Genetic analysis to inform the stock structure of Patagonian toothfish (*Dissostichus eleginoides*) (SIOFA SER2022-TOP1 report)

Literature and data review, Sampling strategy proposal, Feasibility study, and Sampling, laboratory, and shipping protocols

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5 JUNE 2023

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Funded by the European Union

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Executive summary

Genetic stock structure discrimination is an important step in understanding the population dynamics of a stock, especially in cases where stocks are jointly managed by several countries or where multiple RFMOs may have overlapping mandates, as is the case with the Patagonian toothfish. The SER2022-TOP1 project aims to design a genetic stock discrimination project to understand the stock structure of Patagonian toothfish in the SIOFA Area, including linkages to Patagonian toothfish in the CCAMLR Convention Area. This project reviews the literature and existing data held by SIOFA to propose an informed experimental design and sampling protocol for the genetic discrimination of the toothfish stock in the SIOFA area. The collection of samples, analysis, and a full review of the stock structure of Patagonian toothfish will be conducted under SIOFA Project SER2022-TOP2.

The Patagonian toothfish (*Dissostichus eleginoides* Smitt, 1898) is widely distributed on the continental shelves, plateaux, islands, archipelagos, banks and seamounts throughout the Southern Ocean with a bathymetric range from 10 to 2500 m, restricted by water temperatures >2 °C (Duhamel et al. 1982). The species is known to exhibit a K-selection life history, in that it is relatively long lived, slow-growing, with delayed maturity, large body size and long life span (30-50 years). This species exhibits an ontogenetic migration where after a long pelagic larval phase of ≤ 3 months, juveniles settle in nearshore, shallow areas, and as they continue to grow, individuals migrate downslope. Most tagged adult fish move minimal or short horizontal distances (<25 km), though long-distance migrations of almost 2000 km over waters with depths >4000 m have been observed. The distribution and life history of the Patagonian toothfish are closely linked to environmental and ecosystem influences, including influences due to depth, temperature, light intensity, oxygen, salinity, productivity, prey distribution and predation risk.

Genetic studies of *D. eleginoides* around the Southern Ocean have shown that there is population structure between ocean basins, and some distinction within ocean basins separated by important oceanographic features (i.e. the Antarctic Polar Front), though little differentiation has been found using an array of genetic techniques in the Indian Ocean.

Currently, Patagonian toothfish in the SIOFA area are not assessed, though stock assessments are performed for the adjacent CCAMLR regions and recently, SIOFA catches from Williams Ridge were included in the HIMI TOP assessment. However, stocks are likely straddling and a collaborative approach for toothfish assessments has been recommended.

Three main fishing zones were detected from SIOFA catch data including the Del Cano Rise (DCR), South Indian Ridge (SIR), and William's Ridge (WR). Fishing also occurs east of WR, but the SIOFA scientific committee noted that this zone had low likelihood of future fishing effort, and was not further investigated. While fishing operations occur year-round at DCR and SIR, they are concentrated in the austral winter and summer at WR. Most of the fishing operations occurred at the mid-water depth range of 800 - 1500 m. We found that stable physical features such as bottom depth, slope, and bottom temperature are significantly related to both length and sex ratio, while more seasonal and temporary features (e.g. productivity patterns and mesoscale features) do not appear to be of influence. We find that the bathymetric features straddle the CCAMLR and SIOFA zones that would facilitate ontogenetic migration and length distribution data that indicate that post-settlement larvae, juveniles and smaller individuals from the adjacent, shallower CCAMLR regions likely migrate into the deeper SIOFA waters as they age.

After a thorough review of the literature and data available for this species in the Indian Ocean, we find that to have the highest likelihood of genetically discriminating between populations is when spawning individuals have the highest degree of mixing, which we find for the three fishing zones (DCR, SIR, and WR) to be from November to March, contrary to the presumed spawning period found in the literature, in flat areas (<0.2 radians) of <2000 m depth.

We recommend that a dataset composed of Single Nucleotide Polymorphisms (SNPs) loci using a "reduced representation approach", such as Restriction Site Associated DNA marker sequencing (RADseq) or Genotyping-by-sequencing (GBS), be generated for *D. eleginoides* in the southwest Indian Ocean to provide a more representative sample of the entire genome and a possibly clearer resolution of population structure.

We recommend ideally at least 100 samples per fishing zone, though we note that the budget for the TOP2 project is limited to analyzing about 30 samples per fishing zone. We also recommend collecting and analyzing 50% female and 50% male samples. The number of samples allowed for by SIOFA TOP2 budget is likely not sufficient to precisely define population structure across the SIOFA regions and should be considered as a preliminary, or even a pilot project and the ability to recommend management units will likely be limited.

We have developed a detailed sampling protocol upon which the onboard observers will be trained. We have outlined a shipping protocol that partners should use to send us their samples, and a laboratory protocol for the preparation of the samples for sequencing.

Samples should be collected from November 2023 to March 2024, aligning with the planned fishing for the austral summer season, and we expect that samples will be returned to us in Reunion Island by the end of April 2024 to be prepared and shipped to the selected sequencing company by the end of May 2024. We will require approximately 6 months (end December 2024) for the sequencing, bioinformatic analyses and the generation of the final report, which will fit into the project schedule, and is thus considered feasible.

1. Context

This report addresses the first major task outlined in the SER2022-TOP1 ToRs, which includes a review of the literature and existing data held by SIOFA with the aim to propose an informed experimental design and sampling protocol for the genetic discrimination of the toothfish stock in the SIOFA area. This consultancy will address the global objective of providing advice on a genetic stock discrimination project to understand the stock structure of Patagonian toothfish in the SIOFA Area, including linkages to Patagonian toothfish in the CCAMLR Convention Area (Figure 1).

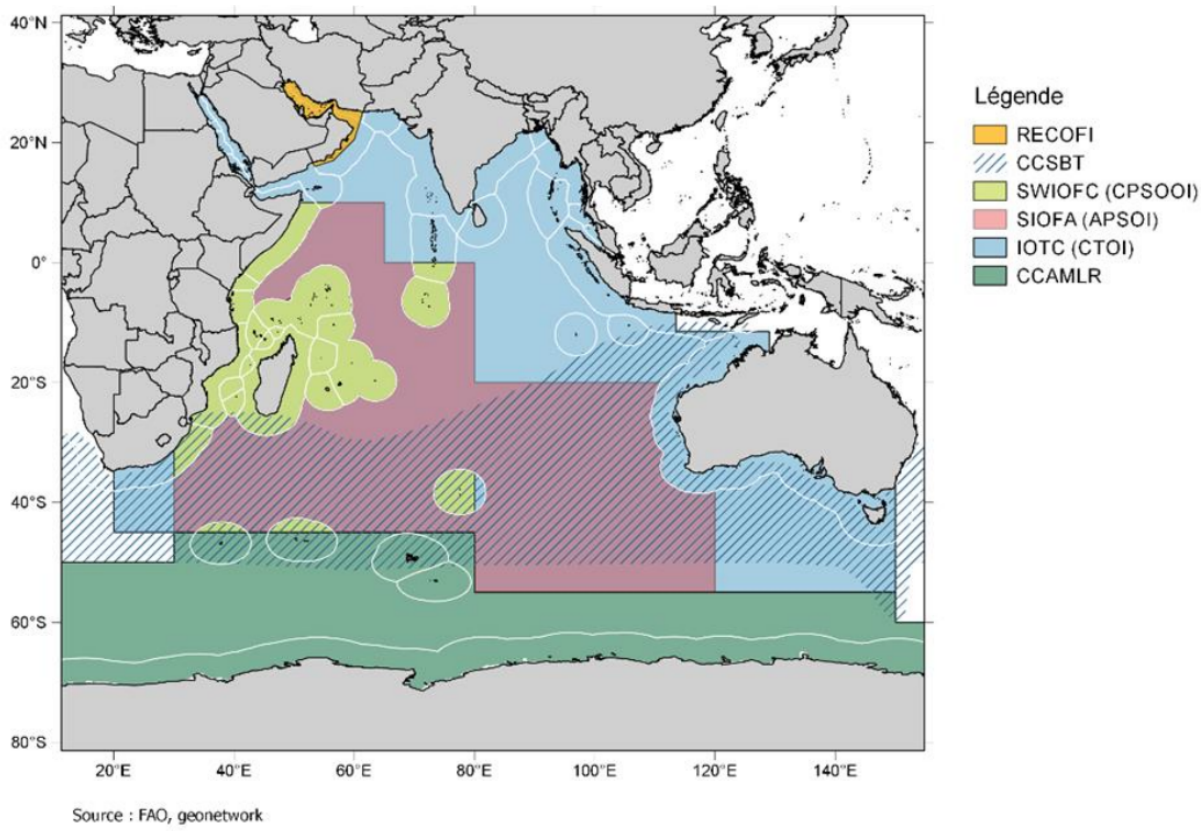


Figure 1: Map of RFMOs and marine conservation organizations in the Indian and Southern Oceans. RECOFI = Regional Fisheries Commission, CCSBT = Commission for the Conservation of Southern Bluefin Tuna, SWIOFC = South West Indian Ocean Fisheries Commission, SIOFA = Southern Indian Ocean Fisheries Agreement, IOTC = Indian Ocean Fisheries Commission. IOTC = Indian Ocean Tuna Commission, CCAMLR = Commission for the Conservation of Antarctic Marine Living Resources.

Genetic stock structure discrimination is an important step in understanding the population dynamics of a stock, especially in cases where stocks are jointly managed by several countries or where multiple RFMOs may have overlapping mandates, as is the case with the Patagonian toothfish. For example, should management measures such as quotas be

introduced, clear delineations of stock structure are key to appropriately allocating the resource, which can impact the state of the resource or the fishing activity (Avise, 1998). Furthermore, management can only be effective if the spatial scale of the measures match that of the target population (Francis et al., 2007). Thus, a key step towards the effective management of important blue resources is to understand the population structure of the resource.

Genetic differentiation of stocks in the same ocean basin can be small, or may not occur where population sizes are large or when migration of individuals causes mixing. The differentiation in many cases can be on the same order of magnitude as the sampling error; therefore, experimental design and sampling protocols must be developed carefully to maximize the signal-to-noise ratio in the data. Furthermore, when evaluating stock structure, it is recommended to draw on all information available, including genetic, demographic, ecological and life history studies (Waples 1998).

The Patagonian toothfish (*Dissostichus eleginoides* Smitt, 1898) is a demersal species known to inhabit waters of the continental slope off of Chile and Argentina from 30 - 35 °S southward, as well as islands, archipelagos, banks and seamounts of the southern Atlantic, Indian and Pacific Ocean sectors of the Southern Ocean (Figure 2, Evseenko et al. 1995; Collins et al. 2010). D. eleginoides is slow-growing and relatively long lived, with an estimated longevity of around 30 years (Andrews et al. 2011). The species also exhibits low fecundity in relation to its length and weight, which indicates that factors other than body size, such as age, may influence its fecundity weight (Kock and Kellermann 1991). D. eleginoides has been recorded as spawning during the austral winter at depths of around 1000 m on the slopes of Burdwood Bank south of the Falkland Islands (Kock and Kellermann, 1991; Laptikhovski et al. 2006), and eggs have been found in <700 m in waters with bottom depths of 2200 - 4400 m north of South Georgia (Evseenko et al. 1995). D. eleginoides displays ontogenetic shifts in habitat, with juveniles and sub-adults generally inhabiting shallower waters (< 400 m) during an estimated period of 7 - 10 years and shifting to depths of 1000 - 2000 m after maturity (Figure 3, Laptikhovsky and Brickle 2005).

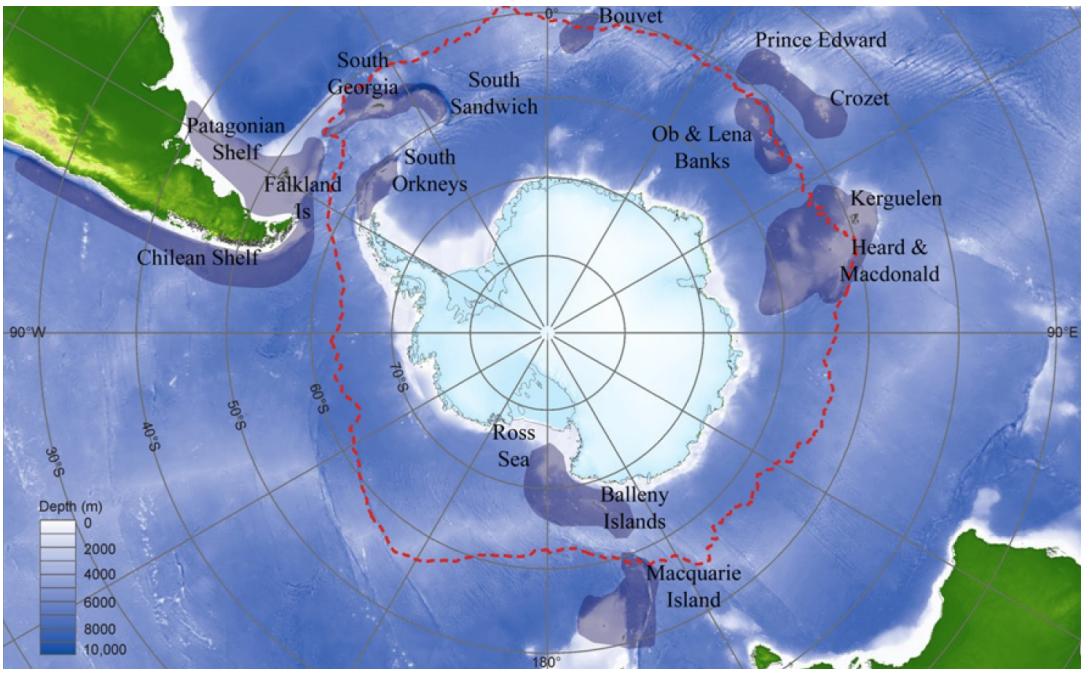


Figure 2. Map showing the known distributions of the Patagonian toothfish (*Dissostichus eleginoides*) in gray shading with the Polar Front indicated by the red dotted line. Illustration is from Collins et al. 2010, The Patagonian toothfish: biology, ecology and fishery, Figure 4.2.

D. eleginoides is not considered to be highly migratory, as the majority of individuals retrieved during tracking experiments were found within 15 n miles from their initial release, though four tagged individuals weren found over 1000 n miles from their release zone (Williams et al. 2002). Genetic studies have identified distinct populations existing on the Patagonian shelf (including the Falkland Islands: Smith and McVeagh 2000; Shaw et al. 2004; Rogers et al. 2006; Canales-Aguirre et al. 2018), the southern Atlantic Ocean (Shag Rocks, South Georgia and South Sandwich Islands: Appleyard et al. 2002; Shaw et al. 2004; Rogers et al. 2006), the western Indian Ocean (Crozet, Kerguelen, Heard, Prince Edward and Marion islands: Appleyard et al. 2004) and the southwest Pacific (Macquarie Island: Smith and McVeagh 2000; Appleyard et al. 2002) (Figure 2). The gene flow restrictions between these regions have been attributed to the separation by deep ocean basins and the deep water trough between the South American and Antarctic peninsulas, which limit adult migration, as well as the Antarctic Polar Front (APF), a powerful jet of the Antarctic Circumpolar Current (ACC), that likely serves as a barrier to larval dispersal (Clark et al. 2005; Shaw et al. 2004; Rogers et al. 2006). In the western Indian Ocean, however, individuals may use bathymetric features such as ridges and seamounts as stepping stones to access other areas (Rogers et al. 2006). Additional genetic analyses of the species inhabiting waters of the southwest Indian Ocean may provide further information on finer scale population structure of this species and how it relates to established fishing zones.

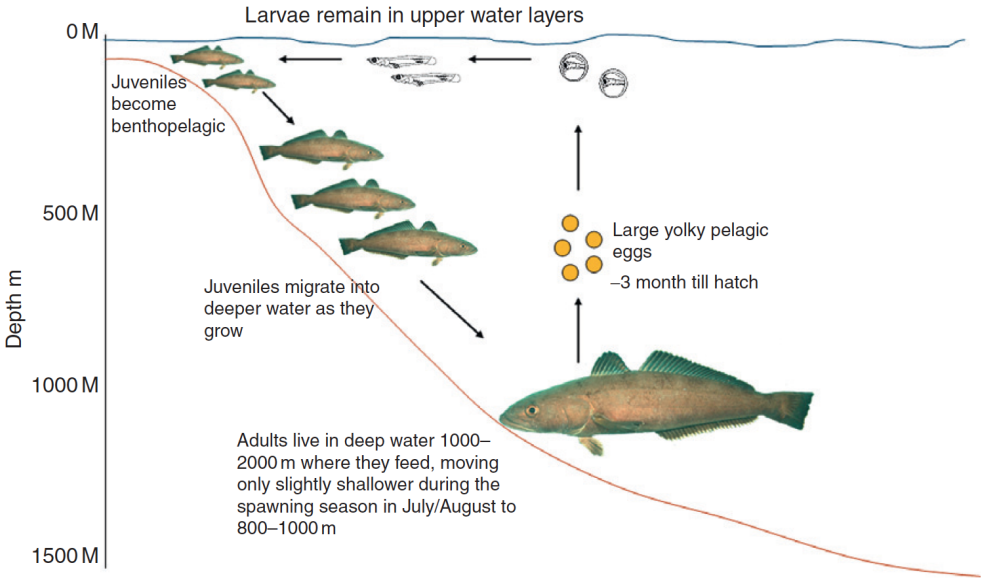


Figure 3. The life cycle of the Patagonian toothfish (*Dissostichus eleginoides*). Illustration is from Collins et al. 2010, The Patagonian toothfish: biology, ecology and fishery, Figure 4.3.

1.1 Project objectives

This review (below) aims to summarize known information relevant to developing a genetic stock discrimination project, including information on the species' biogeography, reproductive biology and population structure, and life history characteristics. The available catch-effort and scientific observer data (age, length, other biological data) shared by SIOFA were reviewed and summarized to inform the spatial distribution and relative abundance of Patagonian toothfish in the SIOFA area and along with biological information from observer data (e.g. length, maturity, sex) to inform the sampling strategy (see section 2.3). Tagging data were also shared by SIOFA as movement information can give insights into stock structure and can be key in developing a genetic stock discrimination project. We have also reviewed the literature to investigate any linkages between recruitment variability, distribution or abundance of Patagonian toothfish and environmental features and variability. We use the results of this review as a starting point from which to analyze the impact of the environment on Patagonian toothfish distribution, with the aim to inform the sampling strategy and give insights towards the population structure. This analysis also includes the Indian Ocean section of the CCAMLR Convention Area to identify if there are environmental linkages between these areas that may influence toothfish distribution.

2. Literature review

2.1 Life History

2.1.1 Habitat preference

The Patagonian toothfish (*Dissostichus eleginoides* Smitt, 1898) is a species of fish that is widely distributed on the continental shelves, plateaux, islands, archipelagos, banks and seamounts in the southern basins of the Atlantic, Pacific and Indian Oceans and throughout the Southern Ocean (Evseenko et al. 1995, Collins et al. 2010, CCAMLR 2021). *D. eleginoides* is a member of the sub-order Notothenioidei and the family Nototheniidae (Antarctic cods), which are the dominant fish fauna in the Southern Ocean (Eastman and Eakin 2000). As they lack antifreeze glycoproteins present in other species of the Nototheniidae (Eastman and DeVries 1986), *D. eleginoides* is restricted by water temperature, preferring >2 °C found in the subantarctic and northern Antarctic zones (Duhamel et al. 1982). Along with its sister species *D. mawsoni*, *D. eleginoides* is the largest member of the Nototheniidae, able to reach over 2 m in length and 100 kg in weight (Duhamel 1991, Péron et al. 2016).

As with all notothenioids, *D. eleginoides* lacks a swim bladder important for facilitating buoyancy and as a result, it has adapted a primarily deepwater benthic lifestyle (Eastman 1991, Kock and Kellerman 1991). *D. eleginoides* has a bathymetric range that extends from 10 to 2500 m, one of the largest of teleost fish (Fischer and Hureau 1985, Evseenko et al. 1995, Collins et al. 2010).

2.1.2 Diet

In terms of diet, *D. eleginoides* is a mixed-species carnivore that is primarily piscivorous, but is an opportunistic feeder that also consumes cephalopods and crustaceans as a secondary food source (García de la Rosa et al. 1997, Troccoli et al. 2020).

2.1.3 Growth

The species is known to exhibit a K-selection life history, in that it is relatively long lived, slow-growing, with delayed maturity, large body size and long life span (30-50 years) (Péron et al. 2016). Earlier age estimates ranged from 27 to over 50 years, varying with

geographic location (Horn et al. 2002, Belchier 2004, Ashford et al. 2005; Andrews et al. 2011).

Females appear to dominate the older age classes, with males older than 17 years rarely encountered (Welsford et al. 2011). Females were found to be larger and grow at a faster rate than males (García de la Rosa et al. 1997, Horn et al. 2002, Ashford et al. 2005). This species is moderately fast growing until it reaches sexual maturity, at which point its growth rate slows (Horn et al. 2002, Candy et al. 2007).

An investigation of length and sex ratio in the southwest Indian Ocean south of Madagascar and north of Prince Edward and Marion Islands in the Del Cano Rise (DCR)[1] region was recorded as nearly 1:1 (male:female) up to 85 cm L_T. Above 95 cm L_T, the sex ratio increases with length with females dominating (López Abellán et al. 2005). In the waters over the Kerguelen Plateau, however, the sex ratio appears varied with spatial distribution and size. Larger, more mature fish of both sexes were recorded in the west of the plateau, though large males dominate the Skiff bank north west of the Kerguelen Islands as well as west of Heard and McDonald Islands, whereas females dominated east of Heard and McDonald Islands (Lord et al. 2006, Welsford et al. 2011, Péron et al. 2016). The sex-ratio was recorded to be more balanced east of Kerguelen Islands which has been described as a juvenile recruitment zone where the fish are typically smaller (Lord et al. 2006).

2.1.4 Maturity

Maturity of *D. eleginoides* is classified into five stages: 1) Immature, 2) Developing/Resting, 3) Developed, 4) Gravid/Ripe and 5) Spent (Table 1, Everson 1977, Kock & Kellermann, 1991, Brigden et al. 2017, Yates et al. 2018).

Table 1. Maturity scale originally described by Everson 1977 for Nototheniidae and used for classing *Dissostichus eleginoides*.

Stage	Maturity Status	Female	Male
1	Immature	Ovary small, firm, no eggs visible to the naked eye	Testis small, translucent, whitish, long, thin strips lying close to vertebral column

[1] Named after Juan Sebastián Del Cano (1476-1526), a Spanish pilot sailing with Ferdinand Magellan, who brought the Victoria back to Spain following the death of Magellan. The ship passed near this feature before rounding Cape of Good Hope and completing the first circumnavigation of the globe (1519-1522). Name proposed by Robert L. Fisher, Scripps Institution of Oceanography (SIO), USA, in 1981.

2	Developing/Resting	Ovary more extended, firm, small oocytes visible, giving ovary a grainy appearance	Testis white, flat, convoluted, easily visible to the naked eye, about 1/4 length of the body cavity
3	Developed	Ovary large, starting to swell the body cavity, color varies according to species, contains oocytes of two sizes	Testis large, white and convoluted, no milt produced when pressed or cut
4	Gravid/Ripe	Ovary large, filling or swelling the body cavity, when opened large ova spill out	Testis large, opalescent white, drops of milt produced when pressed or cut
5	Spent	Ovary shrunken, flaccid, contains a few residual eggs and many small ova	Testis shrunk, flabby, dirty white in color

Maturity and length are linked and vary with geographic location (Box 1). Data from the Kerguelen Islands identified that males mature at smaller sizes with an average size-at-first maturity reported as 63 cm L_T (total length) compared to 85 cm L_T for females (Lord et al. 2006). Findings from southern Chile were similar with the average for males measuring 80 cm L_T and 89 cm L_T for females (Arana 2009). In terms of age, the onset of maturity was first thought to occur between 4 - 10 years (maturity stages ≥3-4), with males maturing closer to age 4 and females maturing closer to age 10 (Moreno 1998, Lord et al. 2006). Several studies have used stage 3 as the threshold for which both female and male fish reach maturity (Everson and Murray 1999, South Georgia; Lord et al. 2006, Kerguelen; Arana 2009, southern Chile).

All fish classified between stages 3 - 5 are considered to be engaged in the annual reproduction cycle (Lord et al. 2006). However, data taken during the height of the spawning period at Heard and McDonald Islands (southern Indian Ocean sector) identified some large females (~160cm) still at stage 2 maturity, suggesting that either females rapidly revert to developing/resting after spawning or may not spawn every season (Yates et al. 2018). An earlier study found similar results at South Georgia (southern Atlantic Ocean sector), in which an estimate of at least 25% of mature females did not spawn during a year (Everson and Murray 1999). Together, these results suggest that a maturity threshold of stage ≥2 should be used to avoid overestimations of age at first maturity (Everson and Murray 1999, Yates et al. 2018).

Source	Area	Lm_{50} (mm)	
		Male	Female
CCAMLR (1987)	South Georgia	577	1104
Moreno (1998)	South Georgia	670	860
Everson and Murray (1999)	South Georgia	785	982
Agnew *et al.* (1999)	South Georgia	750	1010
Laptikhovsky and Brickle (2005)	Patagonian Shelf	860	900
Prenski and Almeyda (2000)	Argentina	763	871
Moreno *et al.* (1997)	Chile	1050	1170
Young *et al.* (1999)	Chile		1287
Oyarzún *et al.* (2003)	Chile	780–940	1130–1170
Arana (2009)	Chile	810	890
Duhamel (1991)	Kerguelen	650	800
Lord *et al.* (2006)	Kerguelen	630	850

Box 1. A summary of length at 50% (Lm50) maturity for the Patagonian from different locations, compiled by Collins et al. 2010.

2.1.5 Ontogenetic migration

This species has a long pelagic phase aided by changes in buoyancy throughout their embryonic development, and an estimated incubation time of ≤ 3 months (Koubbi et al. 1990, Kock and Kellerman 1991, Evseenko et al. 1995, Harte 2020). Eggs collected from Kerguelen, Burdwood Bank and South Georgia sectors were observed either close to hatching or hatching in late October and November on the slope (Koubbi et al. 1990, Kock and Kellerman 1991). Studies have estimated the larval stage as occurring over a period ≥ 3 months in the upper water column <250 m (Koubbi et al. 1990, Evseenko et al. 1995, North 2002). When larvae reach between 15-25 cm TL, they become classified as benthopelagic juveniles (Collins et al. 2007, Belchier and Collins, 2008). As they continue to grow, individuals migrate downslope (Figure 3, Collins et al. 2010). In the case of the Patagonian toothfish, *Dissostichus eleginoides*, ontogenetic migration is primarily characterized by vertical shifts in which individuals migrate from shallow to deeper water as they grow (Figure 3, Collins et al. 2010; Welsford et al. 2011; Péron et al. 2016). This movement is thought to be driven by factors linked to ocean currents, seasonal food availability and spawning behavior (Brown et al. 2013, Lee et al. 2021).

As stated prior, females typically reach larger sizes compared to males, regardless of geographic sector (López Abellán 2005, Lord et al. 2006, Arana 2009, Péron et al. 2016, Brigden et al. 2017, Yates et al. 2018), and length is also linked to depth with larger fish

found on steeper slopes (Duhamel 1991, Welsford et al. 2011, Péron et al. 2016); thus, females being larger are more prevalent at deeper depths (Agnew et al. 1999).

On the Kerguelen Plateau around Heard and McDonald Islands, fish of the smaller size classes (< 50 cm mean length) were identified from shallower areas such as Gunnari Ridge (<500 m), compared to larger size classes which were found predominantly on the slopes of the western and eastern parts of the Kerguelen Plateau (Welsford et al. 2011). A later study observed a similar trend around Kerguelen Islands and Skiff Bank, where individuals of the smallest fish class (< 20 cm) are were found at < 300 m, and around Heard Island and Shell and Discovery banks, fish measuring 30 - 40 cm were found < 600 m, compared to individuals of the largest class (>150 cm) that were found between 500 to 2000 m (Péron et al. 2016). Further, predictive modeling identified that between 100 and 600 m, TL increased linearly by 5 cm per 100 m depth, remaining consistent from 600 to 1200 m, and increasing by 2.5 cm per 100 m depth from depths 1200 to 2300 m (Péron et al. 2016).

Despite the trend of increasing size with depth on the Kerguelen Plateau, fish of the larger size classes were not necessarily restricted to slope areas, suggesting that there is a more complex relationship between depth and length; for example, larger fish were found at shallower Pike Bank (< 300 m), located north of Heard Island, compared to areas of equivalent depth where smaller fish are found. This is possibly due to the habitat at Pike Bank providing foraging opportunities for larger fish (Welsford et al. 2011).

The feeding habits of *D. eleginoides* are presumed to be a significant driver of vertical movement and change with fish size, maturity and depth in the water column (Arkhipkin et al. 2003, Troccoli et al. 2020). Around the Falkland Islands, small *D. eleginoides* (16 - 40 L_T) on the shelves were active predators yet had a limited feeding spectrum focusing on a single prey item at a time, whereas medium sized fish (40 - 60 cm L_T) along the upper slopes had a broader spectrum, feeding on more prey item at one time. In this same region, larger fish (>61 cm L_T) found at greater depths preyed on larger species and increased scavenging behavior, but focused on one prey at a time. Further, after their migration to deep continental slope (500 - 1000 m), *D. eleginoides* was found to feed on active and less active species, while at depths >1000 m, it was opportunistic, feeding primarily on inactive fish, cephalopods and crustaceans (Arkhipkin et al. 2003). It has been suggested that these ontogenetic shifts in diet help reduce competition between conspecifics and the possibility that larger fish can attack larger and more difficult prey (Brown et al. 2013).

Brown et al. 2013 followed tagged individuals measuring >127 cm L_T, in the waters > 1000 m around the Falkland Islands, Burdwood Bank and Scotia Ridge in the southern Atlantic Ocean. The authors detected vertical movement patterns, linked to seasonal foraging and

spawning behaviors. *D. eleginoides* individuals were found to move into deeper waters to feed in December after spawning, while daily vertical movements were linked to foraging behavior in which individuals searched for prey. Few tagged fish were observed moving into more shallow waters (900 - 1200 m) during the spawning months between May-August, but this was only recorded at Burdwood Bank, and possibly done to disperse eggs in warmer, more productive waters (Brown et al. 2013). In the waters around Macquarie Island (Pacific), there were also significant inter-seasonal differences in diet but dietary composition was not related to fishing depth or fish size (Goldsworthy et al. 2002).

