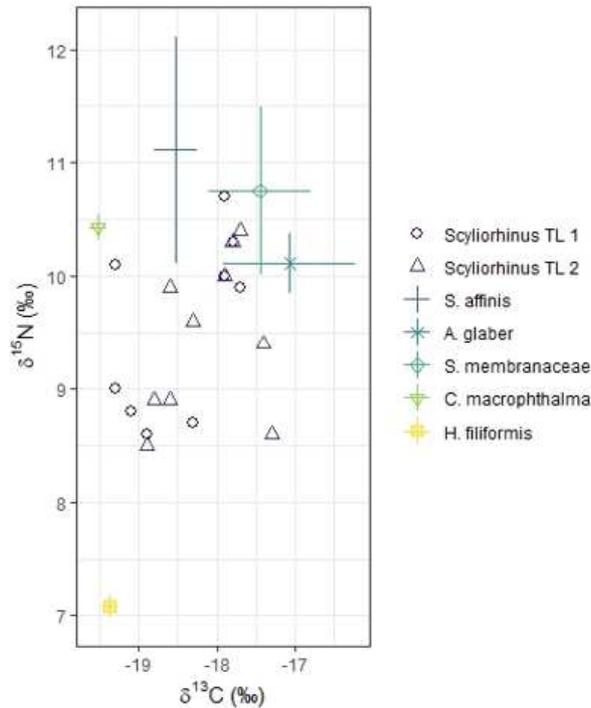


Figure 42. Isospace plot of *Scyliorhinus canicula*, factor = “TL”, TL 1 = juveniles, TL 2 = adults

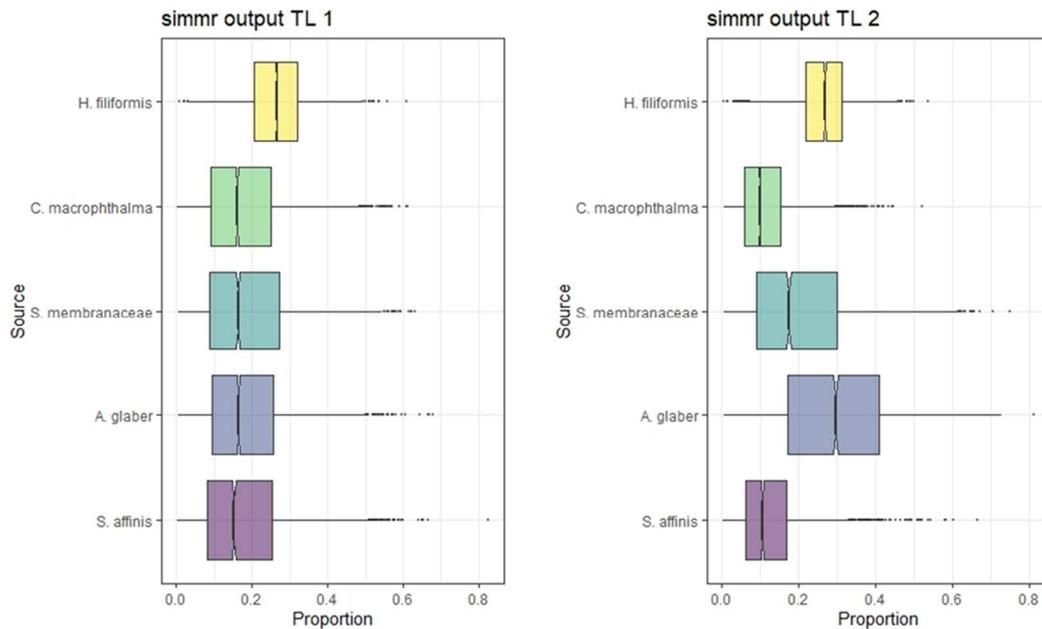


Figure 43. Proportions of each food source in the diet of the catshark collected for TL 1 = Juveniles, TL 2 = Adults. Boxplots were obtained with stable isotope analysis mixing models. Each plot shows proportions for each food source in the specific TL. Boxes indicate 50%, 75% and 95% Bayesian confidence intervals.

Table 15. Summary percentage contribute for juveniles and adults

	Juveniles		Adults	
	mean	sd	mean	sd
deviance	42.35	3.18	41.80	3.45
<i>S. affinis</i>	0.18	0.12	0.13	0.09
<i>A. glaber</i>	0.19	0.12	0.29	0.15
<i>S. membranacea</i>	0.19	0.12	0.21	0.15
<i>C. macrophthalma</i>	0.18	0.11	0.11	0.07
<i>H. filiformis</i>	0.26	0.09	0.26	0.07
sd[d13C_PI]	0.74	0.25	0.56	0.23
sd[d15N_PI]	0.89	0.31	0.78	0.26

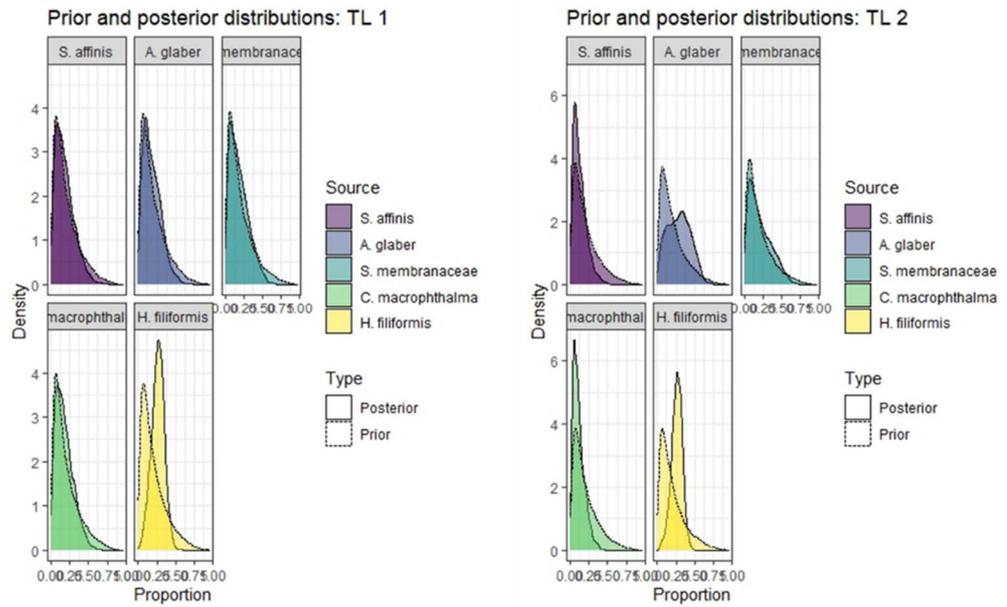


Figure 44. Prior and posterior distribution of the proportional contribute of each source to the diet of *Scyliorhinus canicula* between juveniles and adults. TL 1 = juveniles, TL 2 = adults

Standard ellipses obtained by SIBER show the isotopic niche widths of the groups. These catsharks do not have significant difference in niche widths between juveniles and adults, as confirmed by SEAc values (Figure 45 and Table 16). The color of the column indicates the order of groups, from the top to the bottom.

