APPLICATION OF A MULTI-METHOD APPROACH TO THE STOCK IDENTIFICATION AND DISCRIMINATION OF KINGKLIP, *GENYPTERUS CAPENSIS* (SMITH, 1847), OFF THE SOUTH AFRICAN COAST

by

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Signature: Signed by candidate

Date: 12 February 2023

Dedication

Dedicated to my beloved late

grandmother

Asa (Richards) Mobara

Who encouraged me to constantly seek knowledge and gain experiences.

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Similar to how this study needed a multi-method approach in order to reach a conclusion, the development of this thesis involved a combination of shared knowledge and wisdom as well as the financial, professional, and moral support of many...

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you guys are my backbone

A special thank you to
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Thank you for your motivation, for assisting me, for supporting me, for making me laugh, for being there through the tears..
Thank You for your friendship.
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To the ALMIGHTY, **ALHAMDULILLAH**.

Abstract

Kingklip, *Genypterus capensis*, is a marine demersal fish distributed in shelf and slope waters of southern Africa, from Walvis Bay in Namibia to Algoa Bay in South Africa. This commercially important species is caught by demersal trawling and longlines. Whereas previous analyses of population structure in kingklip gave contrasting results, the most recent genetic and genomic analyses have indicated population structure in this species with three genomic clusters observed: one off the central Namibian coast, one off the southern Namibian coast and the South African West Coast, and one off the South African South Coast. The identification and delineation of sub-populations is necessary for effective management of exploited species, and determination of the stock identity of individual fish is most effectively and cheaply done using phenotypic characteristics. To assess whether the two genomically-differentiated stocks off South Africa are phenotypically differentiated, a holistic and integrated multi-method approach that analysed morphological (body and otolith), meristic, otolith elemental composition, and parasitological data of kingklip from the West and South Coast was applied.

Nine body morphological (standardised for fish size) and three meristic variables were analysed. For both univariate and multivariate analysis, no significant differences between mature *Genypterus capensis* from the West and South coasts were observed in any of the 12 characteristics. For immature specimens, significant differences between regions were observed in four morphological (upper jaw, lower jaw, orbital diameter, and interorbital diameter), but not in any meristic characteristics. Univariate and multivariate analyses indicated that four (rectangularity, roundness, ellipticity, and aspect ratio) of the six otolith shape indices calculated showed significant spatial differences in mature specimens. In immature specimens, circularity, form factor, ellipticity, and aspect ratio, showed significant differences when using multivariate analysis, and roundness was also significantly different between regions when using univariate analysis. Elliptical Fourier Analysis of otolith shape was applied utilising 157 harmonics, of which seven in mature specimens, and nine in immature specimens were significantly different between regions. Seventeen elements were measured in kingklip otoliths and of these barium, phosphorus, and nickel showed significant differences between regions in immature but no differences were observed in mature fish. Regional

differences in four kingklip parasites (*Anisakis* sp., *Diphyllobothrium* sp., *Lecithochirium* sp., *Tentacularia* sp.) previously identified as potential biotags were assessed, with univariate analysis indicating a significant difference for *Tentacularia* sp. for both mature and immature specimens.

A Stock Differentiation Index (SDI) indicated weak (SDI = 0.26) spatial structure for mature, and a moderate (SDI = 0.33) spatial structure for immature *Genypterus capensis* in South African waters. These results indicate that the genomic differentiation of *G. capensis* off the South African coast does not translate into substantial and spatially differentiated phenotypic variation in mature specimens, however moderate stock delineation is observed in immature samples. This suggests that mixing between the two genomically-differentiated kingklip stocks is lowest for juvenile fish and increases with age, which accords with the hypothesis of separated and localised spawning locations for these two stocks off the West and South coasts of South Africa. That otolith shape indices had the strongest discriminatory power of the characteristics examined in this study suggests that analyses of otolith shape at an early stage inferred via measurement of the shape of the first otolith annulus (laid down when fish are 1-year old) may be a useful phenotypic characteristic for stock identity determination, and further research should be conducted in this regard.

Keywords: *Genypterus capensis*, stock structure, morphology, meristics, otolith shape analysis, otolith elemental composition, parasite biotags, Stock Differentiation Index

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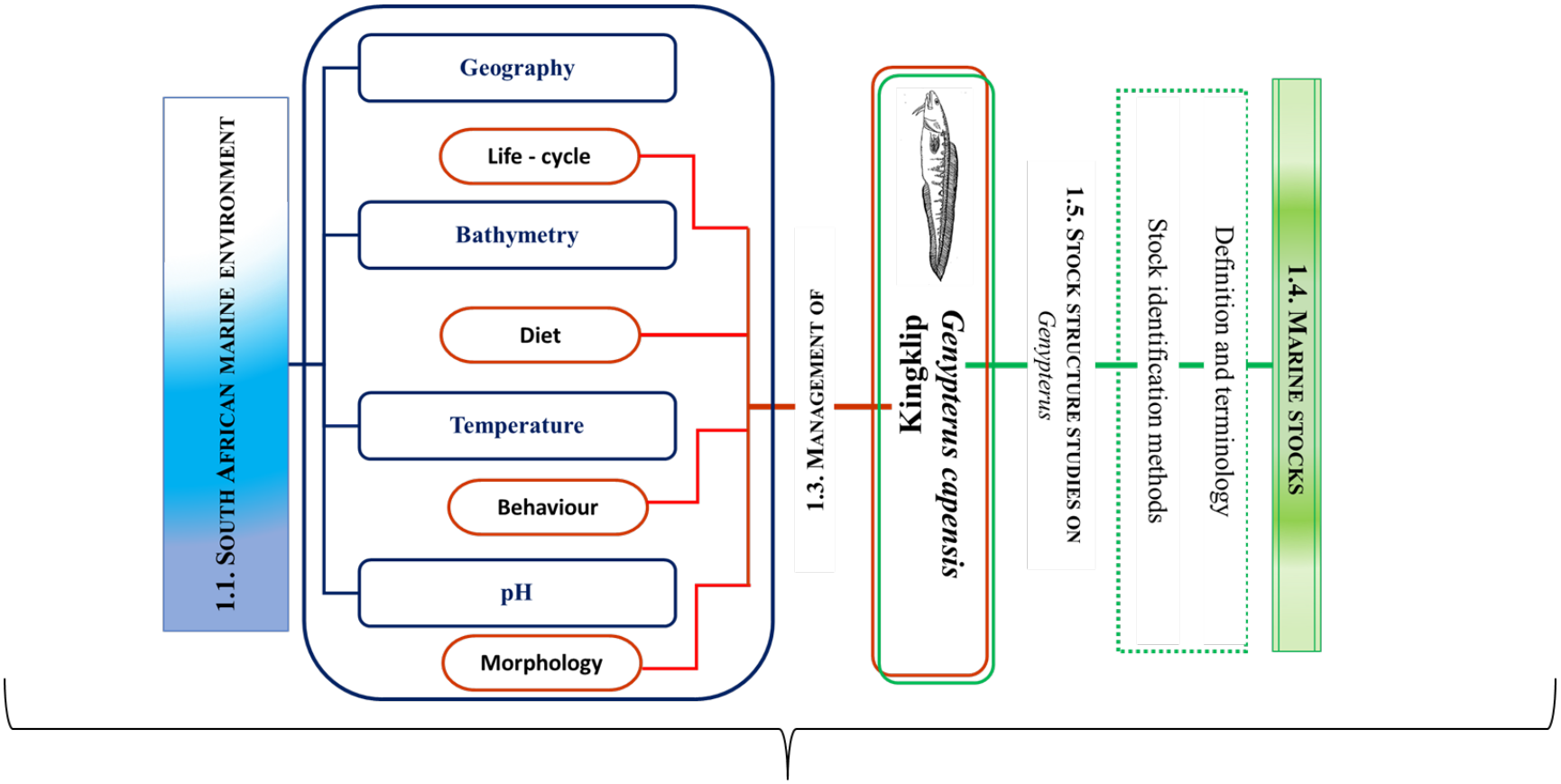
Chapter 1

INTRODUCTION

"Don't forget where you came from, but always remember where you're going."

— Luke Taylor

CHAPTER OVERVIEW



1.6. IMPORTANCE OF THIS STUDY

1.7. THE PURPOSE OF THIS STUDY

1.1. THE SOUTHERN AFRICAN MARINE ENVIRONMENT

The marine environment often gives the illusion of being homogenous, but is, however, highly varied (Branch *et al.,* 2007). The South African coastline is approximately 3650 km in length (Griffiths *et al.,* 2010). Its continental margin covers approximately 165 000 km^2 up to the 200-m isobath, and about 400 000 km^2 up to the 2000-m isobath (Broad *et al.,* 2006). The vast shelf area is narrow on the eastern margin, broader on the southern margin, and very broad and deep on the western margin (de Wet and Compton, 2021). Hydrologically, there is an extreme contrast between the water masses along the east, south, and west coasts. The features described in this section are depicted in Figures 1.1 and 1.2 and listed in Table 1.1.

On the West Coast, the rocky inner shelf outspreads to a narrow, coast-parallel band reaching approximately 130-m in water depth. Three submarine canyons cut across the middle shelf: Cape Canyon, Cape Point Valley and Olifants Valley. Cape Canyon is deeply carved with a longitudinal extent of ±200 km, a maximum local relief of ±900-m, and a water depth of ±3600-m (de Wet and Compton, 2021). The canyon is internationally classified as Vulnerable Marine Ecosystem (VME) and likely plays a vital ecological role in the life history of some commercial resources (DFFE, 2020; MPA SA, 2022).

The Benguela system forms the eastern flank of the South Atlantic Gyre (SAEON, 2021) and is one of the four main eastern boundary upwelling systems of the world (Hutchings *et al.,* 2009). The current moves from the south and swings northward at the Agulhas Retroflection, extending toward the coast of Angola (SAEON, 2021). On the West Coast of South Africa, hydrographic characteristics of the systems are influenced by the upwelling strength, as well as the strength of the intrusion of Agulhas seawater in the form of Agulhas rings. The existence of a thermal and a saline front ultimately controls the oceanography of the southern Benguela system (Branch *et al.,* 2007). The maximum upwelling and maximum infiltration of Agulhas water takes place during spring and summer (August to March), and minimum upwelling and penetration of Agulhas water occurs in winter (Olivar and

Sabatés, 1989). Wind-driven coastal upwelling results in water temperatures inshore being low with relatively small oscillations. In contrast, offshore sea-surface temperatures are higher and include a wide range of values throughout the year (Branch *et al.,* 2007; Olivar and Sabatés, 1989). The "Good Hope Jet" is found between the 200 -500-m isobaths (van der Lingen and Hugget, 2003) close to the shelf-edge, on the western coast. This jet current is relatively narrow and northward flowing, present in zones having substantial southward flow, mainly subsurface (Boyd and Nelson, 1998; Nelson, 1985; Bang and Andrews, 1974). The speed of the jet is dependent on the strength of favourable upwelling winds (Huggett *et al.,* 1998).

On the East and South Coast of South Africa, the Agulhas current is a narrow, rapidly flowing body of tropical water that is formed between 25°S and 30°S (Shannon, 1989). This current carries warm water southwards from the subtropics. In the region of East London, the continental shelf widens, which results in the coastal waters becoming cooler as the Agulhas Current is forced further offshore (Olivar and Sabatés, 1989). South of the continent, this current "retroflects" and reverts water east toward the Indian Ocean. At this point (of retroflection) considerable volumes of water separate from the Agulhas and forms rings/eddies that enter the South Atlantic Ocean, travelling in a north-west direction (SAEON, 2021).

On the edge of the continental shelf at the south-eastern tip of Africa, a rocky ridge (500-m wide, 300-m high and 40 km long) is present at approximately 750-m beneath the surface. The steep decline of the shelf facilitates the movement of the warm Agulhas Current. On the opposite landward side (over 30-nmi off the coast between Gqeberha and Cape St Francis) the ridge forms a natural amphitheatre creating the "Kingklip Spawning Box" (Sink and Japp, 2021) mentioned in Section 1.2.5.

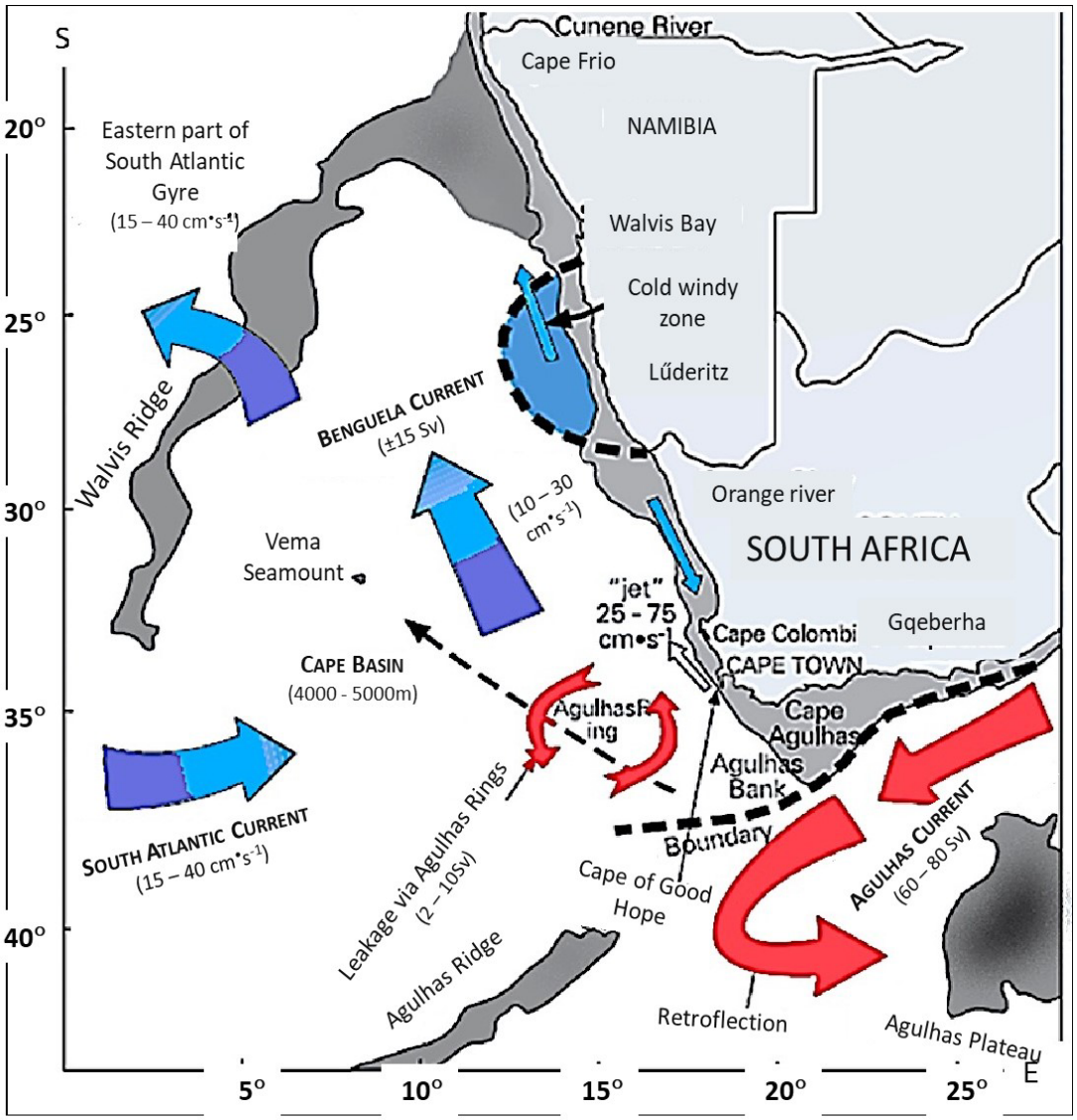
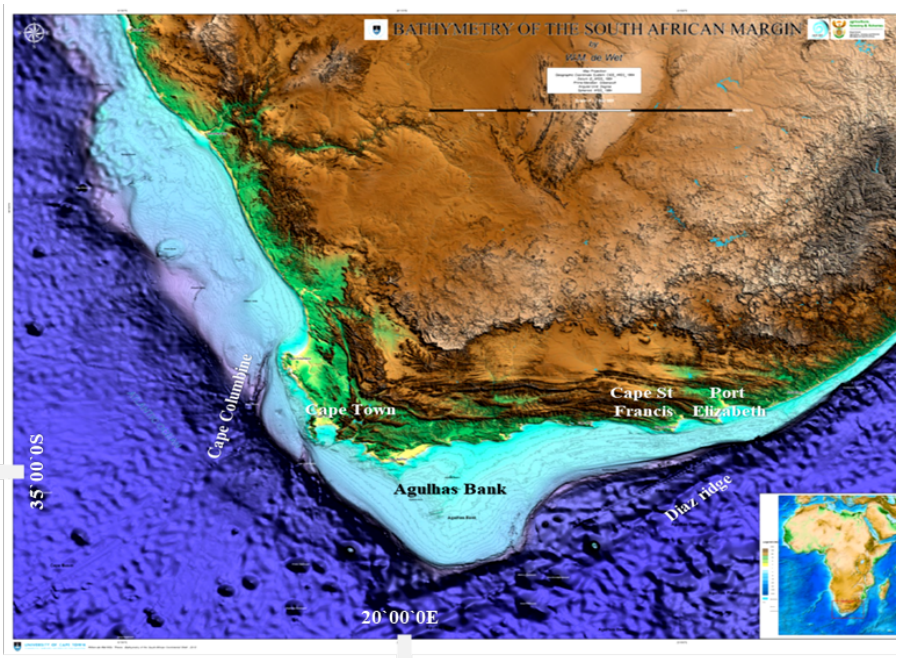


Figure 1.1. Map indicating ocean currents and oceanographic patterns. Blue arrows indicate cold currents and red arrows indicate warm currents (Source: adapted from BCLME, 1999)

(a)



(b)

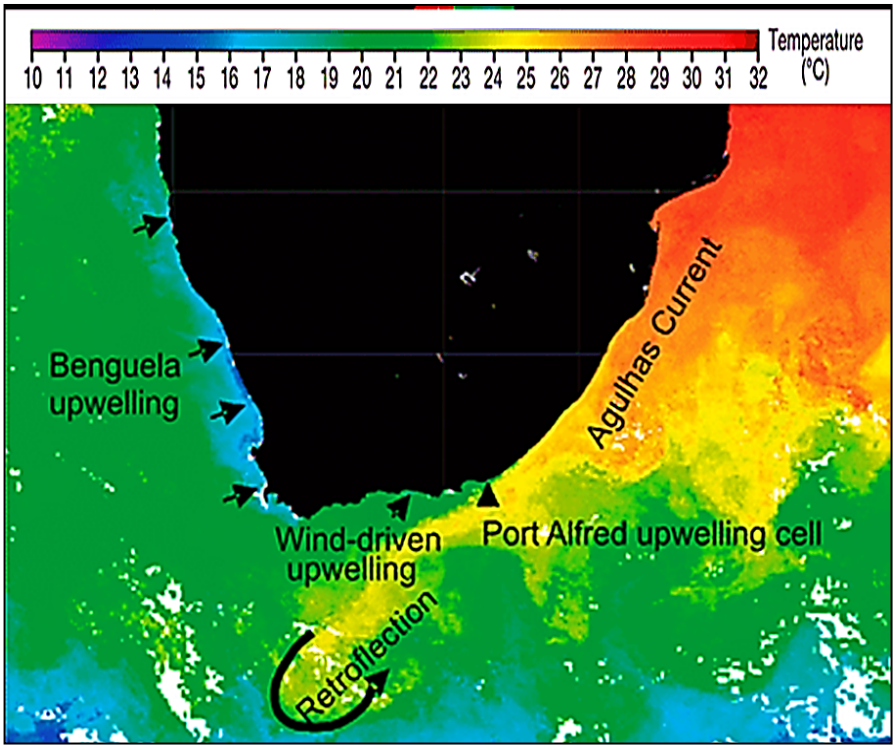


Figure 1.2. Maps indicating **(a)** Relief bathymetric map of the South African continental margin (**Source**: adapted from de Wet and Compton, 2021) **(b)** Map portraying the sea surface temperatures of the Benguela and Agulhas current systems (February 2009). Arrows show the upwelling cells and the Agulhas Retroflection **(Source**: James *et al.*, 2013) .

Note: In February 2021, the name change of Port Elizabeth to Gqeberha was formally gazetted (Government Gazette RSA, 2021)

Table 1.1. Temperature, salinity, and pH along the South African coastline (**Source**: edited from text in de Villiers, 2017)

<table>
<tr><th colspan="4">TEMPERATURE</th></tr>
<tr><td colspan="4">•Temperature regime differs between coasts
•Influenced by anthropogenic sources that are generally linked to the expulsion of cooling water from power stations and a variety of industries</td></tr>
<tr><td colspan="2">West coast
(Cool temperate)</td><td colspan="2">South coast
(Warm temperate)</td></tr>
<tr><td colspan="2">•Largely influenced by wind-generated upwelling (south-easterly and southerly winds) which fluctuates seasonally</td><td colspan="2">•Upwelling can also impact the temperature regime in the coastal zone, although not to the same extent as along the west coast

•During summer, thermoclines are created by the sun heating up the surface water, while during winter months the water column is generally well mixed.</td></tr>
<tr><td colspan="4">Surface temperatures</td></tr>
<tr><td colspan="2">12-16°C
(Dependent on upwelling conditions)</td><td colspan="2">16-18.5°C</td></tr>
<tr><td colspan="4">Average seasonal temperature range</td></tr>
<tr><td>Summer</td><td>Winter</td><td>Summer</td><td>Winter</td></tr>
<tr><td>11 - 18°C</td><td>11-14° C</td><td>18 - 23°C</td><td>13.5 - 17° C</td></tr>
<tr><th colspan="4">SALINITY</th></tr>
<tr><td colspan="4">• Anthropogenic effects on salinity in marine ecosystems can originate from wastewater discharges which, depending on the volume discharged, may result in a short-term decrease in salinity in the immediate vicinity of the discharge.
• More severe impacts may be a result of hypersaline emissions from reverse osmosis plants</td></tr>
<tr><td colspan="4">Average salinity range</td></tr>
<tr><td colspan="2">West coast</td><td colspan="2">South coast</td></tr>
<tr><td colspan="2">• 34.7 to 35.4 PSU with
• Lower salinities associated with cold upwelling water</td><td colspan="2">• 35.4 PSU to 35.0 PSU</td></tr>
<tr><th colspan="4">pH</th></tr>
<tr><td colspan="4">• Anthropogenic sources which may influence the pH of water are usually related to highly acidic or alkaline industrial waste waters</td></tr>
<tr><td colspan="2">West coast</td><td colspan="2">South coast</td></tr>
<tr><td colspan="2">7.9 – 8.2</td><td colspan="2">7.9 – 8.2</td></tr>
</table>

Given the dynamic nature of the ocean and considering the back-and-forth movement of the Agulhas Current, it would be difficult to determine a precise

point where the warm Indian Ocean begins and the cold Atlantic Ocean ends. Cape Agulhas (34.8311° S, 20.0131° E) is commonly considered the boundary between the two ocean masses (Hutchings *et al.,* 2009; Kirkman *et al.,* 2016), and the region between Cape Agulhas and Cape Point (to the west) is a phylogeographic break between cool-temperate and warm-temperate marine biota (Teske *et al.,* 2011).

Temperature (Jobling, 1995), salinity, dissolved oxygen (Nashima and Chilamba, 2013) and ocean depth (Macpherson and Duarte, 1991) significantly impacts the metabolism, reproduction, respiration, and the distribution of fish (Nashima and Chilamba, 2013). These abiotic factors influence both the type of fish present at a locality, in addition to how well the species can survive in that area.

1.2. KINGKLIP: *GENYPTERUS CAPENSIS* (SMITH, 1847)

Kingklip, *Genypterus capensis* (Smith, 1847), is a marine demersal fish (Punt and Japp, 1994; Japp, 1990), found at depths of 50 – 800 meters (SAAMBR, 2021). The species is classified as follows:

1.2.1. Classification

Order: Ophidiiformes
- **Suborder**: Ophidiodei
  - **Family**: Ophidiidae
    - **Subfamily**: Ophidiinae
      - **Genus**: *Genypterus*
        - **Species**: *Genypterus capensis*
        - **Synonyms**:
          Hoplophycis lalandi Kaup, 1858
          Xiphiurus capensis Smith, 1847

This slow growing and long-lived species has a lifespan of over 20 years (Japp, 1990) and is geographically distributed along the coast of southern Africa from Walvis Bay in Namibia to Algoa Bay in South Africa. The species is endemic to this area (Smith and Heemstra, 1986) although there are indications that the distribution may extend further eastwards past KwaZulu-Natal (DFFE, 2020). It is an important commercial species for both South

Africa (Japp 1988, 1989, 1990) and Namibia (Macpherson, 1983; Nashima and Chilamba, 2013).

1.2.2. Morphology, biology, and behaviour

The name of the genus, "*Genypterus*," comes from Greek, where "*geny*" means "jaw/face" and "*pteron*" means "wing/fin" (Froese and Pauly, 2022) emphasising the fish's anatomical complexity. Figure 1.3 depicts a simple labelled drawing of *Genypterus capensis*.

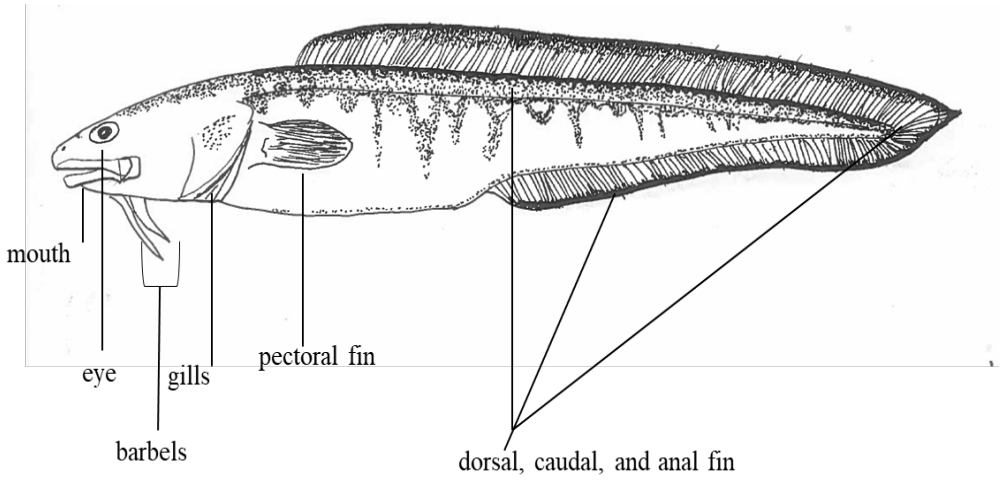


Figure 1.3. Simple line drawing of *Genypterus capensis* with labels for the appendages stated in the text (**Source**: Redrawn from FAO image in EOL, 2021).

Like other Ophidiiform fish, *Genypterus capensis* possesses an eel-like appearance with an extended body with a tapering caudal end (MacPherson, 1983). The species has a huge mouth that reaches below the posterior margin of the eye and has fine teeth on its jaws (Nielsen *et al.*, 1999). The dorsal and anal fins are continuous, but the ventral fins have developed into a forked, barbel-like structure beneath the mouth. Along the posterior side of the fish's body, the caudal fin develops into a long, ribbon-like appendage (Froese and Pauly, 2022). Although the colour of this fish varies, it is often orange-pink with brown areas that are mottled.

Its scales are rounded, small, and arranged in regular rows that are absent on its head (Nielsen *et al.,* 1999). As they are not well adapted for swimming long distances (Japp and Hecht, 1990), adults remain somewhat sedentary,

apart from seasonal inshore spawning migrations (Payne and Badenhorst, 1995).

The species is dioecious, but not sexually dimorphic and there are no obvious external characteristics to differentiate between the sexes (Japp and Hecht, 1990). Payne (1985) and Japp (1990) did however find that females attain larger size than males - a common life-history strategy of deep-sea fish species. Drumming muscles are found to be a reproductive adaptation in benthic species, facilitating sound and enabling communication between individuals at dark depths (Macpherson, 1983). Japp and Hecht (1990) found that the drumming muscle weights for both sexes of *G. capensis* specimens remained low until a length of 52 cm. Thereafter, there was a significant increase in muscle weight relative to body length.

1.2.3. Sexual maturity

Fish become sexually mature at a particular length or age, and once maturity is reached, the gonads go through a seasonal cycle of stages of ripeness. This data can prove useful in recruitment patterns (Mous *et al.,* 1995). For *Genypterus capensis,* the gonads of both sexes can be identified at a total length of ±20 cm (Hecht, 1976). Table 1.2 lists the descriptions that were utilized to macroscopically estimate *Genypterus capensis* maturity age.

According to Payne (1985), females (L_t = 60 cm; L_{50} = 52 cm) grow faster than males (L_t = 78 cm; L_{50} = 48 cm) after reaching maturity. Japp (1989) indicated on the west coast, L_{50} = 81 cm for females and L_{50} = 72.5 cm for males. On the south coast, L_{50} = 65.5 cm for females and L_{50} = 62 cm for males. Japp and Hecht (1990) observed that on the west coast, females mature between 55 - 59 cm (Age: 3.7 years; L_{50} = 80 – 84 cm) and males between 40 - 44 cm (Age: 2.6 years; L_{50} = 70 cm). On the south coast, females begin maturing between 50 – 54 cm (Age: 3.8 years; L_{50} = 70 – 74 cm) and males between 45 – 49 cm (Age: 3 years; L_{50} = 60 – 64 cm).