This migration pattern, thought to play a key role in the distribution and distribution of this species, has been described primarily in the Atlantic; however, there is also evidence that similar patterns exist in the southern Indian Ocean. An older study found that in the northern waters off the Kerguelen Islands, season and depth also played a role in the feeding spectrum of *D. eleginoides* (Pshenichov 1994). Smaller, younger individuals were found in more shallow waters (<500 m) and had a differing feeding spectrum from larger, adult *D. eleginoides* which were found to inhabit greater depths (>500 m) and fed on less active species such as ctenophores, salps, crustaceans, worms and occasional octopus.

The ontogenetic migration of Patagonian toothfish has important implications for the management of the species and the ecosystems in which it occurs. For example, the movement of adult Patagonian toothfish to deeper water habitats may make them less vulnerable to fishing pressure, but it can also lead to changes in their distribution and abundance that are difficult to detect and monitor. Understanding the ontogenetic migration of Patagonian toothfish is therefore important for developing effective management strategies that promote the sustainable use of the species and the preservation of its ecosystem.

Table 2. Summary of Patagonian toothfish ontogenetic migration. Larvae and early juveniles were sometimes reported together. Sizes are in Total Length (T_L) in cm, unless otherwise indicated. * standard length (L_S), originally reported in mm.

Life stage	Locality	Ocean	Size (T_L)*	Depth	Reference
Eggs	Kerguelen Is.	Indian	n/a	< 200 m	Koubbi et al. 1990; Evseenko et al. 1995
	South Georgia	Atlantic	n/a	< 700 m	Evseenko et al. 1995
Larvae/early juveniles	South Georgia	Atlantic	1.9 - 2.2 cm*	< 200 m	Evseenko et al. 1995
	South Georgia,	Atlantic	1.8 - 6.0 cm*	< 250 m (majority)	North 2002

	Shag Rocks & Burdwood Bank			250 - 2900 m (few)	
Juveniles	HIMI	Indian	<50 cm	< 500 m	Welsford et al. 2002
	South Georgia & Shag Rocks	Atlantic	11 - 75 cm	< 400 m	Collins et al. 2007; Belchier & Collins 2008
	Kerguelen Is.	Indian	20 cm	100 – 300 m	Péron et al. 2016
	Skiff Bank	Indian	20 cm	100 – 300 m	Péron et al. 2016
	Heard & McDonald Is.	Indian	30 – 40 cm	< 600 m	Péron et al. 2016
	Shell & Discovery Banks	Indian	30 – 40 cm	< 600 m	Péron et al. 2016
Adults	South Georgia & Shag Rock	Atlantic	> 75 cm	> 1000 m	Belchier & Collins 2008
	W. Kerguelen plateau & Shell Bank	Indian	>150 cm	500 – 2000 m	Duhamel 1985; Péron et al. 2016

2.1.6 Spawning

In addition to differences in distribution for different ages and lengths, Patagonian toothfish also exhibit strong spatial differences in sex composition throughout their distribution where in some zones males are dominant and in other zones, females (López Abellán et al. 2005, Lord et al. 2006, Welsford et al. 2011, Péron et al. 2016). Sexual segregation is common in many fish species, particularly in spawning grounds where males generally arrive first and females arrive later (e.g. Robichaud and Rose 2004).

Two studies observed a spawning behavior whereby mature males appeared to move downslope, while females moved upslope to spawn between 800 and 1 200 m at South Georgia and Shag Rocks (Agnew et al. 1999; Brigden et al. 2017). However, the Brigden et al. (2017) study took place between 1997 and 2014, and only observed this behavior in one year, with no consistent pattern of depth distribution during spawning. Furthermore, while there was spawning activity observed between 800 - 1 200 m, this depth range was not considered to be linked to spawning behavior and instead, spawning depth in this sector was proposed to be linked to biological productivity beneficial to early life-stages, potentially explaining the interannual variability of spawning behavior of *D. eleginoides*

(Brigden et al. 2017). Péron et al. 2016 found no evidence of localized spawning aggregations.

Several studies identified that spawning periods differ with geography. In the southern Atlantic, spawning typically occurs off of the continental slope at Burdwood Bank, as well as at insular shelves of South Georgia (Kock and Kellerman 1991) and off Chile (Arana 2009). Spawning was initially observed during the austral winters from July to September at Burdwood Bank and South Georgia (Kock and Kellerman 1991, Evseenko et al. 1995, Agnew et al. 1999) and from June to August off Chile (Arana 2009). Shag Rocks (and to a lesser extent, South Georgia) was found to be important spawning grounds for *D. eleginoides* where males spawn in early July and females spawned from late June and into July (Brigden et al. 2017). The area's oceanographic positioning at a crossroads of several circumpolar fronts within the Antarctic Circumpolar Current (ACC) and bathymetric complexity together driving the mixing of water masses (Orsi et al. 1995) produce increased phyto- and zooplankton productivity, which is beneficial to newly hatched larva (Young et al. 2011, Brigden et al. 2017).

In the southern Indian Ocean, spawning has been observed off the Kerguelen archipelago in June by Duhamel et al. 1991. Lord et al. 2006 reported the spawning of females occurs from late April to mid-July while males spawn from the end of May to the beginning of August, identifying a synchronous period around mid-June. At Heard and McDonald Islands, gonad growth was the greatest from April to June for both sexes, while mean gonado-somatic index (gonad mass as a proportion of total body mass) declined from July to September, indicating a spawning season from June to September for most individuals (Yates et al. 2018).

2.1.7 Fecundity

D. eleginoides and its high-Antarctic congener *D. mawsoni* are known to be the most fecund among the notothenioid species; based on data from Burdwood Bank, they were recorded as producing from 238 000 to more than 500 000 eggs, depending on the length of the individual and its location (Kock & Kellermann, 1991). The eggs produced by *D. eleginoides* are large, with size estimates ranging from 3.1 - 3.5 mm diameter (Mujica et al. 2016, Chile) to 4.3 - 4.7 mm diameter (South Geogria and Burdwood Bank, Kock and Kellerman 1991).

2.2 Past studies on population structure

Population structure is broadly defined as the manner in which a population is subdivided into local breeding groups ("demes"), the sizes of these groups in terms of the number of breeding individuals and amount of migration or gene flow between each group (King and Standfield 2002). In the context of fisheries biology, population structure refers to whether a species exists as a single and freely interbreeding population (random mating or 'panmixia') or is subdivided into genetically distinct subpopulations across its geographic range (Gaffney 2000). Genetic differentiation, which is responsible for population structure, is an accumulation of differences in allelic frequencies between isolated or semi-isolated populations, while genetic diversity describes how individuals vary within a population, often reflected in the number of different types of alleles in that population and taking into account their frequencies (Gregorius 1987, King and Standfield 2002). At different biological scales (i.e., among, between and within populations), differentiation due to genetic variation is frequently assessed through Wright's hierarchical F-statistical indices, such as the Fixation Index (F_{ST}) which helps identify the extent of gene flow at the level of populations, i.e. understand the degree of population differentiation within a species (Çiftci and Okumuş 2002, Hartl and Clark 2007, Weersing and Toonen 2009). Through the use of F-statistical indices, it is possible to test for the presence or absence of panmictic populations. Panmixia is a scenario in which there exists no barriers to mating, geographic or otherwise, and individuals have an equal chance of mating with any other individual of the population; therefore, there is a full exchange of genetic material throughout the population (King and Standfield 2002). Statistical testing typically applies this scenario as the null hypothesis, whereby significant values indicate a rejection of the null hypothesis of panmixia and a departure from this scenario, which provides support for genetic differentiation of sub-populations (Hartl and Clark 2007).

Genetically distinct populations are generally viewed as independent evolutionary units having distinct biological properties important for managing them (Gaffney 2000). To accurately define distinct populations within a species, it is necessary to recognize how genetic diversity among individuals is distributed across different populations, as well as the biology and ecology of that species, because this information is critical for elucidating dispersal and migration patterns to characterize the connectivity and gene flow between populations. Ultimately, this data can be used to inform conservation and management strategies (Coates et al. 2018).

For fish species of economic importance, an understanding of population structure can be crucial for determining the sustainability of the fishery, defining stocks, understanding the impacts of fishing on a particular population, the recovery potential of such populations,

and the possibility for the exchange of genetic material between populations (Çiftci and Okumuş 2002). Therefore, the examination of population structure and genetic diversity existing within the Patagonian toothfish, *Dissostichus eleginoides*, could provide valuable information for conservation and management efforts, by aiding the identification of distinct genetic groups possibly vulnerable to exploitation or other threats.

2.2.1 Genetic techniques

Several genetic techniques and markers, described below, have been used to investigate the population structure of Patagonian toothfish, *Dissostichus eleginoides*, in the Indian Ocean. These methods sometimes provide differing information about the population structure and genetic diversity of *D. eleginoides*, though their results can be combined to gain a more comprehensive understanding of this species in the region. The use of multiple genetic markers also allows for a more robust assessment of the genetic diversity and structure of populations, and can help to identify potential barriers to gene flow.

1) Allozymes: Allozymes are enzymes (proteins) resulting from varying alleles at the same locus (gene). If a mutation in a DNA sequence coding for a protein results in change in the amino acid, the resulting protein may be modified in terms of its size, shape or electrical charge (but not function), and these differences amongst individual samples can be visualized through gel electrophoresis whereby distinct products represent the distinct alleles. Allozymes have been used in the past to assess population structure of species, in part because they are they are codominant (heterozygotes can be distinguished from homozygotes), and this technique does not require DNA extraction, PCR primers or sequence information, nor associated costs (Berg and Hamrick 1997, Gaffney 2000). While this technique has been superseded by DNA based methods for population genetics investigations (described below) primarily due to their low level of polymorphism leading to a lack of population resolution (Abdul-Muneer et al. 2012), one study used allozymes to investigate population structure of *D. eleginoides* (Smith and McVeagh 2000).

2) Mitochondrial DNA (mtDNA): Mitochondrial DNA markers are DNA segments found within the genome of the mitochondria, which are the energy-producing structures in cells (Box 2). These markers are frequently used for taxonomic resolution as well as to improve understanding of biogeography, speciation and population differentiation of animal taxa. mtDNA markers are based on sequencing analysis and as mitochondria are maternally inherited, these markers provide information on the maternal lineages within a population. Some benefits of mtDNA include a fast

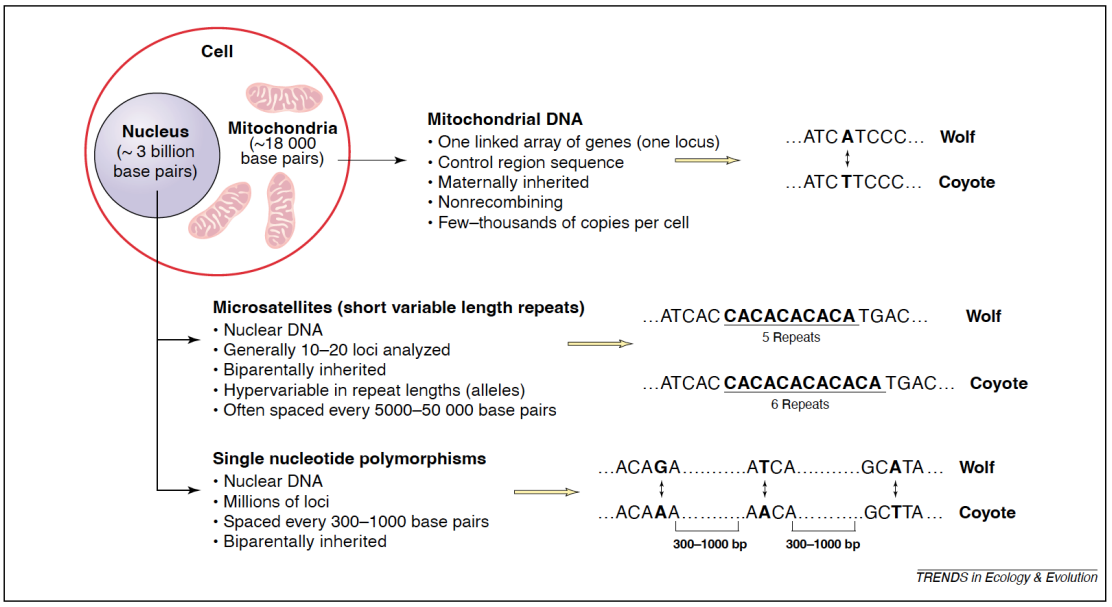
rate of evolution, which produces a higher degree of sequence variation; its clonal (maternal) inheritance, high copy number for ease of amplification, and absence of recombination. Despite these advantages, mtDNA represents only a single locus, and the data obtained from it reflects only the matrilineal heritage. Further, it contains pseudogenes (non-functional genes) and an effective population size that is ¼ of the nuclear genome; this can lead to underestimates of genetic diversity and lack of population detection (Zhang and Hewitt 2003, Nabholz et al. 2008). Regardless, mtDNA markers have helped identify evidence of population structure of Patagonian toothfish across the Southern Ocean (Appleyard et al. 2002, 2004, Rogers et al. 2006. Toomey et al. 2016, Arkhipkin et al. 2022).

3) Nuclear DNA: Nuclear DNA markers (nuDNA) are DNA segments found within the genome of the nucleus of cells, which contains the genetic information from both parents. NuDNA markers, which include microsatellites and DNA fragments containing single nucleotide polymorphisms (SNPs), provide information on both maternal and paternal lineages and have been used to investigate the population structure in a variety of organisms, including the Patagonian Toothfish.

    a) Microsatellites: microsatellites are short tandem repeats (STRs) of 1–6 nucleotides found at high frequency throughout the nuclear genomes of most taxa (Box 2). They are often used to investigate the population structure and genetic diversity of a species due to several benefits: they are codominant (heterozygotes can be distinguished from homozygotes), highly polymorphic, hypervariable and have high mutation rates important for recovering allelic diversity. Furthermore, they can be amplified from a small quantity of tissue and their primers are species-specific, so there is little risk of cross-contamination by non-target organisms. Finally, the resolution and power of a multilocus microsatellite study exceeds that of some other marker types. However, some disadvantages to using microsatellites include the need to develop primers anew for each species, the complexity of the mutational processes of microsatellites, their potential for masking allelic diversity, and their failure to amplify in some individuals (Selkoe and Toonen 2006, Abdul-Muneer 2014). Nonetheless, this technique has been widely used to study the population structure of *D. eleginoides* (Reilly and Ward 1999, Smith and McVeagh 2000, Appleyard et al. 2002, 2004, Shaw et al. 2004, Rogers et al. 2006, Canales-Aguirre et al. 2018).

    b) Single nucleotide polymorphisms (SNPs): SNPs are single base pair positions at which different sequence alternatives (alleles) exist, i.e., single nucleotides

that have changed from one base from another. SNPs are widespread across coding and non-coding regions of the genomes of many species (Box 2) and are present at >1% of the population. SNPs are frequently seen as the marker type of choice for examining fine scale differences within and among differing populations due to several characteristics that include the ease of large-scale automated detection, high data quality, genome-wide coverage and ease of modeling mutational dynamics, which lead to improved estimates of population structure compared to other marker types (Brookes 1999, Morin et al. 2004). A disadvantage to using SNPs is that as they occur as a single base pair (i.e., they only have two alleles, for example, either an A or a T), thus their mutation rate is very low. This leads to the requirement of high numbers of loci compared to other marker types, to achieve a similar level of population resolution. SNP loci are generally recovered by mining the nuclear genome for variation using the “reduced-representation” approach. This approach involves sequencing a subset of locations spread throughout the genome, rather than sequencing the entire genome; this reduces genome complexity (and cost) for genotyping samples using SNP markers. This approach includes Restriction Site Associated DNA marker sequencing (RADseq) and similar techniques such as Genotyping-by-sequencing (GBS). RADseq and GBS use restriction enzymes to divide the genome into DNA fragments that are size fractionated for high-throughput sequencing (Miller et al. 2007, Baird et al. 2008, Andrews et al. 2016). The use of SNPs have been applied to assess potential genetic differentiation of Patagonian toothfish populations across the Southern Ocean (Toomey et al. 2016, Arkhipkin et al. 2022). We note that Toomey et al. 2016 uses the term "SNP" to refer to variations in individual nucleotides occurring within four nuclear gene fragments, while Arkhipkin et al. 2022 used GBS (a broader screening across the genome) to recover a final dataset of ~3 800 SNPs.



Box 2: A comparison of the characteristics of mitochondrial DNA, microsatellites and single nucleotide polymorphisms as genetic markers, with examples from wolf-like canids, from Morin et al. 2004.

2.2.2 Past studies

Genetic studies of *D. eleginoides* around the Southern Ocean have shown that there is population structure within this species, with evidence of subpopulations that are genetically distinct from one another. The earliest study examining population structure in *D. eleginoides* developed five polymorphic microsatellite markers for testing against samples collected from two sites off of Macquarie Island (southern Pacific Ocean) and found evidence for differentiation amongst those sites (Reilly and Ward 1999). This set of microsatellites would be later used in several studies to help discriminate stock structure of the Patagonian toothfish in other oceanic sectors, specifically the Indian Ocean (Smith and McVeagh 2000, Appleyard et al. 2002, 2004, Rogers et al 2006, Toomey et al. 2016, discussion below).

2.2.2.1 Non-Indian Ocean sectors

D. eleginoides in the Atlantic Ocean sector of the Southern Ocean, which includes the Falkland Islands and the South Georgia Archipelago (including the South Sandwich Islands and Shag Rocks), were found to be genetically differentiated from individuals originating from other ocean sectors, supporting the hypothesis of restricted gene flow of this species in the Southern Ocean (Smith and McVeagh 2000, Appleyard et al. 2002). On a finer geographic scale within the Atlantic sector, another study found a distinct genetic division between Shag Rocks/South Georgia and the North Scotia Ridge/Patagonian shelf area

(Shaw et al. 2004), a finding echoed by Rogers et al. 2006. The genetic distinctiveness of *D. eleginoides* originating from the Falkland Islands is possibly due to the Antarctic Polar Front (APF) and a deep-water trough passing between the two locales and creating a barrier to larval dispersal (Shaw et al. 2004, Clark et al. 2005, Rodgers et al. 2006). Most recently, Arkhipkin et al. 2022 confirmed the genetic separation of *D. eleginoides* on either side of the APF and along with other sources of evidence, led the authors to propose that *D. eleginoides* north of the APF be classified as a species separate from populations south of the APF. In the Pacific Ocean sector, no population structure was detected along the South American continental plate (southeast Pacific), though the populations in this area were found to be distinct from those in southwest Atlantic (Canales-Aguirre et al. 2018). Conversely, Touma et al. 2019 used expressed sequence tag simple sequence repeats (EST-SSRs) to identify genetic structure of D. eleginoides inhabiting the South American continental shelf at Iquique, Puerto Montt, Cape Horn and Falkland Islands, while Garcia et al. 2019 employed microsatellites and also found evidence for structure among two locations (Iquique, Puerto Montt) in this region. In the southwest Pacific, the waters around Macquarie Island have been identified as hosting the most isolated *D. eleginoides* population, possibly due to water depths of >4 000 m serving as a barrier between Macquarie Island and the next closest population of *D. eleginoides* at Heard and McDonald Islands (Smith and McVeagh 2000, Appleyard et al. 2002, 2004).

2.2.2.2 Indian Ocean sector

In the southern Indian Ocean, there have been few studies investigating the spatial structuring of Patagonian toothfish as a proxy for species movement in this sector. These studies aimed at contributing to the understanding of stock structure through the analyses of genetic diversity and indices of population differentiation within this species while identifying potential barriers to gene flow and dispersal. Each study employed an array of genetic markers such as allozymes, mitochondrial DNA (mtDNA) and nuclear DNA, which includes microsatellite markers, to test for the presence and absence of panmictic populations and help ultimately resolve population structure.

An early study, undertaken for the purposes of stock identification, employed eleven allozyme loci and eight microsatellite markers, five of which were previously developed by Reilly and Ward (Smith and McVeagh 2000). The analyses of samples obtained from South Georgia and Falkland Islands (southern Atlantic Ocean), the Ross Dependency and Macquarie Island (southern Pacific Ocean), and Prince Edward and Heard Islands (southern Indian Ocean) produced disparate results; allozyme data identified little genetic differentiation amongst *D. eleginoides* in the Southern Ocean, while microsatellites demonstrated slightly significant genetic differentiation among the geographic sectors,

though this depended on the individual locus (Smith and McVeagh 2000). A later study applied isoelectric focusing (IEF), a technique developed to separate proteins based on differences in their isoelectric pH value (Garfin 1990), as a molecular method for differentiating the two species of toothfish *D. eleginoides* and *D. mawsoni* (Smith et al. 2001). The authors found that while this technique was useful for distinguishing the two toothfish species, no distinction was found between *D. eleginoides* samples from the Atlantic ("South Atlantic"), Indian (Heard Island, Prince Edward Island) and Pacific (Macquarie Island) ocean sectors (Smith et al. 2001).

A comparison of fishery localities of the south-central Indian Ocean sector (Heard and McDonald Islands) with those in the south-west Pacific Ocean (Macquarie Islands) and the southwest Atlantic Ocean (Shag Rocks and South Georgia) using mtDNA regions and microsatellite markers found strong evidence of genetic separation by localities and therefore, a rejection of a single panmictic *D. eleginoides* total population in the Southern Ocean (Appleyard et al. 2002). Mitochondrial regions ND2 (NADH dehydrogenase subunit 2 gene, and BCL (control region/D-loop), as well as five microsatellites from Reilly and Ward (1999) and two from Smith and McVeagh (2000) were applied. MtDNA best detected differentiation, with moderate levels of variation within localities yet highly significant heterogeneity between the Indian, Pacific and Atlantic Ocean fishing localities under study, while the microsatellites showed very small differences between fishing localities and no differences within each fishing locality (Appleyard et al. 2002). This finding is contrary to the previous study by Reilly and Ward (1999), which used very few samples and showed low levels of population structure around Macquarie Island (Reilly and Ward 1999).

Appleyard et al. 2002 thus confirmed that the Indian Ocean sector is genetically differentiated from other ocean sectors; however, there was little differentiation recovered between fishing grounds within the Indian Ocean. The authors suggested that the genetic structure recovered by mtDNA could be possibly linked to female philopatry and male dispersal as mtDNA is a maternally inherited locus. Alternatively, the findings more likely reflect mtDNA's sensitivity to bottlenecks in population size and genetic drift (Nei and Tajima 1981).

A follow-up study by Appleyard et al. 2004 focused on finer scale spatial structuring in the southwestern Indian Ocean by applying the same mtDNA markers and microsatellites as in Appleyard et al. 2002 on collections originating from Crozet, Kerguelen, Prince Edward and Marion Islands and comparing the genetic data from these sites to that previously obtained from Heard and McDonald Islands by Appleyard et al. 2002. The mtDNA dataset did not reveal population structuring across the southwest Indian ocean, while the microsatellite dataset revealed weak evidence of population structuring depending on the loci and thus

there was no significant heterogeneity detected among *D. eleginoides* within the southwest Indian Ocean. The authors cautioned, however, that their findings don't necessarily mean that population structure does not exist within the Indian Ocean, but only that the sample sizes and genetic markers employed may not be powerful enough to detect heterogeneity that possibly exists (Appleyard et al. 2004).

Additionally, Appleyard et al. 2004 tested for associations between geographic and genetic distances through mantel testing to determine if the farther the distance, the greater the genetic differences. Neither the mtDNA nor microsatellite datasets were significant for an association between geographic location and genetic distances, despite tagging data identifying *D. eleginoides*' ability to swim long distances and movements between Heard and McDonald Islands and Kerguelen Islands and (>500 km) recorded prior to the study and after (Williams et al. 2002, Welsford et al. 2011).

Rogers et al. 2006 applied two mtDNA markers, 16S rDNA and the 12S rDNA, and seven microsatellites developed by Reilly and Ward (1999) and Smith and McVeagh (2000) to samples obtained from the western and eastern Atlantic, as well as Ob seamount in the Indian Ocean. Both marker types detected a clear differentiation between the Falkland Islands (Patagonian shelf) and South Georgia farther east in the Atlantic); however, the differentiation between South Georgia, Bouvet Island, Meteor and Speiss seamounts (Atlantic) and Ob seamount was weak or non-existent (Rogers et al. 2006). This is in contrast to the study by Appleyard et al. 2002, who identified genetic structure between the Atlantic and Indian Oceans. In the western Indian Ocean, however, individuals may use bathymetric features such as ridges and seamounts as stepping stones to access other areas (Rogers et al. 2006).

More recently, Toomey et al. 2016 examined genetic differentiation across the Atlantic (South Georgia and South Sandwich Islands), Pacific (Macquarie Island) and Indian (Heard & McDonald Islands, Kerguelen Islands, Crozet Islands) ocean sectors using four mitochondrial and four nuclear DNA markers applied to DNA retrieved from dried blood and tissue on the surfaces of *D. eleginoides* otoliths (Table 3). In this study, both mitochondrial and nuclear markers detected differentiation of the Pacific Ocean sector from the Atlantic and Indian oceans, though, only the nuclear markers detected differentiation between the Atlantic and Indian ocean sectors. For example, the mitochondrial dataset did not differentiate Heard & McDonald Islands from South Sandwich Islands, Kerguelen from South Georgia, nor Crozet from South Georgia.

At the scale of the Indian Ocean, the mitochondrial dataset significantly differentiated Heard & McDonald Islands from Crozet, but this was not confirmed with the nuclear

dataset. Also, there was no differentiation found between Heard & McDonald Islands and Kerguelen, nor Kerguelen and Crozet using either marker type (Toomey et al. 2016). Despite the incongruity of the datasets, these findings suggest that populations in the southwest Indian Ocean are not fully panmictic. Additionally, the differentiation seen with mitochondrial markers and the lack of differentiation seen in nuclear markers supports a similar conclusion drawn by Appleyard et al. 2002 (above): that males may disperse farther than females, particularly between Heard & McDonald Islands and Crozet, and this is reflected in the bi-parentally inherited nuclear DNA, while potential female site fidelity/restriction is reflected in the mitochondrial DNA. Further, tagging studies identified that fish moving >500 km included a slightly larger proportion of males (Welsford et al. 2011), though Toomey et al. 216 acknowledges that the differences between the marker types could be explained by the possibility of genetic drift and population bottlenecks accentuating mitochondrial differences (discussed above).

Toomey et al. 2016 also performed mantel testing at the scale of ocean sectors, discovering that the nuclear dataset significantly supported the hypothesis of genetic differentiation as a result of geographical distance. while the mitochondrial dataset did not. Finally, the authors found that it is possible to retrieve important genetic data from *D. eleginoides* otoliths, though the DNA is often degraded and present in low yields compared to the use of DNA from skin or muscle tissue (Toomey et al. 2016).

Table 3. Summary of studies that included an investigation of Patagonian Toothfish population structure in the southern Indian Ocean. Rows highlighted in green represent locations within the Indian Ocean. (*) indicates the dataset from Appleyard et al. 2002 also used in Appleyard 2004.

Study	Molecular Method	Location	Ocean	Sample number	Broad conclusions
Smith & McVeagh 2000	Allozymes and microsatellites	South Georgia	Atlantic Ocean	12	Microsatellites best detected structuring amongst the three oceanic sectors, though the significance was weak.
		Falkland Islands	Atlantic Ocean	50	
		Prince Edward Island	Indian Ocean	50	
		Heard Island	Indian Ocean	50	
		Macquarie Island	Pacific Ocean	50	
		Ross Dependency	Pacific Ocean	50	

Smith et al. 2001	Isoelectric focusing (IEF)	"South Atlantic"	Atlantic Ocean	12	No distinction found between the Atlantic, Indian and Pacific Ocean sectors.
		Prince Edward Island	Indian Ocean	50	
		Heard Island	Indian Ocean	50	
		Macquarie Island	Pacific Ocean	50	
		Ross Dependency	Pacific Ocean	34	
Appleyard et al. 2002	Mitochondrial markers & microsatellites	Shag Rocks	Atlantic Ocean	24	Mitochondrial DNA best detected structuring amongst the three oceanic sectors to reject the null hypothesis that a single panmictic population exists in the Southern Ocean.
		South Georgia	Atlantic Ocean	24	
		Heard & McDonald Islands	Indian Ocean	313	
		Macquarie Island	Pacific Ocean	265	
Appleyard et al. 2004	Mitochondrial markers & microsatellites	Crozet Island	Indian Ocean	54	No population structure detected within the Indian Ocean sector through the use of either marker type; could not reject the null hypothesis that a single panmictic population exists in the Indian Ocean.
		Kerguelen Islands	Indian Ocean	26	
		Prince Edward and Marion Island 1	Indian Ocean	30	
		Prince Edward and Marion Island 2	Indian Ocean	26	
		Heard & McDonald Islands*	Indian Ocean	304*	
Rogers et al. 2006	Mitochondrial markers & microsatellites	Falkland Islands	Atlantic Ocean	87	The two marker types differentiated

		South Georgia	Atlantic Ocean	59	Falkland Islands from South Georgia, but did not differentiate Ob seamount from the other locations in the Atlantic Ocean.
		Meteor & Spiess seamounts	Atlantic Ocean	70	
		Bouvet Island	Atlantic Ocean	11	
		Ob seamount	Indian Ocean	47	
Toomey et al. 2016	Mitochondrial and nuclear markers	South Georgia	Atlantic Ocean	71	Nuclear dataset differentiated ocean sectors, while mitochondrial dataset only differentiated the Pacific sector. Nuclear dataset supports that geographical distance is a major factor in genetic differentiation of populations. In the Indian Ocean, only the mitochondrial dataset significantly differentiated Heard & McDonald Islands from Crozet.
		South Sandwich Islands	Atlantic Ocean	47	
		Heard & McDonald Islands	Indian Ocean	106	
		Crozet Islands	Indian Ocean	59	
		Kerguelen Islands	Indian Ocean	30	
		Macquarie Island	Pacific Ocean	106	

2.3 Tagging and movement studies

Tagging of Patagonian toothfish in the SIOFA zone began from the 2019-2020 season; however, CCAMLR tagging has been underway since 1998, with approximately 350 000 tagged individuals released and about 40 000 recaptured (SIOFA, 2022b). Tagging can help gather information on the distance and speed of migration, the areas occupied by the species, and the time spent in specific regions. This information can provide important

insights into the life history and biology of the species, including its distribution, migration patterns, feeding habits, reproductive behavior, and habitat use.