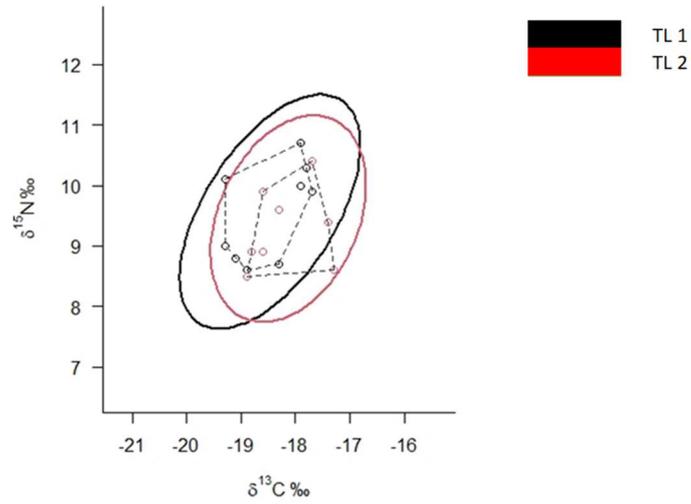


Figure 45. $\delta^{13}\text{C} - \delta^{15}\text{N}$ scatterplot with standard ellipses (p interval = 95%) corrected for small sample size (SEAc) for *S. canicula* collected in the South of the Elba Island. TL 1 = juveniles; TL 2 = adults.

Table 16. Total Convex Hull Area (TA) and Standard Ellipse Areas corrected for small sample size (SEAc) values of *S. canicula* between TLs. 1.1 = juveniles; 1.2 = adults.

	1.1	1.2
TA	2.33	2.09
SEA	1.39	1.21
SEAc	1.59	1.36

5. DISCUSSION

5.1 Spatial variation in the feeding ecology of *Poroderma africanum*

Usually, in catsharks $\delta^{15}\text{N}$ content is greater in animals with higher size (Kim *et al.*, 2012; Di Lorenzo *et al.*, 2020). This means that as the catshark grows, it feeds on prey positioned at a higher trophic level. This means that it can select specimens of the same species but with larger size or it can select prey from species positioned at a higher position within the food web (Di Lorenzo *et al.*, 2020). However, this relationship between $\delta^{15}\text{N}$ and TL is not always true for all the species of sharks (Kim *et al.*, 2012). In fact, sometimes the trophic level, represented by $\delta^{15}\text{N}$, remains fairly constant as the size increases. This happens for the specimens of *Poroderma africanum*, one of the two species of catshark studied in this thesis.

Conversely, the observed trend of $\delta^{13}\text{C}$ that increase with increasing catshark size suggests an ontogenetic dietary shift associated with changes in morphology, physiology or lifestyle (Van der Heeven *et al.*, 2020). The stable isotope value of carbon indicates whether the carbon source is more planktonic or more benthic (Jennings *et al.*, 1997), or marine vs. continental, thus providing indications of an inshore and offshore trend (Rabehagaso *et al.*, 2012). Higher $\delta^{13}\text{C}$ (from -13 to -17‰) indicate that a species is relying on a benthic food web. Conversely, lower $\delta^{13}\text{C}$ values pointed out to a greater dependence on a pelagic food web (Jennings *et al.*, 1997).

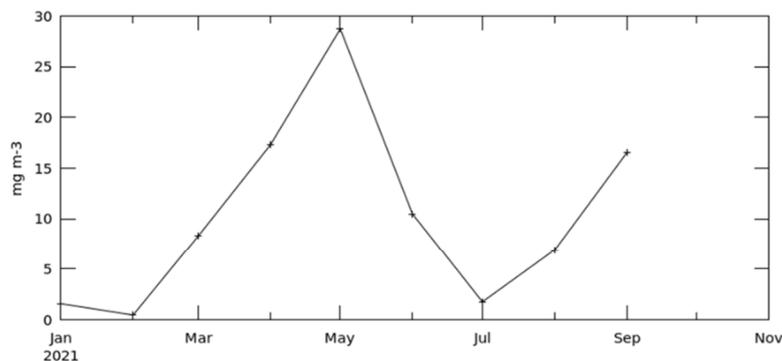
In this study, juveniles have a very low $\delta^{13}\text{C}$ value, while most of the adults have a lower $\delta^{13}\text{C}$ value. So, in this case, there is an increase of $\delta^{13}\text{C}$ with higher TL. There are two hypotheses on why this occurs: i) probably, the position of juveniles and adults in the food web changes, with juveniles eating more benthopelagic shrimps or other prey approaching to the bottom, while adults feed on purely benthic items (Danty, 2002); ii) this shift in the isotopic signal could be due to a horizontal displacement with

adults living more offshore than juveniles (Trueman *et al.*, 2012; Tanaka *et al.*, 2010). However, this catshark is not recognized as a migrator, so it is unlikely that there is an inshore-offshore movement for this species (Graham *et al.*, 2010), particularly in this study area. Moreover, the output of the SIBER model carried out considering TL as discriminant factor shows a higher selective diet of adults (that show a stretched SEAc along the $\delta^{15}\text{N}$ -axis) compared to the more generalist diet of juveniles of the same species. The output of the third SIBER model, which considers the differences between juveniles and adults, confirms that the mean contribution of the gastropod *Phalium craticulatum* (a “strictly benthic” organism) to adults’ diet is greater than that of the same prey for juveniles. Similarly, the pelagic fish *Hyporhamphus capensis* seems to give a bigger contribution to juveniles’ diet. However, since the isotopic data used on the model for prey were from literature, model results have to be taken with caution and further studies are needed on *P. africanum* diet in order to compare SIA to SCA results.