Table 1.2. Characteristics used for the macroscopic estimation of *Genypterus capensis* maturity (**Source:** adapted from Japp, 1989)

Maturity stage			Macroscopic characteristics of gonads	
			Males (♂)Testes	**Females (♀)Ovaries**
Immature	**1**	Inactive	• Almost transparent • Thin and threadlike	• Small, globular, light pink • Tunica transparent • Eggs cannot be distinguished
	2		• Elongated • Cylindrical • Creamy white and opaque	• Larger, speckled with minute eggs that are not very obvious to the naked eye • Tunica opaque (whitish) and in some cases dark red or violet in colour
Mature	**3**	Active	• Larger than the previous stage • Creamy white and opaque • Bulky and lumpy • Often bloodshot	• Distended and filled with eggs • Eggs small but can be clearly seen through tunica • Tunica opaque, has a speckled appearance, often translucent, may have an orange or deep pink colour, is often bloodshot
	4	Ripe and running	• Like Stage 3, but sperm flows with a bit of pressure applied • Testes are bloodshot	• Eggs are large and can be extruded from the ovisac by applying some pressure • Ovisac is bloodshot
	5	Spent	• Deflated and darker in colour	• Deflated and bloodshot

A more recent study by Guerra (2019) indicated a reduction in females' age at 50% maturity when compared to Japp (1989). Guerra (2019) found that for females, L_{50} is at the age of approximately 4 and 4.5 years on both coasts, and it is thought this shift is a result of fishing exploitation. It was found that on

the west coast, for females, L_{50} = 62.8 cm, and for males, L_{50} = 66 cm, whilst on the south coast, for females, L_{50} = 56.3 cm and for males, L_{50} = 59.7 cm.

1.2.4. Diet

Fish growth, and consequently the productivity of a fish stock, may be determined by the quantity and obtainability of food. Information on the food consumption and food composition of a fish species is vital in studies of fish stocks (Mous *et al.,* 1995). *Genypterus capensis* is a nocturnal benthic feeder that captures prey by means of ambush or stalking (Macpherson, 1983). Older fish forage almost entirely on demersal fish, whereas the diet of juveniles also includes crustaceans and squid (DFFE, 2016). As larger individuals occupy deeper waters, there is an increased chance of encountering larger prey species (Merrett & Marshall, 1980). As summarised in Table 1.3., a study by Beukes (2020) indicated differences in the diet of *G. capensis* at different lengths of the fish, and correspondingly at different depths. This noticeable change in diet is characteristic of the *Genypterus* genus and supported by other studies (Macpherson, 1983; Nyegaard *et al.,* 2004; Dunn *et al.,* 2010; Mitchell, 2010).

A study on *G. capensis* in Namibia indicated that part of the species diet is hake (*Merluccius capensis* and *M. paradoxus*), and out of breeding season, they are found to forage extensively on hake trawling grounds (Macpherson, 1983; Japp and Hecht, 1990). Additionally, Watermeyer *et al.* (2016) reported that the subject species was found to predate on horse mackerel (*Trachurus capensis*).

When examining trophic relationships between species using modelling, moderate competition for food resources was found between *G. capensis* and snoek (*Thyrsites atun*), yellowfin tuna (*Thunnus albacares*), as well as the two hake species (Watermeyer *et al.,* 2016). Predators of *G. capensis include Sphyrna zygaena, Arctocephalus pusillus, Carcharhinus brachyurus* and *Rostroraja alba* (EOL, 2021).

Table 1.3. Variations in the diet of *Genypterus capensis* across various depths and size classes (**Source**: edited from text in Beukes, 2020).

		Prey	
		West Coast (WC)	**South Coast (SC)**
Depth (m)	**< 200**	*Pterygosquilla armata capensis* (Mantis Shrimp)	*Cynoglossus zanzibarensis* (Trout)
	200 - 400	*Paracallionymus costatus* (Ladder dragonet)	
		Gadiformes (Cods, grenadiers, hakes and their relatives)	
	> 400	*Coelorinchus braueri* (Shovelnose grenadier), *Helicolenus dactylopterus* (Jacopever), *Coelorinchus simorhynchus* (Grenadiers), *Merluccius paradoxus* (Hake)	
Size class (cm)	**0 - 50**	Callionymiformes (Dragonets)	Callionymiformes (Dragonets)
			Gadiformes (Cods, grenadiers, hakes and their relatives)
			Pleuronectiformes (Flatfish)
	51 - 100	Callionymiformes (Dragonets)	
		Pterygosquilla armata capensis (Mantis Shrimp), *Paracallionymus costatus* (Ladder dragonet), *Cynoglossus zanzibarensis* (Trout)	
	101 - 150	Gadiformes (Cods, grenadiers, hakes and their relatives)	

Note: Common names are listed in brackets

1.2.5. Spawning and life cycle

A migratory life cycle is frequently observed in marine species (Putman *et al.,* 2019) for both denatant (swimming with the water current) and contrantant (migrations against currents) motions. It usually incorporates the use of spatially restricted reproductive areas for spawning after which the larvae drift to the nursery ground. Adolescent fish then disperse and recruit

to the adults on the feeding grounds. Ultimately homing occurs when adult fish migrate against currents (contrantant) from the feeding ground to the spawning ground where reproduction occurs, restarting the cycle (Secor, 2002; Putman *et al.,* 2019). The migration triangle is a diagrammatic representation of this circuit and is portrayed in Figure 1.4. (Harden Jones, 1968; Cushing, 1975; Secor, 1999; Secor, 2002) followed by an account of the life cycle of *Genypterus capensis.*

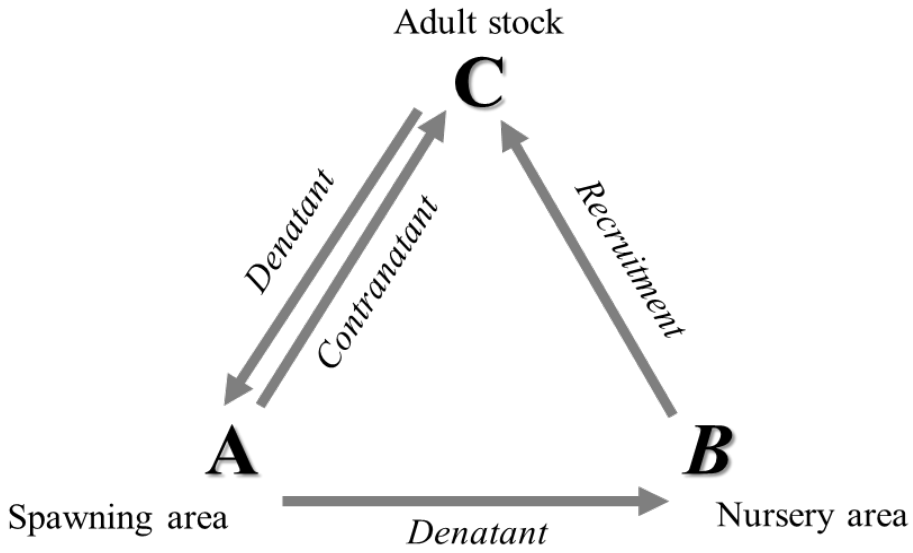


Figure 1.4. Larvae drifts from **(A)** spawning area with the current to the **(B)** nursery area, after which adolescent fish then disperse and recruit to the **(C)** adult stock on the feeding ground. Thereafter, homing occurs, and adult fish migrate against currents to **(A)** spawning ground (**Source:** edited from Putman *et al.,* 2019).

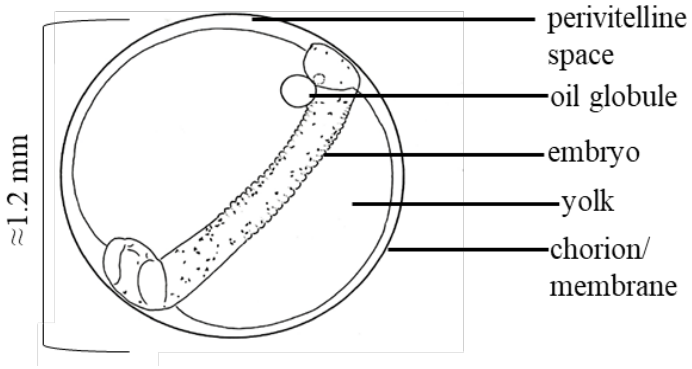
1.2.5.1. Spawning

As depicted in Figure 1.5a., a single, small oil globule combined with a moderately large, spherical egg is diagnostic for the eggs for *Genypterus capensis* (Gillchrist and Hunter, 1919 in Brownell, 1979). Pre-spawning aggregations of *Genypterus* species is common knowledge amongst most anglers and researchers (Roberts, 1987; Japp, 1989). Spawning groups of *G. capensis* can be found off the South African south coast. It is thought that spawning aggregations do occur on the west coast but are difficult to locate due to the broad shelf margin (Japp and Hecht, 1990). Guerra (2019) found high GSI values and reproductive activity in a fraction of the females sampled on the west coast. Additionally, ripe testes were found in males from both

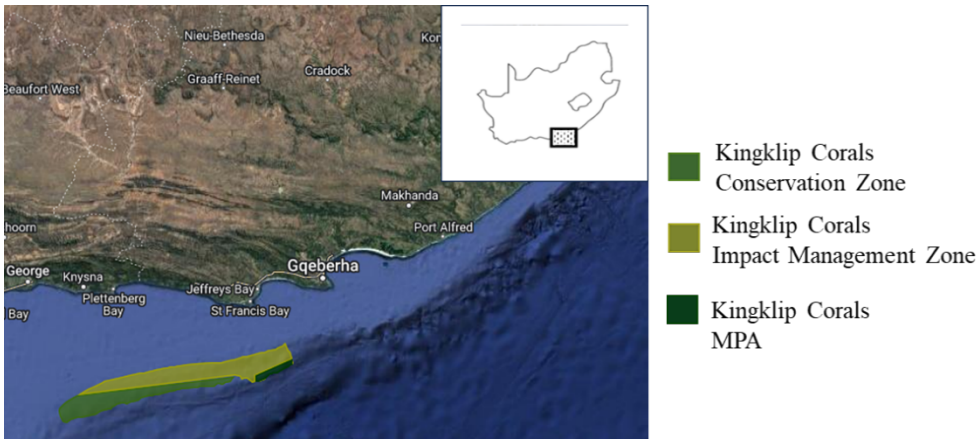
coasts. This suggests that reproductive activity is probable on the west coast as well.

The proposed Kingklip Corals Ecologically or Biologically Significant Marine Areas (EBSA) region encompasses the Kingklip Corals Conservation zone, Impact Management zones, and Marine Protected Area (MPA) zones (CMR, 2024) as portrayed in Figure 1.5b. This MPA is known as" Kingklip Kingdom," a 270 km^2 marine protected area, was established in 2019 to preserve and safeguard the region's biological processes and biodiversity, (MPA SA, 2021). It is situated on the continental slope between Gqeberha and Cape St Francis, the depth of the water spans from 200 to 5000 metres, and is surrounded by a sequence of straight lines that connect four coordinates shown in Figure 1.5c. The “Kingklip Spawning Box” is a seasonal fisheries management area bordering on the Port Elizabeth (Gqeberha) Corals Marine Protected Area (Government Gazette, RSA, 2016; MPA SA, 2021). No fishing is permitted within this area between 1 September - 30 November each year (Norman *et al.,* 2018). This restriction is applied to the offshore demersal trawl fishery to protect spawning aggregations of *G. capensis* which would otherwise be caught at an unsustainable rate (Attwood, 2011).

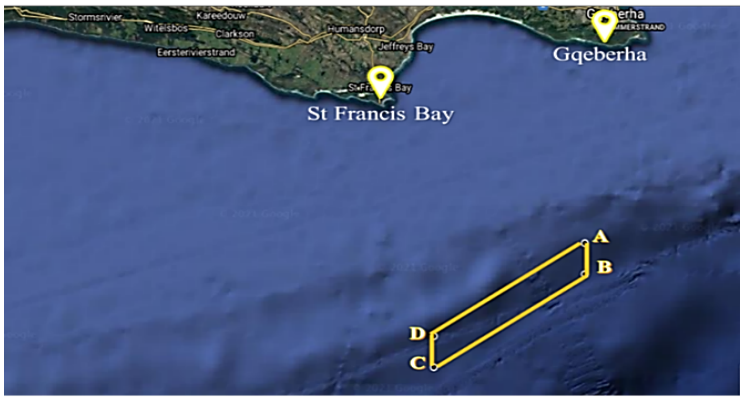
(a)



(b)



(c)



Corner Name	Latitude (S)		Longitude (E)	
	DEG	MIN	DEG	MIN
A	25	27.000	34	37.000
B	25	30.000	34	43.000
C	25	38.400	34	54.600
D	25	0.000	34	50.000

Figure 1.5. (a) *Genypterus capensis* egg (**Source:** reproduced and edited from Brownwell, 1979) **(b)** The proposed Kingklip Corals Ecologically or Biologically Significant Marine Areas zone (**Source:** Adapted from CMR, 2024) **(c)** Map of Kingklip Kingdom - a marine protected area bounded by a series of straight lines sequentially joining the four coordinates listed in enclosed table (**Source:** Adapted from: Government Gazette RSA, 2016).

1.2.5.2. Larvae

Section 1.1 discusses the environmental variations between South Africa's western and southern beaches. The West and South Coasts differ in terms of *Genypterus capensis* larval distribution and spawning seasonality, according to research by Olivar and Sabatés (1989) (Figure 1.6.). On the West Coast, spawning occurred during months in which upwelling was less intense (i.e., June – December). Larvae were only found between May and October, as spawning intensity started declining in October when upwelling resumed, and no larvae were found in December. Surface temperature was linked to larval distribution, with larvae found further offshore when temperatures were low. *Genypterus capensis* larvae show a preference for the central part of the shelf on the West Coast because of the hydrographic complexity of this area.

On the south-western part of the Agulhas Bank (South Coast), spawning intensity was shown to be correlated with/to sea surface temperature, with spawning occurring between April and December when temperatures are lower. Larvae occurred on the whole continental shelf (Olivar and Sabatés, 1989). Japp and Hecht (1990) suggested that *G. capensis* spawning on the South-East Coast is due to the commencement of strong easterly winds in the spring. The resulting inshore advection of water would facilitate the movement of eggs and larvae toward shallower water thereby increasing their chances of survival. Payne (1977) suggested that *G. capensis* may have a similar spawning period to hake (*M. capensis and M. paradoxus*). Analysis by Guerra (2019) established that September is likely the peak month for spawning activity on the south coast.

On the west coast, inshore trawl catches analysed by Japp (1989) indicated movement toward deeper water with increasing size. On the south coast, Japp and Hecht (1990) described how the pelagic larvae settled on the Agulhas Bank and then moved offshore to deeper waters by their fourth or fifth year.

1.2.5.3. Juvenile and adult fish

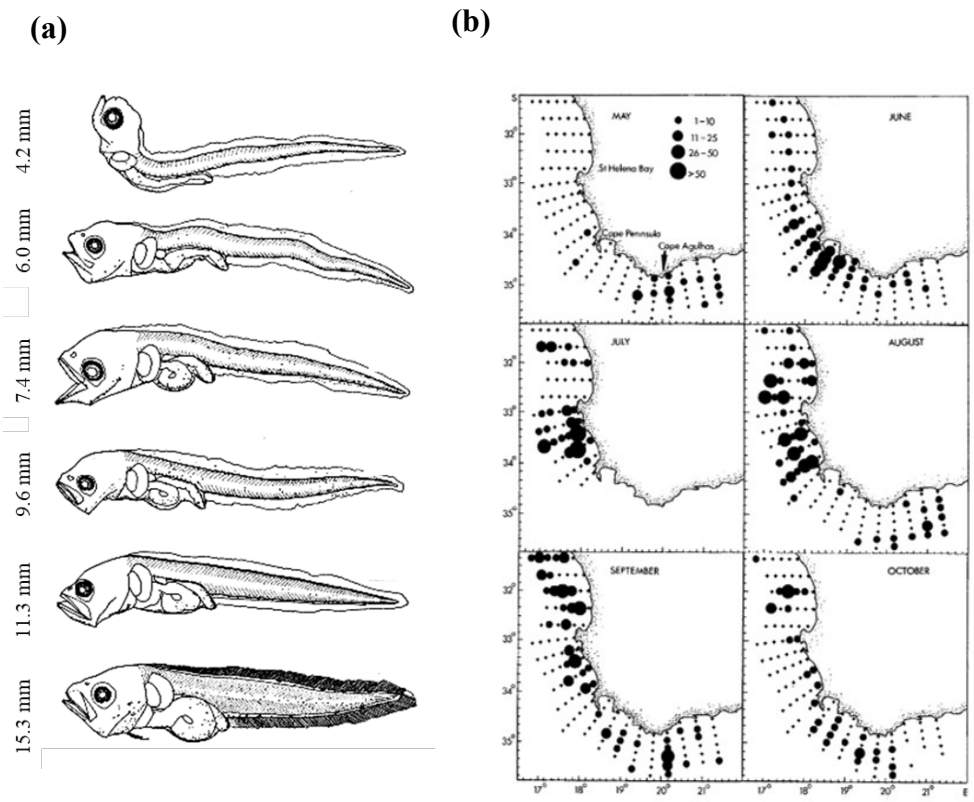


Figure 1.6. (a) *Genypterus capensis* larvae at different lengths **(b)** Distribution of larvae during the main spawning periods (**Source**: Olivar and Sabatés, 1989)

Badenhorst and Smale (1991) noted a trend of increasing age with increasing depth for *Genypterus capensis* on the South Coast, inferring recruitment in shallower water (< 200-m), followed by migration into deeper water as the fish ages. Their study showed that specimens was caught in depths less than 50-m were juveniles, whilst at depths of 51 – 200-m, samples consisted of both juveniles and fish approaching maturity. During the period 1986 – 1988, it was found that 51% of longline catches at depths of 350 – 449-m, were mature specimens (Japp, 1990; Japp, 1989). Badenhorst and Smale (1991) further noted that mature fish were located on the outer shelf and slope.

Additionally, Japp and Hecht (1990) noted that longline catches in deeper waters were composed mostly of large females, whilst trawl catches in shallower water were predominantly males. Only during pre-spawning aggregations does the sex ratio change. This suggests the migration of males to deeper waters when females are ready to spawn. Coast inshore grounds were mainly composed of immature fish or individuals that had just attained sexual maturity. A horizontal depth distribution of *G. capensis* related to size (Figure 1.7a) was previously also suggested by Payne (1986) on the South-East coast, whilst Botha (1985) recorded this detail for the species off the West Coast. Shin *et al.* (2004) and DFFE (2020) reported different spatial distributions for *G. capensis* aged 0 – 3 years (juveniles), compared to those aged 4, as portrayed in Figure 1.7b.

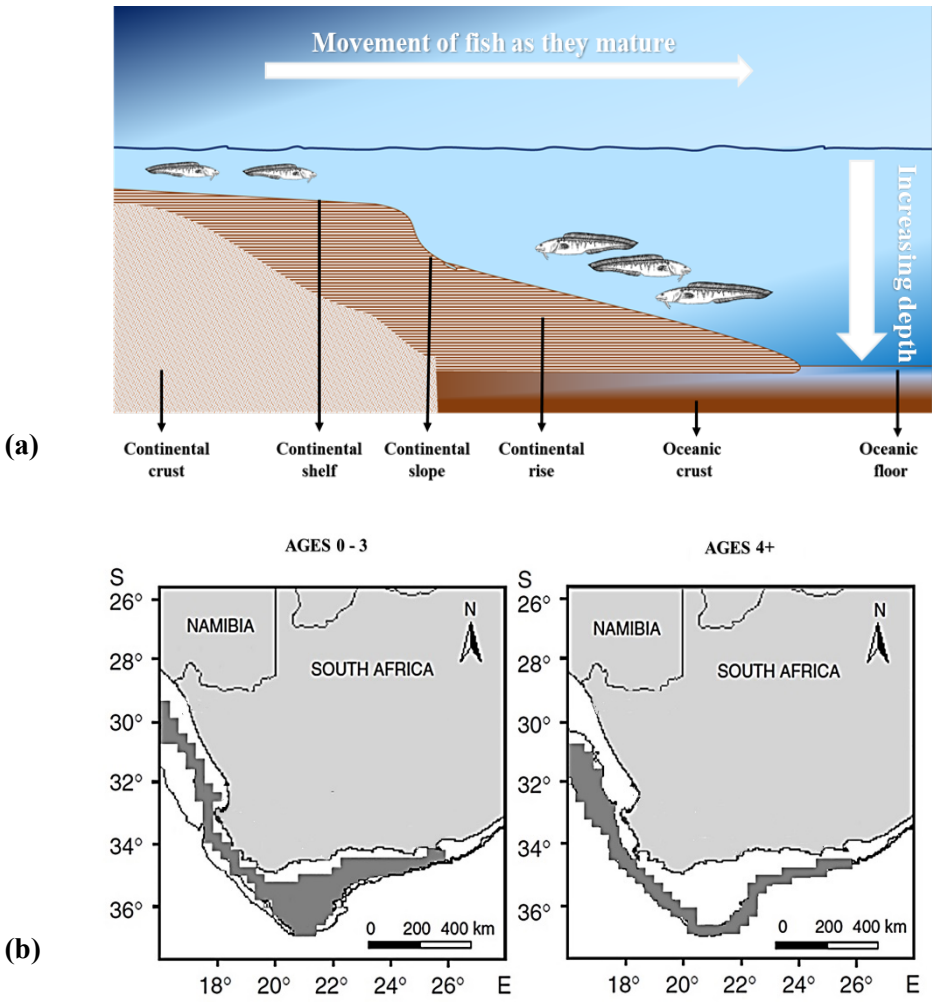


Figure 1.7. (a) Depiction of 'increasing depth with size' (**Source:** adapted from interpretation of text by Japp and Hecht, 1990 and Badenhorst and Smale, 1990) **(b)** Spatial distribution of *Genypterus capensis* at age-classes: 0 – 3 years and 4+ years (**Source:** Modified from Shin *et al.*, 2004) .

1.2.6. Species of the genus *Genypterus*

The genus, *Genypterus,* is unique to Southern Hemisphere temperate waters (Smith and Heemstra, 1986). Cohen and Nielsen (1978) noted the existence of at least five valid species globally, namely: *Genypterus blacodes* (Forster, 1801), *Genypterus brasiliensis* (Regan, 1903), *Genypterus chilensis* (Guichenot, 1848), *Genypterus maculatus* (Tschudi, 1846) and *Genypterus tigerinus* (Klunzinger, 1872).

Another species, *Genypterus microstomus* (Regan, 1903) considered to be a separate species from *G. blacodes* (Regan, 1903; Waite, 1923). The species was known as the banded ling in Australia (Scott *et al.,* 1974) and was listed as a valid species from New Zealand by Hutton (1904), Waite (1907), Phillipps (1927a, 1927b), Whitley (1956), Whitley (1968), Paulin and Stewart (1985), and Paulin *et al.* (1989). Paxton *et al.* (1989) and Nielsen *et al.* (1999) listed *G. microstomus* as a junior synonym for *G. blacodes.* This was however done without justification. A combined genetic and morphological study by Daley *et al.* (2000) indicated that the two are the same species and concluded that *G. microstomus* should be considered a junior synonym for *G. blacodes.* The same conclusion was reached by Smith and Paulin (2003) based on genetic, morphometric and meristic analysis.

1.3. MANAGEMENT OF THE FISHERIES FOR *GENYPTERUS CAPENSIS*

Genypterus capensis is a commercial species in South Africa with a high market value that has considerable economic value to several South African fisheries (Olivar and Sabates, 1989; Japp, 1989; SAAMBR, 2021). The species is commercially significant and caught as incidental bycatch by either the offshore demersal trawls or Hake (*Merluccius* sp.) demersal longline sectors (DFFE, 2020). The annual catches of *G. capensis* made by longline and trawl catches on the Western and southern coasts of South Africa, along with the precautionary upper catch limit (PUCL) for the years 1932 to 2018, is presented in Figure 1.8.

1.3.1. Historical management data

The first trawling fisheries in South Africa took place by steam tugs in Algoa Bay in 1896 (Gilchrist, 1896). The commercial trawl fleet expanded (Payne, 1989) leading to the development of fishing grounds to the west on which hake-focused trawl activity was initiated (Gilchrist, 1922). *Genypterus capensis* grounds were found along the South Coast, and trawling efforts were directed there between 1966 and 1983. Even so, the West Coast catches still constituted more than half the total landings.

The year 1983 was significant since a demersal longline fishery directed at *G. capensis* was introduced on an experimental basis off South Africa, and the first permits were authorised. This led to a severe increase in the total landings of the species. Longline catches of *G. capensis* peaked in 1986, with a large proportion of the catch being taken from the South Coast (Japp *et al.,* 1994; Atkinson, 2010).

In a review of the longline fishery Badenhorst (1988) concluded that despite the increase in longline-directed effort, *G. capensis* stocks off South Africa were not being unfavourably affected. However, following the recommendation of Japp and Punt (1989) to significantly reduce the amount of longline effort directed towards *G. capensis*, both longline and trawl catches experienced a steep decline (Japp 1989).

Warnings of the over-exploitation of the resource led to the closure of directed longline fishery with restrictions eventually being placed on the trawl fishery in 1989 (Punt and Japp, 1994; Brandão and Butterworth, 2013). In 1990, Punt estimated that *G. capensis* had already been biologically overexploited by the trawl fishery.

According to the South African Commercial Fisheries Review, in 1991, the cessation of demersal longlining was followed by evidence of improvement of deep-sea trawl catches, and it was hoped that in the absence of longlining,

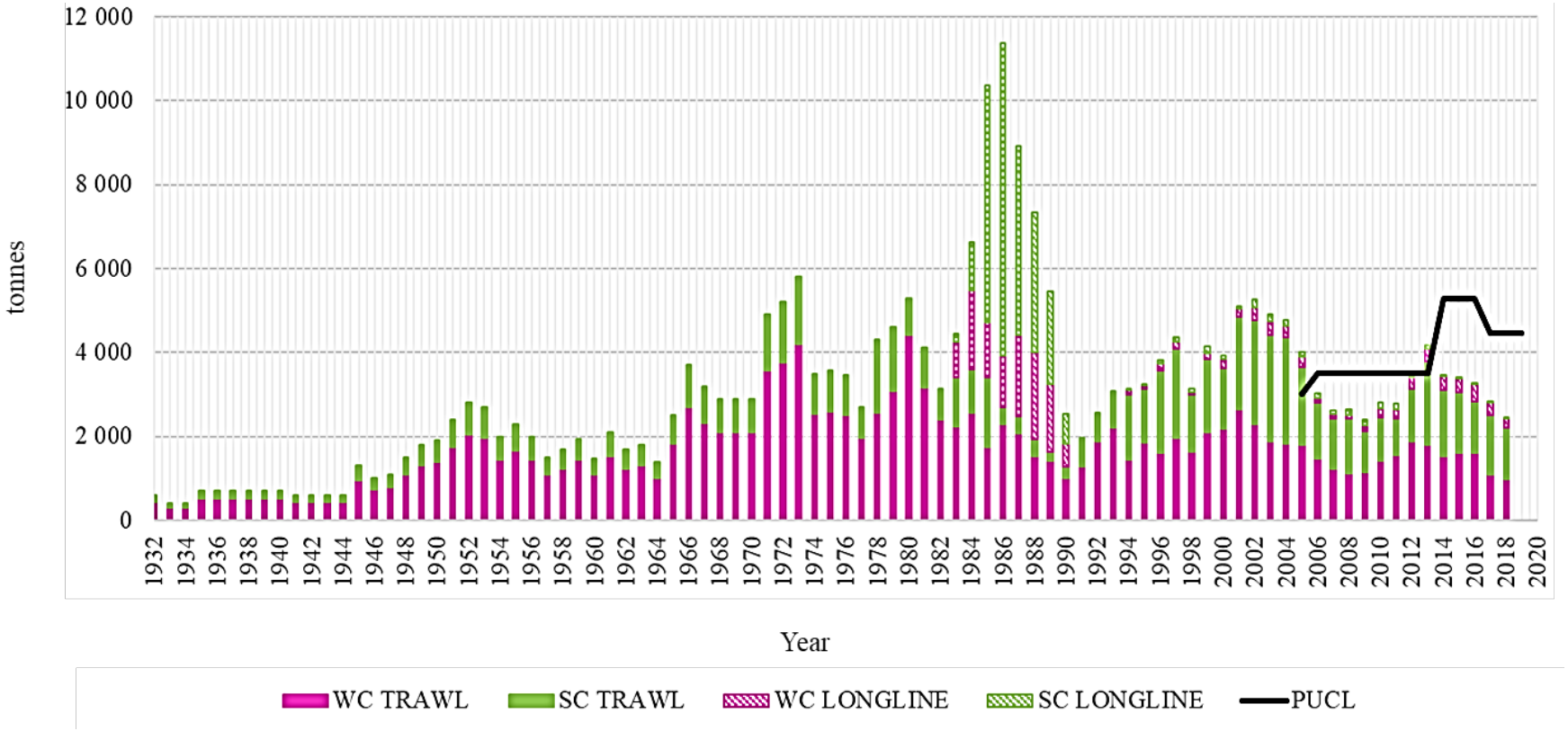


Figure 1.8. shows a stacked bar graph with PUCL (Precautionary Upper capture Limit) showing the longline and trawl capture (in tonnes) of *Genypterus capensis* on the West Coast (WC) and South Coast (SC) of South Africa from 1932 to 2020.

the *G. capensis* stock would recover. Between 1993 and 1997, the resource showed further signs of recovery but landings remained below 3 000 tonnes. Thereafter, landings increased once again, reaching a peak of 5 026-t in 2002.