Throughout the different regions of the species' range (e.g. Macquarie Island, Heard Island, South Georgia and international waters of the Patagonian Shelf between 45 and 47 S), studies on tagged individuals have shown the ontogenetic vertical migration of individuals down the slope with increasing age and length, with minimal or short-distance (<25 km) horizontal migration once fish settle in their preferred depth range (Welsford et al. 2014; Williams et al., 2002; Marlow et al.,2003; Tuck et al., 2003). Some individuals have been shown to have short forays around the slopes (<130 km over a 6 month period) and are still considered to have high site fidelity (Brown et al. 2013). However, there have also been observations of long-distance migrations of almost 2000 km over waters with depths >4000 m. This is possible as adults, while demersal, gain neutral buoyancy through lipid accumulation throughout their lifetimes and are capable of pelagic foraging and movements (Péron et al. 2016). Thus, these long-distance migrations can be made between island chains, for example, between Heard and Crozet Islands (Williams et al. 2002). During commercial gill-net fishing for Greenland halibut in the Davis Strait, there has also been a Patagonian toothfish observed to have traveled into sub-Arctic waters of the northern hemisphere (Møller et al., 2003). Thus, while toothfish are generally sedentary, residential species, these long-distance migrations may contribute to mixing between populations, though at a rate that is unlikely to contribute significantly to the gene flow between neighboring populations.

It is worth noting that while tagging studies provide valuable information on the migration patterns of Patagonian toothfish, they are not without limitations. For example, the accuracy and reliability of the data gathered from tags can be influenced by various factors, such as the durability and attachment of the tags, the behavior of the tagged fish, and the ability to recover tagged fish for data retrieval.

2.4 Oceanographic and ecological influences on distribution

The distribution and life history of the Patagonian toothfish (see Life History) are closely linked to environmental and ecosystem influences. Throughout its life, Patagonian toothfish are limited to waters >2°C (Duhamel et al. 1982), because, unlike other notothenioids, they lack antifreeze glycoproteins (Eastman and DeVries 1986). Previous studies have indicated that the northward distribution of Patagonian toothfish is limited by the Antarctic

Circumpolar Current, Antarctic Polar Front and the Subantarctic Front (Lee et al. 2021, López-Abellán, 2005), though Belchier and Collins (2008) note that they occur both north and south of the Polar Front. These features can act both as barriers to adult migration and dispersal mechanisms for pelagic eggs and larvae (Ashford et al., 2012; Evseenko et al., 1995; Mujica et al., 2016). As noted above, Patagonian toothfish spawn at depth and have a protracted epipelagic egg and larval phase (up to 8 months; Evseenko et al. 1995; Belchier and Collins 2008), which favors dispersal via large, basin-scale oceanographic processes (Evseenko et al., 1995; Koubbi et al., 1990; North, 2002; Doyle and Mier, 2016). This early life strategy is in contrast to the adult phase, which is relatively sedentary and from which many ecological and genetic studies indicate limited dispersal/movement (White 1998; Shaw et al. 2004; Rogers et al. 2006). Mesoscale features can be important due to increased productivity and food availability (via e.g. localized upwelling), and also due to their assistance in increasing larval retention duration in favorable habitat or increasing transport towards recruitment areas (Shelton and Hutchings 1982; Bakun 1996; Tolimieri et al. 2018; Mori 2013; Park et al. 2014) leading to enhanced larval survival and recruitment.

As already reviewed, post-settlement larvae inhabit relatively shallow habitat around seamounts, plateaux, and continental/shelf slopes areas in the Southern Ocean and southern basins of the Indian, Atlantic and Pacific Oceans (Evseenko et al., 1995; Koubbi et al., 1990; North, 2002, Collins et al. 2010, CCAMLR 2021). Increased recruitment in South Georgia appears linked to lower temperatures during the spawning period driven by El Nino-Southern Oscillation events (Belchier and Collins 2008), which may have less to do with the effect of temperature on spawning success and rather a byproduct of increased upwelling which led to increased productivity and food availability (Tolimieri et al. 2018; Lee et al. 2021). In fact, recruitment variability appears to be strongly influenced by changing productivity patterns (Agnew 2002; Arkhipkin et al. 2013, Croxall and Wood 2002; Lee et al. 2021). In the southern Atlantic, mesoscale eddies driven by the Subantarctic Front and upwelling features appear to be closely linked to higher recruitment and subsequent abundance (Lee et al. 2021). Slower current velocities and deeper mixed layer thickness can also be used as proxies for productivity in regions where a deeper mixed layer indicates the mixing of nutrient-rich and productive upwelled water over the shelf (e.g. the south Atlantic region; Lee et al. 2021). The Southern Ocean is characterized by upwelling along the Antarctic Circumpolar Current (ACC) driven by persistent and strong westerly winds and negative wind stress curl (Marshall and Speer, 2012); however it is seasonal, wintertime convective mixing that bring deep, nutrient-rich water to the surface, where they can then be taken up through photosynthetic processes in the surface zone starting from October and then transported through the Southern Ocean by the ACC (Song

et al. 2016). This mixed layer can be as deep as 500 m in the winter, and shallows to 100 m in the summer (Dong et al., 2008; Sallée et al., 2010).

Patagonian toothfish appear to be highly sensitive to oxygen concentration (particularly juveniles and pre-recruits), requiring moderate to high oxygen concentrations (Coppola et al. 2015; Lee et al. 2021). Oxygen concentration is mostly influenced by currents and fronts which bring different water masses. In the south Atlantic, oxygen concentration is influenced by the Falklands Current and Subantarctic Front which carry oxygen-rich water to the Patagonian Shelf. Years with an increased influence of these currents are linked to stronger year classes, whereas years that are more influenced by the relatively warm and oxygen-poor Argentine Shelf Drift branch of the Brazil Current are linked to weak year classes (Lee et al. 2021).

As previously noted, juveniles express an ontogenetic migration pattern whereby they progressively shift into deeper habitats as they age, grow, and mature (Arkhipkin and Laptikhovsky, 2010; Ashford et al., 2012; Evseenko et al., 1995; Mujica et al., 2016; Peron et al. 2016). This behavior is common in deep-sea fishes, and can be related to changing predation habits or predator avoidance behavior (Arkhipkin et al. 2003; Collins et al. 2010; Duhamel et al. 2005). Lee et al. 2021 found that younger fish inhabited zones with high rockcod (prey) and icefish abundance (competitor), but that relationship declined for older fish. Rockcod and other prey species for the juvenile fish that are abundant in the shallower zones suggest more favorable conditions for survival of this life stage at these depths (Collins et al. 2007; 2008; Lee et al. 2021). As the fish grow, their diet changes to other, less active species found at greater depth (Arkhipkin et al., 2003). The importance of icefish in the distribution of juvenile Patagonian toothfish is uncertain in the Kerguelen Plateau and may simply reflect an overlap in habitat preference (Arkhipkin et al., 2003; Pennino et al. 2019), but in South Georgia, this piscivorous species is in lower abundance at shallower depths and juvenile presence at these depths has been linked to lower predation risk (Reid et al. 2007).

Suitable habitat (preferred oceanographic conditions of the different age classes) is not necessarily occupied by the species, as Patagonian toothfish are opportunistic in their distribution, with large recruitment pulses occurring every 4-5 years (Laptikhovsky and Brickle 2005) that are highly influenced by oceanographic processes and large-scale ocean basin events such as El Nino-Southern Oscillation (see above). Lee et al. 2021 note however, that persistent hotspots appear related to quasi-permanent mesoscale frontal zones, which create zones of high productivity that are inhabited by juveniles in the shallower zones (<200m) and larger individuals at greater depths (Agnew 2002; Lee et al. 2021).

Previous studies have investigated the influence of habitat-based oceanographic variables on the distribution of Patagonian toothfish, e.g. sea bottom temperature, sea bottom current velocity, oxygen concentration, mixed layer thickness (Lee et al. 2021; Bouchet et al. 2014; Péron et al. 2016; López-Abellán, 2005). A common conclusion in these studies is that depth is an important environmental feature for Patagonian toothfish as it is highly correlated with differences in temperature, light intensity, oxygen, salinity, productivity, prey distribution and predation risk (Bouchet et al. 2014; Lee et al. 2014; Péron et al. 2016). Shallower waters along shelfs and plateaux can be highly productive, leading to optimal feeding conditions in addition to warmer temperatures which both promote increased growth rates for post-settlement recruits and juveniles (Agnew 2002; Arkhipkin and Laptikhovsky 2010). Smaller individuals may avoid depth due to cannibalism from larger individuals (Péron et al. 2016). Deeper waters can be cooler, darker, more oxygen-rich, and though less productive than shallower habitat (Mbatha et al., 2019) deeper waters may provide respite from predators of larger individuals (Abe and Iwani 1989; Hucke-Gaete et al. 2004; Collins et al. 2010).

Finally, the steepness of the slope is another feature that has been linked to toothfish abundance (Péron et al. 2016), plausibly due to differences in the community make-up of flat plateaux versus steeply sloping shelves (Wilson et al. 2007).

2.5 SIOFA management units, measures, and stock assessments

The CMM 2019/01 (Interim Management of Bottom Fishing) tasks the SIOFA scientific committee with developing and providing advice and recommendations on the status of stocks of targeted deep-sea fishery resources, including the Patagonian toothfish. In addition, CMM 2019/15 (Management of Demersal Stocks) requires that the scientific committee provide annual reports on the status of targeted demersal fisheries resources, relative to available and/or relevant reference points and including where possible, projections of stock status over a period of no less than 20 years. The Meeting of the Parties requested that the scientific committee provide advice based on MSY until specific reference points are adopted (SIOFA 2020).

Currently, Patagonian toothfish in the SIOFA area are not assessed (SIOFA 2022), though stock assessments are performed for the adjacent CCAMLR regions, including the Kerguelen stock (FAO 58.5.1; CCAMLR 2023a; Figure 1), Heard and MacDonald Islands (FAO 58.5.2; CCAMLR 2023b; Figure 1), and Crozet Island (FAO 58.6; CCAMLR 2023c;

Figure 1). Toothfish catches within the SIOFA area were not considered in the CCAMLR assessments (SIOFA 2016); however, SIOFA catches from Williams Ridge are currently included in the HIMI TOP assessment. However, given that the stocks are likely straddling, a collaborative approach for toothfish assessments is recommended, and the possibility of using the data-limited "swept-area" approach as used in CCAMLR was recommended (SIOFA 2018a).

The MoP5 requested the scientific committee provide advice on candidate target and limit reference points for toothfish and develop a framework and workplan for the establishment of their harvest strategy (SIOFA, 2018b). Two management units (MU) have been defined for Patagonian toothfish in the SIOFA area, including one on Del Cano Rise and one on Williams Ridge (Figure 4). The scientific committee noted in 2020 that there appear to be two areas of apparent toothfish fishing outside the Del Cano Rise and Williams Ridge areas, including an area immediately to the northwest of Del Cano Rise MU, and an area to the east of Williams Ridge at the southern boundary of Region 7 around 99°E. These areas are noted covered under CMM 2020/15 and are in close proximity to CCAMLR, thus the MoP recommended that management measures be considered for these two areas.

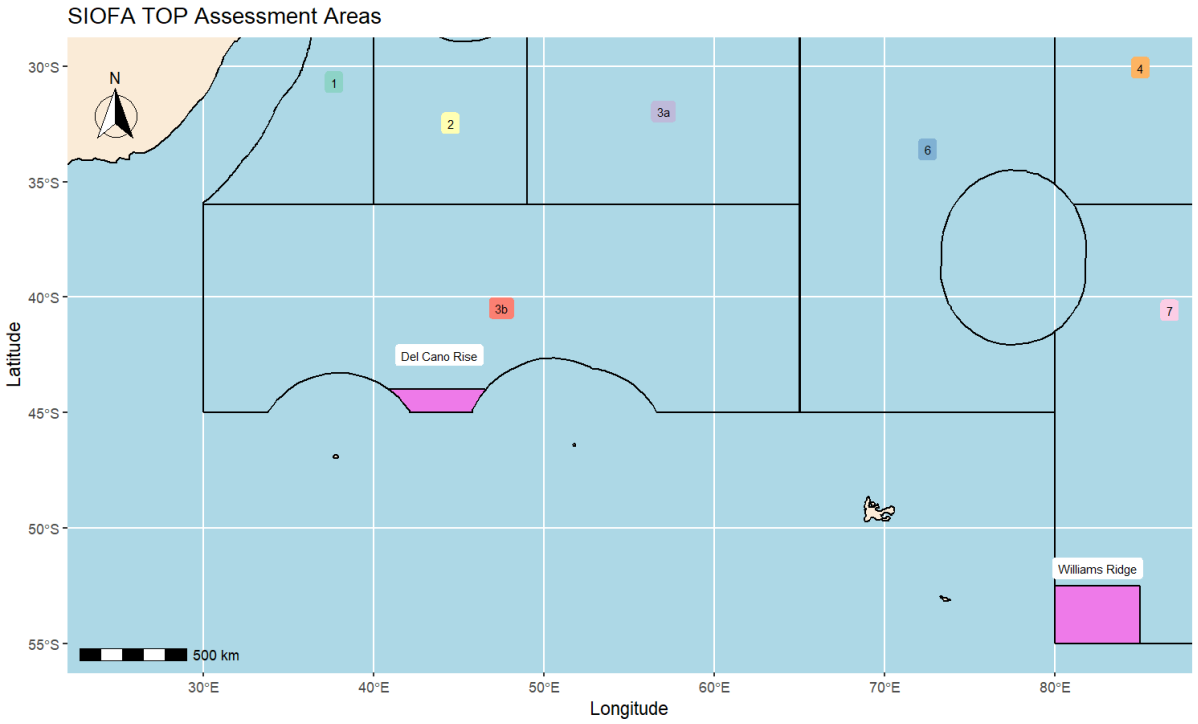


Figure 4. Patagonian toothfish management units (CMM 15; pink polygons) in the SIOFA convention area (black polygons). Figure extracted from SIOFA, 2022a.

An initial evaluation of the state of toothfish stocks in Del Cano Rise was performed by developing proxies of fish biomass using data from vessels flagged to Spain, France, Japan,

and Korea and exploring trends in CPUE. The data-limited methods CMSY, SRA, and JABBA were applied to data collected over the last 17 years. Due to large variability in the depletion analyses, estimates of fish density and virgin biomass were not deemed to be accurate. A specific sampling design and more data were recommended in order to estimate sustainable catch limits (Sarralde et al. 2020).

3. Catch and biological data review

3.1 Catch and biological data review

We submitted an official request to the Secretariat for catch, effort, and observer sampling data for the Patagonian toothfish fishery, which was released to us after agreement from the parties. Catch and effort data are required to be submitted to SIOFA by active fishing countries, which include France (OT), EU-Spain, Australia and Korea. Biological data were collected by observers, who are required on all active vessels and sample a subset of the catch for length, weight, maturity, sex, and otoliths. Data provided by the Secretariat did not include country-level information, but the Secretariat informed us that they received data from France (OT), EU-Spain, Australia and Korea include only longline (LL) and set longline (LLS) fishing operations with data on fishing operations from 2003-2021; observer data were available from 2017-2022. All longline sets were provided by the Secretariat, including autolines, trotlines, Spanish, etc, with most being autoline. However, longline gear details are not always provided by countries and were not provided in the dataset sent by the Secretariat. We removed all data that were not listed as *Dissostichus eleginoides* (i.e. 7 catch records and 30 sampling records were listed for *Dissostichus mawsoni*). We identified 2935 unique fishing operations listed in the catch-and-effort data and 14735 observer samples taken during 510 different fishing operations.

D. eleginoides exhibits an ontogenetic trend of increasing size and maturity with depth in the Indian Ocean and elsewhere (Duhamel 1991, Welsford et al. 2011, Péron et al. 2016). We therefore reviewed the catch and effort data with regards to the depth distribution of the catches. Most fishing operations had bottom depths (n=1632), but for those that did not (n=1303), we used the average of the fishing depth as a proxy for bottom depth where available. Depths >5000 m (n = 2) were removed. A remaining 111 fishing operations had no depth information and were removed from the analyses. We also removed records with no date (n=209). This resulted in a total of 2611 fishing operations with catch and effort data from 2003, 2004, 2007, 2009-2021, with fishing depths between 295 - 2700 m.

Fishing operations related to the observer samples were set at depths between 520 - 2104 m.

3.2 Geographic distribution of catch

Geographic distribution of the fishing operations in SIOFA were mapped using the Catch-Effort data. Three sampling hotspot regions were identified : the Del Cano Rise located between Prince Edward Islands (EEZ South Africa) and Crozet Islands (EEZ France); Williams Ridge located east of Heard and McDonald Islands (EEZ Australia) just off of the Kerguelen Plateau; a set of eastern catch locations. Del Cano Rise, Williams Ridge, and the southernmost eastern location all border the CCAMLR convention area, which is directly to the south of SIOFA (Figure 5).

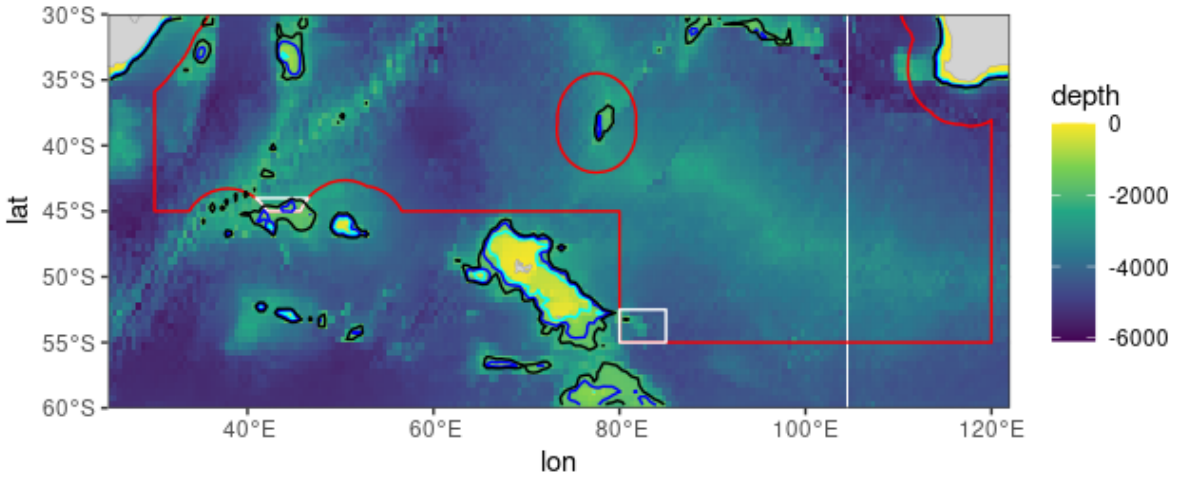


Figure 5. The southern SIOFA region (red polygon) with the Del Cano Rise (white polygon west) and Williams Ridge (white polygon east) management areas. Isobaths represent 2000 m (black), 1500 m (blue), and 800 m (cyan) depth.

Of the 2611 unique fishing operations, the largest number occurred in the waters over Del Cano Rise (n = 2296), followed by Williams Ridge (n = 258), and three sites to the east (n = 57). A closer inspection of the Del Cano Rise region also indicates two distinct fishing zones, one in the southern management area (n=1471 fishing operations) and one further north, following the South Indian Ridge (n=825 fishing operations). The more northerly eastern locations represented only 4 fishing operations in total. The number of individuals sampled from each location is as follows: Del Cano Rise (n=5347), South Indian Ridge (n = 2996), Williams Ridge (n = 5434), and Eastern (n = 958). The data from the eastern locations were recovered during only the recent fishing years (i.e. 2020-2021; except for the three fishing operations reported in 2003), and these locations are relatively distant from the more commonly fished sites. It was suggested that these eastern fishing locations

would not represent a significant fishing zone in the future, and sample collection in this area is unlikely (SIOFA scientific committee, pers. comm). As such, in terms of sampling design for a population discrimination study, we removed data from these sites from downstream data analyses (note that removing these data also removed all data from 2003). Therefore, further analysis focused on three main fishing zones, which we refer to as Del Cano Rise (DCR), South Indian Ridge (SIR) and Williams Ridge (Table 4).

Table 4. The geographic extent of the three fishing zones examined in the spatial analysis.

Region	Longitude (eastern limit)	Longitude (western limit)	Latitude (northern limit)	Latitude (southern limit)
South Indian Ridge (SIR)	39	43.5	-40	-44
Del Cano Rise (DCR)	41	47	-44	-45
Williams Ridge	75	94	-50	-60

Given this information, as well as in accordance with a study investigating *D. eleginoides* from a fishing area south of Del Cano Rise (López Abellán 2005), we created an additional grouping of samples into three depth ranges: <800 m (shallow), 800 - 1500 m (mid-water), >1500 m (deep water) to help better assess the characteristics of the fishing operations and samples retrieved.

3.3 Effort

There are two distinct fishing periods for toothfish in the SIOFA: pre- and post-2016, with only one fishing operation reported in 2016. Fishing prior to 2016 was concentrated in the SIR-DCR region with more fishing occurring in DCR, with a peak in 2012. Operations began at Williams Ridge from 2018, with 98 operations in 2018 and 132 in 2019, but reduced to 28 in 2020 and zero operations were reported in 2021.

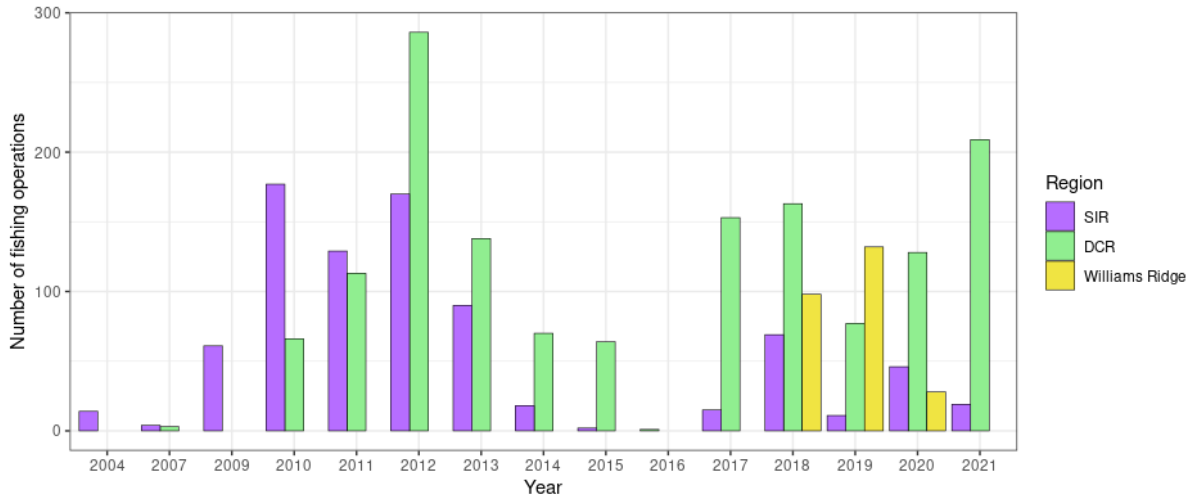


Figure 6. Annual distribution for the number of fishing operations for three regions.

Fishing appears to occur throughout the year in the SIR and DCR regions, with strong effort in the austral summer (Nov-Mar) and in May (Figure 7). Fishing in Williams Ridge has two peaks: one in austral summer (Dec-Jan) and another in austral winter (Jun-July) with minor effort outside these periods.

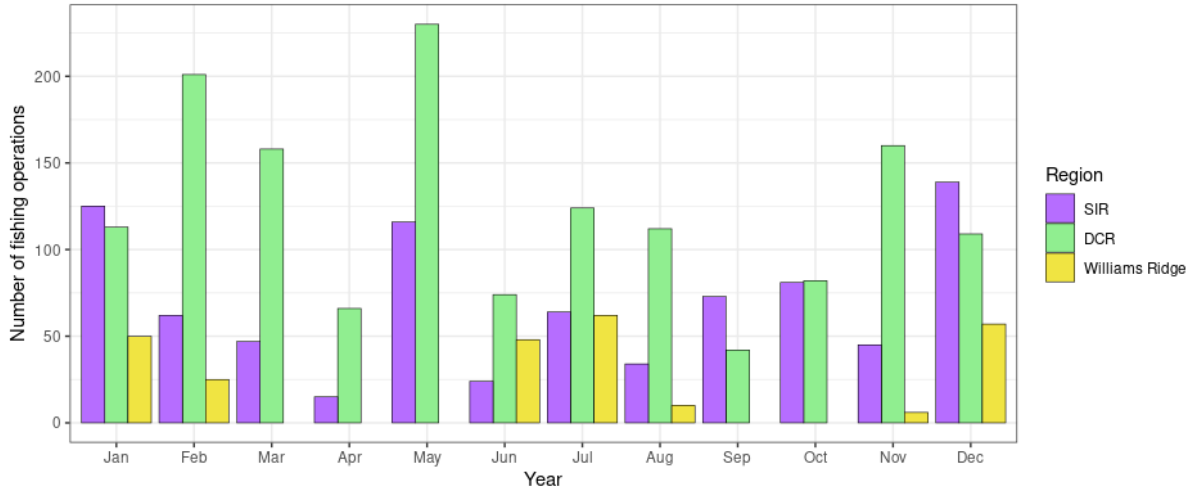


Figure 7. Monthly distribution for the number of fishing operations for three regions.

For all three regions, most fishing operations are set within the 800-1500 depth range, and the fewest operations are set in the shallower range (<800 m). In DCR, no operations are reported for the shallowest range (Figure 8). Fishing in the deep range (>1500) is considerable in DCR and SIR.

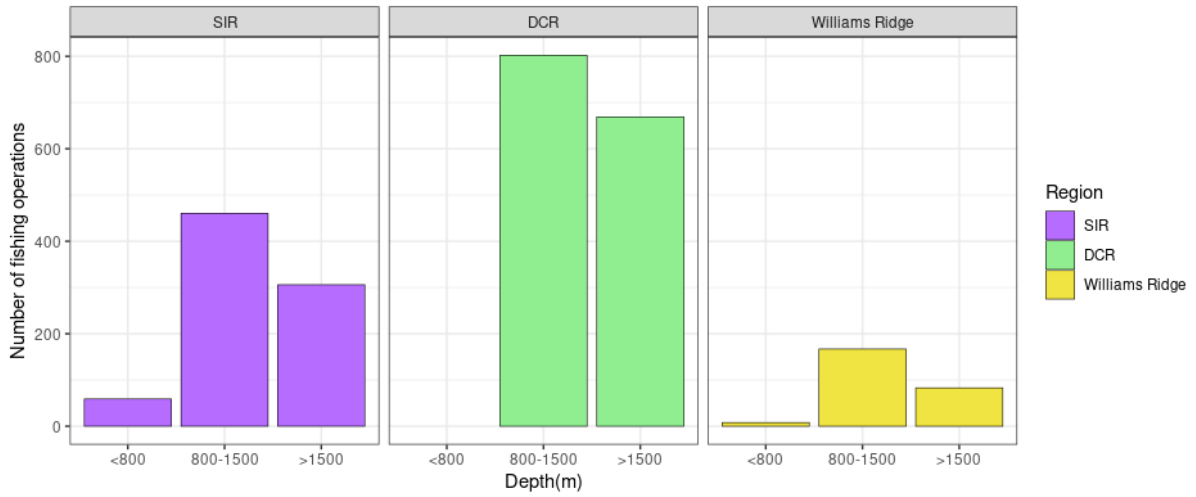


Figure 8. Depth distribution of fishing operations for three regions.

Examining the number of hooks that were deployed over the period of reported fishing,there appears to be substantially more effort after 2016 than before (Figure 9), particularly from 2017-2019, though there is a notable trend of decreasing effort, in terms of number of hooks, across all depths ranges. Effort in shallow areas is low throughout the time series; and highly variable in the deep areas. Fishing in the mid-depths 800-1500 m is more stable, and appears to be the focus of the fishing effort (Figure 9), in accordance with the ontogenetic movement displayed by *D. eleignoides* where mature, larger individuals typically reside in deeper waters. The highest number of hooks was observed in 2017 and 2019 in deep water (>1500 m), however, this decreased drastically in 2020 and 2021 (Figure 9).

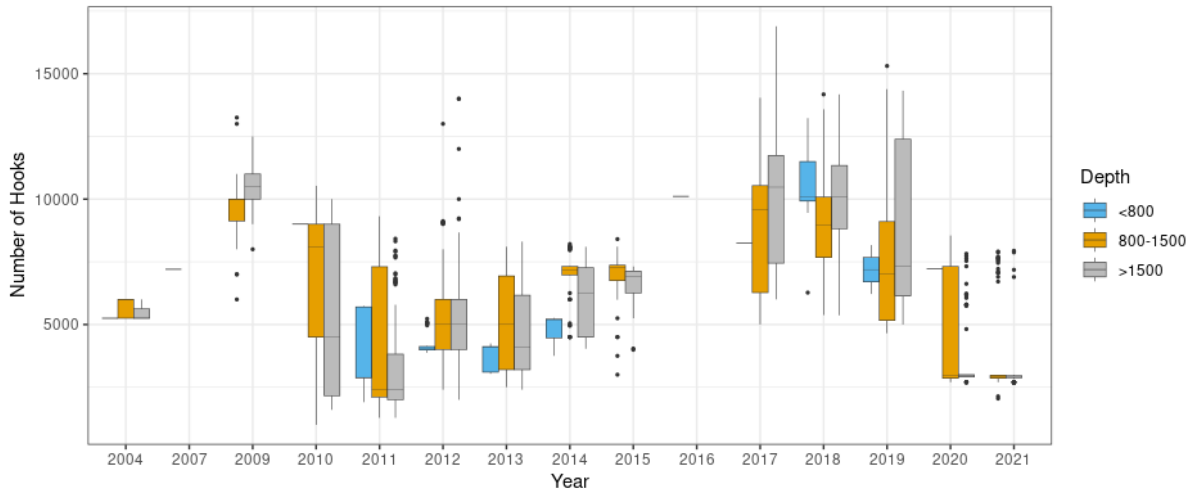


Figure 9. Annual distribution of the number of hooks for three depth ranges.

Throughout the year, the number of hooks and the variability of this number was generally higher in the mid- to deep waters (>800 m, Figure 10) except in February and June where more hooks were set in the shallow zone.