SIBER outputs show also that these catsharks have a wider trophic niche at the TP site, while they are more selective at the HB site, as supported by the outputs of the SIMMR model that considers the sites as discriminant factor. The output of the SIBER model carried out considering the sex and the site as discriminant factor shows a high selectivity of females at GB, while both males and females maintain a wide niche width at TP. Three environmental variables may explain the observed pattern: the river runoff, the chlorophyll-a concentration, as a proxy of primary production and the particulate organic carbon (POC) concentration. Data downloaded by Giovanni NASA (nasa.gov) confirmed the difference between the chlorophyll-a concentration and that of POC among the three sites (data up 30-10-2021) (Figure 46; 47). Higher values of these two variables were registered for HB and GB rather than that observed in TP. No differences were registered among the runoff values in the three sites, being in average

1.817e-06 kg m⁻²s⁻¹ (data up to 30-09-2015), in fact $\delta^{13}\text{C}$ values are very similar in the three sites considered (Lee *et al.*, 2018; Davias *et al.*, 2014).

Time Series, Area-Averaged of Chlorophyll a concentration monthly 4 km [MODIS-Aqua MODISA_L3m_CHL v2018] mg m-3 over 2021-01-01 00:10:01Z - 2021-10-01 02:55:00Z, Region 22.1648E, 34.1185S, 22.3282E, 34.0478S



Time Series, Area-Averaged of Chlorophyll a concentration monthly 4 km [MODIS-Aqua MODISA_L3m_CHL v2018] mg m-3 over 2021-01-01 00:10:01Z - 2021-10-01 02:55:00Z, Region 22.1168E, 34.2359S, 22.2328E, 34.1631S

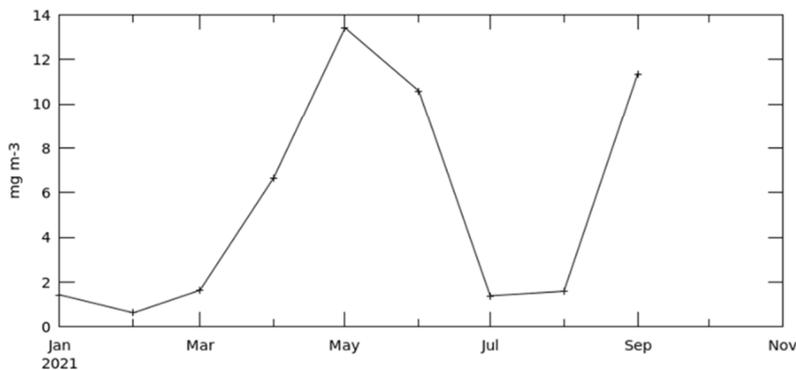
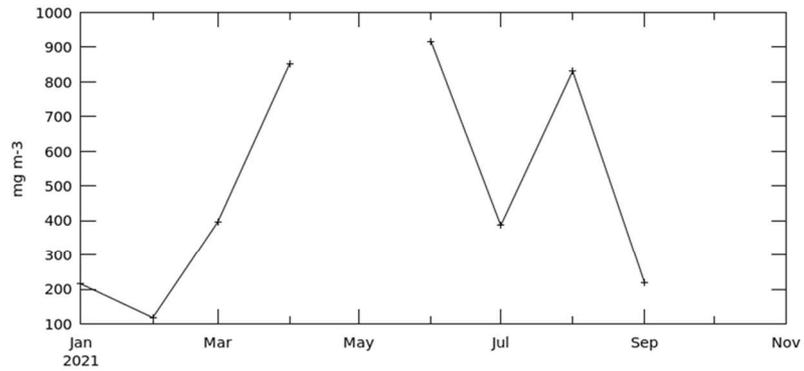


Figure 46. Difference between sites for the concentration of chlorophyll-a (HB and GB, on the top, and TP on the bottom).

Time Series, Area-Averaged of Concentration of Particulate Organic Carbon monthly 4 km [MODIS-Aqua MODISA_L3m_POC v2018] mg m-3 over 2021-01-01 00:10:01Z - 2021-10-01 02:55:00Z, Region 22.1648E, 34.1185S, 22.3282E, 34.0478S



Time Series, Area-Averaged of Concentration of Particulate Organic Carbon monthly 4 km [MODIS-Aqua MODISA_L3m_POC v2018] mg m-3 over 2021-01-01 00:10:01Z - 2021-10-01 02:55:00Z, Region 22.1168E, 34.2359S, 22.2328E, 34.1631S

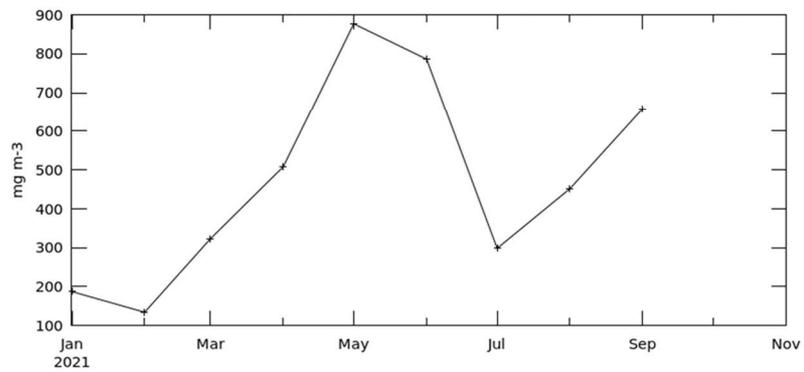


Figure 47. Difference between sites for the concentration of particulate organic carbon (HB and GB, on the top, and TP on the bottom).