In 2006, a Precautionary Upper Catch Limit (PUCL) of 3000 tonnes was introduced for *G. capensis*, which was increased to 3 500 tonnes in 2007. Based on the results of a Replacement Yield Assessment conducted in 2013, the PUCL was increased to 5 264 tonnes in 2014 (DFFE, 2016) and then reduced to 4 450 tonnes in 2017 (DFFE, 2020). As discussed in the following section (Section 1.3.2), the utilization of the resource is considered to be at near optimal levels (DFFE, 2020). However, the by-catch of the fishery is still a management concern (Brandão and Butterworth, 2008; Henriques *et al.,* 2017; Schulze *et al*., 2020).

1.3.2. Current status and management

Genypterus capensis is listed as unclassified on the IUCN (International Union for Conservation of Nature) Red List (IUCN, 2020). According to DFFE (2020), this fisheries stock status and fishing pressure, is at optimal levels.

On the SASSI (South African Sustainable Seafood Initiative) list:

- Offshore demersal trawl caught *G.capensis* fisheries have been designated 'orange'. This is as there are reasons for concern since the impact of the damage caused by trawlers on the seabed is unknown (SASSI, 2018).
- In 2015, WWF-SASSI reassessed the Demersal Longline Fishery sector and *G. capensis* was moved from the orange list to the green list (SASSI, 2018). The green list indicates that the species is of the healthiest and most well-managed fish populations and is fished in a sustainable way that does not cause any damage to the ecosystem (SASSI, 2018).

Sink *et al.* (2012) documented the potential consequences of the demersal hake trawling fishery on benthic habitats. A map showing the spatial distribution of hake landed by the inshore and offshore demersal trawl sectors for the years 2008 to 2016 is shown in Figure 1.9. The same spatial and temporal measures applied to hake trawl, is used for hake directed longline, and distribution of priority areas (Table 1.4) for *G. capensis* is consistent between the two fisheries. Longline fishery does not however form part of the MSC certification. This means that the two fisheries do not share the same restrictions, and longline fishing facilitates catches in areas where trawl cannot e.g., reef, high profile, hard grounds (Norman *et al.,* 2018). Even though *G. capensis* is widely distributed around the South African coast, the species abundance remains fragmented because of habitat preferences. A map showing the distribution of *G. capensis* fishery resource in South African waters, as derived from fishery-independent demersal research surveys (adapted from DFFE, 2020) is presented in Figure 1.10.

Table 1.4. Priority fishing areas for hake longline fishing (derived from Norman *et al.* 2018)

Area	Location	Known as
1	Due west of Hondeklip Bay	Karbonkel
2	Due west of Saldanha Bay	Dassen Hole
3	A widespread area stretching from due west of Cape Town to due south of Danger Point	Browns Bank
4	A region due south of Cape Agulhas reaching towards the southern-most part of the Agulhas Bank	
5	South of Gqeberha and Cape St Francis	Chalk Line
6	A shallow area inshore between Mossel Bay and Struisbaai	

Note: Kingklip (*Genypterus capensis)* is a bycatch of Hake (*Merluccius* sp.) in South African waters

Figure 1.9.

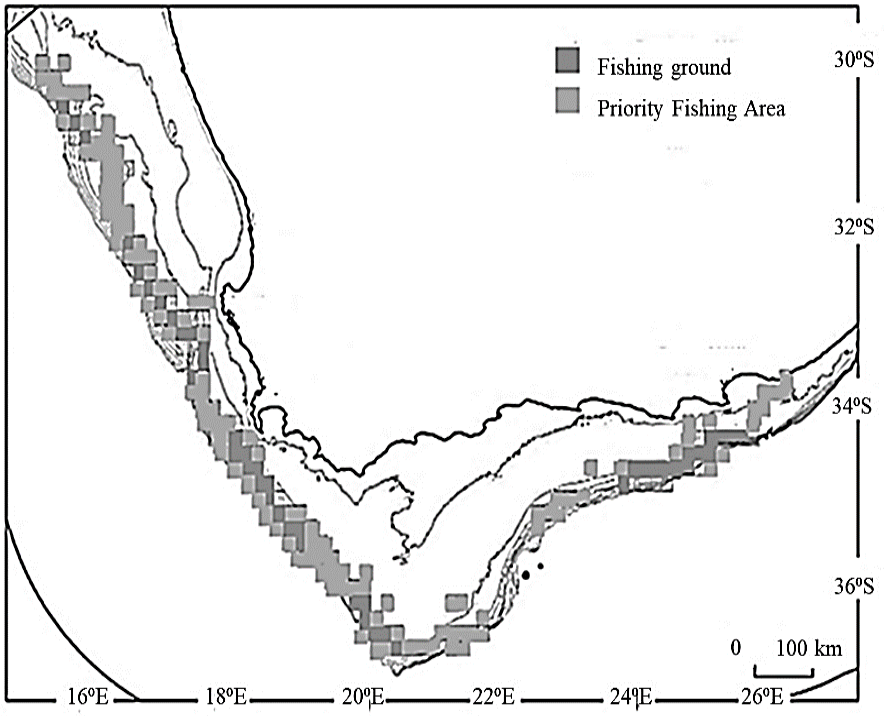


Figure 1.10.

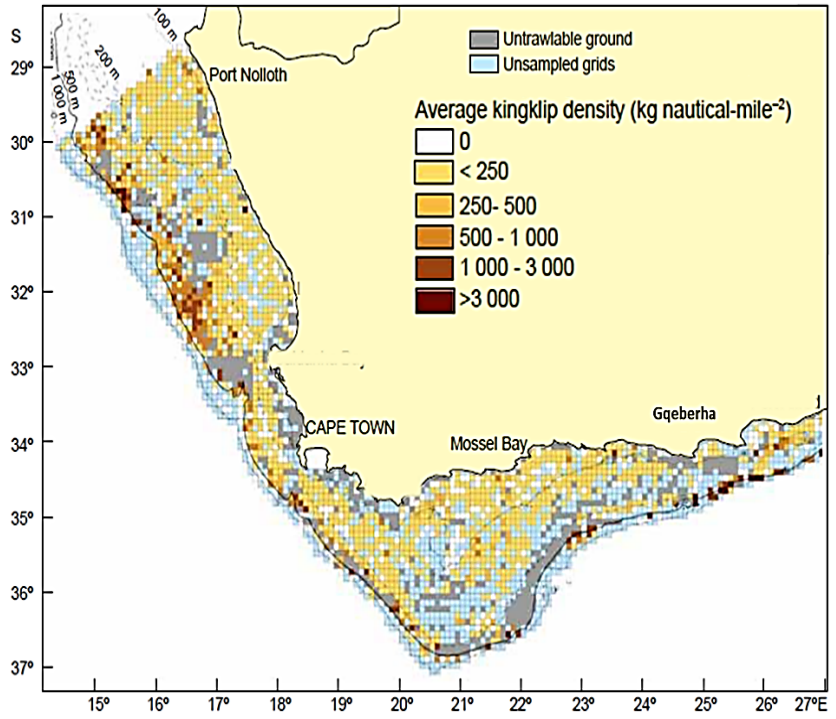


Figure 1.9. Spatial distribution of hake landed by the inshore and offshore demersal trawl sectors for the years 2008 to 2016 (Source: map edited from Norman *et al.*, 2018). **Figure 1.10.** Map showing the distribution of *Genypterus capensis* fisheries resource in South African waters, as derived from fishery-independent demersal research surveys. Untrawlable grounds and unsampled grids are highlighted. Densities (kg nautical-mile^{-2}) are averages over all survey stations within each survey grid block (**Source**: Map edited from DFFE, 2020).

1.4. MARINE STOCKS

1.4.1. Stock assessment

As graphically portrayed in Figure 1.11, stock assessment uses various statistical and mathematical computations to make quantitative projections regarding the reactions of exploited fish populations to alternative management options (i.e., limiting fishing pressure on commercial species). The information required to enable such predictions are based on measures like fecundity, stock size, age structure and recruitment (Hilborn and Walters, 1992). As an alternative to pooling populations into a species-wide management unit, a more refined basis for the setting of harvest quotas may be the identification of genetically and demographically distinct subpopulations (Grant and Leslie, 2005).

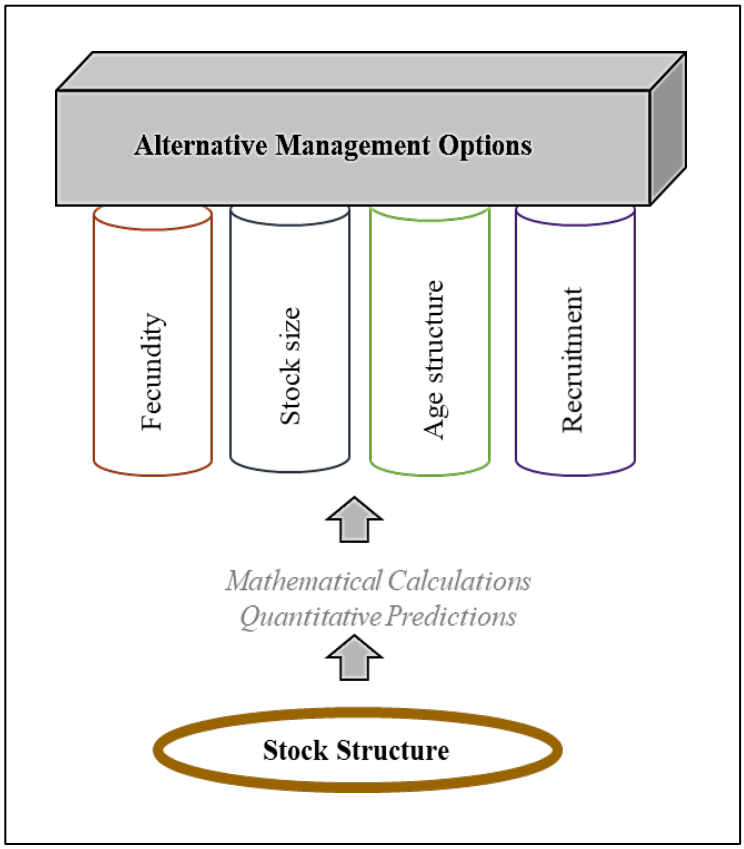


Figure 1.11. The role of stock structure studies in fisheries management (**Source:** based on interpretation of text by Carvalho and Nigmatullin, 1998).

For fisheries management, the understanding of the stock structure of a species is vital (Begg and Waldman, 1999). Fisheries management has a range of objectives which includes forecasting and achieving sustainable yields, assessing recruitment dynamics, conserving threatened and endangered species, evaluating depletion methods, and rebuilding overfished stocks (Carvalho and Nigmatullin, 1998; Cadrin *et al.,* 2005). The identification of stocks allows us to understand how (or why) a fishery might differentially exploit certain components of a population. The stock concept facilitates a better understanding of the vulnerability of subpopulations within specific species to overfishing, as well as the areas and components that are more likely to be overfished (Baldwin *et al.,* 2012).

Unfortunately, information resulting from fish stock structure assessment may also create confusion in management, especially when it contradicts and challenges already established stocks and management boundaries. Misunderstandings in stock structure analysis may arise due to inconsistent terminologies and interpretations, in addition to an extensive range of approaches employed (Cadrin *et al.,* 2005). Overfishing may not necessarily be the sole cause of a stock declining (Perissi *et al.,* 2017), but high exploitation rates in addition to other erroneous and incompetent management strategies are likely to result in the depletion of fish stocks (Begg and Waldman, 1999), as reported for: Anchovy, *Engraulis ringens* (Hilborn and Walters, 1992), Atlantic cod, *Gadus morhua* (Hutchings, 1996), haddock, *Melanogrammus aeglefinus* (Clark *et al.,* 1982), and herring, *Clupea harengus* (Dragesund *et al.,* 1980) , amongst others.

1.4.2. Stock structure: definition and terminology

As species classification may be complex, the identification of a species in a biological community has been described as a "fuzzy art" (Milius, 2017; Zachos, 2016 in Cadrin, 2020). Tawfiq (2010) noted that defining spatial boundaries within or among populations can be challenging because biology is "rather messy." Additionally, the terminology (e.g., population structure, stock identification, stock structure, mixed stocks, and spatial structure) is inconsistent, which is likely the result of the fundamental complexity of

spatial population structure. As such, even though the stock concept is essential for fishery stock assessment, defining stock structure can be difficult (Carvalho and Hauser, 1994; Booke, 1981).

Speciation is defined as the formation of new and distinct species via the course of evolution (Gittleman, 2021 in Encyclopaedia Brittanica). New species can diverge while exchanging genes, hinging on:

- the intensity of disruptive natural selection, and
- factors that impact the linkages of genes under disruptive selection.

This manner of diversification (divergence with gene flow) includes sympatric speciation (Pinho and Hey, 2010). A simple model portraying a scale of population differentiation through divergence, from panmixia to isolation is shown in Figure 1.12. Each circle represents a metapopulation (Waples and Gaggiotti, 2006). The onset of divergence is a result of gene exchange (Pinho and Hey, 2010) and in this initial phase, the metapopulation is panmictic and mating within the subpopulations are random (Waples and Gaggiotti, 2006). A series of situations occurs in which gene flow occurs intermittently and ultimately the subpopulations become completely isolated

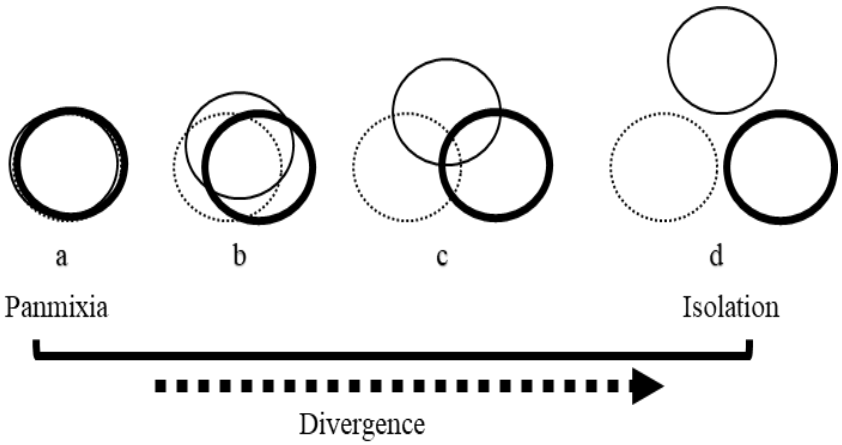


Figure 1.12. The continuum of population differentiation. Each group of circles represents a group of subpopulations with varying degrees of (geographical overlap and/or migration) connectivity **(a)** Panmixia 'subpopulations' are completely congruent **(b)** Substantial connectivity **(c)** Modest connectivity **(d)** Complete independence (**Source:** adapted from Waples and Gaggiotti, 2006)

with no direct genetic or demographic linkages (Waples and Gaggiotti, 2006; Pinho and Hey, 2010).

Numerous definitions of the term ‘stock’ have developed based on different approaches, including contingent stocks (e.g. *I*, Secor, 1999), genotypic stocks (e.g. Booke, 1981; Futuyma, 1986; Carvalho and Hauser, 1994), harvest stocks (e.g., Secor, 1999), operational stocks (e.g., Waldman, 2005) and phenotypic stocks (e.g., Bailey and Smith, 1981; Booke, 1981; Cadrin *et al.,* 2005). Table 1.5. lists and defines different stock types, as well as other terminology used to define population structure.

Since a single stock definition encompassing all relevant factors (e.g., environmental, biological, political) is yet to be established, an inclusive stock identification study should consist of several methods (Coyle, 1998) with the choice of technique dependent on the research and management questions under consideration (Begg and Waldman, 1999). Numerous definitions of the stock concept could be adopted, hinging on the scientific question and methods used (Begg *et al.,* 1999; Abaunza *et al.,* 2008; Cadrin, 2020).

Table 1.5. Terminology used to define population structure

Term	Definition	Reference
Population	A group of interbreeding individuals that exist together in time and space	Waples and Gaggiotti, 2006
Metapopulation	A system of interacting biological populations (termed subpopulations) that exhibit a degree of independence in local population dynamics as well as connectivity between subpopulations	Cadrin *et al.,* 2014
Species	A group of individuals that actually or potentially interbreeds Groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups	Milius, 2017; Zachos, 2016; Mayr, 1942
Stock	A biological population or metapopulation, a portion of a population, or multiple populations	Cadrin *et al.,* 2014
	An intraspecific group of randomly mating reproductive individuals with temporal or spatial integrity	Ihssen *et al.,* 1981
Phenotypic Stock	Characteristics which are expressed in one or more ways depending on the type of environment	Booke, 1981
Harvest Stock	Local group of fish that has an independent response to fishing, regardless of genetic or phenotypic similarities to adjacent resources	Gauldie, 1984
Genotypic Stock	A group of conspecific organisms that occupy a (more/less) well-defined geographic region and exhibit reproductive continuity from generation to generation.	Futuyma, 1986
Operational Stock	A specific portion of a population that is influenced by an anthropogenic activity that affects population productivity.	Waldman, 2005
Contingent Stock	This hypothesis considers multiple life cycles of a single population constrained to utilize a common spawning ground.	Secor, 1999

1.4.3. Assessing Stock Structure

Stock identification plays a significant role in determining the boundaries of specific populations of a species, and indicating what regulates subpopulation abundance, reproduction, growth, and survival (Baldwin *et al.,* 2012). In contrast to being viewed as a homogenous group which differs from other groups, a stock should instead be viewed as a heterogenous group of individuals that are distinguished by portraying a specific range of variation. This range includes differences in morphological, meristic, molecular, or reproductive characteristics. Once the distinction is established, the biological diversity among populations assigned to recognisable groups can then be compared (Carvalho and Nigmatullin, 1998). Several methods have been employed for stock structure analysis (Carvalho and Nigmatullin, 1998; Cadrin *et al.,* 2005).

Some of the methods summarised by ICES (2021) in the Stock Identification Methods Working Group (SIMWG) reviews included: Morphometrics/meristics, otolith shape, otolith chemistry, parasitology, and interdisciplinary approaches, as some of the focal areas of research. These methods have been applied in this thesis and detailed descriptions of each are included in Chapters 2 to 5. Other methods used for stock discrimination, which are not applied in this study, include fatty acid profiles (Cadrin 2005), genetics, genomics, artificial tagging, and life history parameters (Cadrin 2005; ICES, 2021), and are succinctly described below.

1.4.3.1. Fatty Acid Profiles

In the lipids of marine biota, approximately 20 fatty acids appear in relative amounts of more than 1% (Grahl-Nilsen, 2005). Fatty acid (FA) profiles have been widely studied in a range of tissues of aquatic organisms and for a variety of reasons (Prabu *et al.,* 2015). Since FA profiles are influenced by the nutritional habits of the species, they prove to be significant quality parameters for species with a similar genetic or geographic origin (Grahl-Nilsen, 2005; Almeida and Silva, 2016) and different species of fish have different fatty acid profiles (Ackman, 1980). The FA profile of muscle tissue

(*Lutjanus* sp., Almeida and Silva, 2016), heart tissue (*Sebastes mentella* , Joensen and Grahl-Nielsen, 2004) and fish scales (*Catla*, Prabu *et al.,* 2015) are amongst studies in which this method has been used for the identification of fish stocks and species.

1.4.3.2. Genetic and genomic analysis

Genetics

The potential for high gene flow in marine fishes makes it difficult to genetically determine the number of sub-populations managed in specific fishery (Baldwin *et al.,* 2012). The quality of the information contributed to stock identification by molecular genetics depends on the method used (e.g., allozyme, mitochondrial, *etc.*), the study objective (e.g., biological, fisheries-related, genetic, etc.), and the time scale of interest *i.e*., short *vs.* long term management (Coyle, 1998).

For stock identification (and in any parallel applications that hinge on population genetics theory) three main factors must be deliberated upon in the selection and usage of molecular markers:

1. The type of marker
    - Neutral markers → non-functional genomic regions are employed to investigate genetic drift and gene flow
    - Adaptive markers → functional regions that can be used to measure the influence of natural selection
2. The variability of the chosen marker must be such to provide sufficient statistical power for the detection of the effect of interest.
3. The number of markers used must guarantee a realistic and unbiased view of the process investigated.

Some of the genetic tools used in phylogenetic relationship studies include allozymes (Koljonen and Wilmot, 2005), microsatellite loci (Hampton, 2014) and mitochondrial DNA (Magoulas, 2005; Hampton, 2014). The resolution potential of these techniques is portrayed in Table 1.6 (Bloomer, 2004):

Table 1.6. Summary of the genetic tools available and their potential applications (**Source**: Bloomer, 2004).

Technique	Allozyme	Microsatellite	mtDNA
Individual identity			
Relatedness			
Population genetics			
Stocks			
Between closely related species			
Species identity			
Deep phylogenetic relationships			

Legend

Highest potential resolution
Intermediate potential resolution
3 Not appropriate

Other genetic markers available for studies of differentiation, within and between populations of living marine resources, and include: AFLP: Amplified Fragment Length Polymorphism (Liu, 2005), chromosome morphology (Philips, 2005), nuclear DNA: Single Copy and Repetitive Sequence Markers (Wirgin and Waldman, 2005), and RAPD: Random Amplified Polymorphic DNA (Smith 2005).

A study on pink ling (*Genypterus blacodes*) around New Zealand that used allozyme and microsatellite DNA population markers found that allozyme markers could define subpopulation (Smith and Francis, 1982). However, the divergence of stocks and the degree of reproductive isolation is largely dependent on time (see Figure 1.12). Genetic markers can detect genetic heterogeneity and accordingly, determine genetic differentiation amongst stocks (Begg and Waldman, 1999). If a recent divergence, substantial mixing, or the exchange of genetic information between stocks is determined, it can obscure heterogeneity in genetic markers (Clayton 1981; Carvalho and Hauser, 1994; Begg and Waldman, 1999). Due to lack of statistical power, neutral markers may not expose slight levels of genetic divergence (Carvalho and Hauser, 1994; Ward *et al.,* 1994; Hauser and Carvalho, 2008; Nielsen *et al.,* 2009; Milano *et al.,* 2011; Schulze *et al.,* 2020). This is where next-generation sequencing (NGS) has come into play, by enabling the application

of single nucleotide polymorphisms (SNPs) to study both genome-wide (neutral), and allele-specific (adaptive) patterns of diversity in any organism. The use of these genomic tools facilitates moving from fisheries genetics to fisheries genomics (Valenzuela-Quiñonez, 2016).

Genomics

Genomic analysis makes use of the fact the genome of a teleost fish contains over one billion nucleotides (Gregory *et al.,* 2007). Single Nucleotide Polymorphisms (SNPs) allows for the detection of finer-scale population differentiation by increasing the number of markers used in high gene flow systems, or between populations formed by recent divergence (Waples and Gaggiotti, 2006; Reiss *et al.,* 2009; Bernatchez *et al.,* 2017; Schulze *et al.,* 2020; D'Aloia *et al.,* 2020). To improve the resolution of population sub-structuring, putatively adaptive (statistical outlier) loci was found to be useful (Reiss *et al.,* 2009; Milano *et al.,* 2011; Bradbury *et al.,* 2012; Nielsen *et al.,* 2012; Schulze *et al.,* 2020) and genetic variation at the genomic level has proven to be invaluable in fisheries management (Zhang *et al.,* 2020).

Genetics and genomics

Genomic applications facilitates a new paradigm for fisheries management by enabling the integration of adaptive diversity in order to understand vital aspects of fisheries resources (Valenzuela-Quiñonez, 2016). The ICES (2021) scientific report indicated that whilst studies using microsatellite data are slightly declining, the number of papers with SNPs have doubled since 2020. A study by McKeown *et al.* (2020) using RAD-seq-derived SNPs revealed a lack of structure among the Northeast Atlantic samples of European sprat (*Sprattus sprattus*), signifying connectivity across current management boundaries. Another study using SNPs involved the comprehensive analysis of the white anglerfish (*Lophius piscatorius*) in the Northeast Atlantic. One of the key results from this study indicated that the species is likely panmictic, which differed from the current three-stock management approach (Aguirre-Sarabia *et al.,* 2021). According to Attard *et al.,* (2022) genomics surpasses genetics for the management of mistakes in fisheries stocking of threatened species.

The differences between of Mitochondrial DNA, microsatellites, and Single Nucleotide Polymorphisms (SNPs) found in the DNA of a diploid species is listed in Table 1.7 and portrayed in Figure 1.13.

Table 1.7. Differences between of Mitochondrial DNA, microsatellites, and Single Nucleotide Polymorphisms (SNPs) found in the DNA of a diploid species **(Source**: adapted from Morin *et al.*, 2004).

Mitochondrial DNA	Microsatellites	Single Nucleotide Polymorphisms
One linked array of genes (one locus)	Nuclear DNA	Nuclear DNA
Control region sequence	≈10 - 20 loci analysed	Millions of loci
Maternally inherited	Biparentally inherited	Spaced after every 300 - 100 base pairs
Non-recombining	Hypervariable in repeat lengths (alleles)	Biparentally inherited
Few -thousand copies per cell	Often spaced every 5000 - 50 000 base pairs	

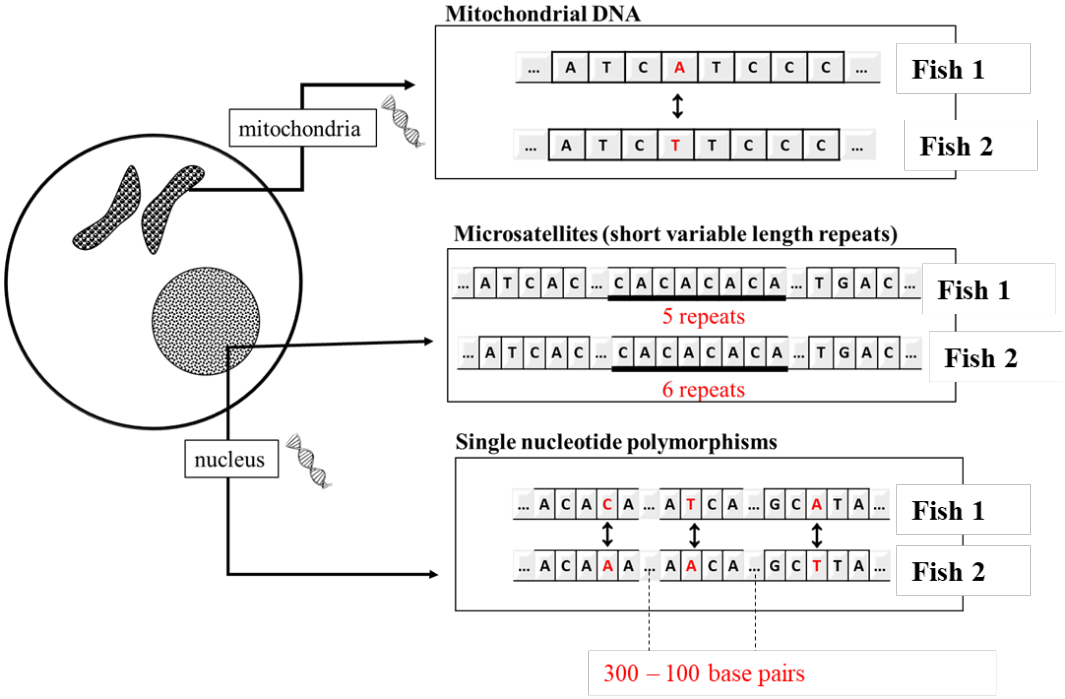


Figure. 1.13. Depictions of Mitochondrial DNA, microsatellites and Single Nucleotide Polymorphisms found in the DNA of a diploid species. **Source**: Adapted from Morin *et al.* (2004).

1.4.3.3. Reproductive life history parameters

According to Ihssen *et al.* (1981), fish adapt their reproductive strategy and life history to the physical environment in which they reside. Life history parameters (e.g. distribution, abundance, age, growth, mortality, reproduction, recruitment, etc.), reflect the fundamental population dynamics of the stock (Ihssen *et al.,* 1981; Pawson and Jennings, 1996; Begg *et al.,* 1999). Differences in life history traits have been used in the fish stock differentiation (Griffiths, 1996; Griffiths, 1997; Coetzee *et al.,* 2008) and may serve as a foundation for the initial recognition and delineation of geographic regions that represent these individual stocks (Pawson and Jennings, 1996). Whilst life history parameters cannot give distinct evidence of genetic differences amongst fish stocks (Ihssen *et al.,* 1981), they may indicate phenotypic variations arising from different environmental conditions and/or fishing pressures (Campana *et al.,* 1995, Begg *et al.,* 1999; Begg, 2005).

Some of the reproductive life history parameters that have been used to describe the dynamics of a fish stock and provide the basis for stock differentiation (Begg, 2005), include:

- Timing, duration, and location of spawning
- Egg and larval distributions
- Proportion of recruit and repeat spawners
- Median or mean age, length, and weight at maturity
- Egg weight, size, viability, and fecundity-size relationships
- Maternal effects and reproductive potential

In this dissertation, the life cycle characteristics of *Genypterus capensis* are considered for background information and the interpretation of results, but they are not discussed as a separate technique.