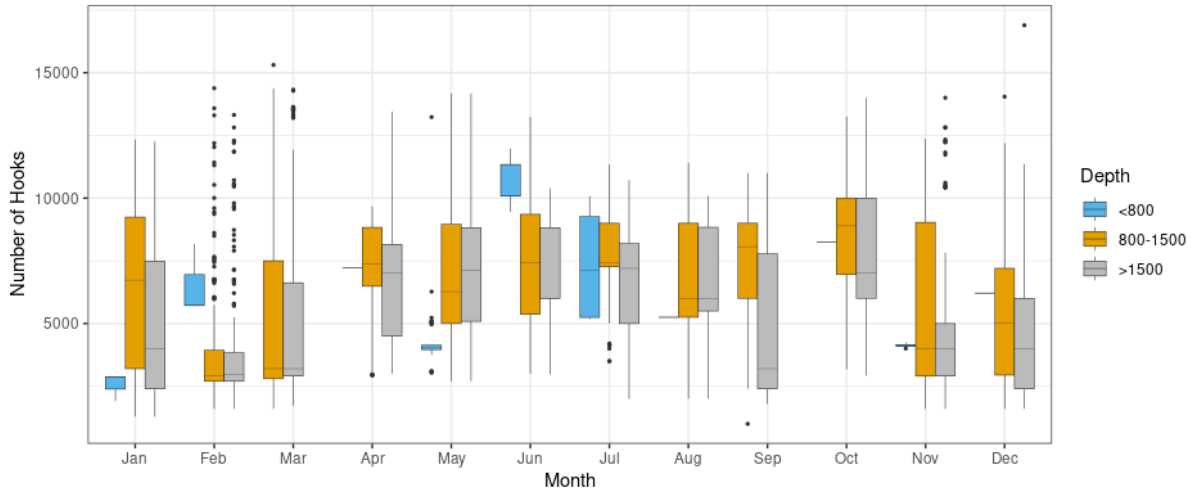


Figure 10. Monthly distribution for the number of hooks across years for three depth ranges.

The average number of hooks deployed was relatively similar between the three regions, with Williams Ridge deploying the most hooks across all depth ranges (Figure 11). This aligns with the SIOFA CMM 2021/15 whereby a significantly higher number of hooks are allowed per set in Williams Ridge (n=6250) than in Del Cano Rise (n=3000). In both SIR and DCR, the highest mean number of hooks were deployed at 800-1500 m, while at Williams Ridge, the highest mean number of hooks was deployed in depths <800 m. Variability in the number of hooks was much greater at all sites in the mid- to deep depth ranges.

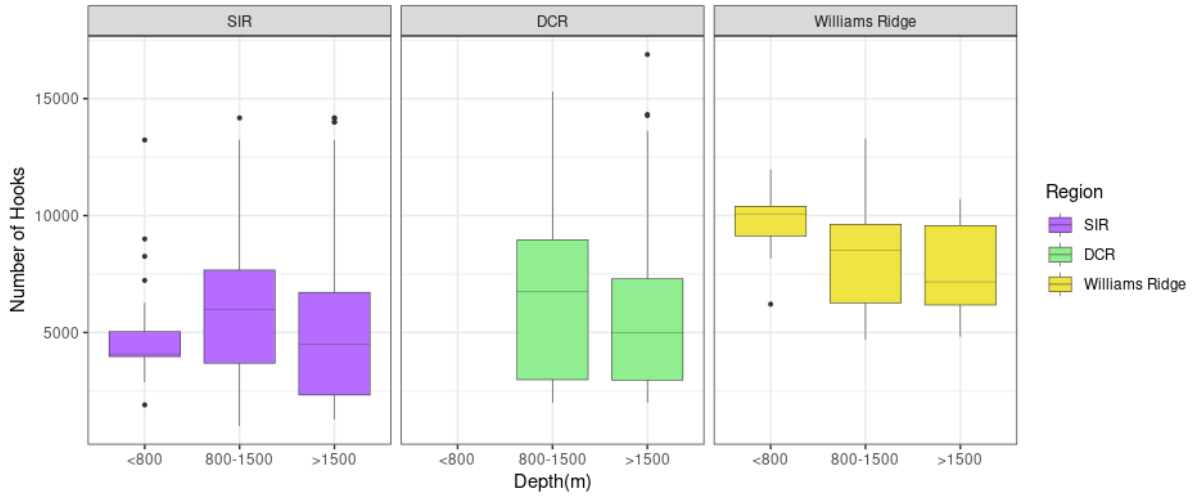


Figure 11. Depth distribution of number of hooks for three regions.

3.4 Catch Per Unit Effort (CPUE)

Figure 12 illustrates nominal CPUE for each region for the years with available records, 2004, 2007 and 2009 - 2021, calculated as the catch in kg per 1000 hooks (kg/1000 hooks). Prior to 2018, all fishing effort was concentrated in SIR and DCR. Fishing effort is reported for Williams Ridge starting in 2018.

CPUE was relatively low in all areas until 2018, where at William Ridge it is observed to be almost 10 times higher than in the SIR and DCR regions with more variability in the hauls. After 2018, effort in Williams Ridge reduced somewhat, and from 2019, CPUE began to increase at SIR, becoming substantially higher than both DCR and Williams Ridge by 2020. Effort in DCR remained relatively low and constant throughout. No data were reported for Williams Ridge in 2021.

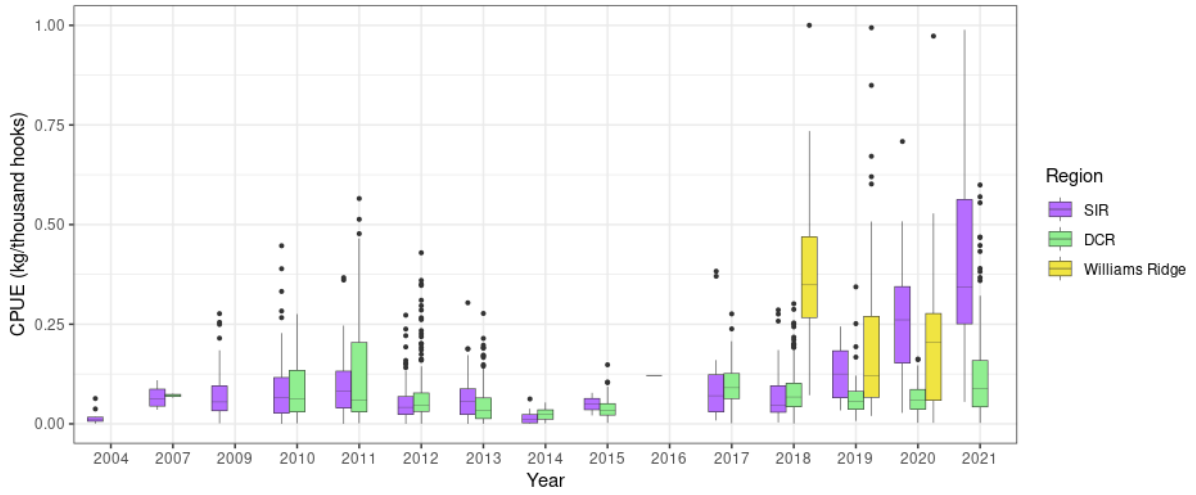


Figure 12. Annual distribution of relative catch per unit effort (CPUE) for three regions.

CPUE was highest in SIR in late austral summer to early autumn (March-April). DCR shows relatively consistent CPUE throughout the year, with slight rises in the austral spring and summer period. Relatively high variability is observed in the hauls from Williams Ridge (Figure 13), with two distinct peaks in effort in the austral winter and austral summer The June to August time period with higher CPUEs at Williams Ridge corresponds to the presumed spawning period identified in previous studies and discussed above (Section 2.1.6).

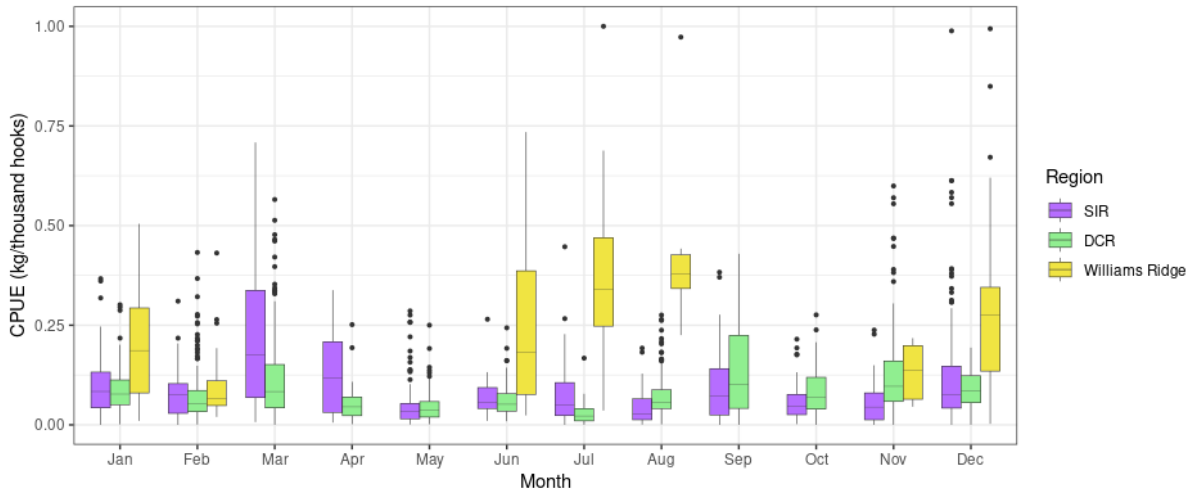


Figure 13. Monthly distribution of relative catch per unit effort (CPUE) for three regions.

Plotting CPUE by depth across all years, months and regions confirmed that fishing is concentrated between 1000 - 2000 m, with the highest values (up to 1000 kg /1000 hooks) also found within this depth range (Figure 14).

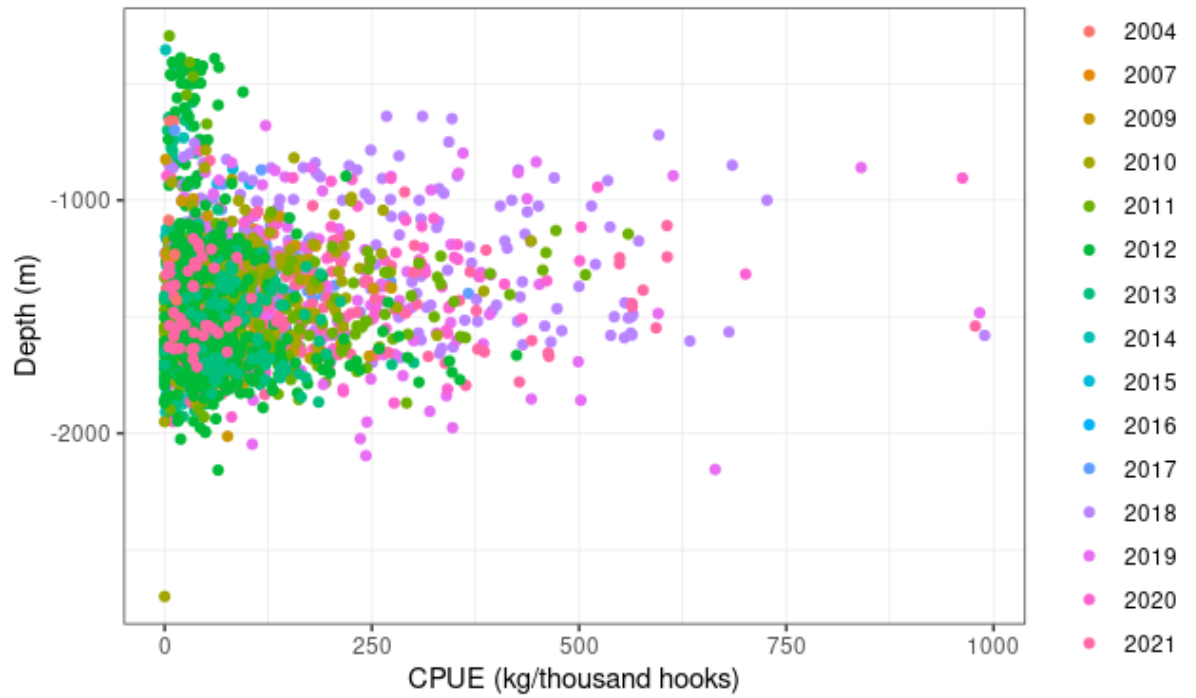


Figure 14. Relative catch per unit effort (CPUE) distribution by depth for years 2004, 2007 and 2009 - 2021.

A closer inspection of CPUE by depth and region identified that lower CPUE was obtained from <800 m at SIR, indicating that more fish are available to the fishery at 800 - 1500 m and >1500 m at this region (Figure 15). CPUE was very similar between the mid- and deep depth ranges at DCR. At Williams Ridge, CPUE was high for all depths relative to the other

regions and the highest mean CPUE was in <800 m, however, there was higher variability in the hauls from 800 - 1500 m and >1500 m.

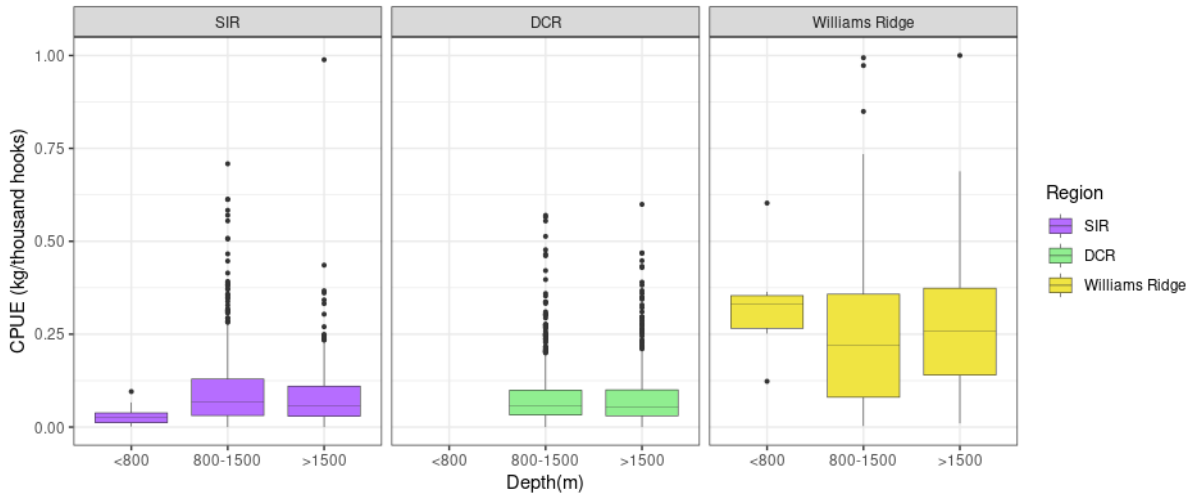


Figure 15. Depth distribution of relative catch per unit effort (CPUE) for three regions.

3.5 Length distributions in SIOFA

Overall, more length samples were obtained from DCR followed by Williams Ridge, then SIR with the fewest samples (Figure 16). Individuals of all size classes were found in the mid- and deep depth ranges, but the majority were 70-120 cm. Larger individuals (>90cm) were more present in the deep zones in DCR. Few to no samples were retrieved from <800 m, but those that were showed that this depth was dominated by smaller individuals (40-90 cm), supporting the idea that ontogenetic migration occurs in the zones.

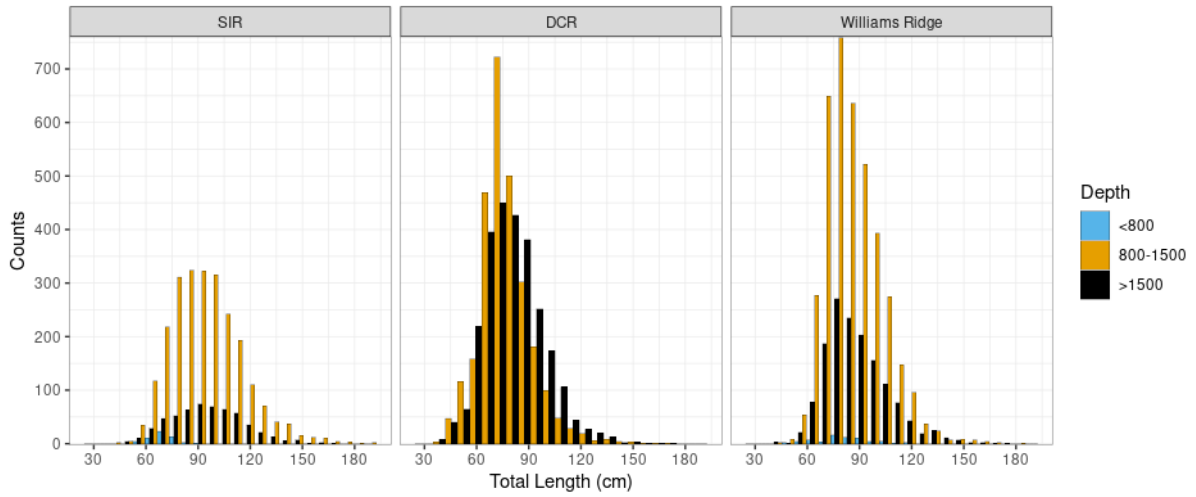


Figure 16. Length distributions across regions for depth (n = 13777).

While in general, increasing size with depth and maturity is noted for this species (Duhamel 1991, Lord et al. 2006, Welsford et al. 2011, Péron et al. 2016), few catches were made of larger (>130 cm) individuals in the deep-water range (>1500 m) at DCR and Williams Ridge. In fact, the largest individuals were more often encountered in the 800-1500 m range (Figure 16). Welsford et al. 2011 noted that observations of larger fish in shallower waters may be due to preferable foraging opportunities in these regions, suggesting a more complex relationship between depth and length. Similar to the current dataset where smaller fish (<80 cm) were found <800 m, Péron et al. 2016 identified individuals around Heard Island, Shell and Discovery banks measuring 30 - 40 cm from < 600 m, while individuals measuring >150 cm were found between 500 to 2000 m. These observations may be an artifact of gear selectivity targeting larger individuals in the shallow zones.

The distribution of length was also plotted by region and sex (Figure 17). At SIR and DCR, males were more numerous than females, whereas females dominated the catch at Williams Ridge. Both sexes exhibited relatively norma length distribution across regions, with females generally making up the larger size classes, particularly Williams Ridge (Figure 17).

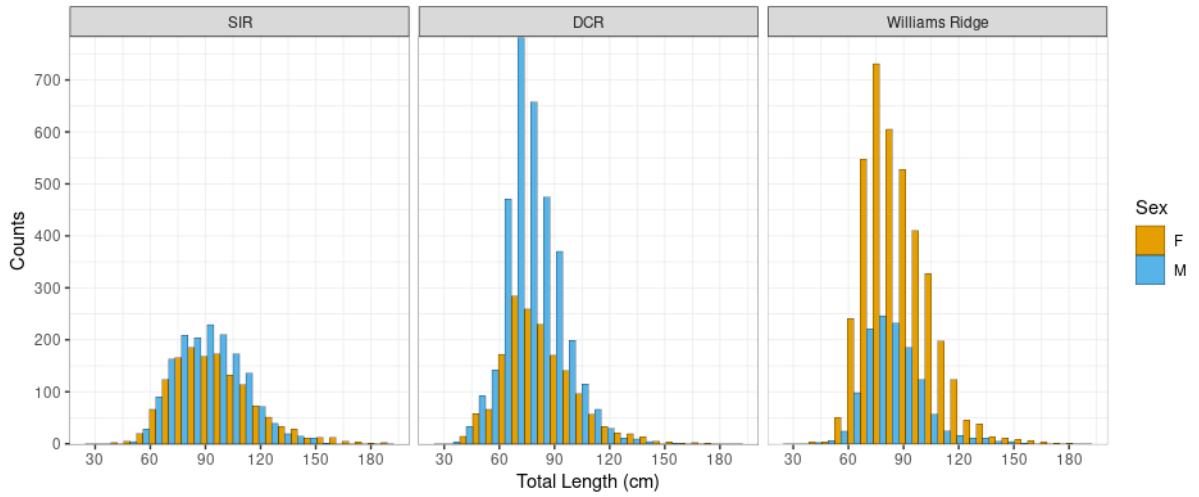


Figure 17. Length distributions across regions for sex (n = 13263).

The length-weight relationship was similar across all three regions, with females more numerous at the larger size classes (Figure 18). Collectively, females measured 41 - 187 cm and weighed 0.4 - 114 kg, while males measured 37 - 156 cm and weighed 0.35 - 50 kg. These findings are in line with previous studies where mature females were typically larger, compared to males, regardless of geographic sector (López Abellán 2005, Lord et al. 2006, Arana 2009, Péron et al. 2016, Brigden et al. 2017, Yates et al. 2018).

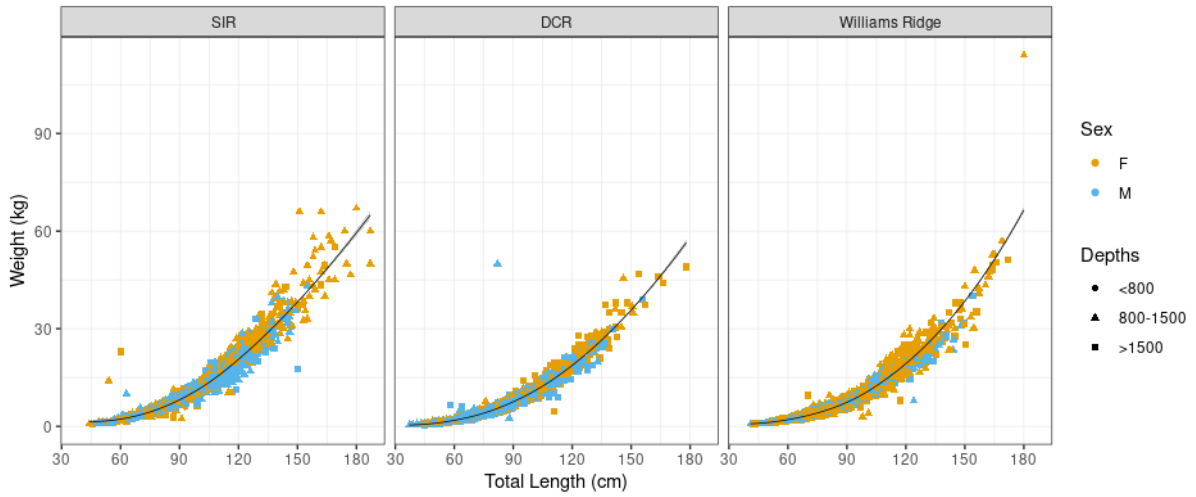


Figure 18. Length and weight distributions across regions by sex and depth (n = 13263).

3.6 Length distributions in CCAMLR

We examined annual CCAMLR fisheries reports for the regions of Prince Edward and Marion Islands (PEMI), Crozet Islands (CI) and Heard and McDonald Islands (HIMI) available here. Our focus was particularly on the years that encompass the catch dataset for the regions SIR, DCR (2017-2021) and Williams Ridge (2018 - 2021, Figures 20,21,22); however, we searched for CCAMLR literature from other years to help assess historical trends. PEMI and CI sandwich DCR and lie to the south of SIR, while HIMI lies on the Kerguelen Plateau, west of Williams Ridge; therefore, we focused our attention on the CCAMLR reports and literature for these areas to compare against the catch dataset of our regions of interest.

We calculated the length-frequency distribution of SIR from 2017 - 2022. Lengths ranged from 44 - 188 cm with modes from 75 - 112 cm (Figure 19). The year 2020 had the highest number and frequency of these lengths, with a mean of 95.35 cm and a mode of 81 cm. At DCR from 2019 - 2021, lengths ranged from 37 - 178 cm with modes from 66 - 73 cm (Figure 20). Similarly, the year 2020 exhibited the highest number and frequency of these lengths, with a mean of 79 cm and a mode of 67 cm. As previously identified (Figure 17), the majority of the catch was retrieved from mid-water depths (800 - 1500 m), followed by deep water (>1500 m) for all years at both SIR and -S (Figures 20,21), though there were no fish captured from shallow water (<800 m) at DCR. There is a clear difference in lengths by depth at DCR, with individuals captured from deep water being larger on average. In 2020, for example, the length distribution for 800 - 1500 m was 37 - 150.5 cm, with a mean of 74.6 cm and a mode of 69 cm. For >1500 m, the distribution ranged from 42 - 154 cm, with a mean of 82.31 cm and a mode of 77 cm.

Comparatively, length distribution of CCAMLR catches from CI longline fisheries report the majority of individuals caught ranging from 30 - 150 cm between the years of 2012 - 2022, with a mode of 70 for all seasons (CCAMLR CI 2022, page 13). Length distributions for PEMI longline fisheries report lengths ranging from 50 - 150 cm between the years of 2013 - 2022, with a mode of 75 cm (CCAMLR PEMI 2022, page 12). Inspection of these specific datasets support that the SIR and DCR regions exhibited a wider length range compared to those individuals caught at CI and PEMI, and at DCR - deep water and SIR, the most frequently caught lengths were larger compared to those from the CCAMLR regions. This may indicate that CCAMLR individuals are smaller and potentially younger. Due to the proximity and continuity of the bathymetry straddling the SIOFA and CCAMLR regions and noting that the SIOFA area has few nearshore, shallow areas appropriate for the settlement of post-larval juveniles (see below; Figure 28), it could be supposed that younger individuals that settled in the relative shallows of the CCAMLR region continue their ontogenetic migration into the deeper waters of SIOFA.

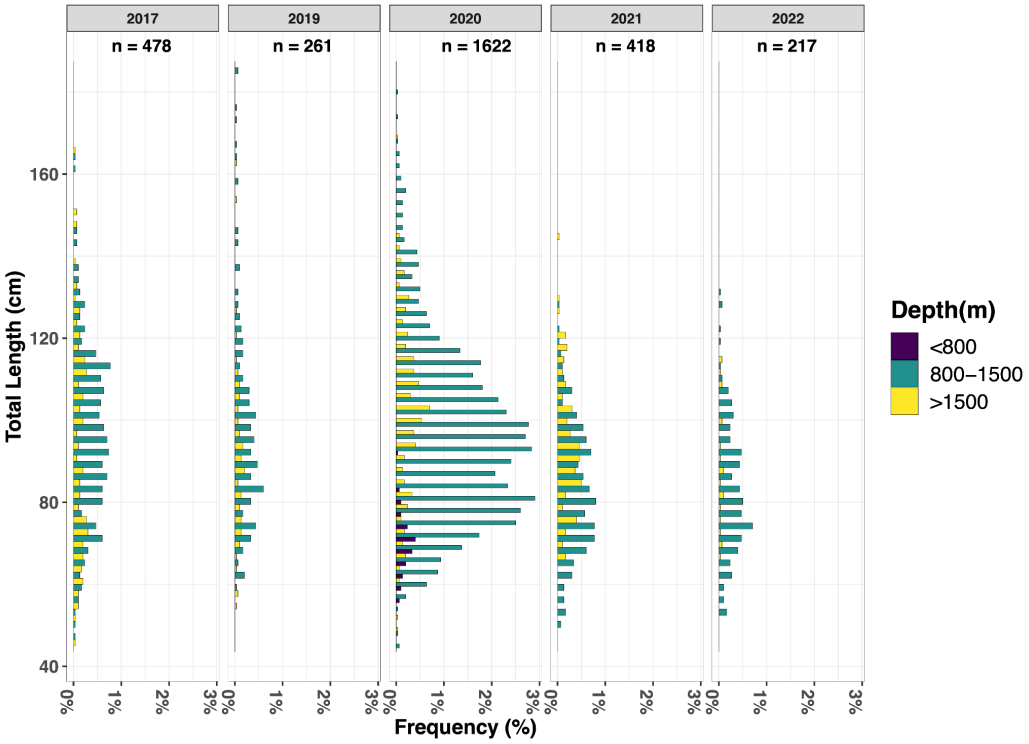


Figure 19. Length frequency by depth at South Indian Ridge for the years 2017 - 2022, based on the *Dissostichus eleginoides* data provided by the SIOFA secretariat.

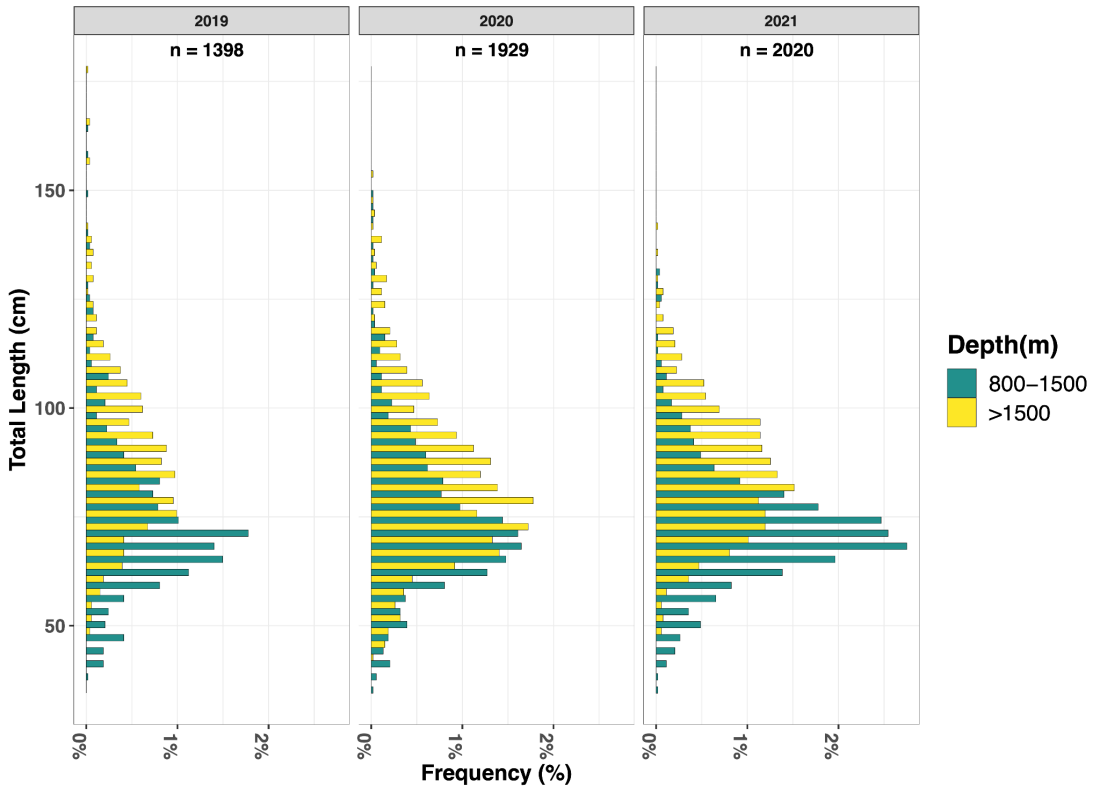


Figure 20. Length frequency by depth at Del Cano Rise for the years 2019- 2021, based on the *Dissostichus eleginoides* data provided by the SIOFA secretariat.

The SIOFA catch dataset does not have age information, nor have we obtained information regarding depth and age from the shallower and relatively adjacent CCAMLR regions of PEMI and CI, and thus we cannot make inferences on the direction of movement of differing life stages, i.e. movement of juveniles from the CCAMLR regions to deeper regions managed by SIOFA as they grow into adults.