5.2 Ontogenetic shift in the feeding ecology of *Scyliorhinus canicula*

Differently from what reported from other studies in the Mediterranean Sea (Barria *et al.*, 2018), here we did not find any positive correlation of $\delta^{15}\text{N}$ values with increasing size, that remains fairly constant as the total length increases. This suggests that during the ontogenetic development, this species continues to feed on similar trophic level prey. Differently, the correlation between $\delta^{13}\text{C}$ and the total length is significant, with $\delta^{13}\text{C}$ values increasing with TL. This likely points out to an inshore-offshore displacement towards more benthic prey (Rabehagaso *et al.*, 2012; Trueman *et al.*, 2012; Tanaka *et al.*, 2010). Similarly to the Pyjama

catshark, also this species does not face up extensive migrations (Rodríguez-Cabello *et al.*, 2014), thus it is more likely that changes in $\delta^{13}\text{C}$ are related to ontogenetic shift in the diet. This suggests a behavioral change in the type of predation, from juveniles feeding in the water column or close to the bottom on suprabenthic and benthopelagic species, such as the Atlantic mud shrimp *Solenocera membranacea*, which moves along the water column near the bottom. Adults, on the other hand, preferably live closer to the seabed and select benthic prey, as reported in literature (Lyle, 1983; Barria *et al.*, 2018).

In this case study, there are few collected samples and they were captured during a single survey. The absence of seasonality and the low number of individuals makes difficult to well defined the diet based on SCA, thus such approach is here used only in a qualitative way and as an integration of SIA results. Polychaetas seem to be the most represented food items along with crustaceans such as *Alpheus glaber*, which is benthic species. Juveniles show a preference for the fish *Cepola macrophthalma*. These results may demonstrate an ontogenetic shift in the diet of this catshark too (Šantić *et al.*, 2012).

5.3 Comparison between *Poroderma africanum* and *Scyliorhinus canicula*

These two catsharks live in two completely distant, but in some features similar, places. The Cape province of South Africa is characterized by a Mediterranean climate and therefore has some similarities with the Mediterranean Sea (Rundel *et al.*, 2018). Moreover, they belong to the same family, i.e., the Scyliorhinidae. Our results showed that these two species have a very similar feeding behavior. Species of the genus *Scyliorhinus* usually live in shallow waters, although they reach deep bottoms up to ca. 400 m depth. Conversely the genus *Poroderma* encompasses more coastal species (Muus *et al.*, 1999; Compagno *et al.*, 1989) the two species are similar in size and morphology and the position

of the mouth confirms them as benthic feeders (Barria *et al.*, 2018, Van der Heeven *et al.*, 2020). They are both mesopredatory sharks (Baum & Worm, 2009; Van der Heeven *et al.*, 2020).

Although *Poroderma africanum* has an important ecological role in the local food web, as also highlighted by our results, catch data are scarce. This means that probably *Poroderma* does not have a well-defined management plan that allows its adequate conservation or, at least, an adequate monitoring of its population status in south-African waters, as reported also by the IUCN assessment for this species. On the other hand, in the Mediterranean Sea, the pressure on shark populations is well documented (Giovos *et al.*, 2021). *Scyliorhinus canicula*, accounts in this basin for ca. 21.6% of the total catch of elasmobranchs of all fisheries combined (Giovos *et al.*, 2021). This figure could be underestimated as national authorities register elasmobranchs in aggregate landing categories (Giovos *et al.*, 2021). The lack of detailed fishery catch statistics hampers the conservation of elasmobranchs in the Mediterranean region (Cashion *et al.*, 2019; Giovos *et al.*, 2020).

The population of *S. canicula* in the Mediterranean is not considered in decline, rather the species has shown an increase in both density and biomass over the past 10 years (Ramírez-Amaro *et al.*, 2020). Early maturity, rapid turn-over time and continuous reproductive cycles have led to greater resilience and recover of this species. Early maturity could also be an adaptive response that increases the likelihood of offspring reaching maturity. Secondly, they are opportunistic scavengers so they can modify their diet benefiting, for example, the discard generated by trawling (Abella & Serena, 2005; Ramírez-Amaro *et al.*, 2020). Finally, large predators (especially sharks) have drastically declined in the last decades due to directed fishing on the species and to by-catch. In addition, their reduction is likely due to the general decrease of fish as prey, due to overfishing (Bearzi *et al.*, 2006; Lotze *et al.*, 2011). All this may have led to the disappearance of large predators, thus resulting in a decrease of

predatory and competition pressure on mesopredators (Dell’Apa, 2012), thus supporting the “mesopredator release hypothesis”.

In conclusion, notwithstanding the different sites the two species living in, many similarities of these scyliorhinids were found. Both act as mesopredators occupying the intermediate level of the food web they belong to. If mesopredators increased, they could lead other species, which share the same resources, such as benthic prey, to modify their trophic niche and possibly also the habitats (Ritchie & Johnson, 2009; Elmhagen *et al.*, 2010), thus avoiding competitive exclusion. Furthermore, an increase in mesopredators would even have the potential some prey to extinct, particularly the more sensitive ones, with low population growth rates or more easily attacked by mesopredators (Ritchie & Johnson, 2009). Future studies on the prey of *P. africanum* could shed more light on its feeding ecology and trophic position, together with its ecological role.

7. BIBLIOGRAPHY

- Abella A.J., Serena F. (2005) Comparison of elasmobranch catches from research trawl surveys and commercial landings at port of Viareggio, Italy, in the last decade. *Journal of Northwest Atlantic Fishery Science*, 35: 345-356.
- Anderson M., Gorley R., Clarke K.P. (2008) for PRIMER: guide to software and statistical methods. *Primer-e, Plymouth, UK*, 32.
- Awruch C.A. (2015) Reproduction strategies. In *Fish physiology* (Vol. 34, pp. 255-310). Academic Press.
- Bada N., Da Ros Z., Rindi F., Busi S., Azzurro E., Derbal F., Fanelli E. (2021) Seasonal trophic ecology of the invasive crab *Percnon gibbesi* (Brachyura, Plagusidiidae) in the southwestern Mediterranean: insights from stomach contents and stable isotope analyses. *Marine Environmental Research*:105513. <https://doi.org/10.1016/j.marenvres.2021.105513>.
- Baker R., Buckland A., Sheaves M. (2014) Fish gut content analysis: robust measures of diet composition. *Fish and Fisheries*, 15(1): 170-177.
- Barría C., Navarro J., Coll M. (2018) Trophic habits of an abundant shark in the northwestern Mediterranean Sea using an isotopic non-lethal approach. *Estuarine, Coastal and Shelf Science*, 207: 383-390.
- Bascompte J., Melián C.J. (2005) Simple trophic modules for complex food webs. *Ecology* 86 (11): 2868-2873.
- Baum J.K., Worm B. (2009) Cascading top-down effects of changing oceanic predator abundances. *Journal of animal ecology* 78 (4): 699-714.
- Bearzi G., Politi E., Agazzi S., Azzellino A. (2006) Prey depletion caused by overfishing and the decline of marine megafauna in eastern Ionian Sea coastal waters (central Mediterranean). *Biological Conservation*, 127(4), 373-382.