1.4.3.4. Artificial tagging

Both external and internal artificial tags have been used for centuries as markers on fish with the aim of identifying and gaining insight into both freshwater and marine species (Jacobsen and Hansen, 2005). The conventional approach involved catching, marking, and then releasing wild

fish, with their recapture providing a wealth of information such as growth rates and distances moved. Since the early twentieth century, this approach has been extended to stock identification studies. Putative stocks were marked, and subsequent recoveries allowed for the range and pattern of movement, as well as the extent of mixing among stocks (if any), to be inferred (Cushing, 1981; Coyle, 1998; Jacobsen and Hansen, 2005). The effective use of this method, however, requires that a representative sample must be tagged and then successfully recaptured (Cushing, 1981; Begg and Waldman, 1999).

Recent published studies are based on a range of tagging techniques including conventional tags (e.g., Randon *et al.,* 2021), electronic data storage tags (Gatti *et al.,* 2021), acoustic tags and telemetry (Hessenauer *et al.,* 2021), and satellite pop-up tags (e.g., Armstrong *et al.,* 2020).

1.4.3.5. Multi-method approach

Several marine species have a mixed stock structure which is more complex than what is currently recognised by management (Stephensen, 1999). The objective of stock identification studies is to obtain management units that complement distinguished biological entities, thereby reducing uncertainty in the assessment models and/or bettering the management of fish resources (Abaunza *et al.,* 2008). The identification of management units of the same species is essential, yet accurate classification is unlikely to be accomplished with a single technique. Given this limitation, a holistic solution for identifying stock structure studies should be established (Edmonds *et al.,* 1989; Campana and Gagne, 1995; Begg and Waldman, 1999). Using multiple methods could produce different results or increase the probability of correctly defining different fish stocks (Pawson and Jennings, 1996; Begg and Waldman, 1999; Begg *et al.,* 1999; Turan, 2004). The combined use of two or more stock identification techniques i.e., interdisciplinary analyses, has increased in recent years and is becoming a standard approach (ICES, 2021). In addition to the identification and delineation of stock boundaries, the techniques used in stock structure studies also provide useful information to better understand and manage fish species (Baldwin *et al.,* 2012).

1.5. STOCK STRUCTURE STUDIES

The stock structure of *Genypterus* species has been the subject of numerous research studies employing a range of methodologies. Tables 1.8 and 1.9 provide summaries of some of the published research.

Debate has always surrounded the stock structure of *Genypterus capensis*. The studies conducted on morphology, capture history, and spawning data revealed the existence of either single- or multi-stock structures (Badenhorst, 1988; Japp, 1989; Isarev, 1976; Payne, 1977; Payne, 1984; Payne, 1985; Olivar and Sabates, 1989). According to genetic data, a single genetic population of *G. capensis* was found by Grant and Leslie's (2005) study employing allozyme markers. However, Henriquez *et al.* (2016)'s study using mtDNA and microsatellites suggested that there were at least two genetic units, and Schulze *et al.* (2020) used the most recent genetic markers (SNPs) to identify two distinct stocks of the species (West and South Coast), with some gene flow between the two components.

In Australia (Daley *et al.*, 2005) and New Zealand (Horn, 1993; Colman, 1995; Horn, 2005), morphology and meristics on *G. blacodes* are among the techniques used. *Genypterus blacodes* has been subjected to otolith shape study in Chile (Wiff *et al.*, 2019) and New Zealand (Colman, 1995; Ladroit *et al.*, 2017). Furthermore, *G. blacodes* in New Zealand (Smith and Paulin, 2003) and *G. chilensis* in Chile (Córdova-Alarcón *et al.*, 2019; González *et al.*, 2019) were the subjects of genetic applications for stock structure. Research on the use of parasites as biotags includes efforts on *G. brasiliensis* in Brazil/Argentina (Alves *et al.*, 2002).

Table 1.8. Table summarising published literature on the stock structure of *Genypterus capensis* off the southern African coastline, showing the number of stocks, their distributions, and techniques used for stock differentiation.

Number of stocks	Year	Reference author(s)	Distribution of stocks	Technique
2	1976	Isarev	1. Lüderitz northwards 2. Lüderitz to Cape Agulhas	Morphology Meristics
2	1977	Payne	1. Off Walvis Bay 2. Lüderitz to south of Cape Town	Morphology Meristics
3	1985	Payne	1. Lüderitz northwards 2. Lüderitz to Cape Agulhas 3. South-eastern coast	Morphology Meristics
1	1988	Badenhorst	2. Southern African coastline	Continuity in longline catches
1	1989	Japp	1. Southern African coastline	Morphology Meristics
2	1989	Olivar and Sabatés	1. Western stock 2. Southern stock (of South Africa)	Spawning strategies
1	2005	Grant and Leslie	Southern African coastline	Genetics
2	2016	Henriques *et al.*	1. Western stock 2. Southern stock (of South Africa)	Genetics
2	2019	Guerra	1. Western stock 2. Southern stock (of South Africa)	Suggestion based on L_{50} values
3	2020	Schulze *et al.*	1. Northern Benguela 2. Southern Benguela 3. South Coast stock (of South Africa)	Genomics

Table 1.9. Table summarising published literature on the stock structure of *Genypterus species* with concluding remarks and techniques used

Species	Number of stocks	Distribution of stocks	Technique	Reference
Genypterus blacodes	5	1. Chatham Rise, 2. Campbell Plateau and Stewart-Snares shelf , 3. Bounty Plateau, 4. West Coast South Island and 5. Cook Strait	Morphology (Growth, age, spawning…)	Horn, 2005
	2	1. Northern and 2. Southern	MtDNA control region haplotypes	Smith and Paulin, 2003
	≈2	1. Northern (comprising Chatham Rise and West Coast South Island and 2. Southern (Otago, Southland coasts, and the entire Campbell Plateau) (Otaga = area of mixing; Bounty Plateau = separate stock)	Cephalic and otolith morphometrics	Colman, 1995
	1	Australian waters	Meristic and morphometric characters	Daley *et al.,* 2000
	3 ("groups")	1. Chatham Rise 2. Bounty Plateau 3. West Coast South Island	Growth characteristics	Horn, 1993
	2	Chilean Patagonia	Otolith contour shape analyses	Wiff *et al.,* 2019
	2	1. Chatham Rise fish are distinct from 2. Sub-Antarctic fish	Otolith shape analyses	Ladroit *et al.,* 2017
Genypterus brasiliensis	2	1. Rio de Janeiro 2. Argentina	Parasitology	Alves *et al.,* 2002
Genypterus chilensis	2	1. Northern (Coquimbo, Zapallar) 2. Southern (Laguna Verde, Lebu)	Genetics: Microsatellite loci	Córdova-Alarcón *et al.,* 2019
	3	1. Brood stock 2. Wildfish 3. Commercial fish (Coquimbo Region)	Genetics: Microsatellite markers	González *et al.,* 2019

Note: Not all available literature is summarised here; Publications on the stock structure of *Genypterus maculatus* and *Genypterus tigerinus* was unavailable

1.6. IMPORTANCE OF THIS STUDY

PROBLEM STATEMENT

Historically, there was disagreement regarding the stock structure of *Genypterus capensis.* Morphological, catch history, and spawning data indicated the existence of both a single- (Badenhorst, 1988; Japp, 1989), and multi-stock structure (Isarev, 1976; Payne, 1977, 1985; Olivar and Sabatés, 1989). Furthermore, conflicting genetic data also indicated both single (Grant and Leslie, 2005) and multiple (Henriques *et al.,* 2016; Schulze *et al.,* 2020) stocks.

Recent genetic and genomic studies (Henriques *et al.,* 2016; Schulze *et al.,* 2020) have shown strong evidence for a two-stock management approach off South Africa, having some spatial distinctiveness (West and South Coasts).

Regarding stock-specific assessment/management, there is now an imperative need to develop clear-cut ways of identifying fish from the different stocks, to which genomically exclusive stocks can be phenotypically differentiated.

The aim of this study is to resolve the existence of one or multiple stocks of *G. capensis* using a multi-method phenotypic approach.

JUSTIFICATION

According to Rawat *et al.* (2017) and Bal *et al.* (2021), the identification of stocks that have distinguished phenotypic and genetic differentiation, may help to:

- Administer the stocks separately
- Attain biologically sustainable productivity
- Regulate stock-wise population abundance
- Assess how individual stocks respond to fisheries exploitation
- Accomplish the goals of fishery stock assessment by modelling

According to Sissenwine (2005) in Cadrin *et al.* (2005), there is a growing acceptance in fisheries management for the need to (i) conserve biodiversity including genetic diversity, (ii) apply a precautionary approach, and (iii) operationalize the ecosystem approach to fisheries management. Each of these points are deliberated upon, in context to *Genypterus capensis* off the coast of South Africa.

(i) Conservation of the genetic diversity of *Genypterus capensis*

The protection of genetic resources has become a significant component of conservation (Kenchington, 2003). According to Frankham (2005), "Conservation genetics is the concept and practice of genetics for the protection of species as ever-changing individuals capable of evolving to survive environmental change to minimize their extinction risk." From a genetic standpoint the long-term objective is not to "conserve" or "preserve", but to facilitate adaptation to environmental change and for continued speciation whereby evolutionary flexibility for the future can be maintained (Stephenson and Kenchington, 2000). Overexploitation has the potential to eliminate genetic diversity (Smith *et al.,* 1991; Schulze *et al.*, 2020), as demonstrated by the disappearance of many populations of salmon on the USA's West Coast, and the beluga sturgeon (*Huso huso*) stock in the Adriatic Sea (Kenchington, 2003).

Global demand for fish as a food source is rising as a result of population growth and economic expansion (Limburg *et al.,* 2011). Compared to other exploited fish populations in South Africa, the *G.capensis* resource is small, but still holds a high market value and is of appreciable economic importance. This species is endemic to southern Africa (Smith and Heemstra, 1986; See Section 1.2) and has commercially exploited in the past (Payne, 1986; Badenhorst, 1988; Japp, 1989; See Section 1.3.1).

Brandão and Butterworth (2008) showed that a single stock hypothesis for *G. capensis* appears a more optimistic status, based on ASPM, however, further investigation is required. If the west and south coast are treated as two separate stocks, then projections indicate that south coast catches would have

to be reduced to 1000 tons. Currently, an assessment by DFFE (2020) indicates that a two-stock hypothesis would mean that the SC stock is decreasing (≈0.8% per annum) in abundance, whilst the WC stock is increasing (≈2.4% per annum) in abundance (DFFE, 2020).

The risk of unsustainable exploitation of marine resources are further amplified by incongruities between populations and stocks (Section 1.4). To prevent the potential loss of biodiversity, it is necessary to know *what* needs to be protected, this is where stock structure proves vital.

(ii) The application of a precautionary approach for of *Genypterus capensis*

When discussing the issue of the implementation of a precautionary approach and associated "acceptable impacts", Garcia (1996) emphasized that the impacts should be reversable. The significance in preservation of the evolutionary potential of aquatic species, as well as the capability of an exploited population to react and adapt to both natural and anthropocentric challenges, was also deliberated upon.

For the *Genypterus capensis* resource, difficulties in the application of a replacement yield (RY) assessment led to the implementation of a conservative approach regarding the resource in 2016. As such, the PUCL is currently maintained at 4 450 tons for this fishery, around the whole coastline (DFFE, 2020). Additional precautions include the closure of *G. capensis* spawning box, as discussed in Section 1.2.5. Management of the *G. capensis* fishery, South Africa adopts a single-stock approach through the implementation of a PUCL that is not spatially apportioned (DFFE, 2020; See Section 1.3).

If spatial variability is necessary but ignored, it could lead to subunits with lower productivity in stocks to be overharvested and may even lead to their collapse (Ying *et al.,* 2011 e.g., Wooster, 1992; Hutchings, 2005). Concrete information regarding the stock structure of *G. capensis* and identification

thereof would prove vital in determining how this commercial species is managed.

(iii) Operationalizing the ecosystem approach whereby greater emphasis on spatial distributions and place-based management (such as Marine Protected Areas for areas) in which *Genypterus capensis* species contribute to the ecosystem:

South Africa has committed to implementing an Ecosystem Approach to Fisheries (EAF) management, which aims to ensure sustainable and productive ecosystems and fisheries. It considers the interactions that take place between different species and other elements making up an ecosystem, as well as the fisheries operating in that ecosystem and other sectors impacting on it (Petersen *et al.,* 2015). As a bycatch in the hake (*Merluccius spp*.) directed fishery, high priority was given to *Genypterus capensis,* with limited information available on the spatial distribution and stock structure in the "Ecological Risk Assessment Review of the South African Hake Fishery" (DFFE, 2011).

Whilst designated green for longline fishing, the orange allocation of *G. capensis* in offshore trawl nets by SASSI is partially due to the damage trawling is known to have on the seabed (Section 1.3.2). Regarding trophic structure, the species has a role as both predator and prey (as discussed in Section 1.2.4). As a nursery area for *G. capensis,* the Port Elizabeth (Gqeberha) Corals MPA covers unique geological features and coral ecosystems (MPA SA, 2020; Section 1.2.5). The presence of the species along the whole Southern African coastline was shown by continuity in longline catches (Japp, 1988), and details of the coast, including upwelling systems, Vulnerable Marine Ecosystems, and ocean currents, are described in Section 1.1. An increased understanding of the entire ecosystem knowledge regarding the stock structure of *G. capensis*, and how the species biological characteristics and ecological roles differ in the case of multiples stocks, would prove both useful and important for EAF management.

1.7. THE PURPOSE OF THIS STUDY

1. This study aims to assess whether the two genomically-differentiated *Genypterus capensis* stocks from the West and South Coast off South Africa (Henriquez *et al.,* 2016; Schulze *et al.,* 2020) are phenotypically differentiated by using a multi-method approach of different techniques:
    - Morphological- and meristics-based stock delineation studies for the species was done by Japp (1989), Payne (1985), Payne (1977), and Isarev (1976). In Chapter 2 of this thesis, the use of these methods are re-evaluated by examining the morphological (pectoral fin ray and cephalic measurements) and meristic (number of vertebrae, number of gill rakers, and number of pectoral fin rays) characters of the specimens under study.
    - Even though there has been research on otolith shape characteristics (Payne, 1985; Japp, 1990), the *G. capensis* stock structure has never been subjected to otolith shape indices, otolith microchemistry analysis, or Elliptical Fourier analysis, even though these techniques have all been applied in previous studies of this genus (Colman, 1995; Ladroit *et al.*, 2017; Wiff *et al.*, 2019). Chapter 3 evaluates otolith shape indices (circularity, form factor, rectangularity, roundness, ellipticity, and aspect ratio), otolith size parameters (length, width, area, and perimeter), and otolith specimen evaluation using elliptical Fourier analysis. Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS), Chapter 4 assesses the elemental composition of otoliths at various life stages and across stocks.
    - The parasite assemblage of the species has been studied previously (Payne, 1986). Sibanda (2015) conducted a preliminary investigation to assess their potential as biotags and, based on the findings of that investigation, the potential of four selected parasites as biotags for *G. capensis* stock delineation is examined in Chapter 5.

2. Since it may not be possible to apply all techniques of stock identification at any given time on fish samples, a ‘best-fit’ single technique might prove useful in the distinction of *Genypterus capensis* stocks. Hence, the study seeks to determine the best technique that produces results which best discriminate between *G. capensis* stocks on the west and south coasts of South Africa.
    - In Chapter 6 the Stock Differentiation Index (SDI) (Izzo *et al.,* 2017) is applied to the results compiled, hereby testing the hypothesis of independent western and southern *G. capensis* stocks. This chapter also contains a general discussion, synthesis, conclusions, and recommendations based on research findings for stock discrimination.

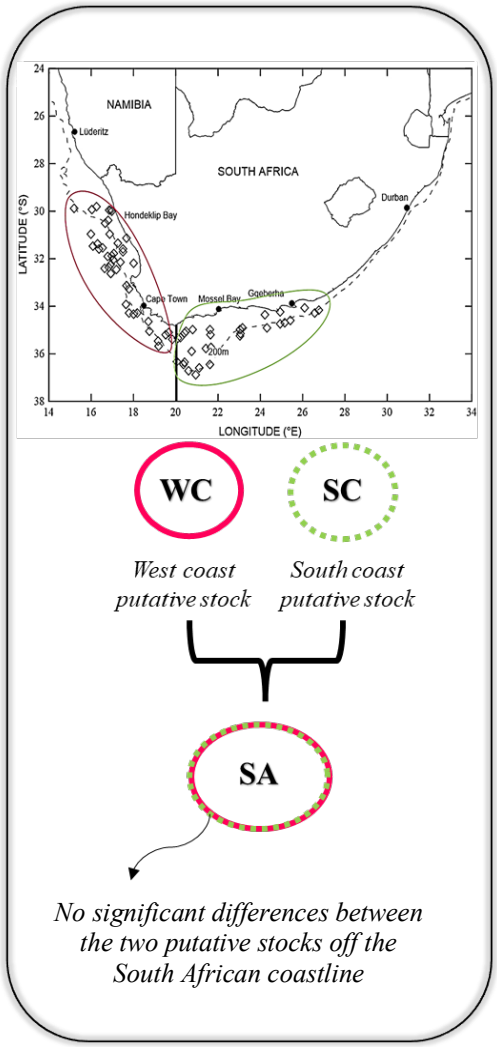
Chapter 2

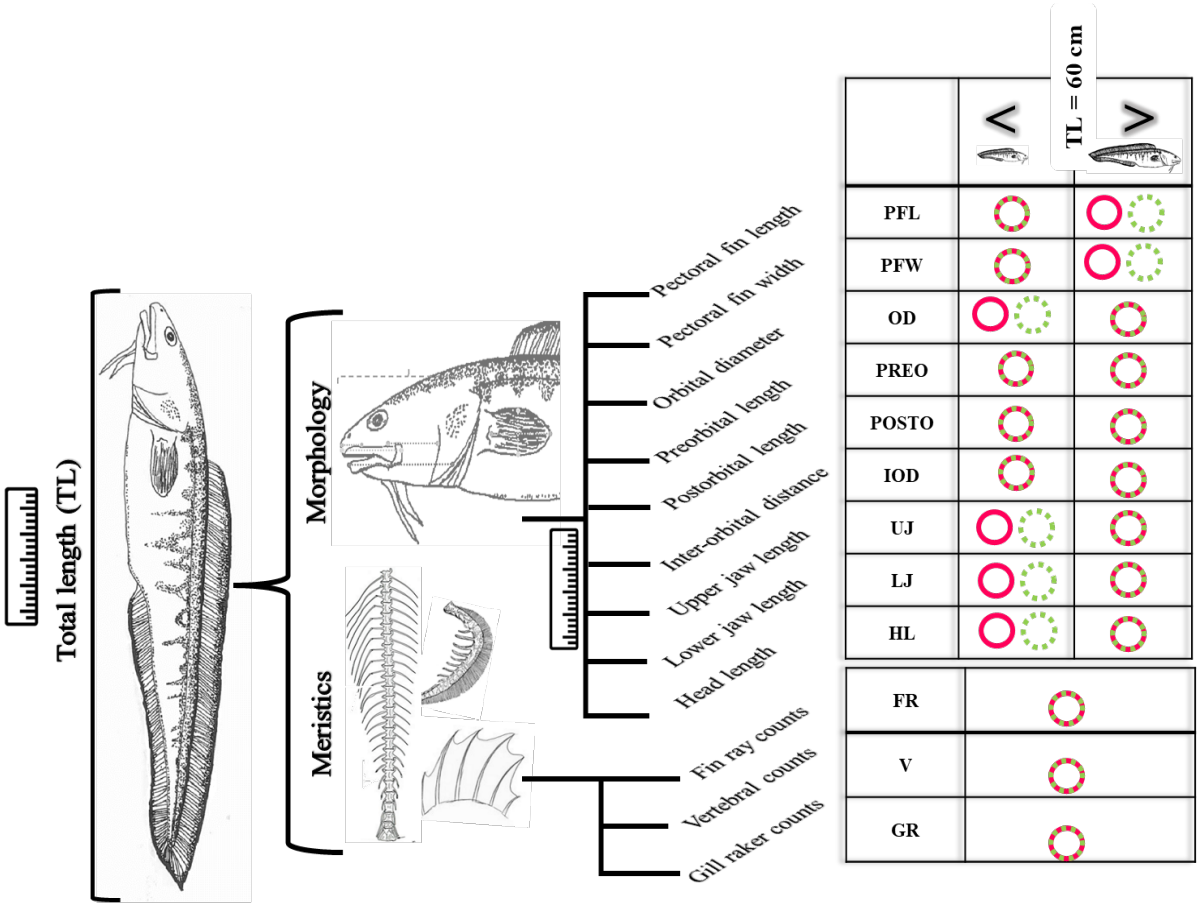
Morphology & Meristic Analsyis

"I am not a product of my circumstances. I am a product of my decisions."

-Stephen Covey

GRAPHICAL ABSTRACT





2.1. INTRODUCTION

2.1.1. Background

Morphological and meristic variants have long been used for fish stock differentiation (Swaine and Foote, 1999). Although there is a genetic underpinning to variations, developmental plasticity determines how these features manifest. This is as meristic features are established during the larval and early juvenile stages, whereas morphological qualities are determined by individuals during the post-recruitment phases (Smith *et al.,* 2005).

Since genetics and/or environmental characters form the bases of morphometric differences, variations in body form, as well as the shapes of fish scales and otoliths, may differentiate between groups that have similar ontogenetic rates or reproductive dynamics, hereby inferring phenotypic stocks (Swaine and Foote, 1999; Cadrin, 2005; Cadrin and Friedland, 2005). In the past, studies concluded that characters influenced by environmental differences could be just as reliable for stock structure as genetically based characters (Casselman *et al.,* 1981). More recently, Groenewald *et al.* (2019) determined that spatial variability in phenotypic characteristics of a fish population may be used to infer the existence of multiple stocks. The phenotypic stock definition is less conservative than the genetic stock definition (See Section 1.4.2) as it allows for some mixing amongst stocks (Cadrin, 2005).

Morphometric characters to identify phenotypic stocks, have been used for over a century (Cadrin, 2005) and are heavily affected by environmental conditions (Ihssen *et al.,* 1981). The environmental variables that modify phenotypic (morphological) characters, e.g., water temperature, salinity, oxygen, pH, and food availability (Corral and Aguirre, 2019), may also influence the evolutionary trajectories of these characters (Georgakopoulou, 2007). For the southern African coastline, environmental differences are described in Section 1.1 of this thesis. The use of morphological differences in a study by Groenewald *et al.* (2019) on *Sardinops sagax* supported a multi-stock hypothesis. Samples of *S. sagax* caught off the south coast had thinner

bodies and smaller heads than those caught off the west coast. Furthermore, *S. sagax* caught off Namibia had thicker bodies and more contracted tail regions compared to South African fish. For *Genypterus blacodes*, cephalic measurements used in a study by Colman (1995) concluded the existence of three different stocks on the coast of New Zealand.

Meristic traits also infer phenotypic stocks, however these features are fixed early in life during the larval or juvenile stage (Barlow, 1961) and do not change over time. Unlike morphometric data, the data obtained from meristic analysis is discrete and not continuous (Waldman, 2005). Meristic characters are serially repeated morphological features of fishes (Ihssen *et al.,* 1981) which includes gill rakers, vertebrae (Alho, 2011) and fin rays, amongst others. These characters were used in pioneer marine stock relationship studies (Heincke, 1898).

2.1.2. The purpose of this study

Among the established techniques for stock identification, the analysis of morphometric and meristic features is one of the more popular and cost-effective approaches (Mahfuj *et al.,* 2023). Relatively recent works using these two approaches for stock delineation on *Genypterus blacodes* includes works by Chick and Lowry (2020) in Australia, as well as Horn (2005), Smith and Paulin (2003), Colman (1999), Daley *et al.* (2000) in New Zealand. For *Genypterus capensis,* stock delineation studies using morphology and meristics is that of Japp in 1989, with prior works by Payne (1985), Payne (1977), and Isarev (1976).

Horn (2005) reported ambivalent results when using morphology and meristics as classifiers for stock differentiation for *G. blacodes* on New Zealand specimens. These results echoed that by Daley *et al.* (2000) on Australian *G. blacodes.* Previous studies showed that genetic differences were found between *G. blacodes* and *G. tigerinus,* however the morphological and meristic characteristics overlapped between species of *Genypterus*, including *G. capensis* (Daley *et al.,* 2000; Smith and Paulin, 2003). Additionally, As was found by Japp (1989), a comparison in the gross morphology of *G. capensis* putative stocks indicated a single stock with small

but insignificant differences in shape between West and South Coast catches.

Aim

The purpose of this chapter is to revisit the possibility of stock differentiation using the morphological and meristic traits of *Genypterus capensis* using data gathered from specimens captured in the years 2014 to 2017.

Objectives

1. The impact of sex on the morphological characteristics of the specimens will be assessed in order to rule out its potential as a contributing factor.
2. Using morphological data, allometric growth patterns between stocks will be evaluated.
3. Each morphological (for immature and mature) and meristic characteristic of *Genypterus capensis* specimens will be subjected to univariate analysis in order to ascertain whether variations between stocks are noticeable per variable.
4. To determine whether morphological and meristic factors needed for stock delineation exist, and what the best combination is, classification models with a stepwise selection method will be employed. Thereafter, MDS and PCA will be computed to illustrate the variances.

2.2. MATERIALS AND METHODS:

2.2.1. Sample locations

Samples of *Genypterus capensis* were collected during research surveys (following the methodology of Yemane *et al.,* 2008, and Atkinson *et al.,* 2010) conducted by the Department of Forestry, Fisheries, and the Environment (DFFE). The samples were separated and tagged according to the location in which they had been caught, namely:

1. West coast (WC) - belonging to the putative western stock, and
2. South coast (SC) - belonging to the putative southern stock,

with the boundary at Cape Agulhas (34.8163° S, 20.0152° E), as shown in

Figure 2.1.

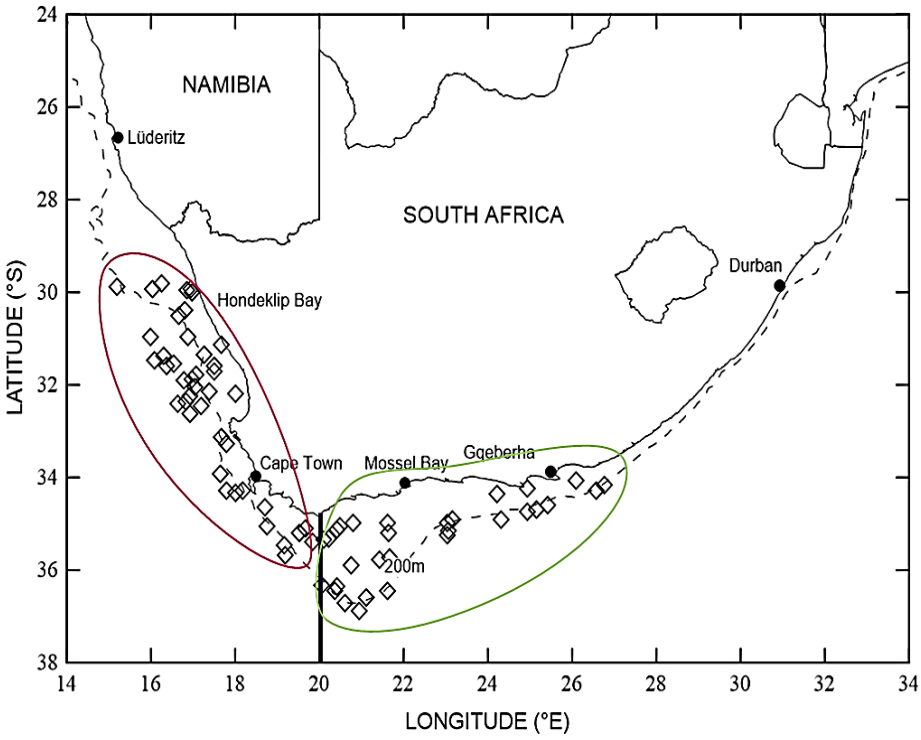


Figure 2.1. Locations where *Genypterus capensis* samples were collected during research surveys conducted by the Department of Forestry, Fisheries, and the Environment (DFFE) from 2014 to 2017. The 200m depth isobath is shown with the black dashed line. West coast (WC) samples are outlined in pink, and south coast (SC) samples are outlined in green. The boundary at Cape Agulhas (34.8163° S, 20.0152° E) is shown with a solid black line.

Specimens were collected at the same time each year, over a period of four years (2014 – 2017), with WC samples being caught in the January - February demersal surveys, and SC samples in the April - May surveys. The latitude and longitude of the stations that captured the specimens in this study, as well as a corresponding bar graph showing the number of fish per station, is shown in Appendix A: Table A1.

The fish were stored in a freezer until they could be dissected in the laboratory at the Department of Forestry, Fisheries, and the Environment (DFFE) or the University of Cape Town (UCT). Prior to taking the measurements, the frozen samples were thawed overnight and then positioned on their right side for analysis. Body posture and fins were moved into a natural position and each specimen was examined for physical damage. Samples and body structures that showed any physical damage were not included in analysis.

2.2.2. Morphological analysis

A wooden board with an embedded two-meter stainless steel ruler (having both centimeter and millimeter increments) was used to measure each specimen's total length (TL) in centimeters. The wet body weight (WBW) was measured using an electronic balance with a resolution of 5000-g. The sex of each fish was determined by reviewing their gonads under a dissecting microscope (See Table 1.2). The fish specimens' exterior morphology was analysed following the methodology of Hubbs and Lagler (1949). Some of the measurements and counts employed by Japp (1989) and Isarev (1976) for *Genypterus capensis*, and those of Smith and Paulin (2003), Daley (2000), Nielsen *et al.* (1999), Gomon *et al.* (1994), Last *et al.* (1983), and Regan (1903), for *Genypterus blacodes,* were used as a guideline for analysis. Two pectoral (left) fin and seven cephalic measurements per specimen was assessed. The measurement, abbreviation, and details of the method used for each are described in Table 2.1. All morphometric measurements were taken using a 150mm Plastic Vernier Sliding Gauge.