The length-frequency distribution by depth and over several years was also examined for Williams Ridge from 2018 - 2021; lengths ranged from 41 - 180 cm with modes from 71 - 89 cm (Figure 21). The year 2019 exhibited the highest number and frequency of these lengths, having a mean of 85.4 cm and a mode of 74 cm. There were no fish captured from shallow water (<800 m) at Williams Ridge, nor was there a clear difference by depth in terms of length at Williams Ridge as was seen at DCR (Figure 20).

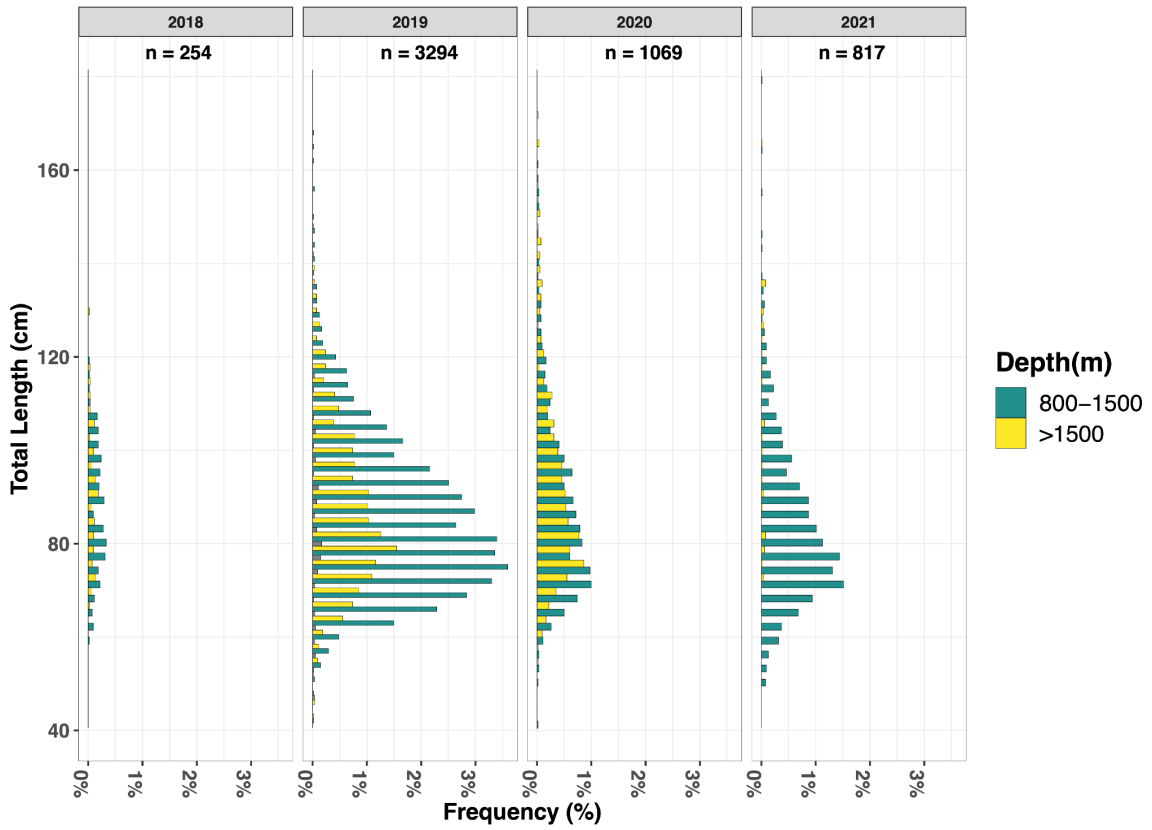


Figure 21. Length frequency by depth at Williams Ridge for the years 2018- 2021, based on the *Dissostichus eleginoides* data provided by the SIOFA secretariat.

The latest CCAMLR report from HIMI (CCAMLR HIMI 2022, page 15) confirmed that fish <60 cm are distributed in depths <500 m where areas of high local abundance have been recorded; these shallow areas are the main fishing grounds. As these fish grow, move into deeper water (>1,000m depth), a finding supported by Péron et al. 2016. It should be noted that both trawling and longline fishing occur at HIMI; however, examination of just the longline captures from 2013 - 2022, we note that fish measured between 50 and 125 cm with a mode around 75cm (CCAMLR HIMI 2022, page 15). Based on the catch data, individuals recovered at >800 m from Williams Ridge from 2018 - 2021 exhibited a wider length range and the most frequently caught lengths were larger compared to those from the HIMI. This is in accordance with the study by Péron et al. 2016 who identified that smaller individuals were found in the shallower waters of the Kerguelen Plateau, which includes HIMI, and that they increase in size with depth from the plateau, which would include Williams Ridge whose depth is > 3000 m. Nonetheless, the SIOFA catch data is composed primarily of individuals caught from 800 - 1500 m, particularly in 2021 and no age data is available for this dataset to fully confirm these findings (Figure 21).

3.7 Maturity and life stages

Individuals at the maturity stage of <2 are generally considered immature (juveniles), while individuals >2 may be considered mature (adults) (Table 1, Everson 1977). Everson and Murray (1999) and Yates et al. (2018) suggested that size-at-first maturity of stage ≥2 be applied to avoid overestimations of age at first maturity. We therefore defined the "life stage" of an individual of either sex as immature (juvenile) if their maturity stage was <2 and mature (adult) if it was ≥2.

Furthermore, Lord and colleagues (2006) reported an average size-at-first maturity of 63 cm L_T (total length) for males and 85 cm L_T for females at Kerguelen Islands. In the current dataset, we used sex and length to estimate maturity for those individuals whose maturity stage was listed as uncertain, such that males measuring <=63 cm and the females measuring <=85 were classified as immature, while those measuring above these values were considered mature. Some of these individuals whose maturity stage was uncertain also had no sex listed and therefore, they remained unidentified in terms of life stage.

We found that maturity stages varied by depth and month, but that the pattern amongst the fishing zones were relatively similar. In general, we found that females were larger than males across all maturity stages (Figure 22). Size increases with maturity, except in males whereby at stage 5 (spent), they are measured smaller than stage 3 (developed) and 4 (spawning).

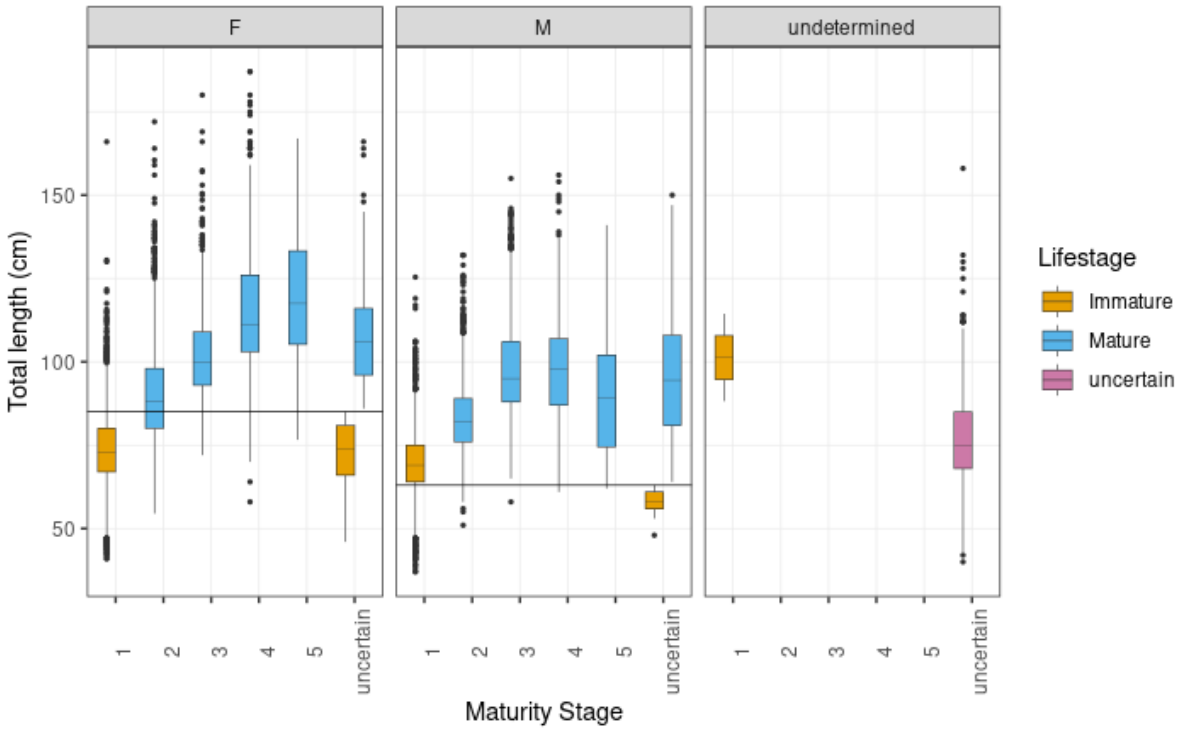


Figure 22. Total length (cm) by maturity stage (1-5; see Table 1 for stages) by sex. Horizontal lines indicate the length at first maturity for females (Lm50=85 cm) and males (Lm50=63 cm).

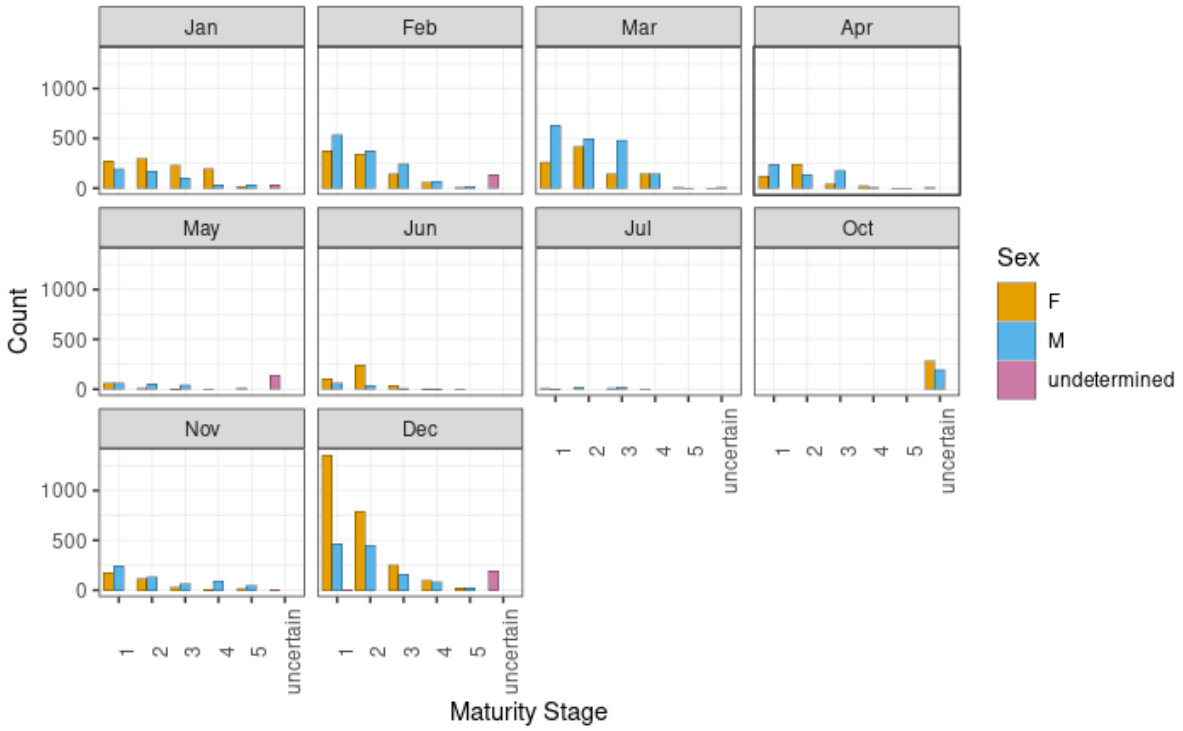


Figure 23. Maturity stages (1-5; Table 1 for maturity stages) by sex (F=female, M=male).

We looked at maturity stages at depth over the year to determine if there were patterns in spawning behavior that could be detected. We found that spawning males (stage 4) were sampled more than spawning females in November and spawning females were sampled more than spawning males in December, January and April (Figure 23). The sex ratio of spawning individuals appears most equal in February and March. We also find that spawning individuals in DCR and Williams Ridge are concentrated at the border of the SIOFA and CCAMLR areas in the austral summer, giving further evidence that these are straddling stocks between the two areas.

We found that individuals of all maturity stages were present in the mid- and deep zones, but that the shallow depth zone only had maturity stage 1 (immature) individuals in January and April and maturity stage 2 individuals in April (Figure 24). Stage 1 and 2 individuals are most frequent in December to March in the mid and deep zones. Spawning individuals (stage 4) are present from November to April in the mid- and deep water zones.

Figure 24. Maturity stage by depth over all the months with observer data aggregated by fishing zone.

This appears to indicate that the spawning season in the Indian Ocean is over the austral summer period (November to April), with more spawning activity by males early in the season (November), more female spawners from (December-January), mixed sex ratios in February and March, and finally more spawning females at the end of the spawning season in April. This seasonality is at odds with what has been previously described, which indicates spawning in the southern Indian Ocean from late April to September, identifying a synchronous mixed sex period around mid-June (Duhamel et al. 1987; Lord et al 2006; Yates et al. 2018). In contrast, we find very few stage-4 spawning individuals from May to July and October, and there are no sampling data for August or September.

3.8 Sex distribution and ratio

D. eleginoides sex ratio by total length (L_T) varied by region and depth (Figure 25). At both SIR and -S, females dominated the smaller size classes (<45 cm). The sex ratio is roughly equal in SIR until about 120cm when females then take over and from about 97 cm in DCR (Figure 25 top). This pattern was previously seen at Del Cano Rise (López Abellán et al. 2005). At Williams Ridge, males dominated when L_T < 100 cm and females dominated when L_T > 150 cm. In the middle size classes, females dominated from 100-120 cm and the sex ratio was balanced from 130-140 cm (Figure 25 top). Males have been previously

observed to dominate larger size classes northwest of the Kerguelen Plateau and west of Heard and McDonald Islands, while females dominated larger size classes east of Heard and McDonald Islands, where Williams Ridge lies (Lord et al. 2006, Welsford et al. 2011, Péron et al. 2016). With respect to depth across all the sites, males were more represented when L_T < 97 in the <800 m and 800 - 1500 m depth ranges, after which females dominated (Figure 25, bottom). In the deeper waters, females dominated both the lower and upper size classes.

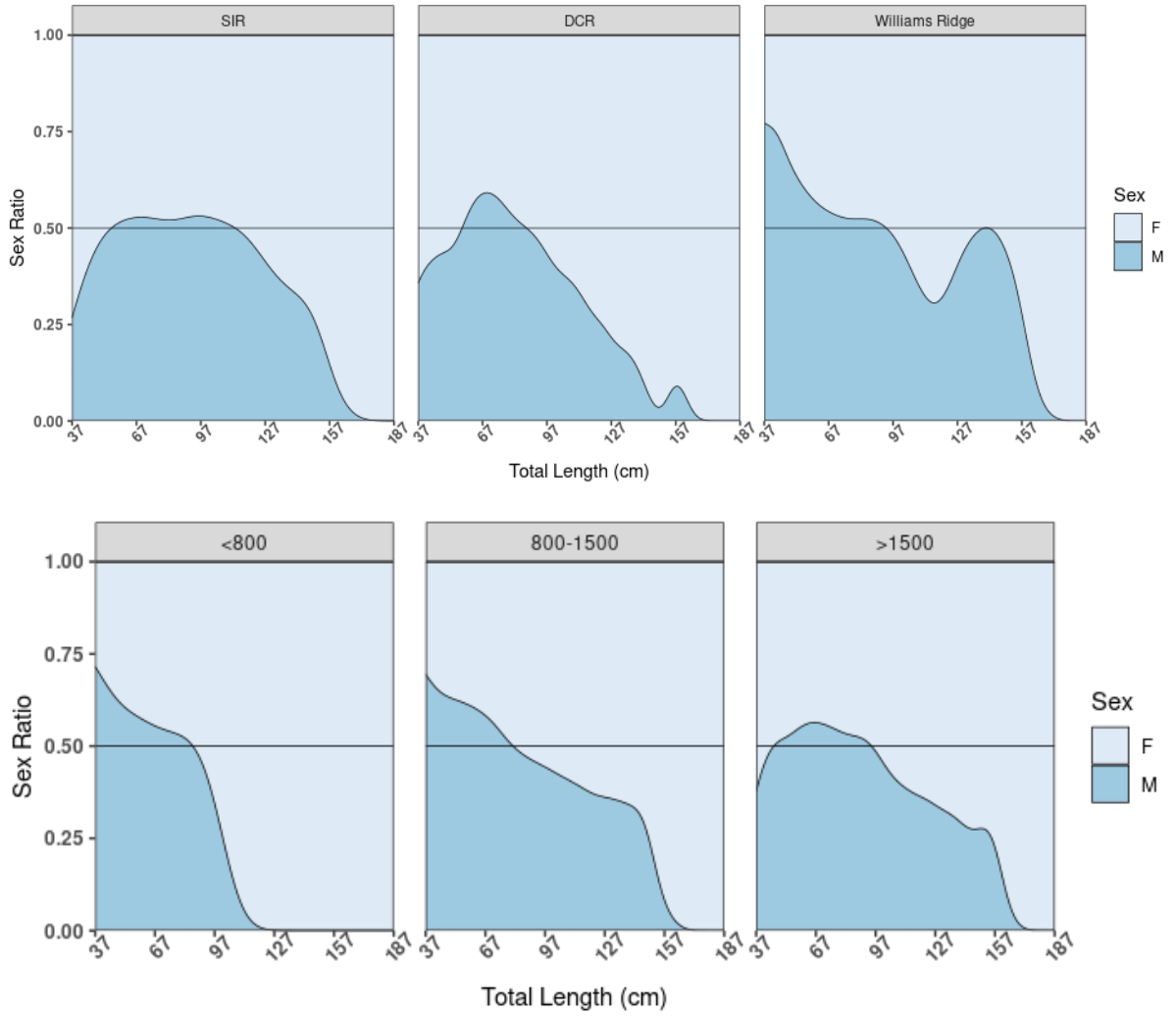


Figure 25. Sex ratio for *D. eleginoides catch* by (top) region and (bottom) depth. These illustrations were produced using R (R development Team), specifically the ggplot2 package (v. 3.4.2, Wickham 2016) and geom density with an adjust value=2 for the smoothing effect.

3.9 Tagging data review

In CCAMLR, over 350 000 tags have been released over the last 25 years. The SIOFA tag database indicates that to date 1134 tags have been released in SIOFA from 2019 to 2022 (1129 have data associated with their release position). These have been released mostly in the Del Cano Rise region (n=799; south in the management area and north along the South Indian Ridget) and along Williams Ridge (n=304) (Figure 26). Twenty-six tags were also released in the eastern SIOFA zone.

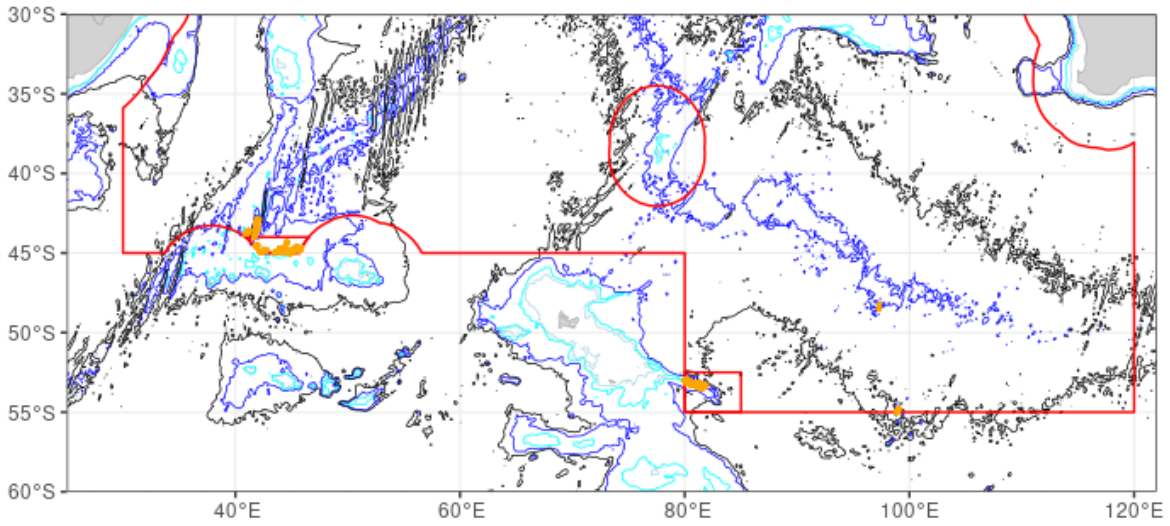


Figure 26. Toothfish tag releases (orange dots) in SIOFA from 2019-2022. The red polygon outlines the SIOFA convention area and management areas. Isobaths are delineated at -4000 m (black), -1500 m (blue), -800 m (cyan), and -600 m (gray).

To date, the SIOFA database indicates that 14 tags have been recovered in the convention area from those released in CCAMLR over an 18-year period (Figure 27). CCAMLR and SIOFA have a tag data sharing protocol, within which includes an algorithm to extract and share the tag data from those tags released in CCAMLR and recovered in SIOFA. These CCAMLR data were not made available for our study, but the recent summary by Sarralde and Barreiro in their confidential SIOFA SC report (SC-08-14-Rev2) give more detailed information on 12 of these tags recovered by a Spanish longliner.

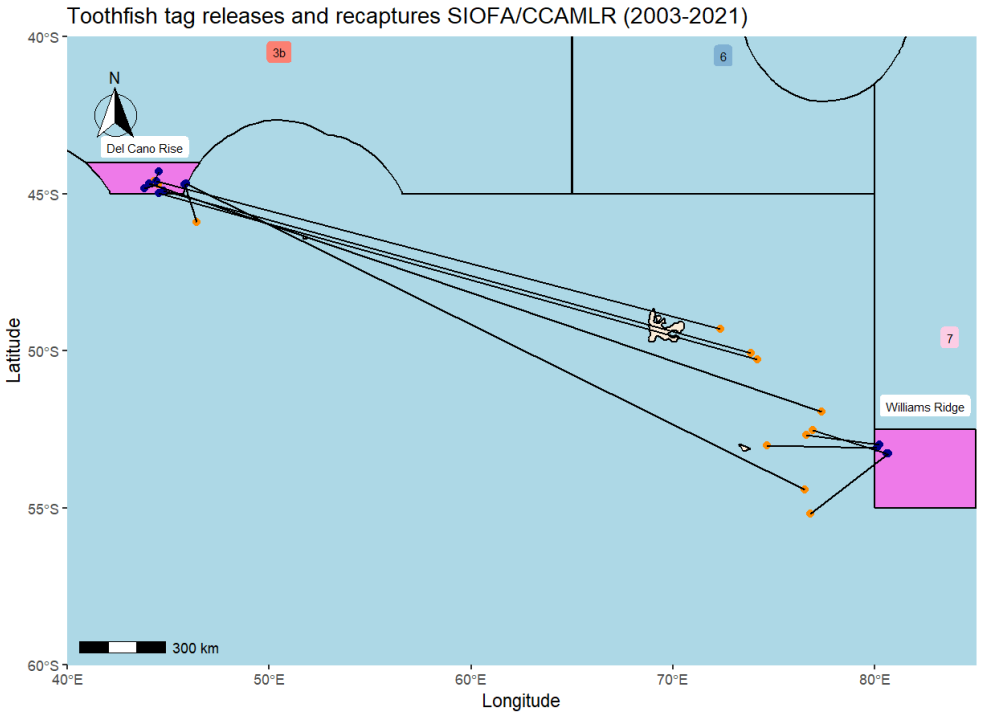


Figure 27. Toothfish tag releases (orange dots) in CCAMLR that were recaptured (black dots) in SIOFA with their minimum-distance trajectory indicated by the black line. The pink polygons indicate the management areas Del Cano Rise-South in the northwest and Williams Ridge in the southeast.

The Spanish tag data show that tagged individuals moved over distances ranging from short forays of <20 km/yr to long distance migrations >750 km/yr. One tag was released in the Crozet EEZ that was later recovered a short distance away (<20 km/yr) in Del Cano Rise after a 2-year period. However, the majority of tags that were released in CCAMLR that were then found in SIOFA were released from the eastern and southeastern Kerguelen Plateau. Six of these individuals moved relatively short distances of <50 km/yr from the southern and eastern Plateau to Williams Ridge, and five others made long distance migrations of on average 777 km/yr from the southern and eastern Kerguelen Plateau to Del Cano Rise. The release data were not provided, but it appears that those fish that traveled short or relatively short distances would not necessarily have to cross deep water areas to reach the site where they were recovered and they could likely travel along the same or similar isobaths (Figure 26,28) The continuity of the depth range indicates that these are likely individuals of the same population. Details of the release data would allow us to investigate if there were changes in depth, which may also support this claim by showing the ontogenetic vertical migration with age.

The five fish that traveled long distances must have crossed a deep water divide to reach Del Cano Rise (>4000 m; Figure 26) and it is possible that these fish have traveled between two distinct populations. Though the percent of recovered fish that traveled long distances into the SIOFA is relatively large in this dataset (i.e. 5/12 = 41%); it is minor percentage

relative to the complete CCAMLR database (40 000 recovered tags); and thus unlikely to be a major source of genetic diversity at the population level.

With the data provided by Sarralde and Barreiro (2022), we found that females undertake shorter distance movements (135 km/yr, n=5), while males undertake longer distances (579 km/yr, n=6).

We note from a review of SIOFA meeting documents that other recovered tag data may be available as CCAMLR holds tagging details from 496 Patagonian toothfish tagged by a Uruguay flagged vessel in 2008 in the SIOFA Del Cano Rise region (in a region between 44.3 to 45.0 S and 43.5 to 45.3 E) and eight tag recaptures that were reported by a Korean-flagged vessel in 2016 with five of these recaptures linked to the 2008 tagging event. Korea has also noted two other recaptures linked to fish tagged in the Crozet Island EEZ and recaptured to the north (SIOFA 2017).

4. Environmental analysis

The environmental analysis took place in two parts. The first was to characterize the main fishing grounds by the key environmental variables and investigate their variability in space and time. The second was to explore the relationship between these variables and both length and sex ratio to assist in developing a strategy to identify the environmental features and timing with sampling the most mixed population, which we postulate to be when spawning individuals of both sexes meet.

4.1 Environmental data

The environmental variables we investigated were determined from the review of oceanographic and environmental influences on distribution and included depth which is importantly linked to many different environmental features and is known to be important to structuring the distribution of toothfish across a wide depth range; steepness of the slope which linked to environmental barriers such as water channels, and different species and community composition; bottom temperature as toothfish are limited to >2°C; chlorophyll as a proxy for primary productivity; eddy-kinetic-energy (EKE) as a proxy for dynamic mesoscale features linked to ocean currents, frontal features, and localized productivity; mixed layer depth indicative of convective mixing (leading to enhanced productivity by October) and bottom oxygen concentration.

For depth we used NOAA bathymetry data extracted at 0.1° resolution via the get.NOAAbathy() function in *marmap R* package. The steepness of the slope was calculated from 0.1° bathymetry using the terrain() function of the *raster R* package. Steepness of slopes ranges from flat (0-0.05 radians) to very steep (0.6-0.75 radians)(Table 5).

Table 5. Slopes were calculated and categorized by their steepness following Peron et al. 2016.

Category	Steepness	Range radians	Range degrees
1	Flat	0 to 0.05	0 to 2.9
2	Slight	0.05 to 0.15	2.9 to 8.6
3	Moderate	0.15 to 0.3	8.6 to 17.2
4	Steep	0.3 to 0.6	17.2 to 34.4
5	Very steep	0.6 to 0.75	34.4 to 43

Bottom temperature, EKE, and mixed layer depth were extracted from the CMEMS global ocean eddy-resolving (1/12° horizontal resolution, 50 vertical levels) GLORYS12V1 monthly mean product, with data available from 1993 (E.U. Copernicus Marine Service Information; https://doi.org/10.48670/moi-00021). Monthly climatologies were calculated from 2017-2022. Chlorophyll and oxygen concentration were extracted from the Operational Mercator Ocean biogeochemical global ocean analysis and forecast system (1/4° horizontal resolution, 50 vertical levels) mean monthly sliding window product, available from May 2019 (E.U. Copernicus Marine Service Information; https://doi.org/10.48670/moi-00015). Monthly climatologies were calculated from 2019-2022.

4.2 Characterizing the fishing grounds

4.2.1 Bathymetry

Bathymetry over the DCR region shows a plateau of <2000 m that straddles the SIOFA and CCAMLR convention areas (Figure 28 left). This plateau is relatively shallow for the SIOFA region, though the shallowest parts of this plateau are <600 m but are to the southwest in the CCAMLR area. In the SIR, bathymetry indicates a series of ridges and canyons extending from the south west to the north east from CCAMLR into SIOFA. Regions shallower than 1500 are few, and several deep trenches (>4000 m) separate the ridge lines.

Williams Ridge is a small finger of the Kerguelen Plateau that extends in the southeast from the CCAMLR area into SIOFA (Figure 28 right). The ridge is between 800-2000m, with the shallowest areas straddling the CCAMLR area in the east. The Kerguelen Plateau, including Williams Ridge, is surrounded by relatively deep waters of >3000 m with the majority of the Williams Ridge management area >4000 m.