- Bergamino L., Dalu T., Whitfield A.K., Carassou L., Richoux N.B. (2014) Stable isotope evidence of food web connectivity by a top predatory fish (*Argyrosomus japonicus*: Sciaenidae: Teleostei) in the Kowie Estuary, South Africa. *African Journal of Marine Science*, 36(2): 207-213.
- Bongiorno L., Nasi F., Fiorentino F., Auriemma R., Rampazzo F., Nordström M.C., Berto D. (2018) Contribution of deltaic wetland food sources to coastal macrobenthic consumers (Po River Delta, north Adriatic Sea). *Science of the total environment*, 643: 1373-1386.
- Bucking C. (2015) Feeding and digestion in elasmobranchs: tying diet and physiology together. In *Fish physiology* (Vol. 34, pp. 347-394). Academic Press.
- Bustamante R.H., Branch G.M. (1996) Large Scale Patterns and Trophic Structure of Southern African Rocky Shores: The Roles of Geographic Variation and Wave Exposure. *Journal of Biogeography* 23 (3): 339–51.
- Cashion M.S., Bailly N., Pauly D. (2019) Official catch data underrepresent shark and ray taxa caught in Mediterranean and Black Sea fisheries. *Marine Policy*, 105: 1-9.
- Caut S., Jowers M.J., Michel L., Lepoint G., Fisk A.T. (2013) Diet-and tissue-specific incorporation of isotopes in the shark *Scyliorhinus stellaris*, a North Sea mesopredator. *Marine Ecology Progress Series*, 492: 185-198.
- Clarke K.R., Warwick R.M. (2001) Change in marine communities. An approach to statistical analysis and interpretation 2: 1-168.
- Compagno, L.J.V. (1984) FAO Species Catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 2 - Carcharhiniformes. FAO Fish. Synop. 125(4/2):251-655. Rome: FAO.
- Compagno L.J., Ebert D.A., Smale M.J. (1989) Guide to the sharks and rays of southern Africa.

- Compagno L.J.V. (1990) Alternative life-history styles of cartilaginous fishes in time and space. *Environmental Biology of Fishes* 28 (1): 33-75.
- Compagno L.J.V., Dando M., Fowler S. (2005) *Sharks of the World*, Princeton University Press.
- Cortés E. (1999) Standardized diet compositions and trophic levels of sharks. *ICES Journal of marine science* 56 (5): 707-717.
- Dainty A. M. (2002) Biology and ecology of four catshark species in the southwestern cape, south Africa.
- Danovaro R. (2019) *Biologia marina. Biodiversità e funzionamento degli ecosistemi marini*; 161-162.
- Davias L.A., Kornis M.S., Breitburg D.L. (2014) Environmental factors influencing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in three Chesapeake Bay fishes. *ICES Journal of Marine Science*, 71(3): 689-702.
- De Lecea A.M., Fennessy S.T., Smit A.J. (2013) Processes controlling the benthic food web of a mesotrophic bight (KwaZulu-Natal, South Africa) revealed by stable isotope analysis. *Marine Ecology Progress Series*, 484: 97-114.
- Dell'Apa A., Kimmel D.G., Clò S. (2012) Trends of fish and elasmobranch landings in Italy: associated management implications. *ICES Journal of Marine Science*, 69(6): 1045-1052.
- De Necker L. (2017) The trophic dynamics of the broadnose sevengill shark (*Notorynchus cepedianus*) in False Bay, South Africa, using multiple tissue stable isotope analysis. MS thesis. University of Cape Town.
- Di Lorenzo M., Vizzini S., Signa G., Andolina C., Palo G.B., Gristina M., Mazzoldi C., Colloca F. (2020) Ontogenetic trophic segregation between two threatened smooth-hound sharks in the Central Mediterranean Sea. *Scientific Reports*, 10(1): 1-15.
- Domi N., Bouquegneau J.M., Krishna D. (2005) Feeding ecology of five commercial shark species of the Celtic Sea through stable isotope

- and trace metal analysis. *Marine Environmental Research* 60 (5): 551-569.
- Dulvy, N.K., Reynolds J.D. (1997) Evolutionary transitions among egg-laying, live-bearing and maternal inputs in sharks and rays. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* 264:1309-1315.
 - Dulvy N.K., Sadovy Y., Reynolds J.D. (2003) Extinction vulnerability in marine populations. *Fish and fisheries*, 4(1): 25-64.
 - Dulvy N.K., Pacoureau N., Rigby C.L., Pollom R.A., Jabado R.W., Ebert D.A., Finucci B., Pollock C.M., Cheok J., Derrick D.H., Herman K.B., Sherman C.S., VanderWright W.J., Lawson J.M., Walls R.H.L., Carlson J.K., Charvet P., Bineesh K.K., Fernando D., Ralph G.M., Matsushiba J.H., Hilton-Taylor C., Fordham S.V., Simpfendorfer C.A. (2021) Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Current Biology*, 31(21): 4773-4787.
 - Ellis J.R., Shackley S.E. (1997) The reproductive biology of *Scyliorhinus canicula* in the Bristol Channel, U.K. *J. Fish Biol.* 51(2):361-372.
 - Ellis J.K., Musick J.A. (2007) Ontogenetic changes in the diet of the sandbar shark, *Carcharhinus plumbeus*, in lower Chesapeake Bay and Virginia (USA) coastal waters. *Environmental biology of fishes* 80 (1): 51-67.
 - Elmhagen B., Ludwig G., Rushton S.P., Helle P., Lindén H. (2010) Top predators, mesopredators and their prey: interference ecosystems along bioclimatic productivity gradients. *Journal of Animal Ecology*, 79(4), 785-794.
 - Emanuel B. P., Bustamante R.H., Branch G.M., Eekhout S., Odendaal F.J. (1992) A zoogeographic and functional approach to the selection of marine reserves on the west coast of South Africa. *South African Journal of Marine Science* 12 (1): 341-354.
 - Escobar-Porras J. (2009) *Movement patterns and population dynamics of four catsharks endemic to South Africa* (Doctoral dissertation, Rhodes University).