Table 2.1. Description of cephalic and pectoral fin measurements, abbreviations and methods used for morphometric analysis.

Measurement		Abbreviation	Method
Total length		TL	the greatest length of the fish from its anterior extremity to the end of its tail fin
Pectoral fin ray	Pectoral fin length	PFL	measured from the base of fin to the longest extending tip of the fin
	Pectoral fin width	PFW	measured on the base of the fin, from one end to the other
Cephalic	Orbital diameter	OD	After removing the eyeball, the eye socket's horizontal distance was measured.
	Preorbital length	PREO	the length from the beginning of the snout to the beginning of the eye socket was measured
	Postorbital length	POSTO	the posterior end of the eye socket to the beginning of the operculum was measured
	Inter-orbital distance	IOD	the dorsal distance between the eye sockets from left to right was measured
	Upper jaw length	UJ	the entire length of the maxilla was measured
	Lower jaw length	LJ	the entire length of the mandible was measured
	Head length	HL	the foremost snout end to the end of the operculum was measured

2.2.3. Meristic Characteristics

Meristics relates to counting quantitative features and forms a significant part of the identification of ophidiiform fishes in general (Cohen and Nielsen, 1978). For this study, the fin ray (FR), vertebral (V), and gill raker (GR) counts were noted from each individual specimen.

Counts of FR were done on the left pectoral fin. The skin was peeled away from the base of the fin using callipers and dissecting scissors, and the fin rays were then counted. After dissection, the vertebrate of each fish was removed and placed in a bath of boiling water for 30 minutes after which excess tissue was removed with tweezers. The vertebrae (V) were then dried under a cooling fan and counted. All counts were repeated at least twice and if results differed between the first two counts, a third count was done. The branchial basket of each fish was removed and GR counts were done on the first left gill arch. These counts were made separately for the upper limb (U), and lower limb (L), of the gill arch. As per Carpenter (2002), if a raker straddled the angle of the arch, the count was included as belonging to the lower limb. Upper and lower limb counts were further separated into developed (d) and rudimentary (r) rakers. Rudimentary rakers are defined as being wider than they are high. The general pattern of the gill-raker was expressed in the following format (NOAA, 2021):

$$\mathbf{A{\sim}B + C{\sim}D = E{\sim}F}$$

where A~B is the range of the raker count on the upper limb, C~D is the range of the raker count on the lower limb, and E~F is the range of the total raker count.

All data was recorded in Microsoft Excel for further statistical analysis.

2.2.4. Statistical methods

2.2.4.1. Analysis of variance (ANOVA)

The Analysis of Variance (ANOVA) attributes variation in one variable to variations in one (or more) explanatory variables. The degree of total variability in a set of data is produced by the sum of squared differences of

the observations from their total mean. Each row of the table is in accordance with one or more of the components of the observed variation. The entry per row includes the sum of squares (SS), the corresponding number of degrees of freedom (dF) and the mean square (MS) (Oxford Reference, 2021). In this study, two-way ANOVAs were performed in IBM SPSS[26] to evaluate for significant differences ($p < 0.05$) between the two putative stocks.

2.2.4.2. Shapiro-Wilk Test for Normality

The Shapiro-Wilk test for normality (Shapiro and Wilk, 1965) tests that the data being analysed has a specified distribution and compares the ordered sample values with the corresponding order statistics from the specified distribution. The test is used to assess whether a distribution shows a normal pattern (Upton and Cook, 2008). When interpreting results, the null hypothesis assumes that the population is normally distributed. If the p-value is less than the chosen alpha level, then the null hypothesis is rejected proving that the data is not normally distributed. If the p-value is greater than the chosen alpha level, then the null hypothesis is not rejected, proving that the data is normally distributed (JMP, 2021). In this study, the Shapiro-Wilk test was computed in IBM SPSS[26] with an alpha level of 0.05.

2.2.4.3. Correlation test: Kendall's tau b

Correlation is a bi-variate analysis that measures the strength of association between two variables and the direction of the relationship. Kendall's tau-b (τb) correlation coefficient is a non-parametric assessment of the strength, and direction of association that exists between two variables measured on at least an ordinal scale. The strength of relationship is determined by the correlation coefficient that varies between +1 and -1:

- A value of $\pm 1 \rightarrow$ indicates a perfect degree of association between the two variables.
- As the value goes towards $0 \rightarrow$ the relationship between the two variables will be weaker.

The direction of the relationship is indicated by the sign of the coefficient:

- a "+" sign → indicates a positive relationship and

- a "-"sign indicates a negative relationship (LAERD, 2021).

This test was computed in IBM SPSS[26] and a p-value with an alpha level of 0.05 was used to indicate the statistical significance of the coefficient.

2.2.4.4. Mann-Whitney U test

The Mann-Whitney U test (Mann and Whitney, 1947) compares differences between two independent groups that are not normally distributed, and when the dependent variable is either ordinal or continuous (LAERD, 2021). The null hypothesis assumes that the two samples have the same median (Shier, 2004). The tests were computed in IBM SPSS[26] for an alpha level of 0.05.

2.2.4.5. Machine learning techniques: Boosted Tree and Random Forest

Ensemble methods are learning tools that use multiple learning algorithms to make better predictions. The potential of machine learning techniques to improve the quality and extent of marine research was extensively discussed in ICES (2020).

Two commonly used machine learning algorithms are 1. Boosted Trees (BT) and 2. Random Forest (RF). Both methods use the same weak learner, with different algorithms, the main difference being how they combine decision trees:

- For BT analysis, decision trees are connected sequentially to obtain a strong learner. The goal is to minimize the errors of previous tree(s), so the trees are not fitted to the entire dataset. Thus, each tree fits to the residuals from the previous one leading to a gradual increase in the overall accuracy and robustness of the model (Yıldırım, 2021).
- For RF analysis, a method called 'bagging' is used. Each decision tree is created as a parallel estimator that is fitted to a subsample taken from the entire dataset. For the classification task, the overall result is determined by taking a majority vote among the results of all decision trees.

Both BT and RF analysis was performed in STATISTICA[13]. For each

combination, 75% of the sample set was randomly chosen to train the classifiers (the training set) and the remaining 25% (the validation set) was used to measure the quality of the prediction. Using predictor importance, the significance of each response variable could be computed, with a higher percentage indicating a higher rank of predictive significance. Stepwise Model Builder was applied by progressively adding predictor variables (from the highest to lower ranking), until all the variables were used. The set that had the highest overall accuracy was used as an indication of the best variable combination.

2.2.4.6. Principal Component Analysis

Principal Component Analysis (PCA) is a dimension reduction technique. It assesses relationships between a set of variables and then uses those relationships to produce a smaller set of components (Howlin, 2018).

PRIMER[6] (Plymouth Routines in Multivariate Ecological Research) was utilized for this assessment. Prior to analysis, raw data was transformed (using an overall transformation: square root) and normalised to place several types of variables onto a common measurement scale (Clarke and Gorley, 2006). Results were expressed on a PCA plot, and interpreted using two tables:

- The first table showing the eigenvalues for each principal component (PC) and using the Kaiser (1960) rule to drop all components with eigenvalues under 1.0 (Kaufman and Dunlap, 2000).
- The second table displays the correlation values between PCs and the original variables.

2.2.4.7. Multidimensional scaling (MDS)

Multidimensional scaling (MDS) is a set of data analysis techniques employed to assess the structure of (dis)similarity data. MDS portrays a set of objects as points in a multidimensional space in a manner by which the points corresponding to similar objects are located close together, and those corresponding to dissimilar objects are located far apart (Zhang and Takane, 2010). PRIMER[6] was used to create MDS plots.

2.2.5. Statistical analysis

The morphometric (continuous) and meristic data (discrete) were analysed separately.

2.2.5.1. Morphometric Analysis

As the species follows a migratory life cycle, reaching sexual maturity at a TL around ±60 cm (as discussed in Section 1.2.5), specimens were separated for analysis into:

- IMM → immature specimens - having a TL smaller than 60 cm.
- MAT → mature specimens - having a TL longer than 60 cm.

The grouping of fish specimens into size classes and the establishment of length-frequency distributions, is a customary practice in fisheries research (Mous *et al.,* 1995). Frequency graphs showing the range of the morphometric variables measured were therefore created.

To determine the effects of sex on analysis, ANOVAs were computed for the different morphometric variables, at 10cm TL-intervals, for specimens from each coast. For comparative purposes between fish from each coast, as well as other species in the *Genypterus* genus, the mean (μ) and standard deviation (σ) of morphometric measurements were then expressed as a percentage of TL.

Since discrimination depending solely on allometric morphometric traits may be inaccurate, it is important to eliminate size- or growth-related shape changes before elucidating shape differences amongst separate groups of fish (Okomoda *et al.,* 2018). Thus, to avoid biases due to the size of the morphometric variables, all were standardized following the methodology of Elliot *et al.* (1995) using the equation:

$$\mathbf{M_{adj} = M(L_s/L_o)^b,}$$

where M = the original value of the morphometric measurement, M_{adjs} = adjusted size of the measurement, L_o = the total length of the fish, and L_s = the mean of the total length of all fish. Parameter b was calculated for each characteristic from the data observed from the regression curve of Log(M) in Log(L_o), using all the fish.

This method normalized the individuals of a sample with a unique arbitrary size common to every sample, whilst respectively maintaining individual variation. The adequate application of this adjustment has been reported by several authors (Turan *et al.,* 2006; González *et al.,* 2016; Caez *et al.,* 2019; Gonzalez-Martinez *et al.,* 2020). Kendall's tau b indicating the relationship between TL and adjusted morphometric measurements of *G. capensis* samples, was then computed.

The Shapiro-Wilk Test was applied to verify the normality of the distribution for both adjusted and non-adjusted variables.

For univariate analysis, ANOVA's and Mann-Whitney U tests were computed. For multivariate analysis included machine learning techniques – Boosted Tree (BT) and Random Forest (RF) analysis. The best variable combination from BT and RF results were visually portrayed on an MDS plot. Principal Component Analysis was also executed and PCA plots with associated tables were read.

2.2.5.2. Meristic Analysis

The final counts of meristic characters are set prior to growth of the larvae into the juvenile phase (Waldman, 2005) and are therefore independent of size. Data was therefore not separated into IMM and MAT subsets. Frequency plots were created, and the Shapiro-Wilk Test was applied to verify the normality of the distribution.

For univariate analysis, ANOVAs were computed, and multivariate analysis made use of PCA's, BT and RF analysis.

2.3. RESULTS

2.3.1. Morphological Analysis

A total of (n = 376) specimens were measured for morphometric analysis. Most samples (approximately 75%) belonged to the putative WC stock, and the remainder were from the putative SC stock. The percentage of IMM and MAT sample sets per coast, is shown in Figure 2.2.

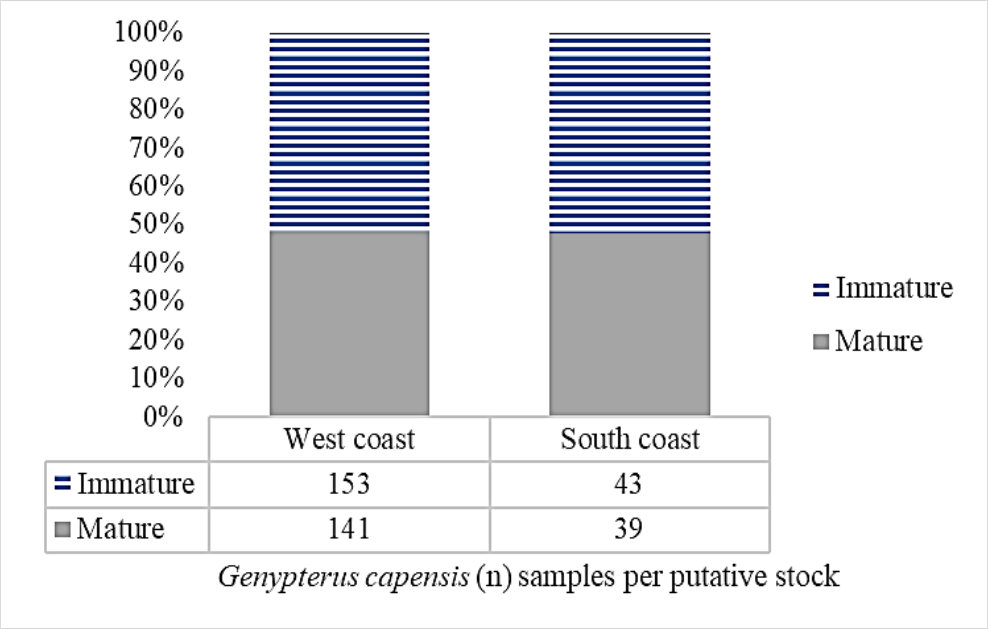


Figure 2.2. Stacked column graph with associated table portraying percentage of immature and mature *Genypterus capensis* samples used in this study.

2.3.1.1. Frequency graphs

The ranges of all measured variables was determined for each putative stock and these results are portrayed as frequency graphs in Figure 2.3.

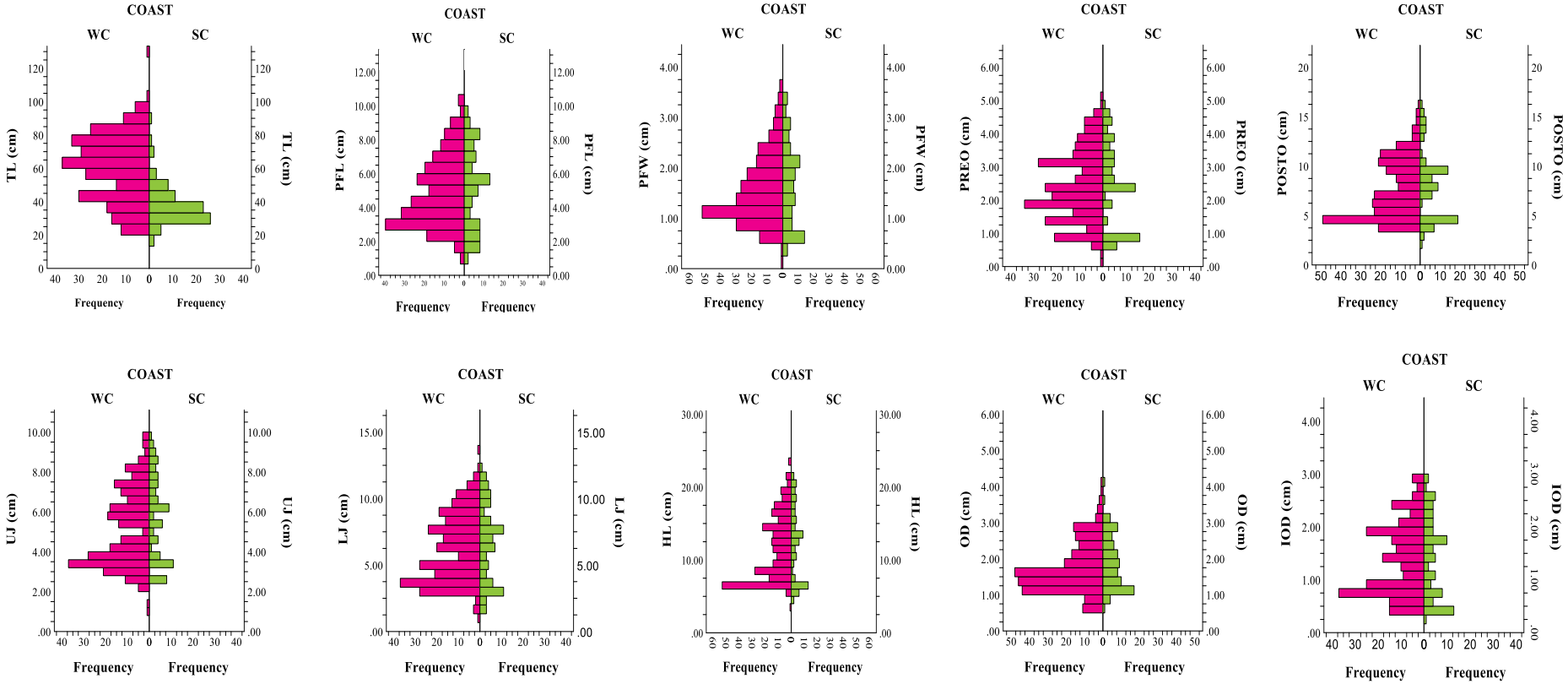


Figure 2.3. Frequency for the total length (TL), pectoral fin length (PFL), pectoral fin width (PFW), preorbital distance (PREO), postorbital distance (POSTO), upper jaw (UJ), lower jaw (LJ), head length (HL), orbital diameter (OD) and interorbital distance (IOD) of all specimens of Genypter*us capensis* from the putative west (WC) and south (SC) coast stocks, for the years 2014 – 2017.

2.3.1.2. The effect of sex on the fish

On each coast, females accounted for approximately half (WC = 54.83%; SC = 46.34%) and males a quarter (WC = 28.28%; SC =26.83%) of the total sample set, with remainder being juveniles (WC = 16.90%; SC = 26.83%).

An ANOVA was applied to test for morphometric differences between males and females, at 10 cm TL-intervals, within each putative stock. A summary of the resulting p-values can be seen in Table 2.2. Most cases showed no significant differences ($p > 0.05$) and fish of different sexes were combined for further analysis.

Table 2.2. Resulting p-values of an ANOVA for different morphometric variables, at 10cm total length intervals, for *Genypterus capensis* specimens from the putative west (WC) and south (SC) coast stocks, for the years 2014 - 2017.

TL (cm)	COAST	PFL	PFW	PREO	POSTO	UJ	LJ	HL	OD	IOD
		p-value								
30	**WC**	0.894	0.856	0.321	0.903	0.839	0.165	0.324	0.714	0.410
	SC	0.667	0.667	0.088	0.083	0.154	0.959	0.121	0.333	1.000
40	**WC**	0.468	0.516	0.722	0.190	0.082	0.797	0.266	0.915	0.064
	SC	0.246	0.822	0.311	0.935	0.956	0.063	0.830	0.869	---
50	**WC**	0.827	**0.007**	0.950	**0.005**	0.513	0.509	0.948	0.693	**0.013**
	SC	0.556	0.344	0.584	0.711	0.799	0.651	0.502	0.546	0.493
60	**WC**	0.715	0.635	0.750	0.992	0.116	0.64	0.294	0.784	0.293
	SC	0.281	0.792	0.356	0.333	0.552	0.859	0.971	**0.014**	0.182
70	**WC**	0.557	0.835	0.597	0.330	0.763	0.859	0.274	0.867	0.802
	SC	0.082	0.145	0.191	0.856	0.967	0.535	0.43	0.806	0.797
80	**WC**	**<0.001**	0.190	0.822	0.897	0.355	0.924	0.928	0.128	0.282
	SC	0.325	0.423	0.566	0.113	0.182	0.871	0.738	0.380	**0.040**
90	**WC**	---	---	---	---	---	---	---	---	---
	SC	**0.004**	0.374	0.815	0.703	0.15	0.978	0.511	0.863	---

Note: *PFL - pectoral fin length; PFW - pectoral fin width, PREO - preorbital distance; POSTO - postorbital distance; UJ - upper jaw; LJ - lower jaw; HL - head length; OD - orbital diameter; IOD - interorbital distance. Significant values ($p < 0.05$) are highlighted in bold, (---) = no data available*

2.3.1.3. Morphometric variables as a percentage of total length (TL)

Morphometric measurements were expressed as a percentage of TL. As can be seen in Table 2.3a., comparable results were found on both coasts for IMM and MAT sample sets.

An ANOVA was performed to evaluate significant differences of these allometric growth patterns between coasts (p-values shown in Table 2.3b). For IMM specimens, significant differences were found in PREO($F_{(1,193)}$ = 16.468, $p < 0.001$), LJ ($F_{(1,193)} = 8.643$, $p = 0.014$), OD($F_{(1,193)} = 8.474$, $p = 0.004$) and IOD($F_{(1,144)} = 7.067$, $p = 0.009$). For MAT specimens, significant differences were found in fin measurements: PFL($F_{(1,144)} = 5.49$, $p = 0.02$) and PFW($F_{(1,144)} = 5.621$, $p = 0.019$).

2.3.1.4. Correlation test: Kendall's tau b

A Kendall's tau b test was used to indicate the relationship between TL and morphometric measurements (adjusted and non-adjusted) of samples, as shown in Table 2.4.

Non-adjusted variables were shown to be highly correlated with TL (($\tau_\beta > 0.8$; $p < 0.001$). Adjusted variables showed a weak relationship with TL ($\tau_b < 0.2$; $p < 0.001$). As such, the adjusted morphological variables were used in further analysis, and TL was not included as a contributing factor.

2.3.1.5. Shapiro-Wilk Test for normality

The data was assessed for normality using the Shapiro-Wilk Test. As presented in Table 2.5. Most variables showed a non-normal distribution ($p < 0.05$).

Table 2.3a. The Mean ± SD of morphometric measurements expressed as a percentage of total length (TL) of *Genypterus capensis* specimens from the putative west (WC) and south (SC) coast stocks, for the years 2014 – 2017.

		Mean ± SD (%)								
	Coasts/Variable	**PFL**	**PFW**	**PREO**	**POSTO**	**UJ**	**LJ**	**HL**	**OD**	**IOD**
IMM	**COMBINED**	8.19 ± 0.85	2.64 ± 0.27	3.92 ± 0.41	13.68 ± 1.42	9.11 ± 0.95	10.94 ± 1.14	19.22 ± 2.021	3.41 ± 0.35	1.91 ± 0.2
	WC	8.16 ± 0.86	2.63 ± 0.28	4.03 ± 0.43	13.86 ± 1.46	9.17 ± 0.97	10.82 ± 1.14	19.37 ± 2.06	3.3 ± 0.35	1.83 ± 0.19
	SC	8.25 ± 1.00	2.71 ± 0.33	3.54 ± 0.44	13.04 ± 1.61	8.93 ± 1.10	11.36 ± 1.37	18.71 ± 2.31	3.81 ± 0.46	2.18 ± 0.26
MAT	**COMBINED**	8.45 ± 0.27	2.66 ± 0.09	4.54 ± 0.06	12.81 ± 0.45	9.54 ± 0.05	11.78 ± 0.089	21.11 ± 0.28	2.91 ± 0.09	2.48 ± 0.09
	WC	8.13 ± 0.31	2.55 ± 0.11	4.58 ± 0.04	12.46 ± 0.52	9.44 ± 0.05	11.75 ± 0.07	21.38 ± 0.13	2.84 ± 0.09	2.45 ± 0.09
	SC	9.32 ± 0.93	2.97 ± 0.29	4.43 ± 0.44	13.76 ± 1.37	9.65 ± 0.96	11.88 ± 1.18	20.39 ± 2.03	3.11 ± 0.311	2.57 ± 0.257

Note: *PFL - pectoral fin length; PFW - pectoral fin width, PREO - preorbital distance; POSTO - postorbital distance; UJ - upper jaw; LJ - lower jaw; HL - head length; OD - orbital diameter; IOD - interorbital distance, IMM – immature samples, MAT – mature samples*

Table 2.3b. ANOVA results showing p-values of morphometric measurements expressed as a percentage of total length (TL) for *Genypterus capensis* specimens from the putative west (WC) and south (SC) coast stocks, in the years 2014 - 2017.

	Variables: p-value								
Sample set	**PFL**	**PFW**	**PREO**	**POSTO**	**UJ**	**LJ**	**HL**	**OD**	**IOD**
Immature	0.833	0.600	**<0.001**	0.069	0.532	**0.014**	0.458	**0.004**	**0.009**
Mature	**0.020**	**0.019**	0.197	0.133	0.057	0.458	0.065	0.115	0.484

Note*: PFL - pectoral fin length; PFW - pectoral fin width, PREO - preorbital distance; POSTO - postorbital distance; UJ - upper jaw; LJ - lower jaw; HL - head length; OD - orbital diameter; IOD - interorbital distance. Significant values ($p < 0.05$) are highlighted in bold.*

Table 2.4. Correlation**:** Kendall's tau_b (τ_β) indicating the relationship between total length (TL) and morphometric measurements (adjusted and non-adjusted) of *Genypterus capensis* samples from the putative west (WC) and south (SC) coast stocks, for the years 2014 – 2017.

Variable		Non-adjusted		Adjusted	
		τ_β	p-value	τ_β	p-value
		Total length (TL)		Total length (TL)	
Total length	TL	1	.	1	.
Pectoral fin length	PFL	0.840	**<0.001**	-0.191	**<0.001**
Pectoral fin width	PFW	0.759	**<0.001**	-0.179	**<0.001**
Preorbital distance	PREO	0.880	**<0.001**	-0.198	**<0.001**
Postorbital distance	POSTO	0.821	**<0.001**	-0.095	**<0.050**
Upper jaw	UJ	0.838	**<0.001**	-0.197	**<0.001**
Lower jaw	LJ	0.882	**<0.001**	-0.173	**<0.001**
Head length	HL	0.855	**<0.001**	-0.166	**<0.001**
Orbital diameter	OD	0.568	**<0.001**	-0.146	**<0.001**
Interorbital distance	IOD	0.874	**<0.001**	-0.196	**<0.001**

Note*: τ_b = Kendall's tau_b correlation coefficient; Significant values (p < 0.05) are highlighted in bold.*

Table 2.5. Shapiro-Wilk Test for normality of cephalic and fin ray measurements of *Genypterus capensis* samples from the putative west (WC) and south (SC) coast stocks, for the years 2014 – 2017.

Variable		Sample set	Immature	Mature
		Coast	p-value	
Pectoral fin length	PFL	**WC**	0.072	0.254
		SC	**0.040**	0.345
Pectoral fin width	PFW	WC	**0.030**	**0.025**
		SC	**<0.001**	0.163
Preorbital distance	PREO	WC	0.123	0.056
		SC	0.184	0.117
Postorbital distance	POSTO	WC	**<0.001**	**<0.001**
		SC	0.581	**<0.001**
Upper jaw	UJ	WC	**<0.001**	0.398
		SC	**0.020**	0.057
Lower jaw	LJ	WC	0.347	**<0.001**
		SC	0.011	**<0.001**
Head length	HL	WC	**<0.001**	**<0.001**
		SC	**<0.001**	**<0.001**
Orbital diameter	OD	WC	**<0.001**	**<0.001**
		SC	0.928	**0.029**
Interorbital distance	IOD	WC	**<0.001**	0.208
		SC	**0.005**	0.165

Note*: WC – west coast; SC – south coast; Significant values (p < 0.05) are highlighted in bold.*

2.3.1.6. Univariate analysis: ANOVA and Mann-Whitney U

An ANOVA indicated significant differences between the two putative stock for UJ ($F_{(1.186)} = 19.573$, $p < 0.001$), LJ($F_{(1.193)} = 10.663$, $p = 0.001$) and OD ($F_{(1.087)} = 7.021$, $p = 0.009$) in IMM samples only. The Mann-Whitney U test showed significant differences for the same variables UJ ($U = 1860$, $p = 0.002$), LJ ($U = 2544.5$, $p = 0.027$), OD($U = 2313.5$, $p = 0.009$) and IOD ($U = 2183$, $p = 0.037$), of IMM samples. For MAT samples, no significant differences ($p > 0.05$) were shown by both and ANOVA and Mann-Whitney U test. A summary of the p-values is shown in Table 2.6., and a complete Table showing results can be found in is shown in Appendix A: Table A2 and A3.

Table 2.6. Univariate analysis: p-values indicating differences in morphometric variables for *Genypterus capensis* samples from the putative west (WC) and south (SC) coast stocks, for the years 2014 - 2017.

	Sample set	**Immature**		**Mature**	
	Test	**ANOVA**	**MWU**	**ANOVA**	**MWU**
Variable		**p-value**			
Pectoral fin length	PFL	0.301	0.377	0.142	0.105
Pectoral fin width	PFW	0.483	0.654	0.687	0.687
Preorbital distance	PREO	0.949	0.98	0.274	0.15
Postorbital distance	POSTO	0.135	0.157	0.697	0.097
Upper jaw	UJ	**0.001**	**0.002**	0.656	0.617
Lower jaw	LJ	**0.001**	**0.027**	0.886	0.496
Head length	HL	0.33	0.656	0.158	0.198
Orbital diameter	OD	**0.009**	**0.009**	0.55	0.394
Interorbital distance	IOD	0.054	**0.037**	0.255	0.178

Note: *ANOVA – Analysis of Variance; MWU – Mann-Whitney U Test. Significant differences ($p < 0.05$) highlighted in bold.*

2.3.1.7. Multivariate analysis: Boosted tree and Random Forest

A variety of classification models are available for discrimination by determining the best variable combination, e.g., Linear Discriminant Analysis, Artificial Neural Network, Classification Tree Analysis, etc., bearing in mind the assumptions required for analysis, Boosted Tree (BT) and Random Forest (RF) were employed for stock discrimination in this study.

The combinations of variables selected for each model was performed using a stepwise selection procedure. The complete table showing results for each step, is included in Appendix A: Table A4 .The variable combinations that gave the highest overall classification accuracy is shown in Table 2.7.