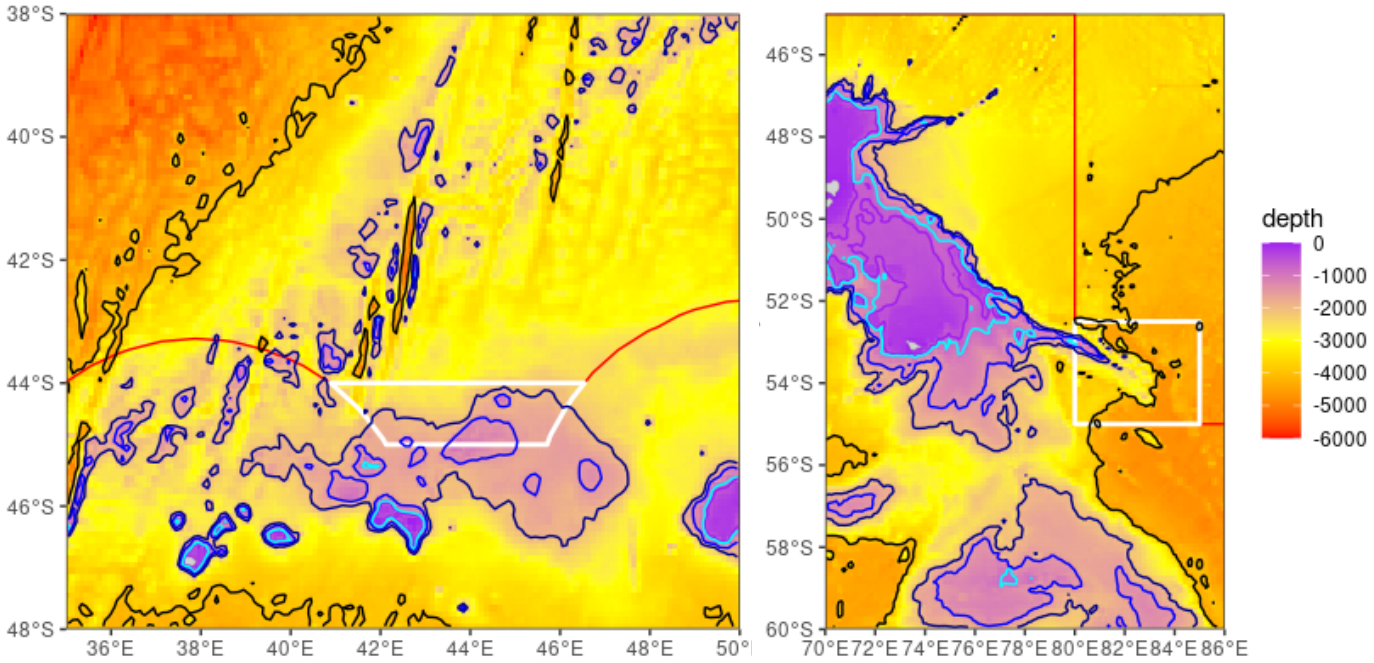


Figure 28. Bathymetry (m) in the South Indian Ridge (SIR) and Del Cano Rise (DCR) regions (left) and Williams Ridge (right). Isobaths indicated at 600 m (purple), 800 m (cyan), 1500 m (blue), 2000 m (dark blue) and 4000 m (black). The DCR and Williams Ridge management areas are indicated by the white polygon with the red polygon delineating the SIOFA convention area. CCAMLR is directly to the south of the DCR in the left plot and to the west and south in the right plot.

4.2.2 Slope

The DCR region is relatively flat with slight and wide slopes around the 1500 m isobath and again at the 2000 m isobath (Figure 29 left). The SIR region shows the majority of the area as very steep, with maximum slope steepness between each ridge and flatter zones on the top of each ridgeline. The Williams Ridge region is also relatively steep throughout the area, with the flatter areas to the north and east following the ridgeline (Figure 29 right).

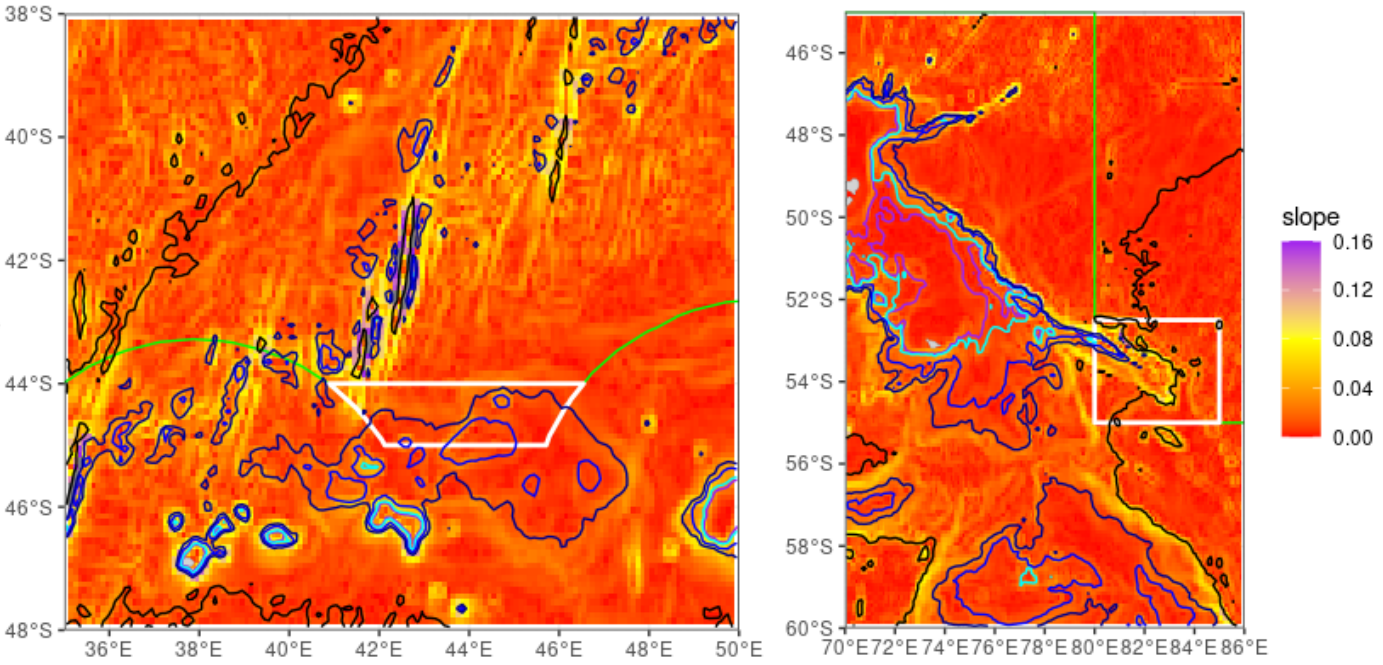


Figure 29. Slope (decimal degrees) in the South Indian Ridge (SIR) and Del Cano Rise (DCR) regions (left) and Williams Ridge (right). Isobaths indicated at 600 m (purple), 800 m (cyan), 1500 m (blue), 2000 m (dark blue) and 4000 m (black). The DCR and Williams Ridge management areas are indicated by the white polygon with the green polygon delineating the SIOFA convention area. CCAMLR is directly to the south of the DCR in the left plot and to the west and south in the right plot.

4.2.3 Bottom temperature

Bottom temperature in the DCR is around 2°C throughout the year, the lower limit of the toothfish's preferred temperature (Figure 30). Slightly warmer temperatures can be found in SIR with up to 3-4°C. Only the shallow areas along the ridge in the Williams Ridge management area has temperatures >2°C, with the majority of the management area having temperatures between 0 and 1°C (Figure 31). Bottom temperature is seasonally stable in all fishing zones though slightly warmer temperatures are observed from January to March in SIR.

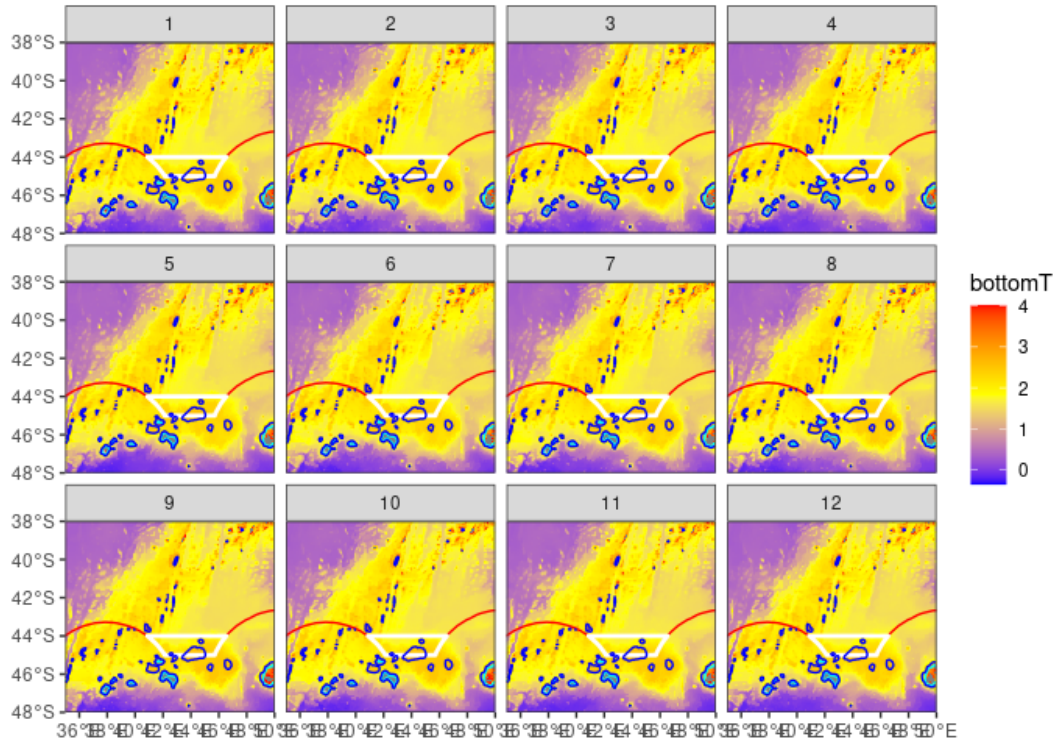


Figure 30. Bottom temperature (°C) per month (January to December) for the South Indian Ridge (SIR) and Del Cano Rise (DCR) regions, with the DCR management area indicated by the white polygon and the red polygon delineating the SIOFA convention area with CCAMLR directly to the south. Isobaths are indicated at 800 m (cyan) and 1500 m (blue).

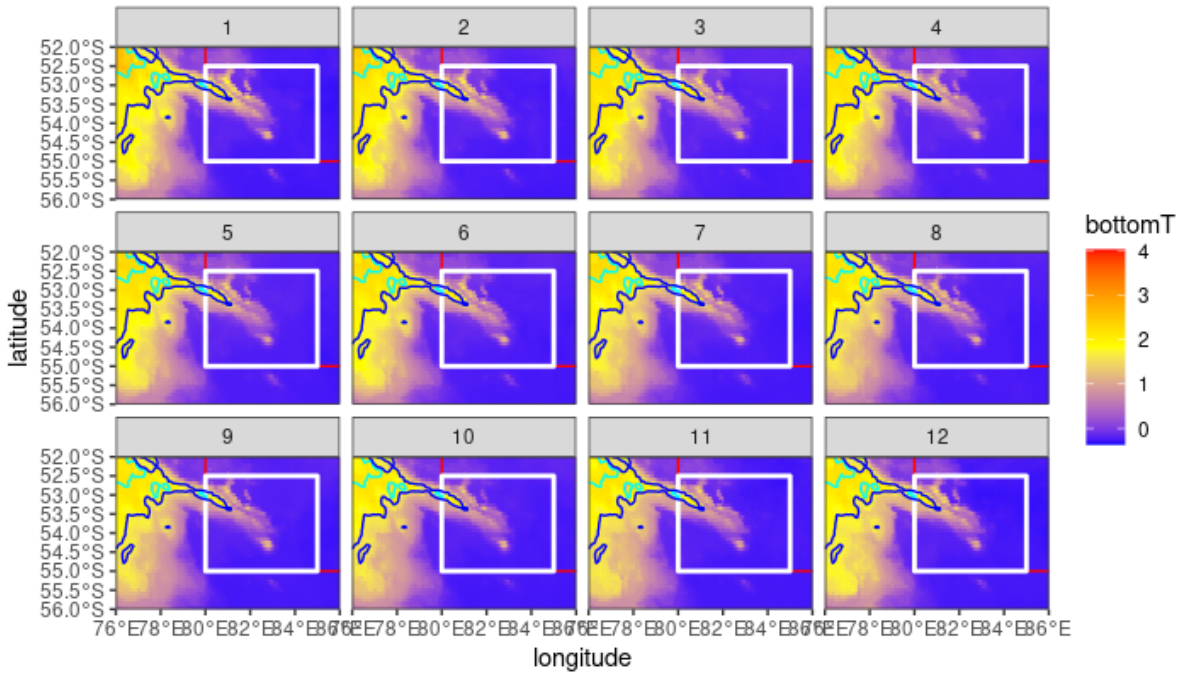


Figure 31. Bottom temperature (°C) per month (January to December) for the Williams Ridge region, with the management area indicated by the white polygon and the red polygon delineating the SIOFA convention area with CCAMLR directly to the south and west. Isobaths are indicated at 800 m (cyan) and 1500 m (blue).

4.2.4 Mixed layer depth

We find mixed layer depth to be deepest in the austral winter (July to September) and shallowest in the austral summer (December to February) in both the SIR and DCR regions and in William's Ridge (Figures 33 and 34). The mixed layer is generally deeper at Williams Ridge than in the SIR and DCR regions throughout the year, but DCR has a stronger seasonal signal (i.e. shallower in the summer and deeper in the winter) than Williams Ridge. The deeper mixed layer is related to winter-time convective mixing of deep waters to the surface, which is then linked to higher productivity from October as photosynthetic processes uptake the upwelled nutrients.

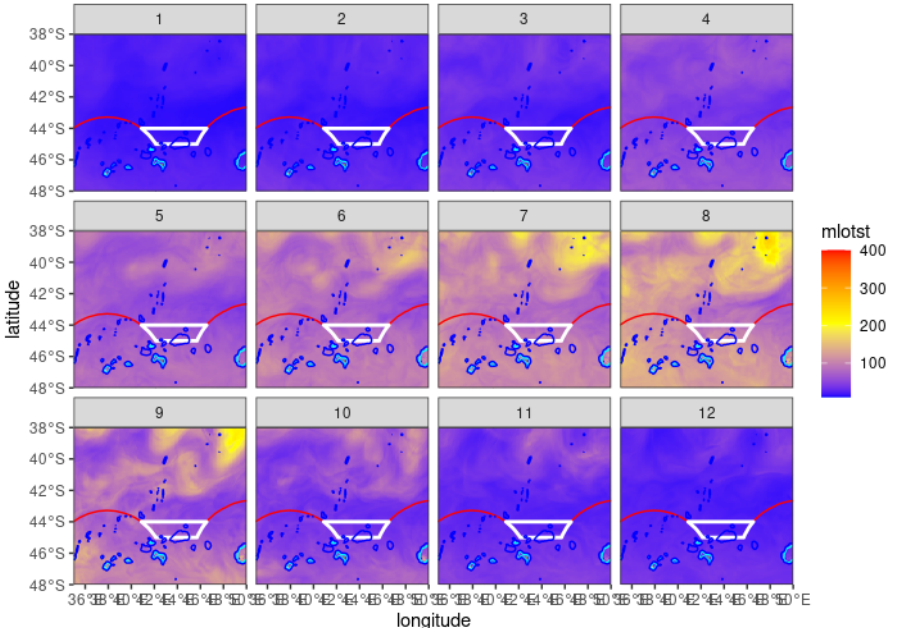


Figure 32. Mixed layer depth (m) in the South Indian Ridge (SIR) and Del Cano Rise (DCR) regions, with the management area indicated by the white polygon and the red polygon delineating the SIOFA convention area with CCAMLR directly to the south. Isobaths are indicated at 800 m (cyan) and 1500 m (blue).

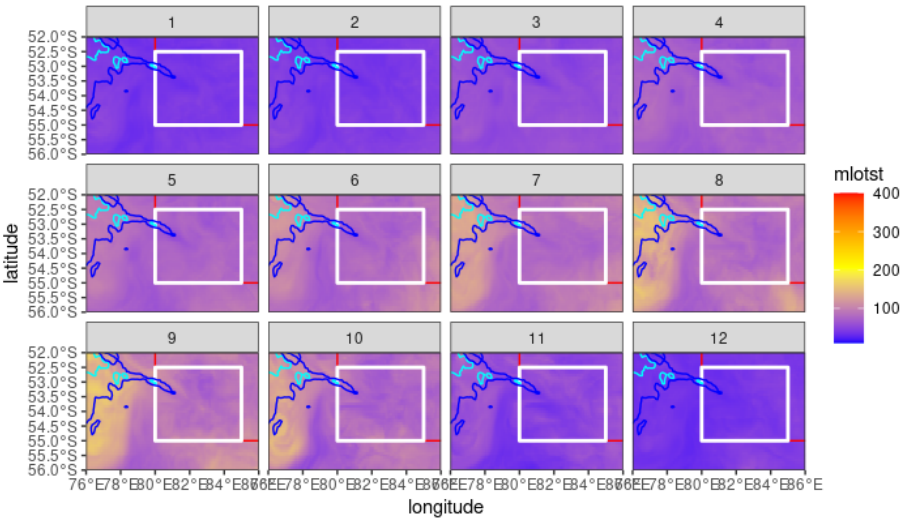


Figure 33. Mixed layer depth (m) in the Williams Ridge area, with the management area indicated by the white polygon and the red polygon delineating the SIOFA convention area with CCAMLR directly to the south and west. Isobaths are indicated at 800 m (cyan) and 1500 m (blue).

4.2.5 Eddy kinetic energy (EKE)

EKE activity is strongest in the SIR, especially in the austral winter (May to July) and the austral summer (November and December) (Figure 34). More EKE activity occurs in the austral spring and summer in the Williams Ridge region, with the strongest activity in the CCAMLR region to the west of the ridge (Figure 35). Winter and springtime cyclonic eddies of the Antarctic Circumpolar Current are related to positive chlorophyll anomalies in the southwestern Indian Ocean and (Frenger et al. 2018).

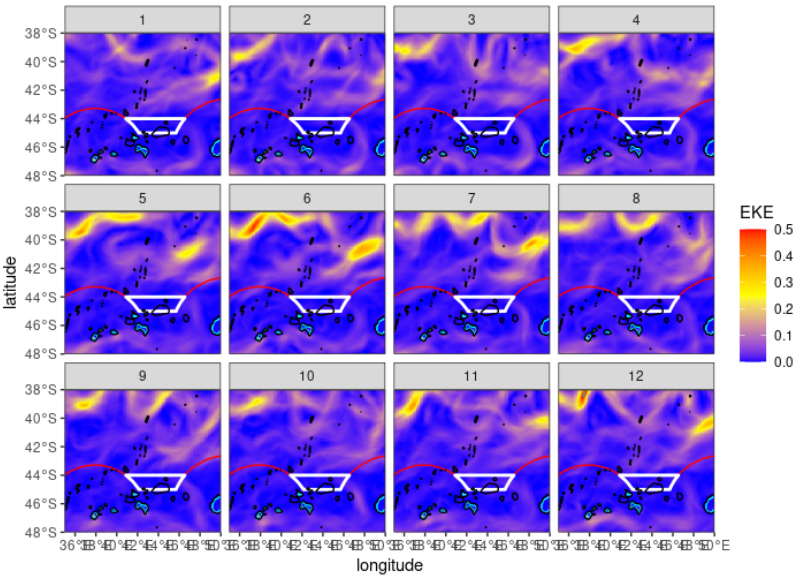


Figure 34. Eddy kinetic energy (EKE) in the South Indian Ridge (SIR) and Del Cano Rise (DCR) regions from January to December, with the management area indicated by the white polygon and the red polygon delineating the SIOFA convention area with CCAMLR directly to the south. Isobaths are indicated at 800 m (cyan) and 1500 m (black).

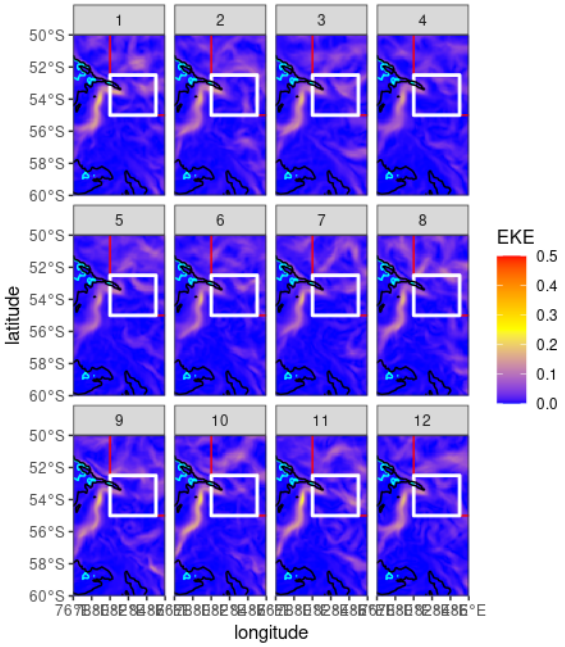


Figure 35. Eddy kinetic energy (EKE) in the Williams Ridge area from January to December, with the management area indicated by the white polygon and the red polygon delineating the SIOFA convention area with CCAMLR directly to the south and west. Isobaths are indicated at 800 m (cyan) and 1500 m (black).

4.2.6 Chlorophyll

Chlorophyll in the DCR region is higher in the fall to spring months (April to November) in the SIR (Figure 36), though the variability is lower than what is seen in other regions in the southern Indian Ocean (e.g. Williams Ridge; Figure 37). This fall to spring chlorophyll peak appears aligned with the EKE in the region (Figure 34), and thus may be driven by mesoscale features associated with variability in the ACC. Chlorophyll in the DCR is relatively constant throughout the year at about 0.25 mg/m3. This region appears to fall between two bands of oceanographic activity that produce chlorophyll. We find chlorophyll to be highly seasonal in the Williams Ridge region, with the highest chlorophyll in the spring and summer, peaking in November. This aligns with the productivity that is generated in October after the winter convective mixing of deep waters (Song et al. 2016).

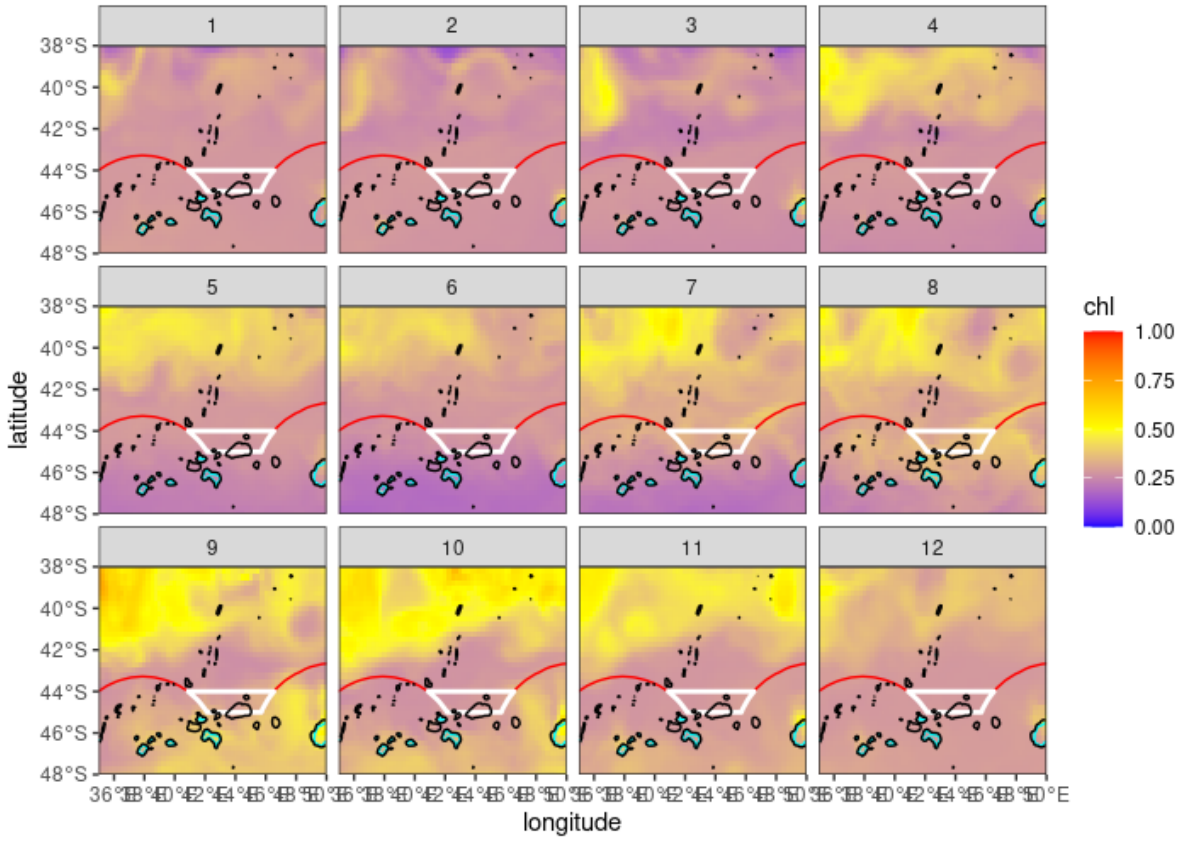


Figure 36. Chlorophyll concentration (mg/m3) in the South Indian Ridge (SIR) and Del Cano Rise (DCR) regions from January to December, with the management area indicated by the white polygon and the red polygon delineating the SIOFA convention area with CCAMLR directly to the south. Isobaths are indicated at 800 m (cyan) and 1500 m (black).

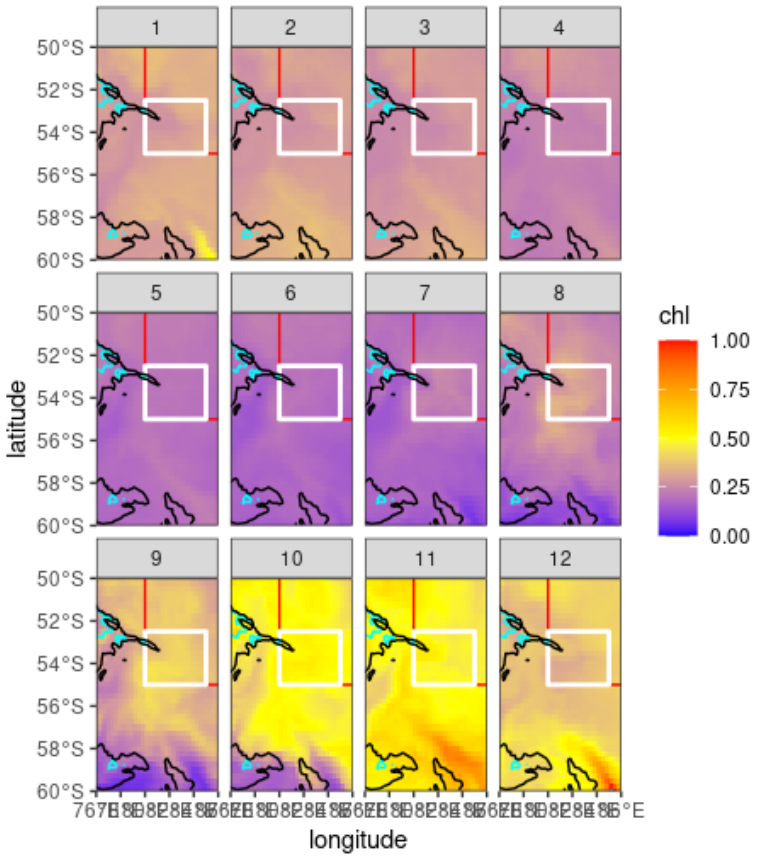


Figure 37. Chlorophyll concentration (mg/m3) in the Williams Ridge area from January to December, with the management area indicated by the white polygon and the red polygon delineating the SIOFA convention area with CCAMLR directly to the south and west. Isobaths are indicated at 800 m (cyan) and 1500 m (black).

4.3 Relationship with environment using Generalized Additive Models (GAMs)

We used spatially-explicit Generalized Additive Models (gams) to investigate the influence of the environment on distribution of different lengths and sex ratios. For the length model, we used length data from observer sampling (n=14727) and explored the environmental variables above, i.e. bottom temperature, bathymetry, EKE, the log(chlorophyll), log(o2 concentration), mixed layer depth, maturity, and sex linked to position (latitude*longitude) and time (month). For the sex ratio model, we used sex ratios grouped by maturity, date, longitude, and latitude (n=510). We explored the same environmental variables as for the length model, and included length data as well.

We assumed a Gamma error distribution and applied a log link function to the GAM of length data. We used a binomial error distribution with a logit link function for the sex ratio data. Models were constructed with all variables included in the first instance and then with the progressive removal of variables we used the minimum value of the Akaike's Information Criterion (AIC; Akaike, 1974) to select the most parsimonious model. We tested interactions between covariates separately, and we used the adjusted r2 to indicate the goodness of fit. The gam.check() function of the *mgcv R* package was used to evaluate the model residuals.

The best model to explain the total length included maturity as a categorical variable, and geographic locations (latitude and longitude) conditioned by month as a smoothed term (spline), slope, bathymetry, and bottom temperature as continuous smooth terms (spline) (Table 6). The AIC between the next competing model was >800. The adjusted r2 was 60.6%.

The best model selected for sex ratio included only the geographic positions (latitude and longitude) conditioned by month as a smooth term (spline), length as a smooth term (spline), and maturity as a categorical variable (Table 6). The AIC between this and the next competing model was >1000, thus we felt this to be strong support for our model selection. The adjusted r2 was 42.6%.

Sex ratio is influenced mostly by length, maturity level, location and season, no other environmental factors were significant. Length was mostly explained by maturity, location and season (month), as well as slope, bathymetry, and bottom temperature. Both slope and bathymetry are static features in time, and bottom temperature shows little variability over the fishing zones (Figures 31,32). Seasonally variable environmental parameters had little or no relationship with length (or sex), indicating that the influence of month on the length (and sex) is likely due to a biologically variable process, rather than a physically variable process.