- Estrada J.A., Rice A.N., Lutcavage M.E., Skomal G.B. (2003) Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotope analysis. *Journal of the Marine biological Association of the United Kingdom* 83 (6): 1347-1350.
- Fanelli E., Azzurro E., Bariche M., Cartes J.E., Maynou F. (2015) Depicting the novel Eastern Mediterranean food web: a stable isotopes study following Lessepsian fish invasion. *Biological Invasions*, 17(7): 2163-2178.
- Fanelli E., Cartes J.E. (2010) Temporal variations in the feeding habits and trophic levels of three deep-sea demersal fishes from the western Mediterranean Sea, based on stomach contents and stable isotope analyses. *Marine Ecology Progress Series*, 402: 213-232.
- Ferretti F., Myers R.A., Sartor P., Serena F. (2005) Long term dynamics of the chondrichthyan fish community in the upper Tyrrhenian Sea. *ICES CM*, 25: 1-34.
- Ferretti F., Worm B., Britten G.L., Heithaus M.R., Lotze H.K. (2010) Patterns and ecosystem consequences of shark declines in the ocean. *Ecology letters* 13 (8): 1055-1071.
- Frisk M.G., Miller T.J., Dulvy N.K. (2005) Life histories and vulnerability to exploitation of elasmobranchs: inferences from elasticity, perturbation and phylogenetic analyses. *Journal of Northwest Atlantic Fishery Science*, 35.
- Fry B. (2006) *Stable isotope ecology*. Vol. 521. New York: Springer.
- Gallagher A.J., Orbesen E.S., Hammerschlag N., Serafy J.E. (2014) Vulnerability of oceanic sharks as pelagic longline bycatch. *Global Ecology and Conservation* 1: 50-59.
- Giovos I., Arculeo M., Doumpas N., Katsada D., Maximiadi M., Mitsou E., Paravas V., Aga-Spyridopoulou R.N., Stoilas V.O., Tiralongo F., Tsamadias I.E., Vecchioni L., Moutopoulos D.K. (2020) Assessing multiple sources of data to detect illegal fishing, trade and

- mislabelling of elasmobranchs in Greek markets. *Marine Policy*, 112: 103730.
- Giovos I., Spyridopoulou R.A., Doumpas N., Glaus K., Kleitou P., Kazlari Z., Katsada D., Loukovitis D., Mantzouni I., Papapetrou M., Papastamatiou Y.P., Moutopoulos D.K. (2021) Approaching the “real” state of elasmobranch fisheries and trade: A case study from the Mediterranean. *Ocean & Coastal Management*, 211: 105743.
 - Gračan R., Zavodnik D., Krstinić P., Dragičević B., Lazar B. (2017) Feeding ecology and trophic segregation of two sympatric mesopredatory sharks in the heavily exploited coastal ecosystem of the Adriatic Sea. *Journal of fish biology* 90 (1): 167-184.
 - Graham B.S., Koch P.L., Newsome S.D., McMahon K.W., Aurioles D. (2010) Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In *Isoscapes* (pp. 299-318). Springer, Dordrecht.
 - Hammer Ø., Harper D.A., Ryan P.D. (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia electronica*, 4(1): 9.
 - Hammerschlag N., Sulikowski J. (2011) Killing for conservation: the need for alternatives to lethal sampling of apex predatory sharks. *Endangered Species Research* 14 (2): 135-140.
 - Heupel M.R., Simpfendorfer C.A. (2010) Science or slaughter: need for lethal sampling of sharks. *Conservation Biology* 24 (5): 1212-1218.
 - Heupel M.R., Knip D.M., Simpfendorfer C.A., Dulvy N.K. (2014) Sizing up the ecological role of sharks as predators. *Marine Ecology Progress Series* 495: 291-298.
 - Hobson K.A. (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120 (3): 314-326.
 - Hussey N. & Dudley, Sheldon & Mccarthy, Ian & Cliff, Jeremy & Fisk A. (2011) Stable isotope profiles of large marine predators: Viable indicators of trophic position, diet, and movement in sharks?. *Canadian*

Journal of Fisheries and Aquatic Sciences. 68. 2029-2045.
10.1139/f2011-115.

- Hussey N.E., Chapman D.D., Donnelly E., Abercrombie D.L., Fisk A.T. (2011) Fin-icky samples: an assessment of shark fin as a source material for stable isotope analysis. *Limnology and Oceanography: Methods*, 9(11): 524-532.
- Hussey N.E., MacNeil M.A., Olin J.A., McMeans B.C., Kinney M.J., Chapman D.D., Fisk A.T. (2012) Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. *Journal of fish biology* 80 (5): 1449-1484.
- Hyslop E.J. (1980) Stomach contents analysis—a review of methods and their application. *Journal of fish biology* 17 (4): 411-429.
- Jackson A.L., Inger R., Parnell A.C., Bearhop S. (2011) Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80 (3): 595-602.
- James B.S., Bester M.N., Penry G.S., Gennari, E., Elwen S.H. (2015) Abundance and degree of residency of humpback dolphins *Sousa plumbea* in Mossel Bay, South Africa. *African Journal of Marine Science* 37(3): 383-394.
- Jennings S., Reñones O., Morales-Nin B., Polunin N.V., Moranta J., Coll J. (1997) Spatial variation in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: implications for the study of trophic pathways. *Marine Ecology Progress Series*, 146: 109-116.
- Josberger E.E., Hassanzadeh P., Deng Y., Sohn J., Rego M.J., Amemiya C.T., Rolandi M. (2016) Proton conductivity in ampullae of Lorenzini jelly. *Science Advances*, 2(5): e1600112. doi=10.1126/sciadv.1600112
- Kardong V.K. (2012) Vertebrates: Comparative Anatomy, Function, Evolution: 6th (sixth) Edition, The McGraw-Hill Companies, pp.765.