The classification accuracy of the predictive performance of the two classification models is shown in Table 2.8. In both IMM and MAT sample sets, BT and RF showed an accuracy of over sixty percent, with BT analysis generally showing a higher classification accuracy than RF. Even so, results from BT analysis for the SC samples were below 56% for all sample sets.

As shown in Figure 2.4, MDS plots portrayed extensive overlap in all cases when using the selected variable combinations,.

Table 2.7. Model selection of variables from *Genypterus capensis* from the putative western and southern stocks off the South African coast based on a stepwise selection procedure.

Sample set	Test	Variable combination	Accuracy	
			Training	Validation
Immature	**BT**	IOD+PFW+POSTO	0.905	0.813
	RF	LJ+UJ+POSTO+IOD+HL+OD+PREO	0.797	0.83
Mature	**BT**	POSTO+PREO	0.819	0.786
	RF	POSTO+HL	0.630	0.641

Note*: PFL - pectoral fin length; PFW - pectoral fin width, PREO - preorbital distance; POSTO - postorbital distance; UJ - upper jaw; LJ - lower jaw; HL - head length; OD - orbital diameter; IOD - interorbital distance ; BT – Boosted Tree Analysis; RF – Random Forest Analysis*

Table 2.8. Comparison of the predictive performance of the two classification models based on variables obtained from *Genypterus capensis* from the putative western and southern stocks off South African coast, for the years 2014 - 2017.

Sample set	Test	(%)		
		OVERALL	SC	WC
Immature	**BT**	71.80	55.81	76.32
	RF	67.69	48.84	73.03
Mature	**BT**	70.75	30.77	85.19
	RF	62.59	51.28	66.67

Note*: BT – Boosted Tree Analysis; RF – Random Forest Analysis*

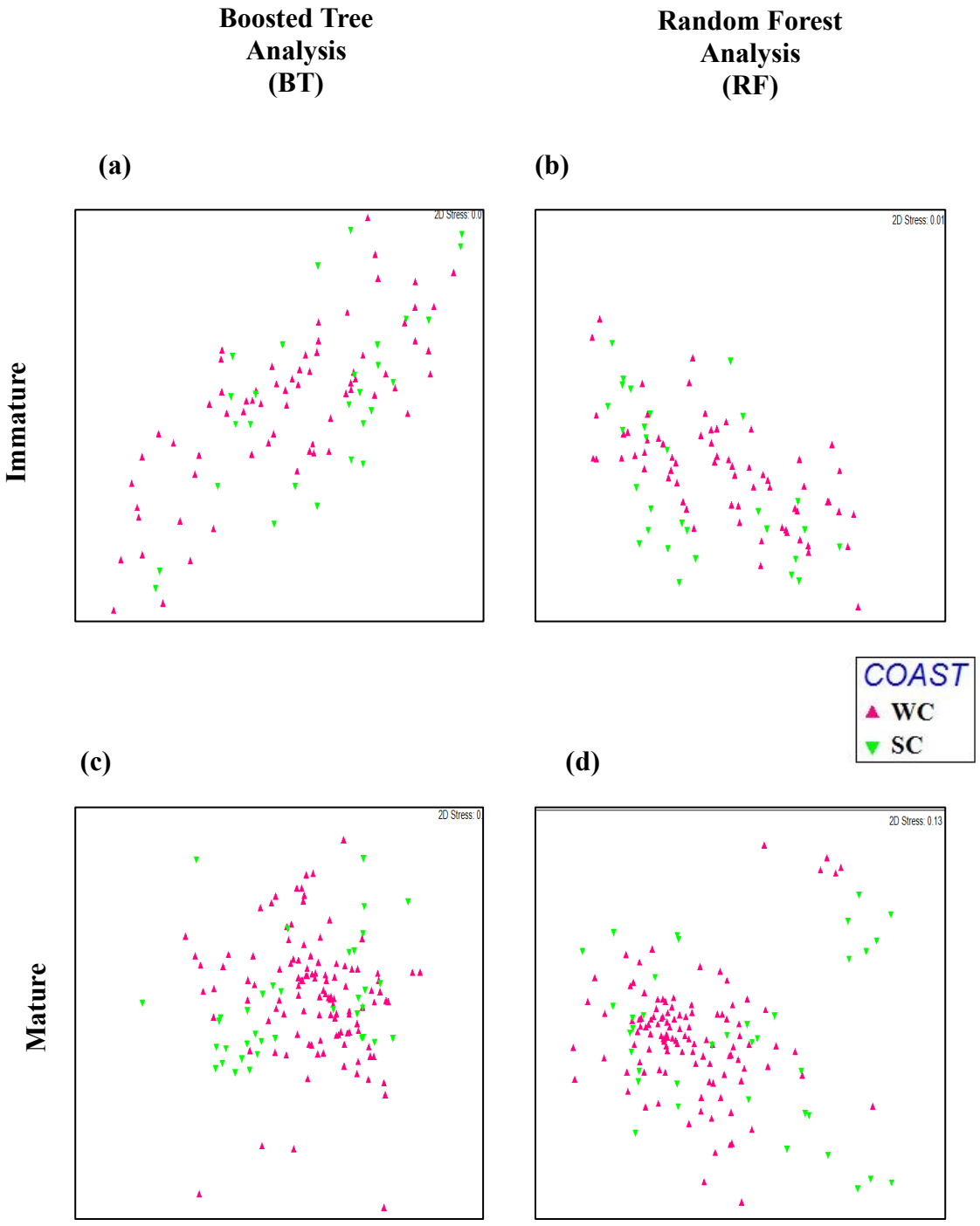


Figure 2.4. Multidimensional scaling plots (MDS) portraying differences shown when using boosted tree (left) and random forest (right) analysis for**: (a and b)** immature and **(c and d)** mature samples from *Genypterus capensis* from the putative western and southern stocks off South African coast.

2.3.1.8. Multivariate analysis: Principal component analysis

Eigenvalues and correlation between principal components and original variables of Principal Components, is shown in Tables 2.9 and 2.10, respectively. PCA plots using morphological data of IMM, and MAT specimens are shown in Figure 2.5.

Together, the first two PCs (PC1 = 44.9%; PC2 = 12.7%) for the IMM *G. capensis* samples had eigenvalues larger than 1, accounting for 57.6% of the variation in the sample. PC1 was not strongly correlated to any of the original variables (< 0.5). In PC2, HL (0.715) was shown to have a strong (> 0.5) correlation. The PCA plot showed extensive overlap between individuals from the two coasts indicating that there is no distinction between the two putative stocks.

For MAT samples, the first two PCs showed eigenvalues greater than 1 and together (PC1 = 38.1%; PC2 = 19.9%) accounted for 58.1% of the total variation. PC1 was not strongly correlated to any of the original variables (< 0.5). In PC2, PFL (0.532) was shown to have a strong (> 0.5) correlation. Extensive overlap between individuals from the two coasts, on the PCA plot, reveals no distinction between the two putative stocks.

Table 2.9. Eigenvalues of Principal Components and the % variation explained for original morphological and meristic characters of *Genypterus capensis* samples from the putative west (WC) and south (SC) stocks, for the years 2014 - 2017.

Sample set		PC1	PC2
Immature	Eigenvalues	**4.04**	**1.14**
	Variation (%)	44.9	12.7
	Cum. Variation (%)	44.9	57.6
Mature	Eigenvalues	**3.43**	**1.79**
	Variation (%)	38.2	19.9
	Cum. Variation (%)	38.2	58.1

Table 2.10. Correlation between principal components and original variables explained for morphometric characters of *Genypterus capensis* samples from the putative western and southern stocks, for the years 2014 - 2017.

Sample set	Variable		PC1	PC2
Immature	PFL	Pectoral fin length	-0.358	-0.28
	PFW	Pectoral fin width	-0.272	-0.371
	PREO	Preorbital distance	-0.359	0.170
	POSTO	Postorbital distance	-0.348	0.136
	UJ	Upper jaw	-0.387	-0.069
	LJ	Lower jaw	-0.428	0.150
	HL	Head length	0.085	**0.715**
	OD	Orbital diameter	-0.233	0.444
	IOD	Interorbital distance	-0.392	0.034
Mature	PFL	Pectoral fin length	-0.296	**0.532**
	PFW	Pectoral fin width	-0.409	0.416
	PREO	Preorbital distance	-0.403	-0.25
	POSTO	Postorbital distance	-0.150	-0.341
	UJ	Upper jaw	-0.446	-0.156
	LJ	Lower jaw	0.299	0.360
	HL	Head length	-0.146	-0.420
	OD	Orbital diameter	-0.255	0.182
	IOD	Interorbital distance	-0.431	0.037

Note*: Variables shown to have a strong (> 0.5) correlation are highlighted in bold.*

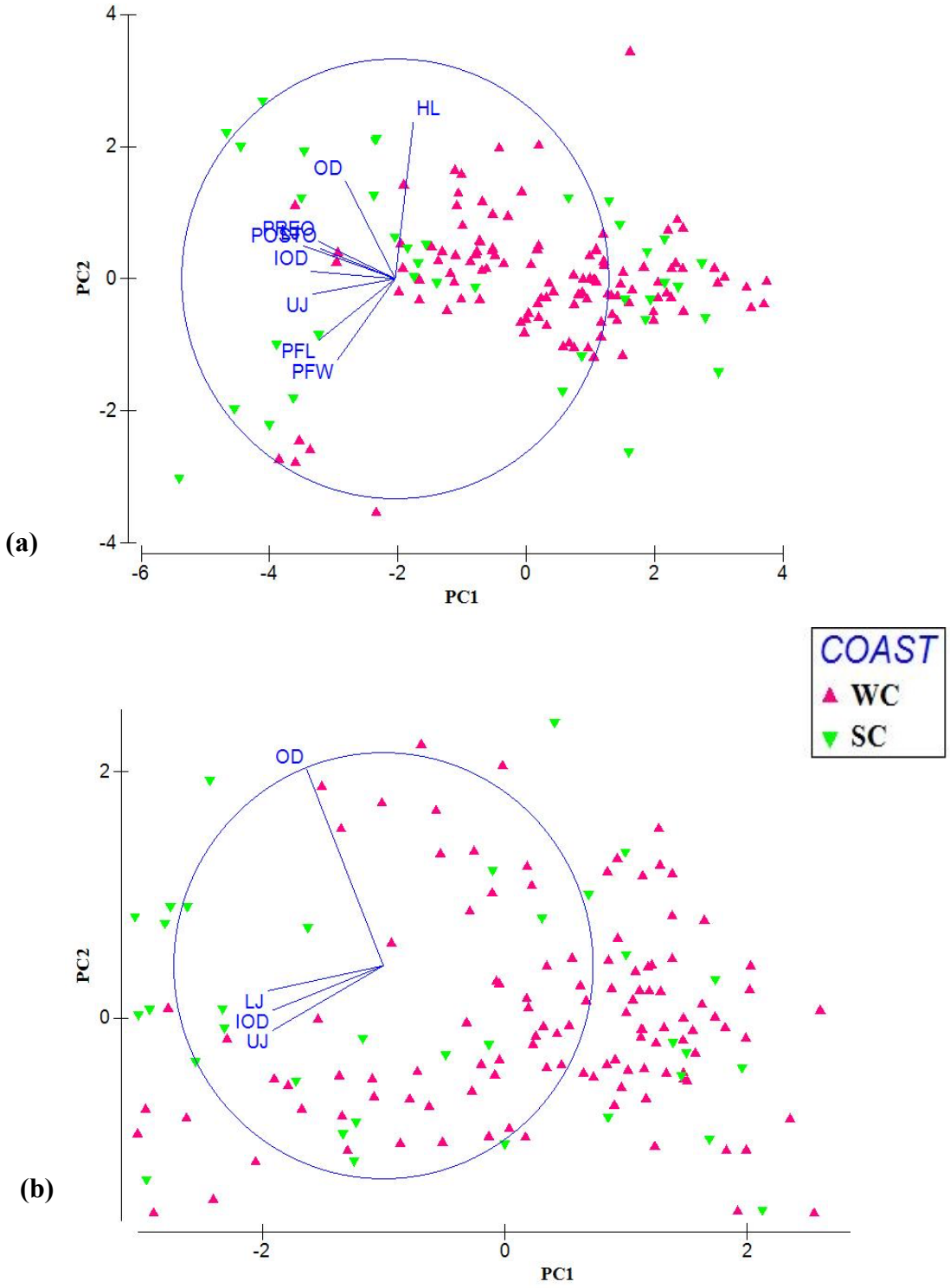


Figure 2.5. PCA plots of **(a)** immature using all variables, **(b)** immature using significant variables as shown by univariate tests of *Genypterus capensis* samples from the putative west (WC) and south (SC) stocks, for the years 2014 – 2017.

2.3.2. Meristic Analysis

Meristic data is not correlated with fish length, as explained in the introduction of this chapter. For this reason, samples were not separated into IMM and MAT subsets.

2.3.2.1. Frequency graphs

Frequency graphs portraying the ranges of fin ray (FR), vertebral (V), and total gill raker (GR) counts, are shown in Figure 2.6.

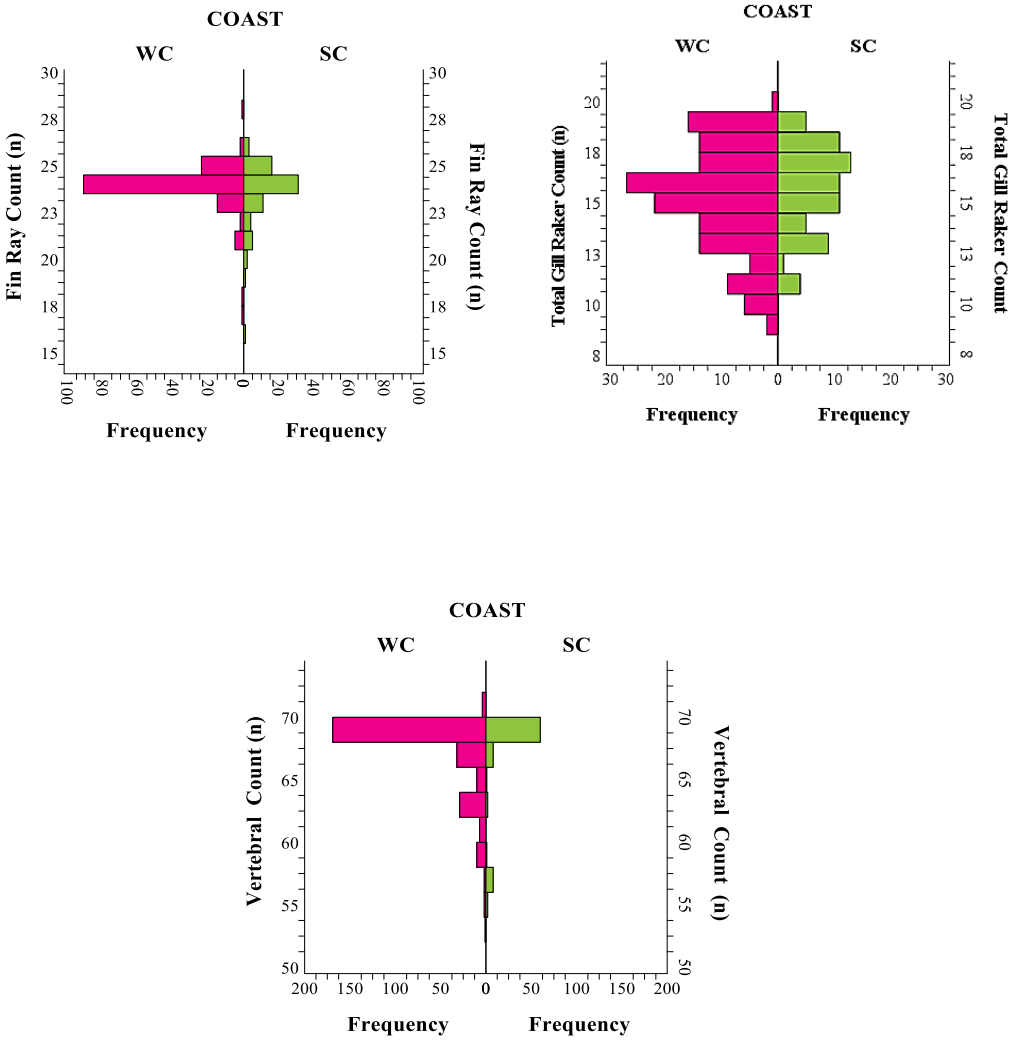


Figure 2.6. Frequency for the fin ray-, vertebral- and total gill raker- counts of *Genypterus capensis* samples from the putative west (WC) and south (SC) coast stocks, for the years 2014 – 2017.

2.3.2.1. Shapiro-Wilk Test for Normality

The data was assessed for normality using the Shapiro-Wilk Test, as presented in Table 2.11. All variables showed a non-normal distribution ($p < 0.05$).

Table 2.11. Shapiro-Wilk Test for normality of meristic character of *Genypterus capensis* samples from the putative west (WC) and south (SC) coast stocks, for the years 2014 – 2017.

Variable	COAST	Statistic	dF	p-value
Fin Ray Count (FR)	WC	0.704	128	**<0.001**
	SC	0.880	70	**<0.001**
Vertebral Count (V)	WC	0.678	128	**<0.001**
	SC	0.643	70	**<0.001**
Total Gill Raker Count (GR)	WC	0.943	128	**<0.001**
	SC	0.941	70	**<0.001**

2.3.2.2. Univariate Analysis: ANOVA

An ANOVA was performed to evaluate differences in the assessed meristic characters. No significant differences ($p > 0.05$) were found for any of the meristic counts between specimens from the two putative stocks.

Table 2.12. ANOVA. Differences in meristic characters of *Genypterus capensis* from the putative western (WC) and southern (SC) stocks, for the years 2014 – 2017.

Variable		SS	dF	MS	F	p-value
Fin ray count (FR)	Between Groups	6.175	1	6.175	3.484	0.063
	Within Groups	405.851	229	1.772		
	Total	412.026	230			
Vertebral count (V)	Between Groups	2.883	1	2.883	0.248	0.619
	Within Groups	4203.807	362	11.613		
	Total	4206.690	363			
Total Gill Raker count (GR)	Between Groups	1.997	1	1.997	0.206	0.651
	Within Groups	3137.591	323	9.714		
	Total	3139.588	324			

Note*: SS = Sum-of-squares, dF = Degrees of freedom, MS =Mean Squares (MS), F =F-ratio and p-values (significance).*

2.3.2.3. Multivariate analysis: Boosted tree and Random Forest

When looking at the best variable combination for meristic variables of *Genypterus capensis,* results differed between Boosted tree and Random Forest classification models. With RF analysis, all variables were used, whilst BT analysis excluded V, thereby only acclaiming FR and GR as having high predictive performance. Regarding the overall classification accuracy, both tests showed an accuracy of over sixty percent, however, results from BT analysis for the SC samples was relatively low (17.14%), as shown in Table 2.13. A complete table showing the stepwise procedure is shown in Appendix A: Table A5.

2.3.2.4. Multivariate analysis: Principal component analysis

For PCA, the first two PCs showed eigenvalues greater than 1 and together (PC1 = 38.6%; PC2 = 37.6%) accounted for 76.2% of the total variation (Table 2.14). In PC1, FR (-0.660) and GR (-0.745) was shown to have a strong (> 0.5) correlation. In PC2, V (-0.825) was shown to have a strong (> 0.5) correlation (Table 2.15). The PCA plot (Figure 2.7) showed extensive overlap between individuals from the two coasts indicating that there is no distinction between the two putative stocks.

Table 2.13. Model selection and predictive performances of variables from *Genypterus capensis* from the putative western (WC) and southern stocks (SC) off the South African coast based on stepwise selection procedure.

Test	Variable combination	Model selection		Predictive performance (%)		
		Training	Validation	OVERALL	WC	SC
BT	FR + GR	0.750	0.603	63.55	86.11	17.14
RF	FR + GR + V	0.773	0.547	63.08	68.06	52.86

Note*: FR - Fin ray count; V - Vertebral count; GR - Gill raker count; BT – Boosted Tree Analysis; RF – Random Forest Analysis.*

Table 2.14. Eigenvalues of Principal Components and the % variation explained for meristic characters of *Genypterus capensis* samples from the putative west and south stocks, for the years 2014 – 2017.

	PC1	PC2	PC3
Eigenvalues	**1.16**	**1.13**	0.714
%Variation	38.6	37.6	23.8
Cum.%Variation	38.6	76.2	100

Table 2.15. Correlation between Principal Components and original variables explained for log-transformed shape indices *Genypterus capensis* samples from the putative west and south stocks, for the years 2014 – 2017.

Variable	PC1	PC2
Fin ray count (FR)	**-0.660**	0.473
Vertebral count (V)	-0.099	**-0.825**
Gill raker count (GR)	**-0.745**	-0.309

Note*: Variables shown to have a strong (> 0.5) correlation are highlighted in bold.*

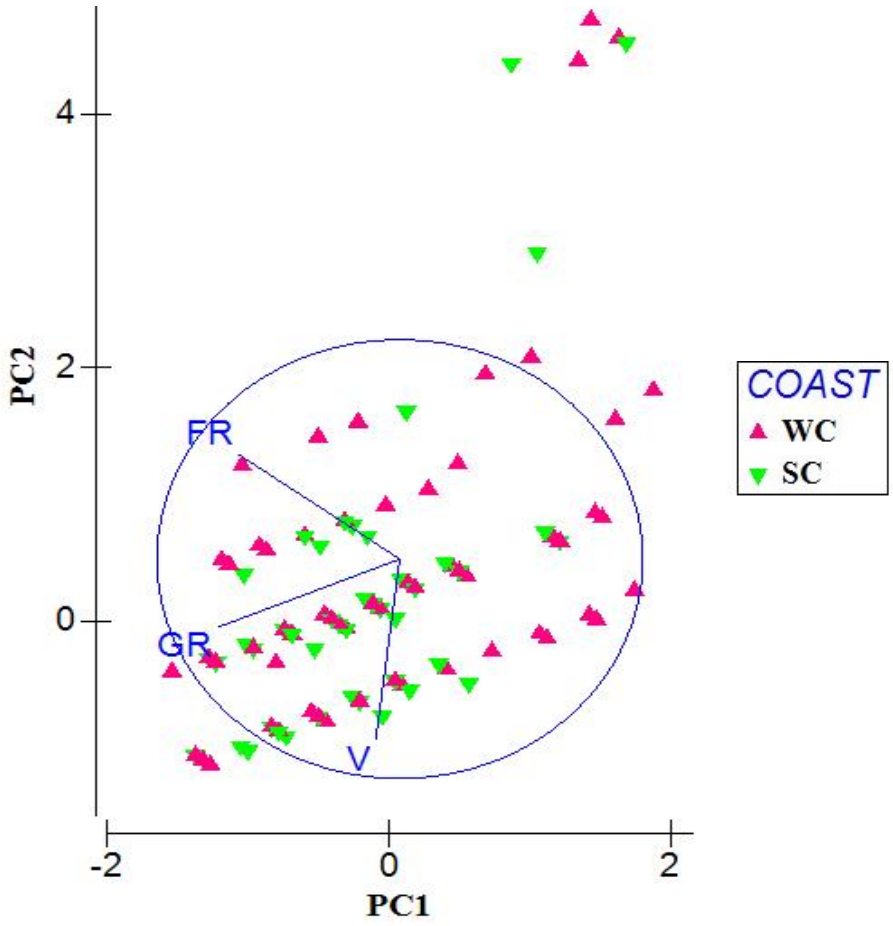


Figure 2.7. PCA plot using meristic variables obtained from *Genypterus capensis* samples from the putative west (WC) and south (SC) stocks, for the years 2014 – 2017.

2.4. DISCUSSION

2.4.1. Morphological Analysis

One of the advantages of morphological data collection is that the characters are easy to measure. However, environmental influences, including spawning, may affect some of the morphometric features used in stock delineation studies (Cadrin, 2005; Cadrin and Friedland, 2005). Additionally, based on prior research, Smith *et al.* (2005) suggested the possibility that morphological variation between stocks is non-adaptive and could reflect feeding conditions and spawning times, rather than stock relationships. Given that fish specimens were collected at the same time every year, and that the gonads of 83.15% (♀ = F2) of females and 97.79% (♂ = M2) of males were found to be inactive (see Table 1.2), the effect of spawning had no bearing on the results in this study. The gonad somatic index (GSI) did not significantly differ between stocks according to preliminary results. No statistically significant differences between the sexes were found in this examination (Table 2.2), as was the case in Japp's study (1989). Therefore, the impacts of spawning and sex were disregarded as potential influencing factors in subsequent research.

Table 2.3a presents non-adjusted morphometric features as a percentage of TL. When compared, the results of ANOVA (Table 2.3b) showed significant differences in some of the variable across stocks, for MAT (PFL and PFW) and IMM (PREO, LJ, OD, and IOD) sample sets. Based on univariate analysis (Table 2.6), there were no significant differences discovered for MAT sample sets, however there were significant variations found for UJ, LJ, OD, and IOD for IMM specimens between the two hypothetical coasts. Since body form is a product of ontogeny, morphometric variation could indicate differences in growth and maturation rates (Sajina *et al.,* 2011). Results from this study demonstrates that for immature samples of *G. capensis*, stock structure delineation is implied by differences in allometric growth patterns and morphology, which are less evident in mature samples. However, before accepting these conclusions, care should be taken in light of previous research.

Based on Payne's (1984) findings, variations may have resulted from *G. capensis* from the putative SC growing more quickly than in other parts of southern Africa. When assessing the differentiation of *Genypterus microstomus* from *G. blacodes* (where the maxilla length/upper jaw length was the defining trait), Roberts (1991) noted that the differences in cephalic measures of immature individuals was attributable to allometric growth rather than speciation. Japp (1989) believed that allometric growth misrepresented differences. This is because using univariate statistics to analyse data implies that the ratios of the initial variates vary regularly and continuously with size, resulting in the variation and correlations between several variables getting ignored.

Laboratory errors also need to be taken into consideration when interpreting results. Mous *et al.* (1995) stated that variation in the pressure applied when putting the jaws into a normal closed position at time of measurement may lead to inconsistencies. The study by Japp (1989) found differences in the OD of *G. capensis* samples between the two putative coasts. He stated that the likely reason for this discrepancy may be due to the difficulty in measuring the parameter, possibly resulting in large differences and, consequently, an unacceptably high variance. This could also account for the differences in characters from specimens between the two putative stocks in this study. Other possible sources of error that can be made when measuring fish specimens includes the fact that muscular tension in living fish relaxes post-mortem, and that fish shrink due to preservation techniques, including freezing (Mous *et al.,* 1995). A study by Sayers (1987) on freshwater fish (*Coregonus hoyi*) indicated that freezing significantly alters the length or weight of fish and the mean change in weight was greater for adults than juveniles. According to Jawad's (2003) research on marine fish (*Alepes djedaba*), TL remained constant until the sixth week of freezing, at which point it shrank at a rate that was statistically significant. In this study, the TL may have been influenced by laboratory procedures as specimens were frozen for different lengths (varying from days to months) of time.

For multivariate analysis, when specific characters were included, variable

combinations for IMM samples produced a rather high predictive value for both BT and RF (Table 2.8). However, PCA (Figure 2.5; Tables 2.9 and 2.10) revealed a significant degree of overlap, suggesting that there is no discernible difference between the two potential stocks, while MDS plots failed to clearly demonstrate any distinctions (Figure 2.4). In MAT samples, POSTO was found to be the best predictor of both BT and RF (Table 2.8), and PFL demonstrated a high correlation between the major components of the PCA (Figure 2.5; Tables 2.9 and 2.10). As a result, no group of morphological features could be firmly identified as a predictive suite for the delineation of the *G. capensis* population.

2.4.2. Meristic Analysis

For vertebral counts, Jordan's rule (Jordan, 1891) describes an association between increasing latitude and vertebral number in fishes: the vertebral number would be higher at lower temperatures, and lower at higher temperatures (This assumes that fish vertebral counts were recorded in the same area during their early development, when the vertebral count was determined). Based on this, and given the differences in temperature (described in Section 1.1) between the two oceans where the putative stocks reside, it seemed reasonable to assume that there would be a notable variation in the number of vertebrae between specimens from each coast. An ANOVA (Table 2.12) however proved this untrue and indicated no significant difference in vertebral counts between the two putative populations and, likewise, there was also no significant difference in fin ray and gill raker counts.

2.4.3. Intraspecific comparison

As shown in Table 2.16., The findings in this study were comparable to morphometric variables written as a percentage of SL for *Genypterus blacodes* in Australia (Daley, 2000), and New Zealand (Smith and Paulin 2003). For meristic analysis, the FR range for this study (WC = 17–28; SC = 16–26) is consistent with results reported for other species in this genus: *G. blacodes* n = 21–26, *G. blacodes*: n = 22–28, and *G. tigerinus*: n = 19–24 (Daley *et al.,* 2000; Smith and Paulin, 2003). The GR (range: WC: 1 ~ 4 +5

~ 16 = 8 ~ 25 and SC: 4 ~ 4+7 ~ 15 = 11 ~ 26) in this study had a ventral developed gill raker count of n = 4, which was the same as other species of the genus, namely *G. blacodes* and *G. tigerinus* (Daley, 2000). This study revealed lower minimum values for the vertebral counts (WC: n = 53 – 71, and SC: n = 54 – 69) than previously reported for this species and other species in the same genus. The South-East stock range (synonymous with SC) was reported by Payne (1985) to be n = 66–75, while the Cape stock range (synonymous with WC) was reported by Payne (1977) to be n = 67–72. The current study's V_{μ}: n = 67 ± 4 differs from the published mean ranges by Daley *et al.* (2000) for *G. blacodes* (n = 67–71) and *G. tigerinus* (n = 68–71).