Table 6. Parameters and fit of the GAM model of total length and sex ratio for Patagonian toothfish. Note that parametric terms have a t statistic and smooth terms have an F statistic estimated.

length ~ maturity + s(lon, lat, by = month) + s(slope) + s(bathy) + s(bottomT); adj r2=0.613				
parametric coefficients	*estimate*	*std error*	*t value*	*p-value*
(intercept)	67.532	0.9123	74.02	<0.0001
maturity2	16.443	0.35	46.98	<0.0001
maturity3	28.9258	0.4777	60.55	<0.0001

maturity4	39.3907	0.7061	55.78	<0.0001
maturity5	34.3335	1.3448	25.53	<0.0001
smooth terms	*edf*	*ref df*	*F*	*p-value*
s(slope)	6.187	6.98	5.224	<0.0001
s(bathy)	6.951	7.711	5.356	<0.0001
s(bottomT)	5.09	5.869	4.651	<0.0001
sex ratio ~s(lon,lat,by=month)+maturity+s(length), adj r2 = 0.459				
parametric coefficients	*estimate*	*std error*	*t value*	*p-value*
(intercept)	0.84923	0.010455	81.228	<0.0001
maturity2	-0.041148	0.009829	-4.186	<0.0001
maturity3	-0.122835	0.012444	-9.871	<0.0001
maturity4	-0.105265	0.01569	-6.709	<0.0001
maturity5	-0.186527	0.017489	-10.665	<0.0001
smooth terms	*edf*	*ref df*	*F*	*p-value*
s(lon,lat):january	5.807	7.041	16.404	<0.0001
s(lon,lat):february	4.7	5.85	23.044	<0.0001
s(lon,lat):march	2.741	3.082	36.096	<0.0001
s(lon,lat):april	3.013	3.456	8.798	<0.0001
s(lon,lat):may	2.001	2.002	12.395	<0.0001
s(lon,lat):june	2.558	2.853	9.998	<0.0001
s(lon,lat):july	2.478	2.729	2.511	<0.01
s(lon,lat):november	5.459	6.528	13.656	<0.0001
s(lon,lat):december	2.898	3.423	18.173	<0.0001
s(length)	1.284	1.523	167.84	<0.0001

5. Summary of data review

Globally, both the SIR and DCR regions have substantial fishing and effort starting from 2003. Fishing operations were recorded at Williams Ridge beginning 2018, however, operations were possibly present at these regions in prior years. While fishing operations occur year-round at DCR and SIR, they were concentrated in the austral winter and summer at Williams Ridge. Notably, the highest number of fishing operations was reported at Del Cano Rise in May, which coincides with the presumed spawning season for *D. eleginoides* at Kerguelen Plateau, also in the southern Indian Ocean (Lord et al. 2006). Most of the

fishing operations occur at the mid-water depth range of 800 - 1500 m, with significant effort in the deep-water range.

Overall, CPUE was lower at DCR and SIR than at Williams Ridge. At mid- and deep depths at DCR and SIR, there was the highest effort in terms of many fishing operations deploying many hooks combined, for less variable and lower CPUEs. At Williams Ridge, at depths <800 m, there were only 8 fishing operations (compared to 58 at the same depth at SIR), but more hooks, exceeding 10 000 hooks on average. The pattern was repeated at Williams Ridge for depths >800 m, in which there was less effort (<300 fishing operations but an average of at least 7000 hooks deployed) but higher CPUEs. Alternatively, the higher CPUEs at Williams Ridge, followed by Eastern, could be a reflection of more abundant fish populations at these regions, whose fishery began more recently than DCR and SIR.

With respect to length distributions, increasing length with weight was seen across the three regions. Further, females tended to be larger and dominated larger size classes at each region, though males were most numerous in the catches from SIR and DCR. At all three regions, mid-sized to larger fish (70 - 100 cm) were the most captured, though few largest individuals (>130 cm) were caught at depths >1500 m, despite length being linked to depth with larger fish found on steeper slopes (Duhamel 1991, Welsford et al. 2011, Péron et al. 2016). Fish of the larger size classes are not necessarily restricted to deeper areas, signaling a more complex relationship between size and could possibly be due to habitat preference or spawning season.

Maturity, measured from stage 1 (immature) to 5 (most mature) was closely linked to length, with a linear relationship between these two factors seen for both sexes at each region. However, size increases for males was limited until stage 3 or 4 at each region, afterwards, more mature male fish tended to be smaller than the previous stage. For females, this pattern was also observed at DCR, though elsewhere, females were, on average, larger with each successive maturity stage. At all three sites, females and males of maturity stages ≤3 (corresponding to ~90 cm) were most numerous in 800 - 1500 m, throughout the year (Del Cano Rise) or austral summer months (Williams Ridge). More mature fish of both sexes in mid-water during a presumed spawning period could indicate that the mature fish are spawning, however a later study at South Georgia and Shag rocks in the Atlantic sector did not observe a consistent pattern of depth distribution during spawning (Brigden et al. 2017), so it is currently unclear when this is occurring at SIR, DCR and Williams Ridge, nor if the months leading up to the austral summer are indeed the spawning period at these regions.

We note that several aspects of the data reviewed indicate a close connection between the CCAMLR and SIOFA areas. We find that the bathymetric features straddle the CCAMLR and SIOFA zones that would facilitate ontogenetic migration. We note that few settlement areas (shallow, nearshore) are available in suitable latitudes in the SIOFA area, indicating that settlement may occur in CCAMLR and ontogenetic migration may bring individuals into the deeper adult habitat in the SIOFA region. This appears to be supported with length frequency data that indicate that juveniles and smaller individuals in the adjacent, shallower CCAMLR regions and larger, more mature individuals in the deeper SIOFA waters. Furthermore, spawning individuals appear to concentrate near the border of the SIOFA and CCAMLR areas in DCR and Williams Ridge, indicating that the population straddles the two zones. Tagging studies show some movement between the Kerguelen Plateau and Del Cano Rise, and between the Kerguelen Plateau and Williams Ridge. The data review points to the conclusion that the fishing areas in SIOFA are likely fishing the same population as in the adjacent CCAMLR region.

The three fishing sites are distinctly defined by their bathymetry, with DCR being relatively flat throughout the management area, SIR defined by relatively shallow ridgelines separated by deep canyons, and Williams Ridge extending from CCAMLR into SIOFA as a narrow, shallow ridge. Bottom temperature of >2°C was common throughout the greater DCR area, and only found along the shallow ridge at Williams Ridge. Mixed layer depth was found to have patterns in line with deep winter convective mixing in the Williams Ridge area that then also showed a peak in chlorophyll in October, as described by Song et al. 2016. Mesoscale features appear to be influential to local productivity for SIR. We found; however, that stable physical features such as bottom depth, slope, and bottom temperature are significantly related to both length and sex ratio, while more seasonal and temporary features (e.g. productivity patterns and mesoscale features) do not appear to be of influence.

6. Proposed methods to determine population structure

6.1 Sampling strategy

6.1.1 Mixed-sex spawning distribution

Synthesizing the information reviewed above, we conclude that to ensure that sampling is performed over the most representative sample of the full population, we recommend targeting mixed-sex spawning grounds.

To identify where and when these mixed-sex spawning grounds could be targeted, we therefore examined the spatial sex ratio of spawning individuals (maturity stage 4; Table 1) in the different fishing grounds (n=204 in SIR, n=450 in DCR, and n=309 in Williams Ridge), calculating the sex ratio at a spatial resolution of 0.1° x 0.1°. We define "well-mixed" sex ratios as those between 0.4-0.6; 'male-dominated' as <0.4 and 'female-dominated' as >0.6. We describe the stable habitat features (i.e. bathymetry, slope) where mixed sex ratios are most commonly distributed, as these features have been shown to be important in influencing the sex ratio (see relationship with environment using Generalised Additive Models) and because they can be easily targeted by observers.

Though previous studies have not shown evidence for spawning aggregations in the Indian Ocean, this study indicates that *D. eleginoides* appear to have a seasonal pattern to the sex ratio of spawning individuals in that they mix on the shallower, flatter areas of the DCR from November to March (Figure 41). Few spawning individuals are found in SIR (see Table 1, maturity stage 4), and they are generally female when they are found (e.g. December, January, March). Similarly, in Williams Ridge, few locations indicate well-mixed sex ratios, but those that are observed in the northeast near the CCAMLR border and southwest at the edge of the ridge.

There are insufficient well-mixed sex ratios to draw conclusions on their distribution in time and space, thus we present the distribution of spawning individuals. We find that spawning individuals are most frequently found in the austral summer (November to March; Figure 40 left) in all three fishing areas. Spawning individuals are more often found <2000 m depth. Spawners are more often on flat zones for all three fishing areas, including SIR (<0.1; Figure 40), despite the habitat made up of shallow ridges divided by deep canyons.

Table 7. Number of spawning individuals (maturity level 4, see Table 1) per site and depth range.

Depth range	SIR	DCR	Williams Ridge
<800 m	1		3
800 m - 1500 m	158	132	209
>1500 m	46	318	97
Total	**204**	**450**	**309**

We therefore conclude that to have the highest likelihood of genetically discriminating between populations, the sampling should be performed in the three fishing zones (SIR, DCR, and Williams Ridge and target spawning individuals from November to March in flat areas (<0.2 radians) of >800 m and <2000 m depth (Figure 41). The number of spawning individuals may be limited (Table 7), and we note that while samples from spawners would have the highest likelihood of capturing a single population, targeting the habitat and season where and when spawners occur should also lead to an increased probability of representative sampling for a single population.

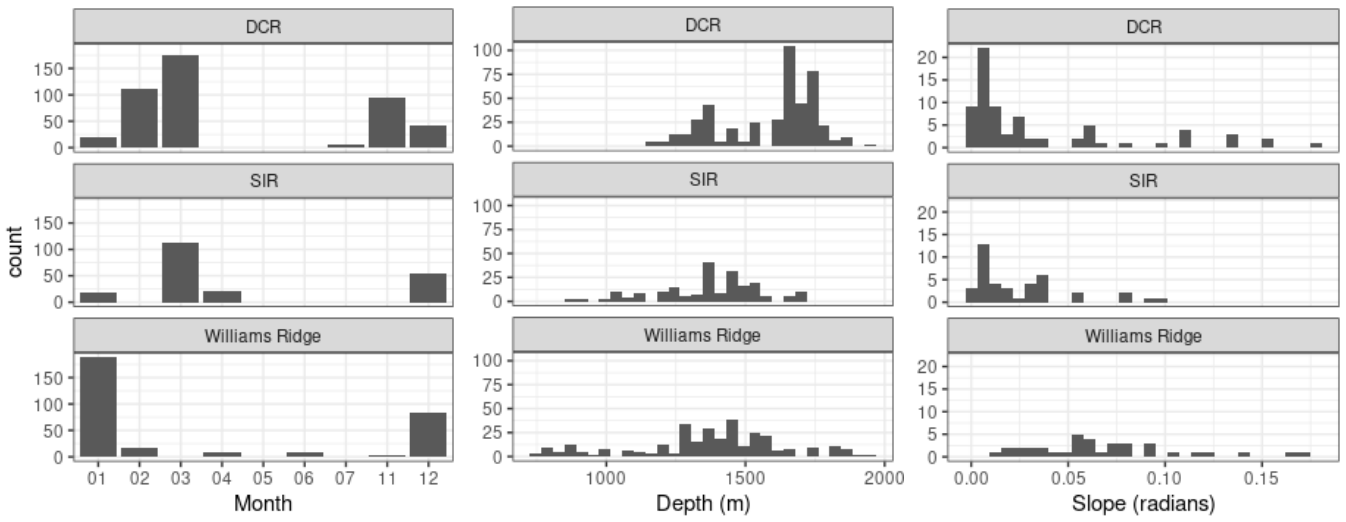


Figure 40. Frequency of spawning individuals in the DCR (top), SIR (middle) and Williams Ridge (bottom) fishing areas for months (left column), depth (m; middle column), and slope (radians; right column).

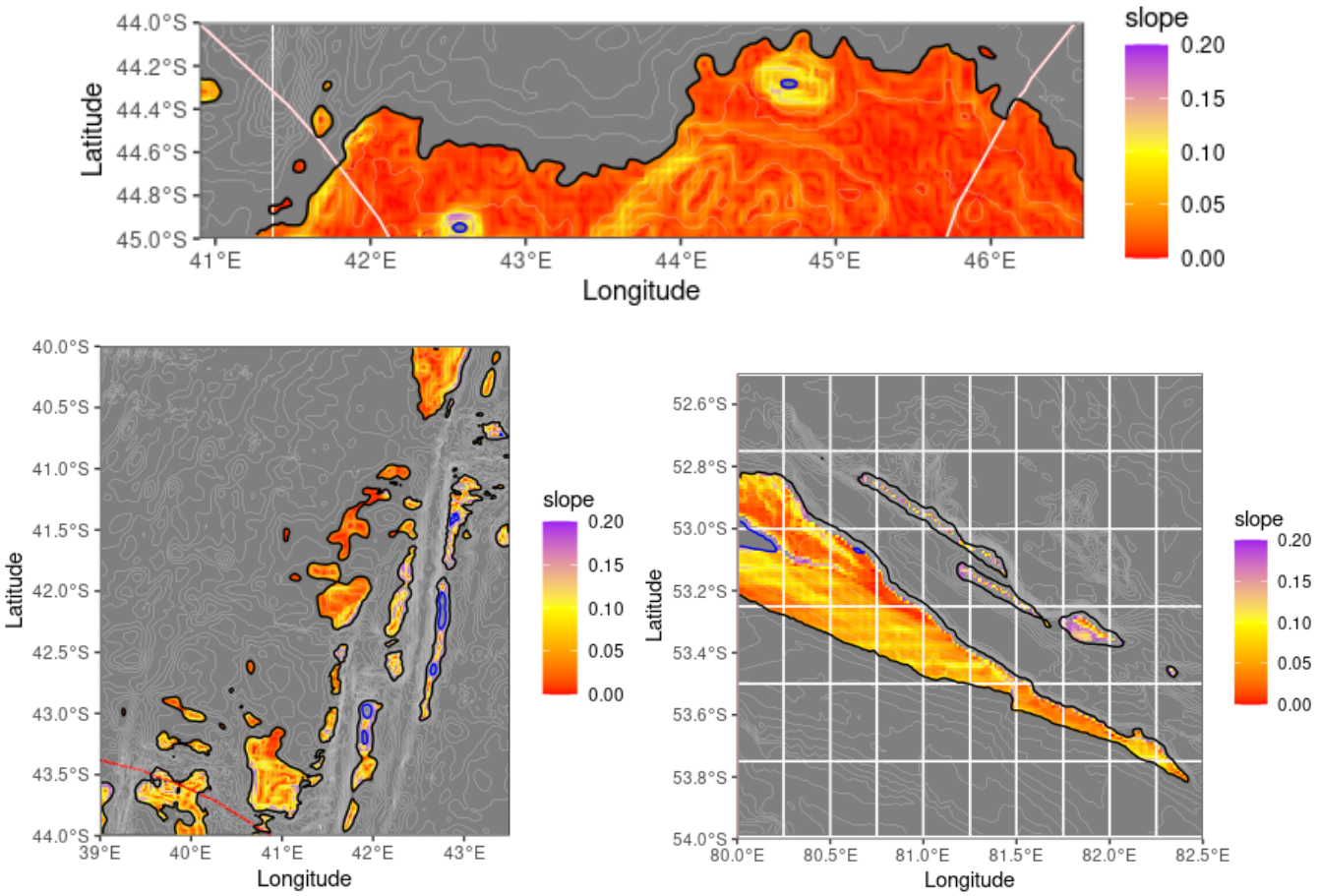


Figure 41. The recommended sampling sites are colored by slope <0.2 radians and bathymetry between 800 m (blue contours) to 2000 m (black contours) depth for each fishing zone in the SIOFA area: DCR (top), SIR (bottom left), and Williams Ridge (bottom right). The DCR and Williams Ridge management areas are indicated in white polygons and the SIOFA boundary is indicated by the red polygon for SIR.

6.2 Genetic analyses

6.2.1 Single Nucleotide Polymorphisms

Toomey et al. (2016) found evidence that populations in the southwest Indian Ocean are not fully panmictic (Table 3). This suggests that a finer level of differentiation may exist in this region which would require additional study to confirm. We recommend that a dataset composed of Single Nucleotide Polymorphisms (SNPs) loci should be generated for *Dissostichus eleginoides* in the southwest Indian Ocean. First, hundreds to thousands of SNP loci can be recovered, compared to microsatellites markers, which provides a more representative sample of the entire genome and a possibly clearer resolution of population dynamics (Morin et al. 2004). We recommend analyzing the samples using a "reduced representation approach" such as RADseq or GBS, described above, for SNP discovery in individuals of *D. eleginoides*. RADseq and GBS are commonly applied methods that enable

relatively low-cost discovery of SNP loci for non-model organisms when combined with high-throughput DNA sequencing technologies (Baird et al. 2008, Andrews et al. 2016). There are companies that provide RADseq services, such as GenoScreen in France and CD Genomics in the USA.

6.2.2 Sample numbers

Additionally, Toomey and colleagues (2016) state that "to further examine these differences, it is necessary to sequence more individuals and have larger datasets (at least 100 individuals per location)." Within their study, they amassed a dataset of 30 - 100 individuals per Indian Ocean location (Table 3). We therefore recommend that the number of samples to be collected should aim for 100 per region, if this fits within the confines of the project budget. Further, there is evidence that males and females may exhibit differing migratory behaviors and that this can result in specific patterns of dispersal depending on sex, ultimately influencing the genetic structure of the species (Appleyard et al. 2002, Shaw et al. 2004, Rogers et al. 2006, Toomey et al. 2016). We therefore recommend striving to collect a 1:1 ratio between females and males (50% female and 50% male) to help determine if we identify any differing patterns linked to sex, as they relate to defining potential population structure.

Based upon review of the catch, effort and biological data held by SIOFA (Catch and biological data review), as well as a project presentation to the SIOFA Secretariat and the Scientific Committee, the geographic sampling regions proposed are 1) South Indian Ridge, located in SIOFA's statistical subregion 3b, 2) Del Cano Rise South located in SIOFA's statistical subregion 3b and 3) Williams Ridge, located in SIOFA statistical subregion 7. These three sampling regions have been proposed as they represent established *D. eleginoides* fisheries, which have regular fishing operations mostly through Spanish and Australian fleets, as well as large catch sizes, as seen within the years 2003 - 2022. The large sample numbers able to be obtained from these locations are needed to aid in deriving meaningful information from population genetic analyses. Another region, Eastern, is also located in SIOFA's statistical subregion 7. However, due to the lack of regular fishing operations that visit this region, we do not suggest Eastern be considered at this time for inclusion in the sampling design, though it is possible that the flag states fishing in this region have well-preserved samples that can be used for the sequencing analyses. A well-preserved sample refers to one that has experienced minimal degradation, minimal contamination, is of adequate quantity, that has undergone optimal collection and preparation protocols and has been properly stored using the appropriate fixative technique for the sample type (Dessauer et al. 1996). For tissue or fins, for example, these samples

should have been sampled while the organism was still alive or soon after death as possible to reduce degradation of the sample. These samples should have been collected using clean tools such as forceps and scalpels to prevent cross-contamination, and in a quantity sufficient for downstream DNA processing, which would be approximately 25 - 50 mg. The samples should have been stored in rigid, plastic and sealable tubes, then placed in a cold environment, or at least away from light, as soon as possible to reduce contamination and prevent further degradation. The most optimal fixation method for these samples would be frozen storage, primarily by flash freezing (liquid nitrogen), but also by storage in -80 or even -20 C conditions, as the freezing method typically provides the highest yield of animal DNA (Dessauer et al. 1996). However, cryopreservation is not absolutely required for DNA studies. Tissue that was submerged and saturated in 95% ethanol (or even 75% ethanol) for two hours and then replaced with fresh ethanol of the same concentration for longer term storage is also acceptable for DNA studies (Dessauer et al. 1996). If samples have been frozen or preserved in alcohol, they tend to be viable longer (timescale: years), as these processes cause less DNA shearing. Younger samples (< 5 - 10 years) are preferred, but viability of younger samples may also depend on the full collection and preservation process (e.g., were the samples moved from one freezer to a cooler freezer, were they transported in light, etc.).

As noted in the proposed sampling strategy, sampling should occur between the November to March season, where spawners appear to be mixed and more abundant. In recent years, we find that biological sampling is especially high in the austral summer in DCR and Williams Ridge, and in the early autumn in SIR (Figure 42). Further, we note that there is much effort in this period during recent years (Figures 7,8,10,11); thus we find that sampling from November to March is feasible, though sampling can only occur when and where feasible for each individual vessel. Further, spawning should occur in flat areas (<0.2 radians) at depths >800 m and <2000 m, where spawners appear to be more abundant (Figure 41). We note that there may be a trade off between the number of samples that should be taken and the restrictions on the times, depths, and maturity stages over which samples are taken.

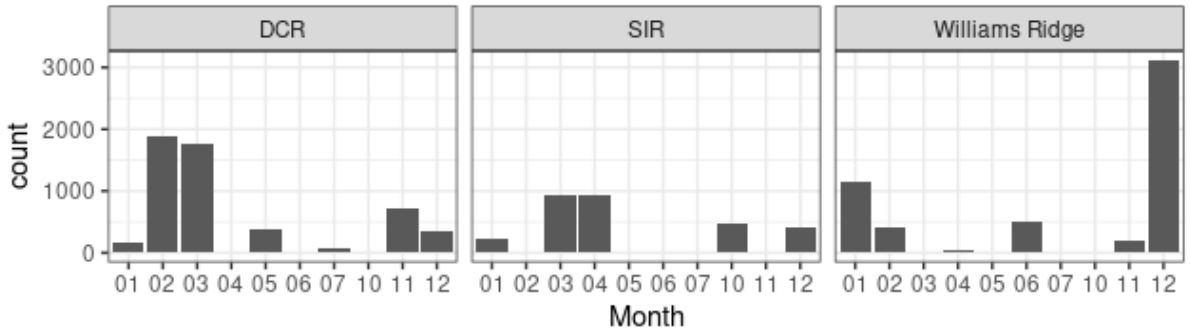


Figure 42. The number of biological samples taken per month per site, summed over all years.

A sequenced, assembled and annotated genome for *D. eleginoides* produced Ryder et al. 2022 is currently available on the National Center for Biotechnology Information (NCBI) under BioProject accession PRJNA864592. This genome was produced by a team from Centre for Environment, Fisheries and Aquaculture Science (CEFAS) in Weymouth, Dorset, United Kingdom and should help facilitate SNP discovery in this species during the sequence data analysis. As few population genetics studies employing SNPs for *D. eleginoides* exist, we plan to use two recent publications as reference: Arkhipkin et al. 2022, who examined the genetic separation of *D. eleginoides* on either side of the Antarctic Polar Front (APF) in the southern Atlantic and Maschette et al. 2023, who identified high genetic connectivity of its sister species, *D. mawsoni*, around the Antarctic continent.

It should be noted that SNPs can potentially detect population structure within smaller sample sets, however, SNP loci are highly subjected to ascertainment bias, defined as when the selection of loci from an unrepresentative sample of individuals yields loci that are not representative of the allele frequencies in the population and can skew population assessments (Morin et al. 2004). Fortunately, ascertainment bias can be reduced by identifying SNPs from a large sample set of individuals across all target "populations" (regions). Further, a SNP loci panel developed for *D. eleginoides* in the southwest Indian Ocean could be implemented on additional sampled individuals from the same locations, noting that biases can still arise when transferring SNPs across population or between studies (Morin et al. 2004, Fitak et al. 2016).

7 Project feasibility

7.1 Sample numbers

We contacted several companies and academic sequencing facilities who provide genomic services for reduced representation genomic approaches such as RADseq and GBS. Ultimately, we received quotes from three companies: GenoScreen in Lille, France, CD Genomics in Shirley (NY) USA and the University of Minnesota in Minneapolis USA. The representatives at each facility provided us with costs for a workflow which includes DNA extraction > library preparation > DNA sequencing > genotyping analyses (Tables 6-8). For GenoScreen (Table 8), the costs are based on the number of well plates that would contain a maximum of 95 samples, while for CD Genomics and Minnesota, the costs are based on sample number. It should be noted that GenoScreen, like most sequencing facilities, must submit DNA extracts to a service provider who is licensed by KeyGene, the company that

holds the patent for the RADseq technology. The service provider that GenoScreen works with is Floragenex, and therefore, GenoScreen requires an additional shipping cost to Floragenex. CD Genomics and Minnesota are licensed by KeyGene and therefore can perform the entire workflow on site and with no additional shipping costs.

CD Genomics (Table 9) and Minnesota (Table 10) have the most competitive pricing for performing the full workflow at approximately 97.40 € and 70.96 € respectively per sample compared to GenoScreen at approximately 117 - 243 € per sample. Based on costs detailed in the TOP-2 budget (Table 11) and the Tables 6-8, we would be able to submit 65 samples through CD Genomics and 90 samples to Minnesota and remain under the budget allocated for SER2022 of 34 000 €. This would mean that 20 - 30 samples per region could be submitted, instead of 100 samples per region (Toomey et al. 2016), as initially suggested. It should be noted that CD genomics provides more data (~6 million sequencing reads per sample and 25x coverage) than Minnesota (~2 - 4 million sequencing reads per sample with 10x coverage). A lack of sequences and coverage can lead to problems producing SNP databases, such as an inability to call polymorphisms and yield homozygous bias in called polymorphisms, therefore, 20 - 30x coverage should be targeted (Rivera-Coloìn and Catchen 2023). The number of samples allowed for by the present SIOFA budget is likely not sufficient to precisely define population structure across the SIOFA regions proposed. The TOP-2 project should therefore be considered as a preliminary, or even a pilot project. Overall, depending on the results of the TOP-2 study, our ability to recommend management units will likely be limited.

A recent study in preparation for publication and led by Chris Darby (CEFAS) analyzed the population structure of Patagonian toothfish throughout the Southern Ocean. We are attempting to contact the project team to determine whether it would be possible to include our samples in their analyses, thereby vastly reducing costs.

Furthermore, it has come to our attention that members of the Scientific Committee may have access to *D. eleginoides* samples stored in ethanol, which may be acquired for processing and aid in the elucidation of population structure in the southwest Indian Ocean. We would need to interact with the committee members to identify the status of these samples (tissue type, date of recovery, origin of recovery, amount of tissue type) to determine if they would be suitable for RADseq processing. However, given the budget constraints of TOP-2, freshly obtained samples should be prioritized for analyses and previously collected samples may be used for future population structure studies for *D. eleginoides*. If our goal of 30 samples minimum is not reached, we could coordinate with the scientific committee to obtain these previously collected samples.

Finally, we note that we have a contact at the armament Cap Bourbon, based in Le Port, La Réunion, who sells *D. eleginoides* from FAO major fishing areas 51 and 58. With the approval of the SIOFA SC, we would like to approach this contact with the delineations of the sampling sites to determine whether he already has samples available that can be exploited for this project.

Table 8. Quote provided by GenoScreen for performing RADseq (GBS) approach to generate a SNP-loci database for *D. eleginoides*. One plate can hold a maximum of 95 samples. Low vs. high density lane refers to the number of sequencing lanes needed to process all sample libraries; more lanes indicates a higher number of DNA fragments have been produced for sequencing, and thus, more SNP loci to be obtained.

GenoScreen workflow	1 plate (95 samples)		2 plates (190 samples)		4 plates (300 samples)	
	~25 mg per sample for submission					
	2 x 150-bp (paired-end) sequencing on NovaSeq 600 - 25x coverage					
	Turnaround time: 12 - 20 weeks					
	Low-density (single lane)	*High-density (triple lane)*	*Low-density (single lane)*	*High-density (triple lane)*	*Low-density (single lane)*	*High-density (triple lane)*
DNA extraction + QC + homogenization	€ 2 000 (per plate)	€ 2 000 € (per plate)	€ 2 000 € (per plate) x 2	€ 2 000 € (per plate) x 2	€ 2 000 € (per plate) x 4	€ 2 000 € (per plate) x 4
Shipment to Floragenex	€ 450	€ 450 €	€ 450 €	€ 450 €	€ 450 €	€ 450 €
Library prep + QC + sequencing	€ 7 720 (per plate)	€ 15 960 € (per plate)	€ 7 720 € (per plate) x 2	€ 15 960 € (per plate) x 2	€ 7 720 € (per plate) x 4	€ 15 960 € (per plate) x 4
Standard genotyping analyses	1 000 € (per plate)	€ 1 000 € (per plate)	€ 1 000 € (per plate) x 2	€ 1 000 € (per plate) x 2	€ 1 000 € (per plate) x 4	€ 1 000 € (per plate) x 4
Total estimate:	€ 11 170	€ 19 410	€ 21 890	€ 38 370	€ 43 330	€ 69 090
Cost by sample	€ 117.60	€ 204.30	€ 115.20	€ 201.90	€ 152.03	€ 242.40

Table 9. Quote provided by CD Genomics for performing RADseq approach to generate a SNP-loci database for *D. eleginoides*. Samples are submitted in tubes. Prices are approximate as they have been converted from USD to EUR with a rate of 0.94 euros.

CD Genomics workflow	90 samples	300 samples
	>50 mg per sample for submission	
	2 x 150-bp (paired-end) sequencing on Illumina - 25x coverage	
	~6 million reads per sample	
	Turnaround time: 8 - 10 weeks	
DNA extraction + QC	20.59 €	18.72 €
Library prep + QC + sequencing	58.03 €	58.03 €
Standard genotyping analyses	18.72 €	18.72 €
Total estimate:	8 760.60 €	28 641 €
Cost by sample	97.34 €	95.47 €

Table 10. Quote provided by the University of Minnesota for performing RADseq (GBS) approach to generate a SNP-loci database for *D. eleginoides*. Samples are submitted in tubes. This facility requires a service agreement to be submitted for international customers. If submitting only 90 samples, the samples will be held until a full flow cell is filled; this may delay processing. Cost minimum and maximum are the same except for sequencing, in which the number of lanes may change depending on how many fragments are produced. Note that if processing 300 samples, six blanks (negative controls) will be added to the DNA extraction and library creation steps. Prices are approximate as they have been converted from USD to EUR.

University of Minnesota workflow	90 samples		300 samples (+6 blanks)
	25 mg per sample for submission		
	2 x 150-bp (paired-end) sequencing on Illumina - 10x coverage		
	2 - 4 million reads per sample		~2 million reads per sample
	Turnaround time: 12 - 20 weeks		
	Cost minimum	Cost maximum	Cost minimum & maximum are the same
DNA extraction + QC	1 056.07 €	1 056.07 €	3 590.62 €
Library creation	2 089.08 €	2 089.08 €	7 102.88 €
Library QC	183.92 €	183.92 €	183.92 €
Sequencing QC	1 001.09 €	1 001.09 €	1 001.09 €
Sequencing (number of lanes)	1 782.98 € (0.5 lane)	3 565.97 € (1 lane)	7 131.93 € (2 lanes)
Bioinformatics - analysis	269.86 €	269.86 €	269.86 €
Bioinformatics - sample	404.15 €	404.15 €	1 347.18 €
Labor for sample prep	195.90 €	195.90 €	783.61 €
Total estimate:	6 983.05 €	8 766.04 €	21 168.09 €
Cost by sample	77.59 €	97.40 €	70.56 €

7.2 Estimated budget

Below we provide a detailed budget, estimating the costs for 90 samples (i.e. 30 samples x 3 sites) with 6 million reads per sample. This represents the minimum recommended number of samples for population discrimination studies, and is the cheapest option found at 8 760.60 € with the quickest turn-around time (max 3 months).

We include the costs of the consumables and equipment needed to collect the genetic samples for observers and to prepare the samples for onward genetic analyses.

COOOL team costs are associated with the time required for our team to perform project coordination, observer training, protocol preparation and printing, sampling kit preparation; sample preparation in the lab; data review and analysis, and report writing.