- Kim Sora & Tinker, Martin & Estes, James & Koch, Paul. (2012) Ontogenetic and Among-Individual Variation in Foraging Strategies of Northeast Pacific White Sharks Based on Stable Isotope Analysis. *PloS one*. 7: e45068. 10.1371/journal.pone.0045068.
- Kim S.L., Casper D.R., Galván-Magaña F., Ochoa-Díaz R., Hernández-Aguilar S.B., Koch P.L. (2012) Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-term controlled feeding study. *Environmental Biology of Fishes*, 95(1): 37-52
- Koehler L., Smith L.E., Nowell G. (2018) Recovered and released-A novel approach to oviparous shark conservation. *Ocean & Coastal Management* 154: 178-185.
- Layman C.A., Arrington D.A., Montaña C.G., Post D.M. (2007) Can stable isotope ratios provide for community-wide measures of trophic structure?. *Ecology* 88 (1): 42-48.
- Lee K.Y., Graham L., Spooner D.E., Xenopoulos M.A. (2018) Tracing anthropogenic inputs in stream foods webs with stable carbon and nitrogen isotope systematics along an agricultural gradient. *PloS one*, 13(7): e0200312.
- Logan J.M., Lutcavage M.E. (2010) Stable isotope dynamics in elasmobranch fishes. *Hydrobiologia* 644 (1): 231-244.
- Lotze H.K., Coll M., Dunne J.A. (2011) Historical changes in marine resources, food-web structure and ecosystem functioning in the Adriatic Sea, Mediterranean. *Ecosystems*, 14(2), 198-222.
- Lyle J.M. (1983) Food and feeding habits of the lesser spotted dogfish, *Scyliorhinus canicula* (L.), in Isle of Man waters. *Journal of fish biology*, 23(6): 725-737.
- MacNeil M.A, Skomal G.B., Fisk A.T. (2005) Stable isotopes from multiple tissues reveal diet switching in sharks. *Marine Ecology Progress Series* 302: 199-206.

- MacNeil M.A., Drouillard K.G., Fisk A.T. (2006) Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. *Canadian Journal of Fisheries and Aquatic Sciences* 63 (2): 345-353.
- Mannini A., Masnadi F., Dott. Ligas A. (2015-2016) Ecologia e valutazione delle risorse alieutiche di ambienti profondi in relazione a fattori ambientali ed impatto di pesca. Tesi di laurea.
- Maruska K.P. (2001) Morphology of the mechanosensory lateral line system in elasmobranch fishes: ecological and behavioral considerations. *Environmental Biology of Fishes*, 60(1): 47-75.
- Matich P., Heithaus M.R. (2014) Multi-tissue stable isotope analysis and acoustic telemetry reveal seasonal variability in the trophic interactions of juvenile bull sharks in a coastal estuary. *Journal of Animal Ecology* 83 (1): 199-213.
- Matich P., Heithaus M.R., Layman C.A. (2011) Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. *Journal of Animal Ecology* 80 (1): 294-305.
- McQuaid C.D., Branch G.M. (1985) Trophic structure of rocky intertidal communities: Response to wave action and implications for energy flow. *Marine ecology progress series. Oldendorf* 22 (2): 153-161.
- McQuaid C.D. (1981) The establishment and maintenance of vertical size gradients in populations of *Littorina africana knysnaensis* (Philippi) on an exposed rocky shore. *Journal of Experimental Marine Biology and Ecology* 54 (1): 77-89.
- Meredith T.L., Kajiura S.M. (2010) Olfactory morphology and physiology of elasmobranchs. *Journal of Experimental Biology*, 213(20): 3449-3456.
- Motivarash Y.B., Fofandi D.C., Dabhi R.M., Makrani R.A., Tanna P.D. (2020) Importance of sharks in ocean ecosystem.
- Muus B.J., Nielsen J.G., Dahlström P. (1999) *Die Meeresfische Europas in Nordsee, Ostsee und Atlantik*. Kosmos.

- Norse E.A., Brooke S., Cheung W.W., Clark M.R., Ekeland I., Froese R., Gjerde K.M., Haedrich R.L., Heppell S.S., Morato T., Morgan L.E., Pauly D., Sumaila R., Watson R. (2012) Sustainability of deep-sea fisheries. *Marine policy*, 36(2): 307-320.
- Oliver S., Braccini M., Newman S.J., Harvey E.S. (2015) Global patterns in the bycatch of sharks and rays. *Marine Policy*, 54: 86-97.
- Pace M.L., Cole J.J., Carpenter S.R., Kitchell J.F. (1999) Trophic cascades revealed in diverse ecosystems. *Trends in ecology & evolution* 14(12): 483-488.
- Parnell A.C., Phillips D.L., Bearhop S., Semmens B.X., Ward E.J., Moore J.W., Jackson A.L., Grey J., Kelly D.J., Inger R. (2013) Bayesian stable isotope mixing models. *Environmetrics* 24 (6): 387-399.
- Parnell A. (2021) simmr: A Stable Isotope Mixing Model. R package version 0.4.5. <https://CRAN.R-project.org/package=simmr>
- Phillips D.L., Inger R., Bearhop S., Jackson A.L., Moore J.W., Parnell A.C., Semmens B.X., Ward E.J. (2014) Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology* 92 (10): 823-835.
- Popper A.N., Fay R.R. (1977) Structure and function of the elasmobranch auditory system. *American Zoologist*, 17(2): 443-452.
- Post D.M., Layman C.A., Arrington D.A., Takimoto G., Quattrochi J., Montaña C.G. (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*. May; 152 (1): 179-89. doi: 10.1007/s00442-006-0630-x. Epub 2007 Jan 16. PMID: 17225157.
- Post D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83 (3): 703-718.
- Rabehagaso N., Lorrain A., Bach P., Potier M., Jaquemet S., Richard P., Ménard F. (2012) Isotopic niches of the blue shark *Prionace glauca* and the silky shark *Carcharhinus falciformis* in the southwestern Indian Ocean. *Endangered Species Research*, 17(1): 83-92.