Table 2.16. List of morphometric and meristics characters recorded for *Genypterus blacodes*

	Location	Australia		New Zealand	
		Tasmania	**Victoria**	**Northern**	**Southern**
Source		Daley *et al.,* 2000		Smith & Paulin, 2003	
Variable		**Mean (range)**			
Morphology	**Head length**	24.72 (23.95-25.86)	22.76 (21.71-24.0)	23 (18.69-24.51)	22.34 (19.27-24.15)
	Snout length	5.77 (4.92-6.29)	5.63 (5.28-6.28)	5.48 (3.85-6.54)	5.26 (3.63-6.15)
	Orbit diameter	3.72 (2.97–4.25)	3.87 (3.45–4.31)	3.92 (3.33- 4.79)	4.05 (3.10-5.12)
	Interorbital width	3.49 (3.17–4.24)	3.36 (2.83-3.91)	2.85 (1.83-3.63)	2.88 (1.88-3.71)
	Upper jaw	10.6 (10.02-11.03)	10.32 (9.60-10.83)	9.91 (8.12-10.89)	9.63 (8.68-10.42)
	Pectoral length	9.59 (6.82 - 10.48)	9.44 (8.65 - 9.89)	9.41 (8.62 - 10.88)	9.04 (7.12 - 10.24)
Meristics	**Fin ray**	23 - 26	22 - 25	21 - 26	23 - 25
	Vertebral	48-50	48-50	48-50	48-50
	Gill raker	3–4r+4-5+0-3r	3–4r+4+1-3r	3–4r+4-5+3-5r	3–4r+4-5+3-6r

2.5. CONCLUSION

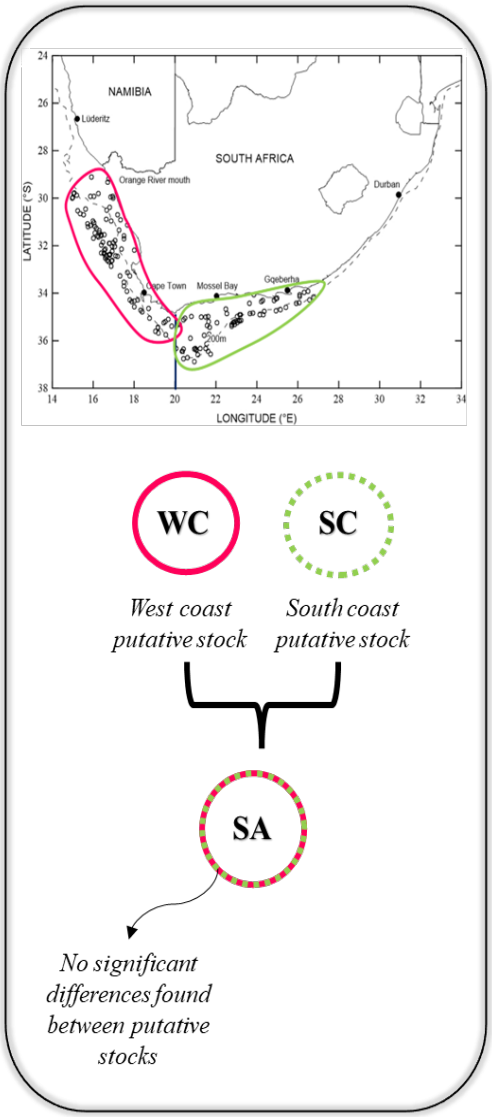
Three meristic and nine morphological characteristics were analysed for this study. The mean allometric growth patterns demonstrated both intraspecific and interspecific similarities when compared between the two putative populations, and with other *Genypterus* species, when differences were tested. Univariate analysis for immature samples revealed variations in the orbital diameter, interorbital diameter, lower jaw, and upper jaw between the two regions. Using both univariate and multivariate analysis, no statistically significant differences were found in the variables evaluated for specimens between the two populations for mature specimens. This means that while variations are evident in immature samples, they are less pronounced in mature samples.

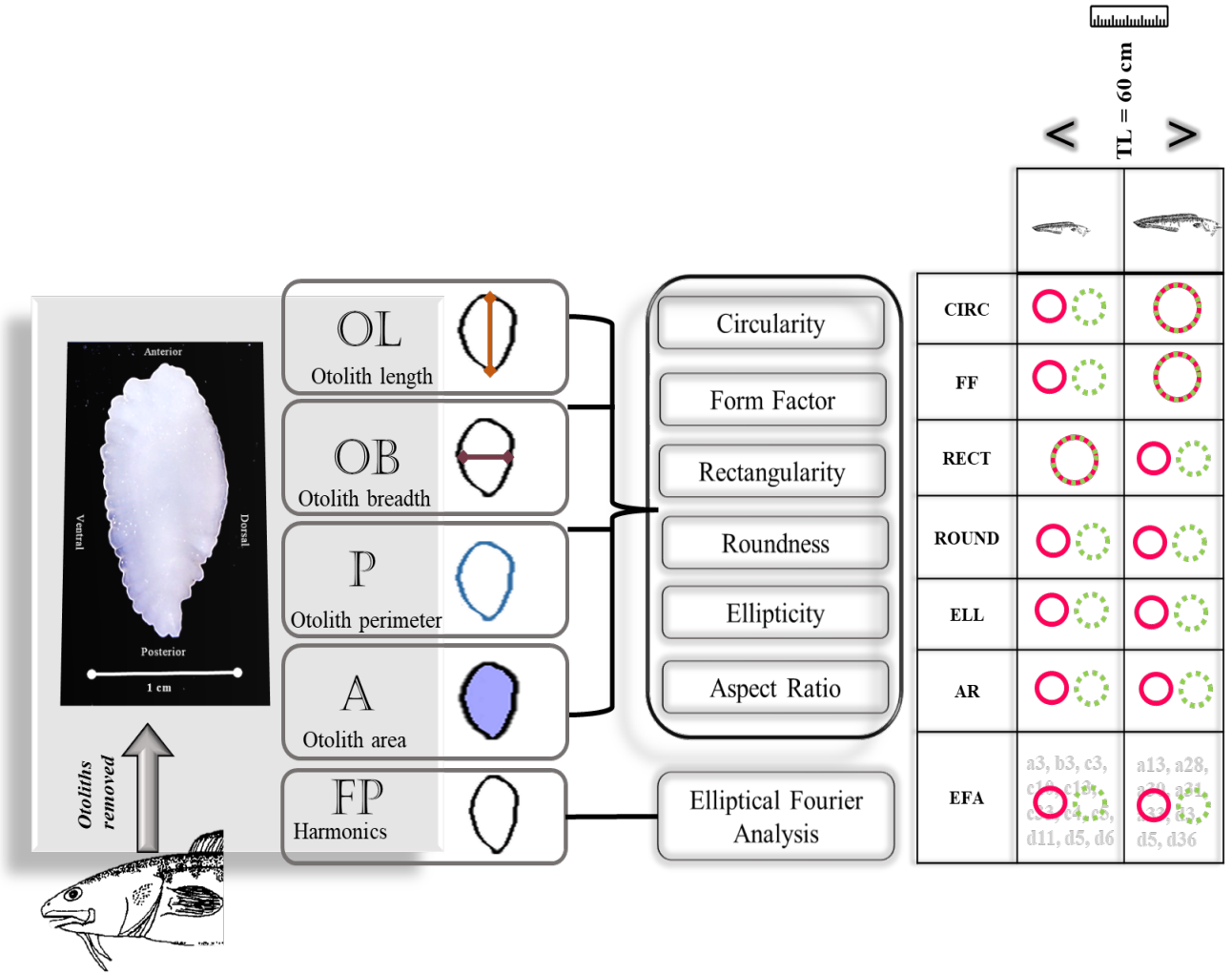
Chapter 3

Otolith Shape Analysis

"We become what we behold.
We shape our tools and then our tools shape us".
-Marshall McLuhan

GRAPHICAL ABSTRACT





3.1. INTRODUCTION

3.1.1. Background

Otoliths are paired mineralised structures found on each side of the brain cavity in the inner ears of teleost fish (Popper and Coombes, 1980; Morales-Nin, 1987; Tanner *et al.,* 2016), with the primary function of aiding in hearing and balance of the fish (Tanner *et al.,* 2015). There are three types of otoliths: the lapillus, the asteriscus, and the sagittae (Morales-Nin, 1987). The sagittae is the largest of the three with unique characteristics exhibiting a species-specific morphology (Campana, 2004).

The otoliths size strongly reflects fish size and this serves as a fundamental basis for its use in growth studies (Campana and Nielson, 1985). The size, shape, and characteristics of otoliths differs among fish species (L'Abée-Lund, 1988; Campana and Casselman, 1993) because of both genetic and environmental effects (Vignon and Morat, 2010; Bourehail, 2015). The combined exogenous and endogenous factors that determine its shape may vary between populations resulting in the stock-specific traits of otoliths (Campana and Neilson, 1985; Nazir and Khan, 2021). Thus, otolith shape analysis has been shown to be a promising tool for stock identification (Campana and Casselman, 1993) and has been extensively, and successfully, used in stock-identification research of various marine fish species (Mérigot *et al.,* 2007) e.g. Begg And Brown (2000), Khemiri (2018), and Vaz *et al.* (2023). Since they facilitate the recognition, classification, and identification of species (L'Abée-Lund, 1988; Campana, 2004; Bourehail, 2015), the classification of some ichthyological taxa is often complemented by morphological descriptions of their otoliths (Tuset *et al.,* 2008; Bostanci, 2009). This method can also detect recent divergence or secondary reproductive interaction between stocks that may not necessarily be reflected in gene frequency (Begg *et al.,* 1998).

Advantages of using this method is that it is relatively easy, cheap, can be performed using archived collections of otoliths (ICES, 2021), otolith samples can be easily stored, operations are easily replicated, and

environmental machinery has minimal impact (Bani *et al.,* 2013). The technique relies on obtaining and analysing images, the extraction of shape variables, and statistical data models (ICES, 2021).

Otolith shape can be explained in various ways:

1. The comparison of linear distances among otolith shapes is one of the simplest methods (Burke *et al.,* 2008; Legua *et al.,* 2013). With otolith morphometrics, the otolith shape is ascribed as a size multivariate that is highly correlated to the fish dimensions (Cadrin and Friedland, 1999). Since fish length and otolith growth have a positive relationship, examining otolith dimensions (for allometric growth patterns) can be useful for detecting physiological changes caused by major events in the life cycle of the fish like reproduction or sexual maturity (Medeiros *et al.,* 2021) e.g., Bervian *et al.,* 2006; Carvalho *et al.,* 2015; Maciel *et al.,* 2019. Evaluating the growth of fish, and fish stocks, is beneficial in fisheries management (Dehghani *et al.,* 2015)
2. Otolith shape indices are size-independent geometric variables. This method reveals geometric complexity by quantifying the variability in otolith shape (Tessier, 1938 cited by Cadrin and Friedland, 1999). Otolith shape analysis is useful in the application of stock, population, and species discrimination, in fish (Medeiros *et al.,* 2021) e.g., Capoccioni *et al.,* 2011; Santos *et al.,* 2017; Assis *et al.,* 2020; Song *et al.,* 2020). Elliptic Fourier Analysis has shown to be an effective method for examining and describing the contour shapes of an otolith (Cadrin and Friedland*.,* 1999; Bani *et al.,* 2013) and is the method most commonly used for this purpose (Medeiros *et al.,* 2021).

3.1.2. The goal of this study

For *Genypterus capensis,* published studies using otolith morphometrics included that of Payne (1977), Payne (1984), Payne (1985), Morales-Nin (1985), Morales-Nin (1987) and Japp (1990). The current study is the first to apply otoliths shape indices and Elliptical Fourier analysis for stock delineation of the species.

Aim

The aim of this chapter is to evaluate the ability of *Genypterus capensis* otolith shapes to distinguish between different stock boundaries within the species.

Objectives

1. To rule out its potential as a contributing element, the impact of the fish's sex, as well as differences between right and left otoliths, will be evaluated.
2. Determine whether the otolith weight relative to otolith length (OW:OL) relationship indicates stock delineation.
3. Examining allometric growth patterns, by using the otolith length (OL) and otolith breadth (OB) shape parameters, in comparison to published studies by Payne (1977 and 1985), and to draw conclusions regarding stock structure.
4. Using the six otolith shape indices (circularity, form factor, rectangularity, roundness, ellipticity, and aspect ratio), univariate analysis will be carried out on both immature and mature specimens to determine whether there are significant differences among stocks for each variable.
5. To determine the best combination of otolith shape indices for stock delineation, five classification models will be used in a stepwise selection process. Thereafter, MDS and PCA will be computed to illustrate the variances.
6. Elliptical Fourier analysis will be applied to otoliths of immature and mature sample sets to evaluate the distinctions between the two possible stocks of *G. capensis*.

3.1.3. General description of the *Genypterus capensis* sagittae otolith

As portrayed in Figure 3.1., the sagittal otolith of *Genypterus capensis* has an oval – ovate shape, is laterally compressed, and is translucent. The dorsal margin has a lobed crenate appearance because of the radial lobes being most prominent in this area. The ventral margin is approximately smooth, and this continues toward the posterior margin which is sharper in comparison to the

bluntly rounded anterior margin. The proximal surface is strongly convex, and its smooth surface is broken only by faint indentations marking the position of the radial lobes. The distal surface is strongly concave except for the thickening in the central core around the nucleus. The *sulcus acusticus*, a groove on the length of the medial surface of the sagittae (Lombarte, 2006), is well marked. No *excisura* or an *anti-rostrum* are present (tailored from information in Payne, 1977). Morales-Nin (1985) noted that calcite crystals (otolith elemental composition is further discussed in Chapter 4 of this dissertation) are constructed by layers with a width of 3µm. Subsequent growth of the crystals followed a dendritic pattern, with a secondary growth in a helicoidal pattern being frequently added. Figure 3.2. portrays an example of an immature and mature *G. capensis* otolith pair from each putative stock.

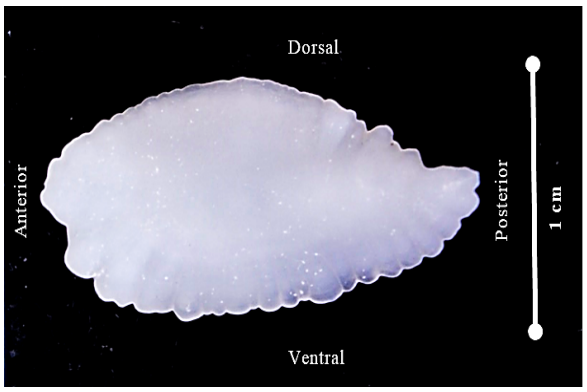


Figure 3.1. Anterior, posterior, dorsal, and ventral sagittal otolith positions shown from the distal surface of a *Genypterus capensis* otolith**.**

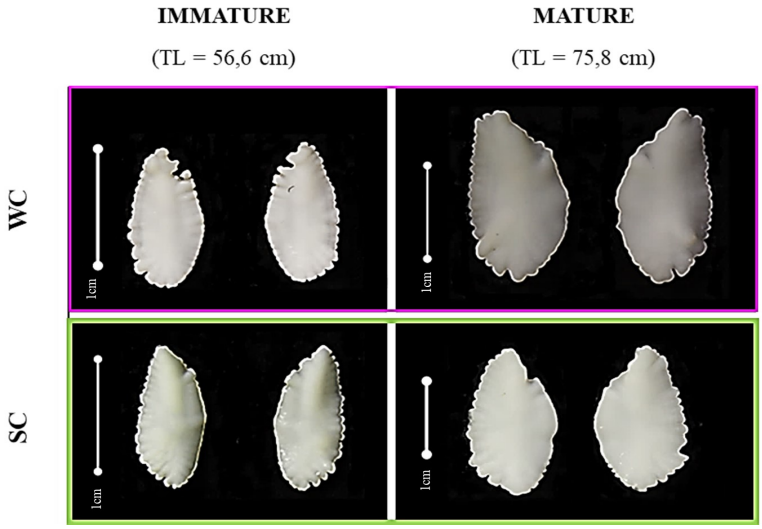


Figure 3.2. Immature and mature *Genypterus capensis* otoliths from the putative west coast (WC; top panel), and south coast (SC; bottom panel) stocks.

3.2. MATERIALS AND METHODS

3.2.1. Sample locations

Otoliths that were examined for shape analysis were collected over a period of six years: 2008, 2009, 2011, 2014, 2015, 2016, and 2017. Otoliths for the years 2008, 2009, and 2011 were obtained from the Department of Forestry, Fisheries, and the Environment (DFFE) as shown in Figure 3.3. These otoliths had been already removed from the fish and were preserved in vials containing specimen details. Otoliths for the years 2014 – 2017, were obtained from fish specimens used in this study upon completion of morphological and meristic analysis (see Chapter 2). As presented in Figure 3.4., all otoliths were assigned to one of two regions off the South African coast, the west (WC) and south coast (SC), based on the location where the fish were captured.

	2008	2009	2011	2014	2015	2016	2017
WC	111	76	146	117	14	37	38
SC	99	68	83	15	7	16	

Figure 3.3. Bar graph with associated table values indicating the number of *Genypterus capensis* otoliths examined (n) from fish caught annually on the west coast (WC) and south coast (SC). Otoliths for the years 2008, 2009 and 2011 were collected by the Department of Forestry, Fisheries, and the Environment and are outlined with a black dashed border. Otoliths for the years 2014 - 2017 were obtained during this study.

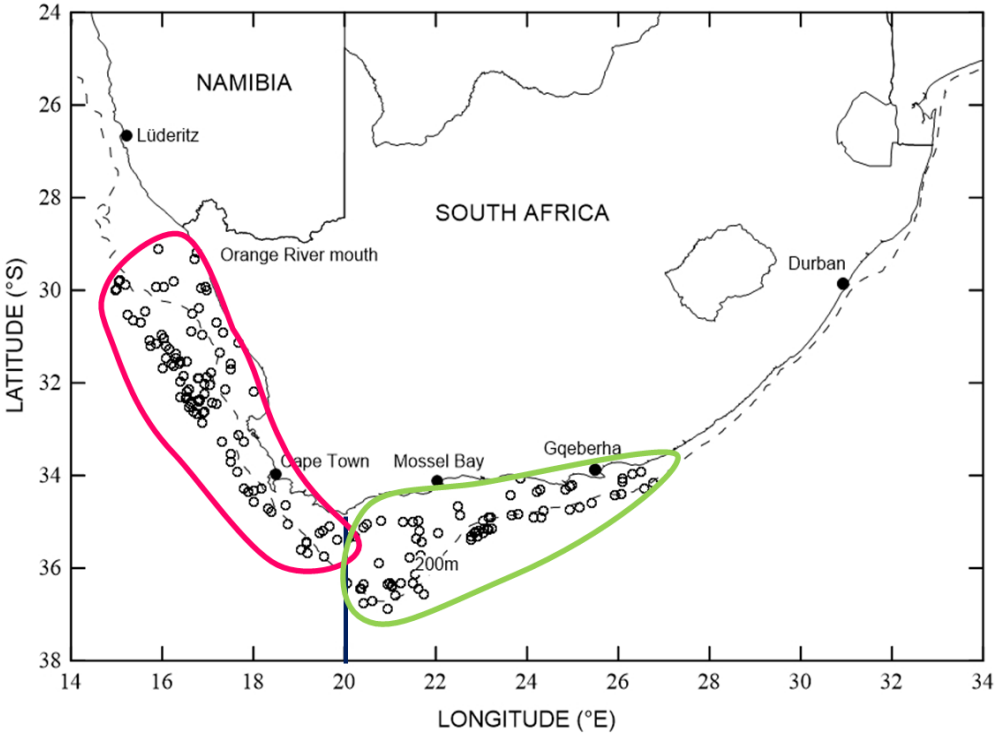


Figure 3.4. Locations where *Genypterus capensis* samples were collected during research surveys conducted by the Department of Forestry, Fisheries, and the Environment (DFFE) during 2008,2009,2011 and 2014 -2017. The 200m depth isobath is shown with the black dashed line. West coast (WC) samples are outlined in pink, and South coast (SC) samples are outlined in green. The boundary at Cape Agulhas (34.8163° S, 20.0152° E) is shown with a solid black line.

3.2.2. Preservation

Excess tissue adhering to the otolith was removed, otoliths were rinsed with reverse-filtered osmotic water, and then dried under a cooling fan at the UCT laboratory. Once dried, otoliths were preserved in labelled glass vials until further examination.

3.2.3. Photographs

Otoliths were photographed using a Nikon D500 camera that was firmly fastened onto a height adjustable camera desk stand at a set distance of 22 cm [Mode: AV; ISO = 100; Exposure compensation: 0; Aperture = 100]. The otoliths were placed against a black background with their distal sides facing upward toward the camera. A ruler was placed next to the otoliths to serve as a measurement guide, as well as a label on which the assigned otolith/specimen number was written. A digital image with a unique identifier

for each otolith was then saved.

3.2.4. Otolith shape parameter measurements

TpsUtil and TpsDigs software was used to determine the otolith shape parameters based on the digital photographs. The ruler in the photographs was used to establish a reference length and outlines were set to designate precise measurements. Otolith breadth (OB) was defined as the greatest distance from the dorsal the ventral edges. Otolith length (OL) was demarcated as the greatest distance between anterior and posterior edges. The otolith area (A) was the extent of the surface which the otolith enclosed, and its perimeter (P) was the continuous line that formed along boundary of the otolith.

After photography, the weights of the otolith collected in the years 2014 – 2017, was determined by weighing the otolith on Metler AE100 precision scale (0.0001-g). Microsoft Excel was then used to calculate the six shape index values using the otolith size parameters in formulae listed in Table 3.1.

Table 3.1. Equations used to calculate otolith shape indices

Shape indices	Formula
Form factor (FF)	$FF = (4\pi A)/P^2$
Circularity (CIRC)	$CIRC = P^2/A$
Rectangularity (RECT)	$RECT = A/(OL \times OB)$
Roundness (ROUND)	$ROUND = (4A)/(\pi OL^2)$
Ellipticity (ELL)	$ELL = (OL - OB)/(OL + OB)$
Aspect ratio (AR)	$AR = OL/OB$

Note: *OL – Otolith Length; OB - Otolith Breadth; P – Otolith Perimeter; A – Otolith Area*

The form factor (FF) index provides an estimate of the irregularity of the edge or circumference of the otolith, taking values of 1.0 when it is a perfect circle, and < 0.1 when it is irregular. The circularity (CIRC) and roundness (ROUND) indices are a measure of how closely the otolith approaches that of a circular object and equals 1 for mathematically perfect circle. The ellipticity (ELL) is the same as the circular variance, except that an ellipse is fitted to the shape (instead of a circle) and the mean-squared error is

measured, indicating whether the changes in the axes are proportional. The rectangularity (RECT) describes the variation in length and width in relation to the total area, having a value of 1 for perfectly rectangular object. Aspect ratio (AR) refers to the degree of otolith elongation (Tuset *et al.,* 2003; Volpedo and Echeverría, 2003; Bani *et al.,* 2013; Medeiros *et al.,* 2021).

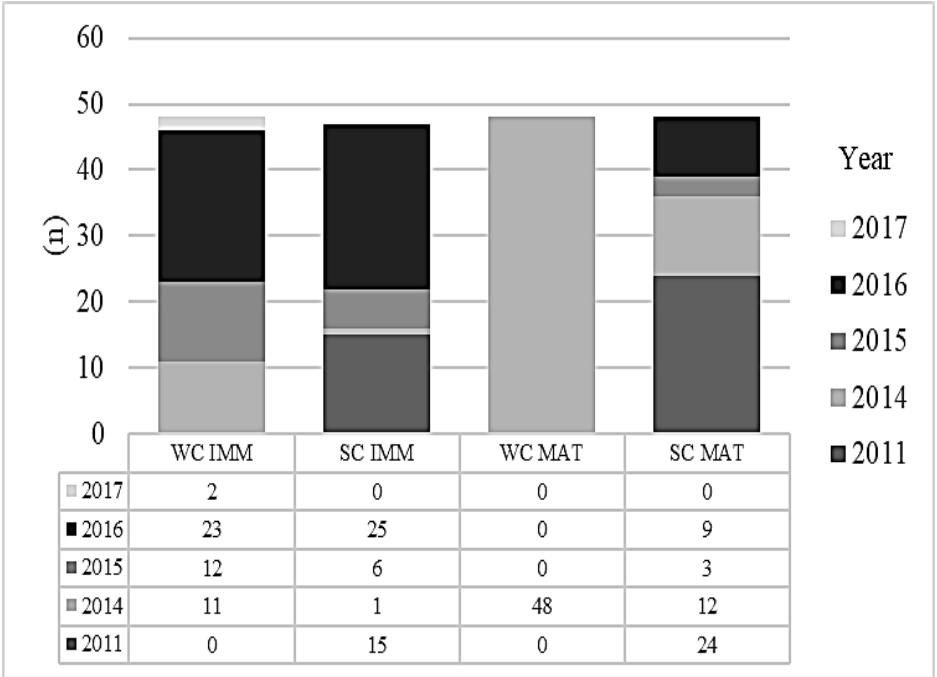
3.2.5. Elliptical Fourier analysis

With Fourier analysis, the contour of an irregular 2-dimension image is decomposed into simpler components known as harmonics. Each successive harmonic can add more information about shape morphometrics (Campana and Casselman, 1993).

In this study, data was separated into samples caught from the west coast (WC) and those from the south coast (SC). These were further separated into mature (MAT) and immature (IMM) sample sets at TL = 60 cm (See Section 2.2.4.1). In total, 200 otolith images were selected (50 per group – WC IMM, SC IMM, WC MAT, SC MAT), the otoliths in the images were from fish caught in the years 2014 – 2016. When there was not enough otoliths in a group, images from the year 2011 was used to compensate the deficit. Some otoliths could not be analysed due to technical mishaps and these were excluded in results. The resultant sample groups (WC IMM: n = 48, SC IMM: n = 47; WC MAT: n = 48, and SC MAT: n = 48 otolith images) and the years in which the fish were captured is shown in Figure 3.5.

For preparation of Fourier analysis, the contours of the sagitta otolith outline was extracted using SHAPE $^{1.3}$ suite. The otolith images were converted to BITMAP (24-bit, *.bmp) format, loaded into the ChainCoder program for image analysis, and several coordinates (x, y) were generated to describe the outline of the otolith. This data was loaded into the Elliptic Fourier transformation program (CHC2NEF) and 40 harmonics for each otolith was composed. Each harmonic was characterized by four coefficients (a, b, c, d), but the first three (a1, b1, c1) were constant for every sample (Steer and Fowler, 2014), therefore, the total number of shape descriptors for each otolith was: (4 * (number of harmonics) - 3) = 157. Each descriptor was treated as an independent variable. Principal component analysis program

(PrinComp) and contour visualization program (PrinPrint) was then used to generate linear outline images of the otolith contours.



	WC IMM	SC IMM	WC MAT	SC MAT
2017	2	0	0	0
2016	23	25	0	9
2015	12	6	0	3
2014	11	1	48	12
2011	0	15	0	24

Figure 3.5. The number of otolith images used for Elliptical Fourier Analysis, and the years in which the host *Genypterus capensis* fish was caught. **Note**: WC – putative west coast stock; SC – putative south coast stock; MAT – mature; IMM - immature

As per Crampton (1995), the cumulative percentage of Fourier Power ($FP_n\%_C$) was calculated to determine the necessary number of harmonics required to construct the silhouette of the otolith. This is obtained at the 99.99% value of the $FP_n\%_C$ point. Fourier Power (FP), the percentage of Fourier Power (F.P.%), and the cumulative percentage of the Fourier Power ($FP_n\%_C$), was calculated using the following equations:

- $\boldsymbol{FP} = \frac{a^2+b^2+c^2+d^2}{2}$
- **FP%** = $(FP_n/\sum_1 nFP_n) \times 100$
- $\mathbf{FP_n\%_C} = \sum_1 nFP_n\%$

Where a,b,c, and d = harmonic coefficients, FP = Fourier Power, C = Cumulative, n = number of specimens

Principal component analysis (PCA's) and Multi-Dimensional Scaling (MDS) plots were then created using PRIMER[6] to illustrate differences in data obtained from specimens from the two putative stocks.

3.2.6. Statistical Analysis

An explanation of the Shapiro-Wilk test, ANOVA's, Mann-Whitney U tests, Random Forest (RF) Analysis; Boosted Tree (BT) Analysis, and Principal Component Analysis (PCA), is explained in Section 2.2.4. These methods are used in the current chapter, and additional assessment methods used is explained:

3.2.6.1. Linear Discriminant Analysis (LDA)

Linear Discriminant Analysis (LDA) builds a predictive model for group membership. The model is comprised of a discriminant function established on linear combinations of the predictor variables that postulate the best discrimination between the groups. Independent variables are usually continuous whilst the dependent group is categorical. The functions are generated from a sample of cases of which group membership is known. These functions are then applied to new cases that have measurements for the predictor variables but have undetermined group membership. Thus, LDA is useful in predicting group membership by distinguishing which variables are the best discriminators between groups (IBM SPSS, 2022). In this study, LDA was executed in SPSS[26].