To analyze the minimum recommended number of samples, i.e. 30 per site, the current budget allocated by SIOFA to the SER2022 TOP2 project, i.e. 34 000 € is about 2 400 € too low (Table 11). We will continue to search for lower-cost alternatives for the sample processing, but we also request that additional funds be allocated to allow for this minimum recommended sample processing. If no further funding can be allocated, CD Genomics will allow us to send less than 90 samples and will charge us a price-per-sample of 97.34 €. Therefore, the number of samples that can be processed at budget would be about 65, or 20-22 samples per each of the three sites.

Table 11. Estimated budget for the SER2022 TOP2 project.

Item	Cost before tax and shipping(€)	Quantity	Sites	Total before tax and shipping (€)	Comments	Reference
DNA extraction, shipment to facility for library preparation, DNA sequencing, genotyping analyses, all QC steps sequencing	8761	1	3	8761	This is the price for total processing of 90 samples, with 6 million reads (30 samples x 3 sites = 90 samples), the cheapest option with the fastest processing time. The number of samples (price per sample) can be scaled based on needs/budget limitations	link
Well plates	325	1	3	325	2ml well plate, rounded bottom. This is for the minimum quantity, 30 plates per pack. We need 3 for each region.	link
Strip caps	132	1	3	132	This is for sealing the plates. The	link

Item	Cost before tax and shipping(€)	Quantity	Sites	Total before tax and shipping (€)	Comments	Reference
					minimum quantity, case of 120. We need only 8 for one plate.	
Tubes	€49.60	1	3	€49.60	Each bag contains 1000 1.5-ml vials	link
ice-pack shipping (to la reunion)	€100.00	1	3	€300.00	3 shipments from different CPs to COOOL, using ice-packs to keep samples frozen	
ice-pack shipping (to company)	€95.00	1	1	€95.00	One package from La reunion to metropole with the plate, and ice packs to keep samples frozen	
printing costs - protocols	€3.00	3	5	€45.00	3 observers per 5 different boats	
50mL tubes	€65.50	2	3	€65.50	Two boxes of 100 tubes. These are used to aliquot absolute ethanol for adding ethanol to samples. Also to potentially store samples for transport to La Reunion.	link
Parafilm	€50.04	1	3	€50.04	One roll; to wrap tubes to prevent leakage from cap.	link
Labels	€63.50	1	3	€63.50	1 packet of 480 labels	link
Absolute Ethanol	€54.38	1	1	€54.38	For preserving the samples. This is the price for 1L. We need 500ul per sample. < 100 samples = 50 ml	link

Item	Cost before tax and shipping(€)	Quantity	Sites	Total before tax and shipping (€)	Comments	Reference
					of ethanol; 1L is the most adapted volume for purchasing.	
70% Ethanol	€30.00	1	1	€30.00	5 L - needs to be in squeeze bottles	link
10% Bleach	€16.90	1	3	€16.90	20 L - needs to be in squeeze bottles; this is the most adapted size.	link
1 ml transfer pipettes	€288.62	1	3	€288.62	Each box comes with 200 pipettes; one pipette each sample to avoid cross contamination	link
Disposable gloves	€15.45	2	3	€17.45	Medium, large Nitrile	link
Paper towels	€2.45	1	3	€2.45	Pack of 3	link
Zip bags	€1.80	1	3	€1.80	1 box, one bag for the plate	link
Weigh boats	€73.20	1	3	€73.20	Plastic weigh boats for measuring tissue; count for each pack is 500, which is the most adaptable size.	link
Wash bottles	€21.10	3	3	€63.30	One 750-ml bottle for 10% bleach, one for 70% ethanol. The bottles come in packs of two, so three are needed for three sites	link
JC	€2,900.00	1	n/a	€2,900.00	observer training, protocol preparation and printing (2 weeks),	

Item	Cost before tax and shipping(€)	Quantity	Sites	Total before tax and shipping (€)	Comments	Reference
					sampling kit preparation (1 week)	
AE	€5,200.00	2	n/a	€10,400.00	observer training, project coordination, data review, environmental analysis, management units, report writing	
DC	€4,900.00	2	n/a	€9,800.00	literature review, data review, bioinformatic analysis, spatial structure, management units, report writing	
Subtotal				€33,534.74		
TVA (8.5%)				€2,850.45		
Total				€36,385.19	€2,385.19	

7.3 Timing

The recommended sampling season is between November to March. From discussions with the scientific committee, this will align with the planned fishing for the austral summer season. However, one of the two target vessels will be leaving port in August. Therefore, we must finalize the sampling strategy, prepare the materials, and train the observers before this time.

Samples will be collected from November 2023 to March 2024 (Table 12). We expect that samples will be returned to us in Reunion Island by the end of April 2024. We will prepare the samples and ship them to the selected sequencing company such that they arrive by the end of May 2024.

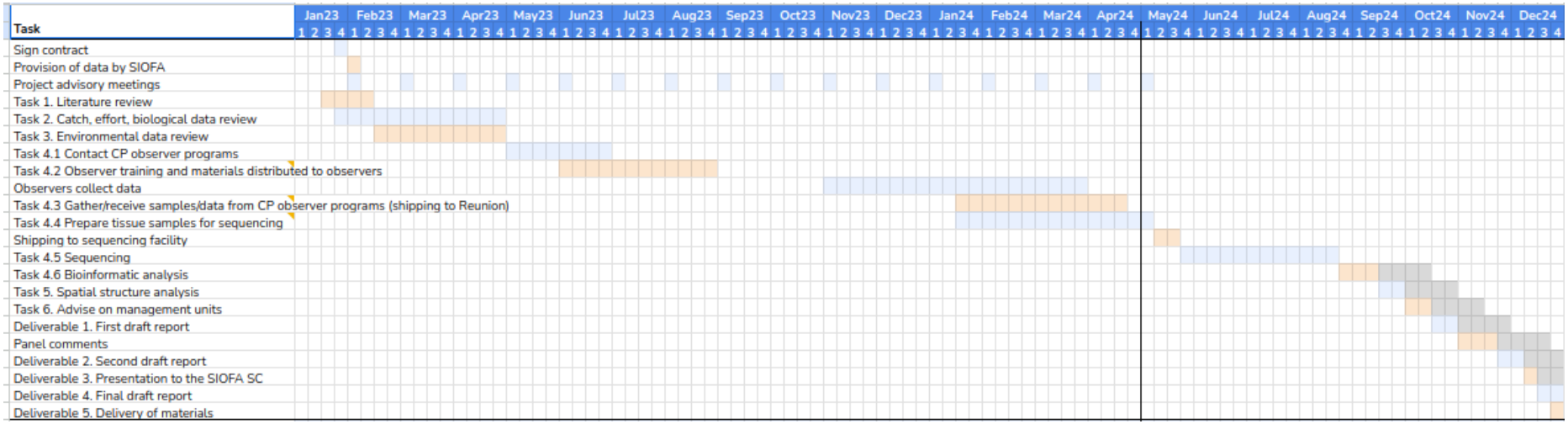
Based on estimates from the three facilities above, the time delay for sample processing can take 2 - 5 months from the reception of the samples to the data output depending on the facility chosen (Tables 6-8). Given the limitations of the sampling season and the

project timeline, we are making our best efforts to choose the most timely and cost efficient company. At the moment, CD Genomics is the most competitive, with a turnaround time of 10 weeks (< 3 months) maximum. It should also be noted that while Minnesota estimates 3 - 5 months turnaround time. Additionally, if we submit only 90 samples to Minnesota, this is not enough to fill a full flow cell on a sequencer. The sequencing run will not proceed until the flow cell is filled, likely with projects from other teams, which may delay our results.

After the data have been generated, the sequencing facility will perform initial analyses on the raw sequencing output, which include sequence quality assessment and read mapping to the *D. eleginoides* reference genome to help identify SNP loci, leading to a final output file that can be used for statistical testing for potential population structure (estimated delivery by end August). We will require approximately 4 months to perform these additional data analyses and generate the final report based on these analyses. This time period also considers that the final report is only submitted after a series of report drafts are examined by the scientific committee for feedback. Therefore, we plan to deliver the final draft, all materials, code, data and reports by the end of December 2024 (Table 12).

We understand from the SIOFA Secretariat that this timeline will fit into the project schedule, and is thus considered feasible.

Table 12. The proposed timeline for the updated sampling strategy.



8. Sampling protocol

We include here the sampling protocol that will be used to communicate with the onboard observers, the lab protocol for preparing the samples for shipping to the sequencing company, and the shipping protocol for sending the samples between facilities. The lab and shipping protocols are subject to change based on the sequencing company finally selected, as protocols may be company-specific.

8.1 Observer protocol

We request that observers collect samples on biometrics as well as taking genetic tissue samples (fin clip) to assist in subsequent population discrimination analyses. We have attempted to align this protocol with the CCAMLR observer manual.

Observers will be asked to measure the fish to the nearest cm for total length, weight (nearest gram), and note the location and date of capture, vessel name and registration number, sex, and maturity stage.

We will ask observers to collect 2 sets of fin clips for each fish to assure the sample processing. Gloves should be worn and tools (forceps and scissors) should be sterilized by rinsing with the 10% bleach solution, followed by rinsing with 70% ethanol. A clean paper towel should be used to dry the tools. The fin clips should be completely submerged in 95% to absolute ethanol and they should not touch the sides of the 5 mL tube. The ethanol should be decanted and replaced after a few days, as water from the fin clips will dilute the ethanol after several days of submersion. We will stress the importance of sterilizing their tools between each fin clip to prevent contamination between the individuals sampled.

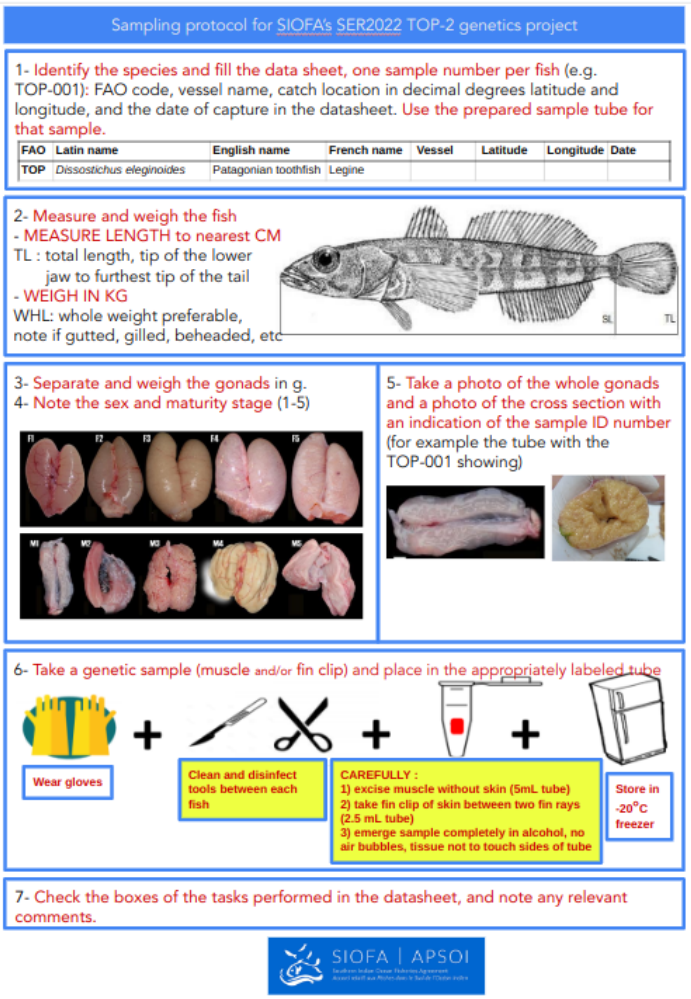


Figure 43. Screenshot of a draft of the simplified observer sampling protocol.

8.2 Lab protocol

Dissostichus eleginoides samples should be prepared using the steps described below. The final packaging and shipment details for these samples must be in accordance with the facility that will perform their processing and sequencing.

8.2.1 Supplies

- Nitrile gloves
- Absolute alcohol (for preservation)
- 70% ethanol (squeeze bottle)
- 10 % bleach solution (squeeze bottle)
- 1.5 ml cryovials
- Plastic weigh boats
- Parafilm

- Paper towels
- Ice bucket
- 1.0 ml transfer pipettes
- Double zip sandwich bags (6 l)
- 50 ml tubes
- One cryobox
- Marker
- Large rubber bands
- Sample labels
- Sample list

8.2.2 Plate protocol

1. Don gloves.
2. Decontaminate the workbench using 10% bleach solution and wipe dry, followed by thorough rinsing with 70% ethanol and wipe dry.
3. Fill a 50 ml tube with absolute ethanol.
    - This will be the aliquot used to fill the plated samples using a pipette.
    - Do not place a pipette in the ethanol bottle, only the 50 ml tube.
    - When the tube is empty, discard it and fill another 50 ml tube with absolute ethanol to continue processing.
4. Prior to handling the sample, disinfect cutting tools.
    - Sterilize forceps, scalpels and scissors by rinsing with the 10% bleach solution, followed by rinsing with 70% ethanol.
    - Use a clean paper towel to dry tools.
5. Prepare a bucket of ice to place the cryovials.
6. A portion of fin (sample) weighing at least 50 mg should be extracted using the tools.
7. Carefully place the sample on a new and clean weight boat on the balance.
8. Record sample name and weight.
9. Carefully place the sample securely in the first cryovial.
10. Use a new transfer pipette to add enough absolute ethanol to the cryovial to fully submerge the sample.
    - The volume to be added is likely between 0.5 - 1 ml
11. Close the cryovial tightly by screwing the cap
12. Wrap a strip of parafilm around the cap of the cryovial to help prevent leakage.
13. Place the cryovial on ice.
14. Discard the weigh boat and the pipette and decontaminate all handling tools before moving on to the next sample.

15. Repeat steps 6 - 13 for the subsequent samples.
16. Once all samples have been collected and closed, place them in the cryobox and close its lid.
17. Secure the cryobox by using two large, thick rubber bands looped over the box.
18. Place the box in a zip lock bag, close and immediately place the bag into the -20 °C freezer until ready for shipping.
19. Decontaminate workbench after use.

8.2.3 Notes

- Always sterilize tools between each individual.
- Do not put a used pipette tip back into any ethanol container.
- Switch a new and clean weight boat and pipette for each individual.
- Be sure to keep the cryovials with samples on ice during the processing.
- Change your gloves if they have been soiled (e.g., a piece of tissue or blood has fallen/spilled on them).
- Be sure to correctly match the sample name and its associated well on the list.
- If writing the sample name on the tube instead of placing a label, do not write the sample name and other information directly on the tube wall or tube cover with an oil pen. It is better to write sample names at the top and the side of each tube with black permanent marker.

8.3 Shipping protocol

The shipping protocol will be exactly defined when the sequencing company is selected, as they will have company-specific protocol to follow. Here we include a generic shipping protocol.

Information on shipping instructions is found on the company website. It is extremely important to precisely follow the instructions for filling and packing the documents outlined below. For those performing the physical shipping of the samples, <email address> can be contacted for further questions.

1) Full sender contact details must be provided on a printed form inside the parcel for the sender of the material and also for the client to be invoiced, if different.

2) Make sure all sender and <sequencing company> contact details are correct and legibly written on outside the package, and include <sequencing company> phone number.
3) Include sender's Service Specification and Sample Tracking File(s) in the package.
4) Package should be sent in a rigid box/container with ample packing material to allow for rough handling during shipment.

8.3.1 Shipping documents (international shipments)

International shipping documents will vary between sequencing companies, but may include forms such as though described below. We recommend printing two copies of the following six (6) documents. Place the first set of the required documents in an envelope attached to the OUTSIDE of the package, clearly labeled "Quarantine and Customs Documentation". Include the second set of the documents inside the package. Clearly/legibly include <sequencing company> Quarantine Officer phone number or contact details.

1) Supplier's Declaration Template for Animal Tissue and Fluids
    a. Be printed on organization letterhead paper
    b. Be in English
    c. Prominently quote the Air WayBill (AWB) number on all pages of declaration
    d. Be issued in and dated in the last six months
    e. Be signed by the sender
    f. Describe samples accurately, stating "Preserved tissue in 70% ethanol from species *Dissostichus eleginoides* for in vitro use only"
    g. The declaration MUST also clearly state that the samples are sent for destructive analysis in the <sequencing company>
2) Shipping document letter
    a. Include as a part of the shipment a letter stating the following: "Contents of this package are non-Infectious, non-Hazardous, not an etiologic agent, not for human consumption. Shipment consists of sterile DNA for scientific analysis only. The material contains small pieces of genomic DNA suspended in sterile water. The material was not generated by microbial fermentation. The product is purified and does not contain animal or cell derived materials or additives. The material is non-toxic, non-hazardous and nonpathogenic. The end use is for research purposes only, and will be used for laboratory research purposes only. Material will be maintained within appropriate biological containment facilities, preventing exposure of material to plants, animals or the public. This package is temperature sensitive and needs to be handled in a timely manner."

b. As may be requested by the sequencing company, we will enter our names and contact information and will not include any other information about the source or about how the samples were packed.

3) Pro Forma Invoice Template
    a. On the organisation's letterhead (Ifremer) stating the value of each plate of 96 samples at 20 Euros.
4) Import Permit
5) Service Specification (will be provided after submitting an order online)
6) Sample Tracking File (.csv file with all samples, to be submitted for order online)
7) Air WayBill (AWB) (from the shipping company)
    a. Accurately describe your samples (space is more limited: "Preserved tissue in 70% ethanol from species *Dissostichus eleginoides* for in vitro use only")
    b. Do not use the words ANIMAL or ANIMAL SAMPLES without also mentioning PRESERVED IN 70% Ethanol.
    c. As may be requested by the sequencing company, we will not include words such as "Human, Tissue, Cell, blood, blue ice, dry ice, etc." on the airway bill, nor the company name on the airway bill.

When the package is shipped, email <email address> the:

- Name of the courier company;
- The tracking number from the courier; and
- The Service Number

We suggest loading samples in tubes and sealing each tube with parafilm for transportation. In order to prevent the tubes from being crushed and broken during transportation (leading to sample loss) it is better to either insert sample tubes into 50 mL centrifuge tubes (or other rigid supports such as a cryobox), which can also be packed with cotton, foam, etc. The 50 ml tubes or cryoboxes should be shipped with ice blocks.

9. Recommendations

9.1 Proposed sampling strategy

To the degree possible, onboard observer sampling should target spawning individuals (maturity stage 4; Table 1) in the three fishing zones (SIR, DCR, and Williams Ridge and target from November to March in flat areas (<0.2 radians) of >800m and <2000 m depth (see section 6.1), and collecting a minimum of 30 samples per site up to 100 samples per site (see section 6.2.2). We note that while sampling of spawning individuals is preferable,

the habitat, area, and the time period of sampling are most important as these samples will likely be sufficient to discriminate between populations.

We note that there may be a trade off between the number of samples that should be taken and the restrictions on the times, depths, and maturity stages over which samples are taken, and what is feasible for the commercial vessel. Therefore, we recommend attempting to source additional samples from alternate sources. Flag states should be approached to request well-preserved samples (see section 6.2.2 for description of the acceptable state of a sample) collected in previous campaigns. The details of the appropriate contacts should be provided by the SIOFA SC to the TOP2 consultants to facilitate this request. Armaments in Reunion Island should be approached to request access to sample fish that have been caught in the three target areas.

9.2 Proposed sampling protocol

We recommend that trip data (vessel, registration number, date, latitude and longitude of catch) and biometric data (weight, length, maturity, sex) be collected along with two fin clips, following the protocol defined in section 8.1. We stress the importance of sterilizing the sampling tools between each fish sampled to avoid contamination between individuals.

9.3 Proposed genetic analyses

We recommend that a dataset composed of Single Nucleotide Polymorphisms (SNPs) loci should be generated for *Dissostichus eleginoides* in the southwest Indian Ocean (see section 6.2). We recommend analyzing the samples using a “reduced representation approach” such as RADseq or GBS, described above, for SNP discovery in individuals of *D. eleginoides*.

To analyze the minimum recommended number of samples, i.e. 30 per site, the current budget allocated by SIOFA to the SER2022 TOP2 project, i.e. 34 000 € is about 2 400 € too low (Table 11). We will continue to search for lower-cost alternatives for the sample processing, but we also request that additional funds be allocated to allow for this minimum recommended sample processing. If no further funding can be allocated, CD Genomics will allow us to send less than 90 samples and will charge us a price-per-sample of 97.34 €. Therefore, the number of samples that can be processed at budget would be about 65, or 20-22 samples per each of the three sites.

Chris Darby (CEFAS), project leader on a recent study to analyze the population structure of Patagonian toothfish throughout the Southern Ocean, should be contacted to determine whether it would be possible to include our samples in their analyses, thereby vastly reducing costs.

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11. Acknowledgements

We thank the SIOFA Secretariat and the Scientific Committee for their support and guidance throughout the development of this report.

Annex 1

Terms of Reference (ToR) for the provision of scientific services to SIOFA Scientific Committee

Project title: Genetic analysis to inform the stock structure of Patagonian toothfish (*Dissostichus eleginoides*)

Project Code: SER2022-TOP1

Introduction

SIOFA CMM2018/01 (paragraph 6a) requires the SIOFA Scientific Committee to provide advice to the Meeting of Parties on the status of stocks of deep-sea fishery resources, including Patagonian toothfish (*Dissostichus eleginoides*). In 2020, the SIOFA Scientific Committee (SC3) conducted the first preliminary analysis of the Patagonian toothfish fishing data from the Del Cano Rise in the SIOFA Area. Those approaches were in early stages and to estimate stock structure in the SIOFA Area, more robust approaches and data would be needed.

This document describes the project Terms of Reference (ToR), milestones, and administrative matters for a consultancy to undertake Patagonian toothfish stock assessments. Once appointed, the Consultant should direct any questions and clarifications to the SIOFA Science Officer (Marco Milardi, marco.milardi@siofa.org) who will coordinate the project and its interactions with the project advisory panel, the relevant SC HoDs and the SIOFA Scientific Committee Chair, as appropriate.

This project aims to design a genetic stock discrimination project. Note that the collection of samples, analysis, and a full review of the stock structure of Patagonian toothfish will be conducted under SIOFA Project SER2022-TOP2.

Terms of Reference

The project objectives and tasks are described below. The Consultant shall undertake these tasks and consult with the project coordinator, to ensure that the project objectives are met.

A project advisory panel consisting of the SIOFA Scientific Committee Chair, selected members of the SIOFA Scientific Committee, and the SIOFA Secretariat will meet periodically with the consultant to assist the consultant access and interpret reports, data, and to provide advice on relevant analyses or data interpretation for the project.

Overall objectives

Objective 1: Provide advice to the SIOFA Scientific Committee on the design of a genetic stock discrimination project to understand the stock structure of Patagonian toothfish in the SIOFA Area, including linkages to Patagonian toothfish in the CCAMLR Convention Area.

Task 1: Literature review

Review the previous stock assessments, SIOFA reports and publications, CCAMLR scientific papers and reports, the general scientific literature, and other relevant information sources, including Patagonian toothfish stocks in other areas, to design and a genetic analysis of Patagonian toothfish stock structure in the SIOFA Area. The outcomes of this project will be used to support SIOFA project SER2022-TOP2: Stock structure of Patagonian toothfish.

Task 2: Review of catch-effort and other relevant data
Review the relevant catch-effort and scientific observer data (e.g., age, length, and other biological data) held by SIOFA, and available bathymetric, oceanographic, and other relevant environmental drivers to design a genetic analysis sampling project of Patagonian toothfish in the SIOFA Area. This will also include consideration of potential linkages with Patagonian toothfish stocks in the Indian Ocean sector of the CCAMLR Convention Area[2].

Task 3: Genetic stock discrimination
Evaluate the feasibility of genetic stock discrimination for Patagonian toothfish, including the development and design of a genetic stock discrimination project to improve the understanding of stock structure in the SIOFA Area, by:

(i) evaluating the feasibility of a genetic stock discrimination project, and
(ii) develop and design a genetic sampling project including specifications of the number of samples, locations and timing for the collection of samples using commercial fishing operations, the contents of a genetic sampling kit for observers and/or vessels, timelines, and costs for the project.
(iii) describe the contents of genetic sampling kits and collection protocols for distribution to SIOFA vessels and observers to enable them to collect samples

Reporting requirements

1. Provide updates and engage with the project advisory panel that will assist the consultant access and interpret reports, data, and to provide advice on relevant analyses or data interpretation for the project

2. Provide a draft report detailing the methods, outcomes of reviews, conclusions, and recommendations to the SIOFA project advisory panel for review by 31 January 2022.

3. Update the draft report in (2) by considering any comments and advice from the project advisory panel and submit this report to SIOFA Secretariat for submission to the SIOFA Scientific Committee meeting in 2023 by 15 February 2023

4. Present the draft report in (3) to the SIOFA Scientific Committee to its meeting in March 2023 by videoconference.

[2] CCAMLR Convention Area includes the South African, French and Australian management areas

5. Provide an amended final report to the SIOFA Secretariat, considering any comments made at the SIOFA Scientific Committee meeting in March 2023, by 15 April 2023

6. Provide all the information collected to the SIOFA Secretariat (including that sourced from the Secretariat) before the final payment of the contract is made to the consultant. Such information includes electronic data files, analysis codes, biological samples, and other relevant data if applicable.

Confidentiality and distribution of project outcomes

The Consultant shall not release confidential data provided for conducting this study to any persons nor any organisations, other than SIOFA Secretariat. The consultant shall delete all the confidential data after the completion of the contract. Any arrangements for ownership, storage, or disposal of physical samples shall be agreed by SIOFA as a part of the contract.

All Intellectual Property generated as a part of this contract shall become the property of SIOFA unless otherwise excluded in the proposal and agreed by SIOFA in the contract.

All reports and presentations will be reviewed by the SIOFA Secretariat prior to any form of further distribution. The Consultant will revise the report according to comments received from the review process before the report or presentation is accepted as a submission against the requirements in the Terms of Reference.

Relevant SIOFA information

1. SIOFA data (provided by the SIOFA Secretariat upon request)
2. SIOFA reports:
    a. SIOFA SC reports and National Reports. Scientific Committee Meeting | SIOFA (siofa.org)
    b. MoP reports. Meeting of the Parties | SIOFA (siofa.org)
    c. SIOFA technical and scientific reports (public reports available from siofa.org, and restricted reports available from the SIOFA Secretariat to the project consultant)

Relevant CCAMLR information

1. CCAMLR papers and reports that consider linkages with Patagonian toothfish stocks in the Indian Ocean sector of the CCAMLR Convention Area
2. Previous studies on the genetic stock structure of Patagonian toothfish in the CCAMLR and adjacent areas
3. Patagonian toothfish management options currently in use for these stocks in the CCAMLR Convention Area

Work plan and payment schedule

The funds for this project are budgeted under General Objective 1 of the SIOFA/EU Grant Agreement SI2837681 - Scientific Work Support, for a total allocated budget of 8,333 euro (including all costs and including any travel related expenses). Any report and/or presentation, in paper or electronic form, must indicate that this task has received EU funding and display the EU emblem.

The consultant shall follow the timeline described in Table 1 below.

Table 1: Timeline for payments, milestones, and report submission

Milestone	Date	Activities
Initiation of contract	6 January 2022	First instalment payment (30% of the total contract sum)
Delivery of draft report	15 February 2023	Submission of draft report to SC8
Delivery of final report	15 April 2023	Submission of final report and project information to SIOFA. Final instalment payment (70% of the total contract sum) on acceptance of the final report and the submission of project information

Submission of applications

The applicants should have appropriate experience and knowledge of developing stock structure hypotheses and preferably on the stock dynamics and life cycle of Patagonian toothfish. The applicants should submit a proposal to the project coordinator (SIOFA Science Officer - Marco Milardi, marco.milardi@siofa.org) containing the following items:

1. A current CV that summarises the applicant(s) relevant educational background and professional experience
2. A brief proposal (indicatively 1-2 pages) outlining the proposed methods and analyses, including a description of how the objectives of the ToRs will be achieved
3. Any proposed exclusions to the intellectual property clause
4. The proposed consultancy price (including all consultant expenses and project related costs), noting that the available budget for this work is a maximum of €8,333
5. Identification of any project risks and associated mitigation and management required to successfully complete the project
6. A statement that identifies any perceived, potential, or actual conflicts of interest of the applicant(s), including those described in paragraph 4 of the SIOFA recruitment procedure (see Box 1), and
7. Any additional relevant information the applicant(s) wish to submit.

8. We note that similar projects for alfonsino and orange roughy in the SIOFA Area are also available, and we encourage consultants to submit combined proposals for these projects if appropriate.

Applications received before 12 AM (9 AM UTC) on Monday the 2nd of January 2023, Reunion Island time, will be considered in the following selection process.

EVALUATION CRITERIA FOR THE SELECTION OF CANDIDATES

The selection criteria will be developed by the evaluation panel along with the project manager, the Secretariat, and the Chairpersons of the relevant subsidiary bodies. The criteria may include following items:

1. Adequate submission of information to allow the panel to evaluate the candidate
2. Evaluation of the proposal from the candidate, including the proposed contract price
3. Ability to undertake and complete the analyses or work required in the ToR
4. The candidate's agreement with confidentiality provisions required for the project
5. Acceptable conflict of interest statement
6. Agreement with the data submission and intellectual property terms required in this ToR, and
7. Financial and resourcing considerations.

Conflicts of interest. Paragraph 4 of SIOFA's Recruitment Procedure
To ensure that situations relating to potential and actual conflict of interests are avoided, persons falling into the following categories may not normally be considered for SIOFA consultancy: (i). any person designated as a designated representative or alternate representative of a CCP to the Meeting of Parties (MOP) as per Rule 3.1 of the Rules of Procedure, and to the SC and any other subsidiary bodies of the MOP, as per Rule 21.3 of the Rules of Procedure; (ii). Any person fulfilling the function of Chair or Vice-Chair of the MOP or Chair or Vice-Chair of a SIOFA subsidiary body or working group; (iii). Any person acting as a member of a delegation involved in the SIOFA decision-making process resulting in recommendations and/or approval for the SIOFA work requiring the engagement of a consultant; and (iv). Individuals who were SIOFA Secretariat staff members at the time when the recommendations and/or approval for the SIOFA works were adopted or who are members of immediate family (e.g., spouse or partner, father, mother, son, daughter, brother, or sister) of any Secretariat staff member or of the persons identified in 4 (i), (ii), and (iii).

CONTACTS
Project Coordinator – SIOFA Science Officer (Marco Milardi, marco.milardi@siofa.org)

Administration – SIOFA Executive Secretary (Thierry Clot, thierry.clot@siofa.org)