- Ramírez-Amaro S., Ordines F., Esteban A., García C., Guijarro B., Salmerón F., Terrasa B., Massutí E. (2020) The diversity of recent trends for chondrichthyans in the Mediterranean reflects fishing exploitation and a potential evolutionary pressure towards early maturation. *Scientific reports*, 10(1): 1-18.
- Richoux N.B., Froneman P.W. (2007) Assessment of spatial variation in carbon utilization by benthic and pelagic invertebrates in a temperate South African estuary using stable isotope signatures. *Estuarine, Coastal and Shelf Science*, 71(3-4): 545-558.
- Ritchie E.G., Johnson C.N. (2009) Predator interactions, mesopredator release and biodiversity conservation. *Ecology letters*, 12(9): 982-998.
- Rodríguez-Cabello C., Sánchez F., Fernández A., Olaso I. (2004) Is the lesser spotted dogfish (*Scyliorhinus canicula*) population from the Cantabrian Sea a unique stock?. *Fisheries research*, 69(1): 57-71.
- Rodríguez-Cabello C., Sánchez F., Velasco F. (2005) Growth of lesser spotted dogfish (*Scyliorhinus canicula* L., 1758) in the Cantabrian Sea, based on tag-recapture data. *J Northwest Atl Fish Sci* 37: 131-140.
- Rundel P.W., Arroyo M.T., Cowling R.M., Keeley J.E., Lamont B.B., Pausas J.G., Vargas P. (2018) Fire and plant diversification in Mediterranean-climate regions. *Frontiers in Plant Science*, 9: 851.
- Šantić M., Rađa B., Pallaoro A. (2012) Feeding habits of small-spotted catshark (*Scyliorhinus canicula* Linnaeus, 1758) from the eastern central Adriatic Sea. *Marine Biology Research*, 8(10): 1003-1011
- Shiffman D.S., Gallagher A.J., Boyle M.D., Hammerschlag-Peyer C.M., Hammerschlag N. (2012) Stable isotope analysis as a tool for elasmobranch conservation research: a primer for non-specialists. *Marine and Freshwater Research* 63 (7): 635-643.
- Sims D.W., Nash J.P., Morritt D. (2001). Movements and activity of male and female dogfish in a tidal sea lough: alternative behavioural strategies and apparent sexual segregation. *Mar. Biol.* 139: 1165-1175.

- Smale M.J., Klages N.T., Cockcroft V.G., David J.H.M. (1994) Predators of the Agulhas Bank. *South African Journal of Science* 90(3): 135-143.
- Smith J.A., Mazumder D., Suthers I.M., Taylor M.D. (2013) To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. *Methods in Ecology and Evolution*, 4(7): 612-618.
- Soares K.D.A., De Carvalho M.R. (2019) The catshark genus *Scyliorhinus* (Chondrichthyes: Carcharhiniformes: Scyliorhinidae): taxonomy, morphology and distribution. *Zootaxa* 4601 (1): 1-147.
- Speers-Roesch B., Treberg J.R. (2010) The unusual energy metabolism of elasmobranch fishes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 155(4): 417-434.
- SQUALI, GLI, and NEL MEDITERRANEO. MEDITerranean Large Elasmobranchs Monitoring.
- Swynnerton G.H., Worthington E.B. (1940) Note on the Food of Fish in Haweswater (Westmorland). *Journal of Animal Ecology* 9 (2), [Wiley, British Ecological Society] pp. 183–87, <https://doi.org/10.2307/1454>.
- Tanaka H., Ohshimo S., Takagi N., Ichimaru T. (2010) Investigation of the geographical origin and migration of anchovy *Engraulis japonicus* in Tachibana Bay, Japan: A stable isotope approach. *Fisheries Research*, 102(1-2): 217-220.
- Tilley A., López-Angarita J., Turner J.R. (2013) Diet reconstruction and resource partitioning of a Caribbean marine mesopredator using stable isotope Bayesian modelling. *PloS one*, 8(11): e79560.
- Trueman C.N., MacKenzie K.M., Palmer M.R. (2012) Identifying migrations in marine fishes through stable-isotope analysis. *Journal of Fish Biology*, 81(2): 826-847.
- Van der Heever G.M., Van der Lingen C.D., Leslie R.W., Gibbons M.J. (2020) Spatial and ontogenetic variability in the diet and trophic

ecology of two co-occurring catsharks (Scyliorhinidae) off South Africa. *African Journal of Marine Science* 42 (4): 423-438.

- Watling R.J., Watling H.R. (1982) Metal Surveys in South African Estuaries. III. Hartenbos, Little Brak and Great Brak Rivers (Mossel Bay). *Water SA* 8 (2): 108-113.
- Whitenack L.B., Motta P.J. (2010) Performance of shark teeth during puncture and draw: implications for the mechanics of cutting. *Biological Journal of the Linnean Society*, 100(2): 271-286.
- Yemiskan E., Navarro J., Forero M., Megalofonou P., Eryilmaz L. (2019) Trophic partitioning between abundant demersal sharks coexisting in the North Aegean Sea. *Journal of the Marine Biological Association of the United Kingdom* 99 (5): 1213-1219.

8. WEBLIOGRAPHY

- [COL | Elasmobranchii \(catalogueoflife.org\)](#), last visited on 21st December 2021
- [Giovanni \(nasa.gov\)](#), last visited on 4th February 2022
- [Poroderma africanum, Striped catshark : fisheries, gamefish \(fishbase.de\)](#), last visited 22nd December 2021
- [Poroderma africanum \(Pyjama Shark\) \(iucnredlist.org\)](#), last visited on 22nd December 2021
- [Poroderma africanum – Discover Fishes \(ufl.edu\)](#), last visited on 22nd December 2021
- [Pyjama Catshark - Poroderma africanum \(sharksandrays.com\)](#), last visited on 22nd December 2021
- [Pyjama Catshark | Poroderma africanum | Shark Database \(sharkwater.com\)](#), last visited on 22nd December 2021
- [Poroderma africanum \(Gmelin, 1789\) \(gbif.org\)](#), last visited on 22nd December 2021
- [Scyliorhinus canicula \(Smallspotted Catshark\) \(iucnredlist.org\)](#), last visited on 15th December 2021
- [Scyliorhinus canicula – Discover Fishes \(ufl.edu\)](#), last visited on 15th December 2021
- [Smallspotted Catshark - Scyliorhinus canicula \(sharksandrays.com\)](#), last visited on 15th December 2021
- <http://www.jstor.org/stable/2845850>, last visited on 20th December 2021
- [Stable Isotope Mixing Models in R with simmr \(r-project.org\)](#), last visited on 7th December 2021
- [Ministero delle politiche agricole alimentari e forestali - Mipaaf 2011, Piano di gestione pesca a strascico, GSA 9 - Mar Ligure, Mar Tirreno Settentrionale e Centrale, Decreto 20 maggio 2011](#), last visited on 23rd December 2021

- [Geographical subareas | General Fisheries Commission for the Mediterranean - GFCM | Food and Agriculture Organization of the United Nations](#) | [General Fisheries Commission for the Mediterranean \(GFCM\) | Food and Agriculture Organization of the United Nations \(fao.org\)](#), last visited on 23rd December 2021