3.2.6.2. Artificial Neural Networks (ANN)

Artificial neural networks (ANNs) are comprised of node layers, containing:

- an input layer,
- one or more hidden layers, and
- an output layer

Each node/artificial neuron, connects to another, having a related weight and threshold. If the output of any individual nodes is above the specified threshold value, that node is activated. Data is either sent to the next layer of the network, or no data is passed along to the next layer. Neural networks are

dependent on training data to learn and advance accuracy over time. If training is successful, ANNs can achieve analytical tasks such as predictions, classification, and function approximations, as well as pattern recognition and completion from input data. Once fine-tuned for accuracy, ANN's are powerful tools in computer science and artificial intelligence, allowing the classification and clustering of data at a high velocity (IBM SPSS, 2022). In this study, ANN analysis was computed in SPSS[26].

3.2.6.3. Support vector machines (SVM)

Support vector machines (SVMs) are specific linear classifiers which are based on the margin maximization principle. Structural risk minimization are executed to improve the complexity of the classifier with the goal of achieving optimum generalization performance. The classification task by creating, in a higher dimensional space, the hyperplane that best differentiates the data into two categories (Adankon and Cheriet, 2015). In this study, SVM analysis was completed in STATISTICA[26].

3.3. RESULTS

3.3.1. Preliminary analysis

The TL range, effect of sex, differences between right and left otoliths, and the relationship between otolith weight and otolith TL were examined, and results are described.

3.3.1.1. Total Length (TL) of Genypterus capensis specimens

Since otolith shape is reflective of phenotypic development, it could be influenced by a range of factors including sex, age, stock, and environmental conditions (Mérigot *et al,.* 2007; Vignon and Morat, 2010; Renán *et al.,* 2016). For TL, n = 792 samples were measured, and a length-frequency distribution graph is shown in Figure 3.6.

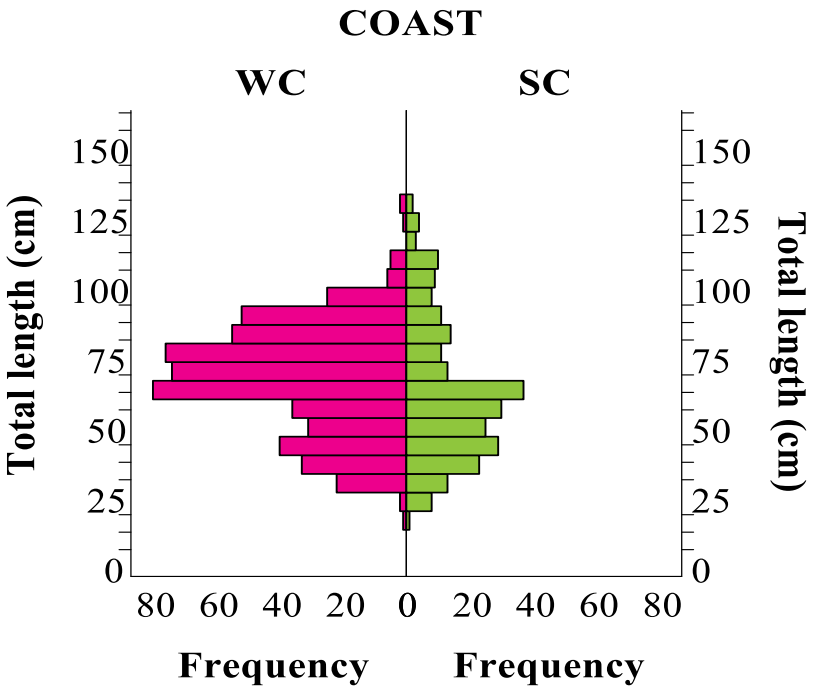


Figure 3.6. Frequency plot showing the range of total length (TL) of *Genypterus capensis* for the putative western (WC) and southern (SC) stocks, for the years 2008, 2009, 2011, and 2014 - 2017.

3.3.1.2.Examine any variations between the left and right otoliths

Both otoliths were visible in every photo, but only the measurements from the right otolith (unless it was broken or missing) was used for analysis. A paired t-test was performed using paired otoliths of comparable lengths coast [n = 30 (WC =15; SC =15); TL = 70 – 90 cm] from each coast to ensure that there were no variations in the data collected. No significant differences ($p > 0.05$) were found when evaluating variations in shape parameters between the right and left otoliths for fish from both putative stocks (Table 3.2)

Table 3.2. T-test: p-values indicating differences in shape indices between right and left otoliths of *Genypterus capensis*, from the putative western (WC) and southern (SC) stocks. [n = 30 (WC =15; SC =15); Total Length (TL) = 70 – 90 cm].

Shape index	p-value	
	WC	**SC**
Area (A)	0.9547	0.9324
Perimeter (P)	0.9862	0.9794
Otolith Length (OL)	0.9360	0.9413
Otolith Breadth (OB)	0.9406	0.9316

3.3.1.3. The effect of sex on otolith measurements

To determine whether the sex of the fish influences the otolith measurements, ANOVA tests were performed at different TL-intervals (Table 3.3). It was only at the TL = 60 cm interval that P ($F_{(1,57)} = 4.649$, $p = 0.035$) and OL ($F_{(1,57)} = 5.018$, $p = 0.029$) showed significant differences between sexes for SC samples. However, no significant differences ($p > 0.05$) were found in any of the measurements at other TL intervals. As such, the sexes were not separated for further analysis.

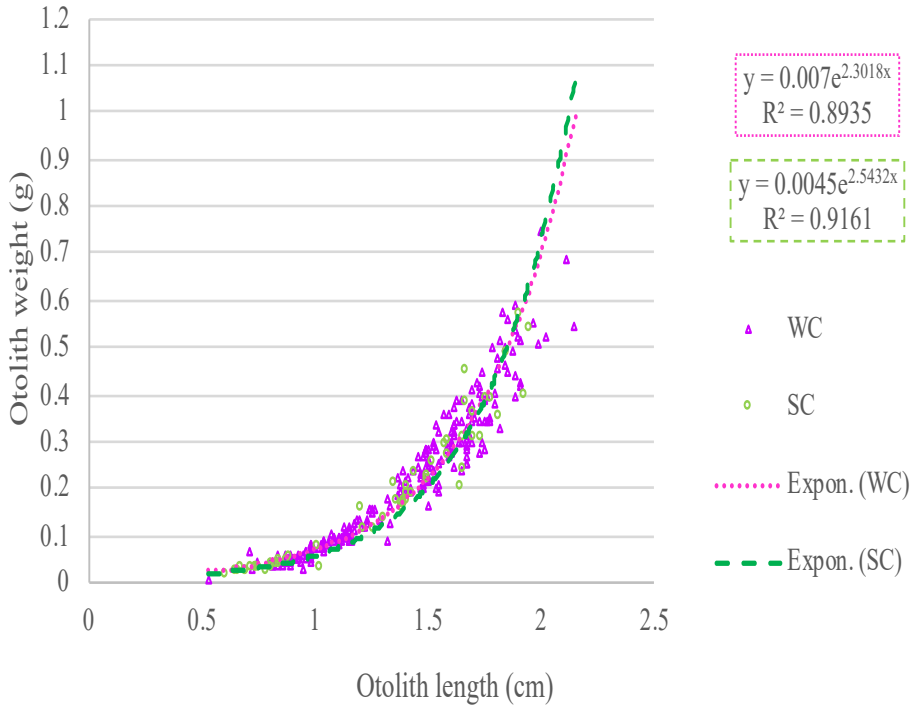
Table 3.3. ANOVA: Summary of p-values signifying differences in shape indices between male and female otoliths of *Genypterus capensis*, from the putative western (WC) and southern (SC) stocks, for the years 2008, 2009, 2011, and 2014 - 2017.

TL (cm)	Area (A)		Perimeter (P)		Otolith Length (OL)		Otolith Breadth (OB)	
	WC	**SC**	**WC**	**SC**	**WC**	**SC**	**WC**	**SC**
30	0.516	0.416	0.705	0.264	0.937	0.31	0.301	0.274
40	0.158	0.363	0.24	0.294	0.924	0.374	0.462	0.303
50	0.066	0.522	0.767	0.341	0.059	0.712	0.076	0.562
60	0.562	0.063	0.92	**0.035**	0.898	**0.029**	0.686	0.319
70	0.195	0.786	0.567	0.722	0.711	0.992	0.195	0.621
80	0.648	0.057	0.336	0.199	0.799	0.063	0.491	0.108
90	0.539	0.62	0.934	0.866	0.558	0.829	0.245	0.539
100	---	0.606	---	0.637	---	0.9	---	0.456
110	---	0.13	---	0.118	---	0.142	---	0.332

Note: (---) = No data available

3.3.1.4. Otolith weight (OW) relative to otolith length (OL)

The exponential relationships between OW and OL of *Genypterus capensis* from the putative western (WC) and southern (SC) stocks off the South African coast is shown in Figure 3.7. After converting exponential equations into linear regression equations, a student's t-test was computed and results showed no significant differences ($p = 0.776$) between the two putative stocks.



Students t-test		
	WC	**SC**
Slope	3.451	3.339
n	291	79
SE(reg)	0.269	0.138
SE(slope)	0.041	0.0340
Difference	0.023	
SE (diff)	0.082	
t-stat	0.285	
df	286	
p-value	0.776	

Figure 3.7. Scatterplots with fitted exponential linear relationships, R^2 values, and a students t-test describing otolith weight (OW) in relation to otolith length (OL) of *Genypterus capensis* from the putative western (WC) and southern (SC) stocks off the South African coast, for the years 2014 - 2017.

3.3.2. Otolith shape parameters

3.3.2.1. Frequency distribution graphs

Frequency distribution graphs depicting the range of each of the otolith size parameters was determined and are shown in Figure 3.8.

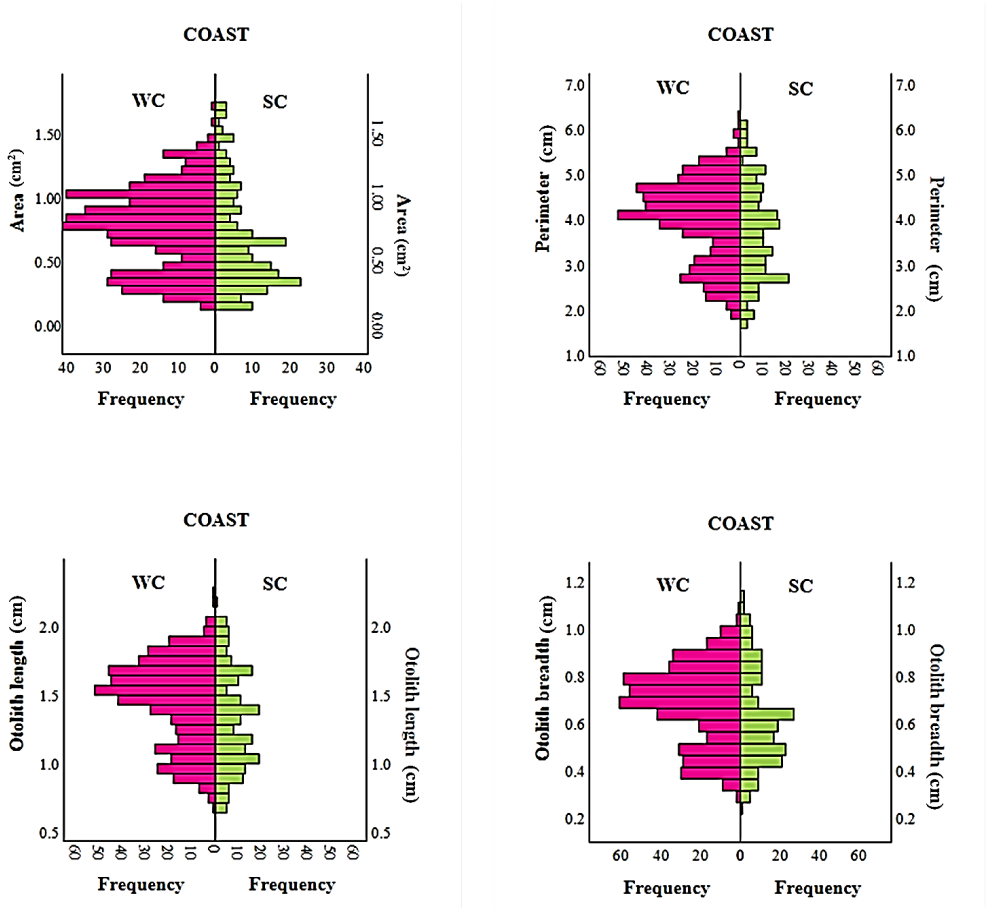


Figure 3.8. Frequency plots of the area, perimeter, length, and breadth of *Genypterus capensis* otoliths from the putative west (WC) and south (SC) stocks off the coast of South Africa, for the years 2008, 2009, 2011, and 2014 - 2017.

3.3.2.2. Relationship: Otolith shape dimensions and total length (TL)

Allometric relationships reveal how morphoanatomical or physiological changes differ with variations in body size (Medeiros *et al.,* 2021). A Kendall's tau b test was computed to indicate the relationship between TL and the shape parameters of *Genypterus capensis* otoliths from the two putative stocks. As shown in Table 3.4., all variables were shown to be highly correlated with TL ($\tau_b > 0.5$; $p < 0.001$).

Table 3.4. Kendall's tau_b test indicating the relationship between total length (TL) and the otolith shape parameters of *Genypterus capensis* from the putative western and southern stocks, for the years 2008, 2009, 2011, and 2014 - 2017.

Variable	Total length (TL)	
	τ_β	p-value
Total length (TL)	1	
Otolith area (A)	0.620	<0.001
Otolith perimeter (P)	0.580	<0.001
Otolith length (OL)	0.600	<0.001
Otolith breadth (OB)	0.585	<0.001

As discussed in Chapter 2, whilst allometric growth may not necessarily be reflective of stock delineation, it plays a significant role in data interpretation. With otolith morphometric studies, larger individuals tend to have larger otolith dimensions (Campana and Casselman, 1993; Begg and Brown, 2000; Renán *et al.,* 2016). A students t-test revealed no significant differences (p = 0.184) between the two putative stocks for the OL linear relation to TL (Appendix B: Table B1).

The otolith dimensions (TL/OL, TL/OB, and OL/OB) were calculated and linear graphs depicting their relationship to TL were created and is shown in Figure 3.9.Linear equations were ascertained for these graphs and a student's t-test was computed to test for differences in linear regression slopes between the two putative stocks. No significant differences (p = 0.525) was found for the OL/OB *vs* TL relationship. Significant differences were found for the TL/OL *vs* TL (p = 0.023) and TL/OB *vs* TL (p = 0.037) relationships (Appendix B: Table B1).

Payne (1985) documented linear equations for *G. capensis* describing the relationship between the otolith length : otolith height (OL:OB) ratio with fish length (TL) for the Cape Stock (synonymous with the putative WC stock in this study) and South-East stocks (synonymous with the putative SC stock in this study) Linear graphs describing the relationship between Payne (1985) and this study is portrayed in Figure 3.10. A students t-test indicated significant differences (West Coast samples: p = 0.0018; South Coast samples: p = 0.0015) between results from the two studies.

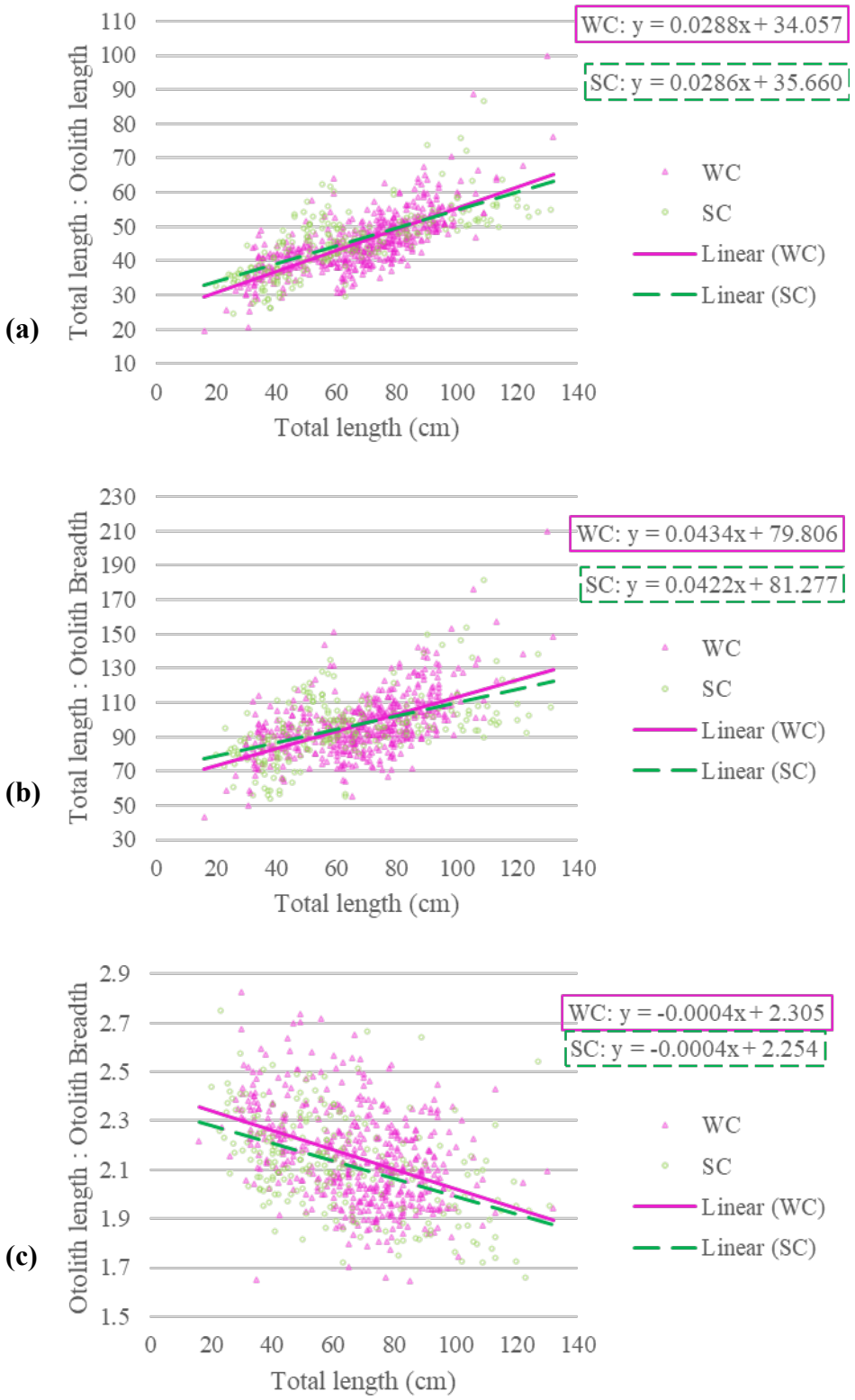
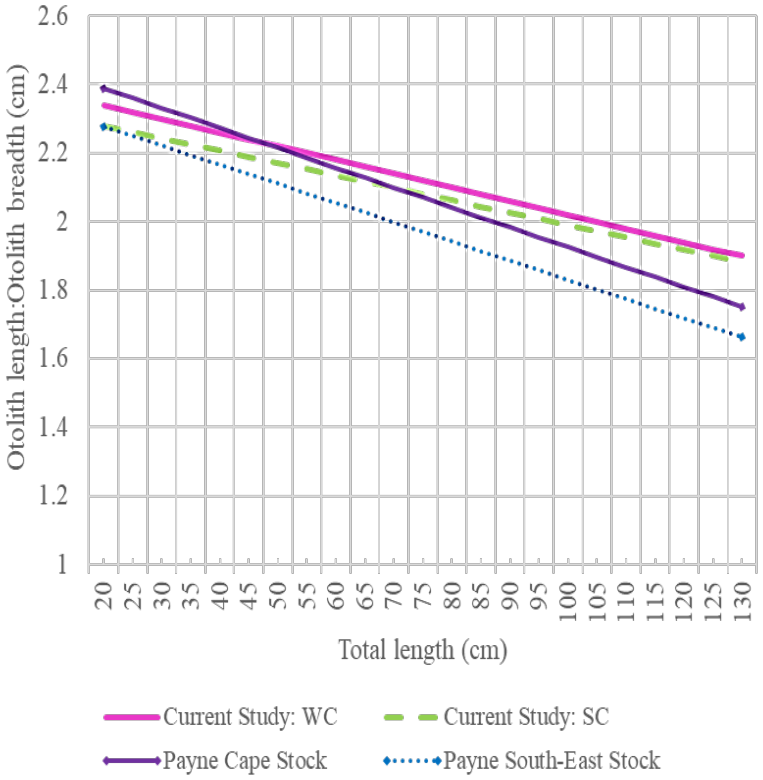


Figure 3.9. Linear graphs showing the **(a)** TL:OL vs TL **(b)** TL:OB, vs TL and **(c)** OL:OB vs TL, with linear regression equations of *Genypterus capensis* from the putative western and southern stocks, for the years 2008, 2009, 2011, and 2014 - 2017. **Note**: TL – Total length, OL – Otolith length, OB – Otolith breadth.



Students t-test				
	West Coast		**South Coast**	
Calculation	**Payne (1985)**	**Current Study**	**Payne (1985)**	**Current Study**
Slope	-0.0058	-0.0061	-0.0056	-0.0036
n	234	234	235	235
SE(reg)	1.96E-09	0.1836	3.9E-09	0.176
SE(slope)	1.06E-11	0.0010	9.92E-12	0.0004
Difference	-0.0003		0.0020	
SE(difference)	0.0010		0.0004	
t-stat	-0.3274		4.4532	
df	464		466	
p - value	0.0018		0.0015	

Figure 3.10. Linear graphs documenting the regression equations that describes the relationship between the otolith length : otolith height (OL:OB) ratio and fish length (TL), with student t-test, for *Genypterus capensis* from the putative western (WC) and southern (SC) stocks in this study, and the Cape Stock and South-East stocks were measured by Payne (1985).

3.3.3. Otolith shape indices

3.3.3.1. Kendall's tau b test

A Kendall's tau b test was computed to show the relationship between TL with shape indices, and log-transformed shape indices, of *Genypterus capensis* otoliths from each putative stock (WC and SC), as shown in Table 3.5. Except for rectangularity ($p < 0.5$) all variables found to have a weak correlation with TL ($\tau_b < 0.34$; $p < 0.001$). As five of the six variables showed a weak relationship, the effect of length (TL) was eliminated for further analysis. Frequency distribution graphs for the shape indices are shown in Figure 3.11.

Table 3.5. Kendall's tau_b test indicating the relationship between total length and the shape parameters of *Genypterus capensis* otoliths from the putative western and southern stocks, for the years 2008, 2009, 2011, and 2014 - 2017.

Variable		τ_β	p-value		τ_β	p-value
Total length	TL	1	--.	TL	1	--.
Circularity	CIRC	-0.143	<0.001	Log(CIRC)	-0.136	<0.001
Form Factor	FF	0.143	<0.001	Log(FF)	0.136	<0.001
Rectangularity	RECT	0.020	0.424	Log(RECT)	-0.001	0.973
Roundness	ROUND	0.222	<0.001	Log(ROUND)	0.305	<0.001
Ellipticity	ELL	-0.195	<0.001	Log(ELL)	-0.316	<0.001
Aspect ratio	AR	-0.195	<0.001	Log(AR)	-0.316	<0.001

Note: *TL – Total length; CIRC – Circularity; FF – Form factor; RECT - Rectangularity; ROUND - Roundness; ELL - Ellipticity and AR – Aspect ratio.*

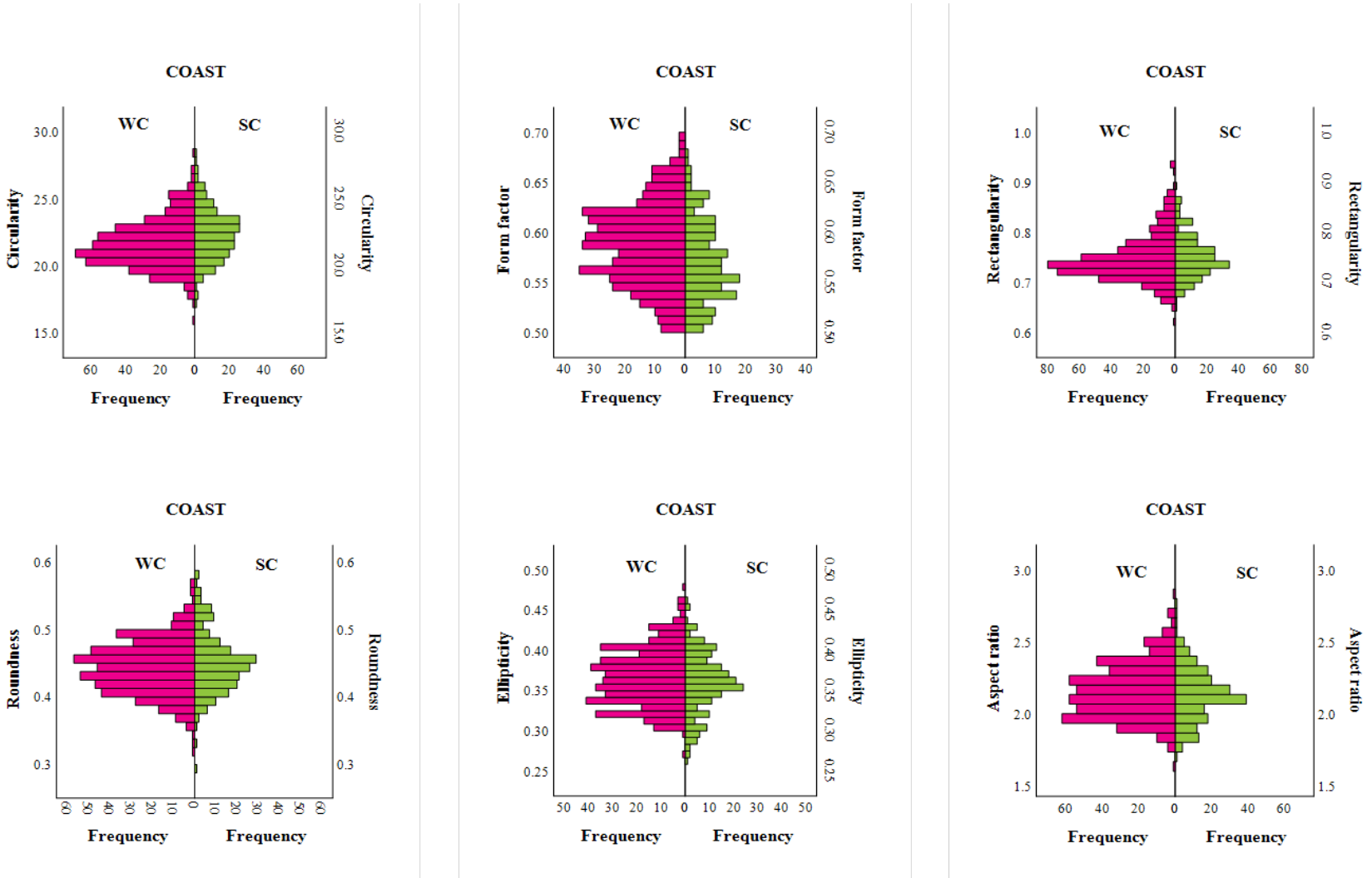


Figure 3.11. Frequency plots of the shape indices (circularity, form factor, rectangularity, roundness, ellipticity, and aspect ratio) of *Genypterus capensis* otoliths from the putative west (WC) and south (SC) stocks off the coast of South Africa, for the years 2008, 2009, 2011, and 2014 - 2016.

3.3.3.2. Kolmogorov-Smirnov (K-S) Test for Normality

A Kolmogorov-Smirnov (K-S) test for normality indicated that most of the otolith shape indices had a skewed distribution i.e., were not normally distributed. To overcome this, the otolith indices were log-transformed. Another Kolmogorov-Smirnov (K-S) test of the now log-transformed otolith shape indices was then computed. For this, data was separated for WC and SC specimens, and applied to IMM and MAT categories. As seen in Table 3.6, even after log-transformation, some of the variables [Log(RECT) in IMM specimens; Log(FF),Log(RECT), and Log(AR) in MAT samples)], did not follow a normal distribution ($p < 0.05$).

Table 3.6. Kolmogorov-Smirnov (K-S) test for normality of otolith shape indices for *Genypterus capensis* samples from the putative west (WC) and south (SC) coast stocks, for the years 2008, 2009, 2011, and 2014 - 2017.

Variable	Coast	p-value	
		Immature	**Mature**
Log(CIRC)	SC	0.200	0.200
	WC	0.063	0.058
Log(FF)	SC	0.200	0.200
	WC	0.189	0.200
Log(RECT)	SC	0.200	0.090
	WC	**0.000**	**0.003**
Log(ROUND)	SC	0.200	**0.000**
	WC	0.172	0.200
Log(ELL)	SC	0.200	0.200
	WC	0.200	0.200
Log(AR)	SC	0.553	0.200
	WC	0.212	**0.032**

Note*: TL – Total length; CIRC – Circularity; FF – Form factor; RECT - Rectangularity; ROUND - Roundness; ELL - Ellipticity and AR – Aspect ratio; Significant differences ($p < 0.05$) are highlighted in bold.*

3.3.3.3. Univariate analysis

Boxplots of the log-transformed shape indices used in analysis, is shown in Figure 3.12. For univariate analysis, ANOVA was computed for variables showing a normal distribution on both coasts. For variables showing a non-normal distribution, a Mann-Whitney U test was performed. Results are summarised in Table 3.7 (ANOVA) and Table 3.8 (Mann-Whitney U). Complete tables are shown in Appendix B: Tables B2 and B3.

Boxplots

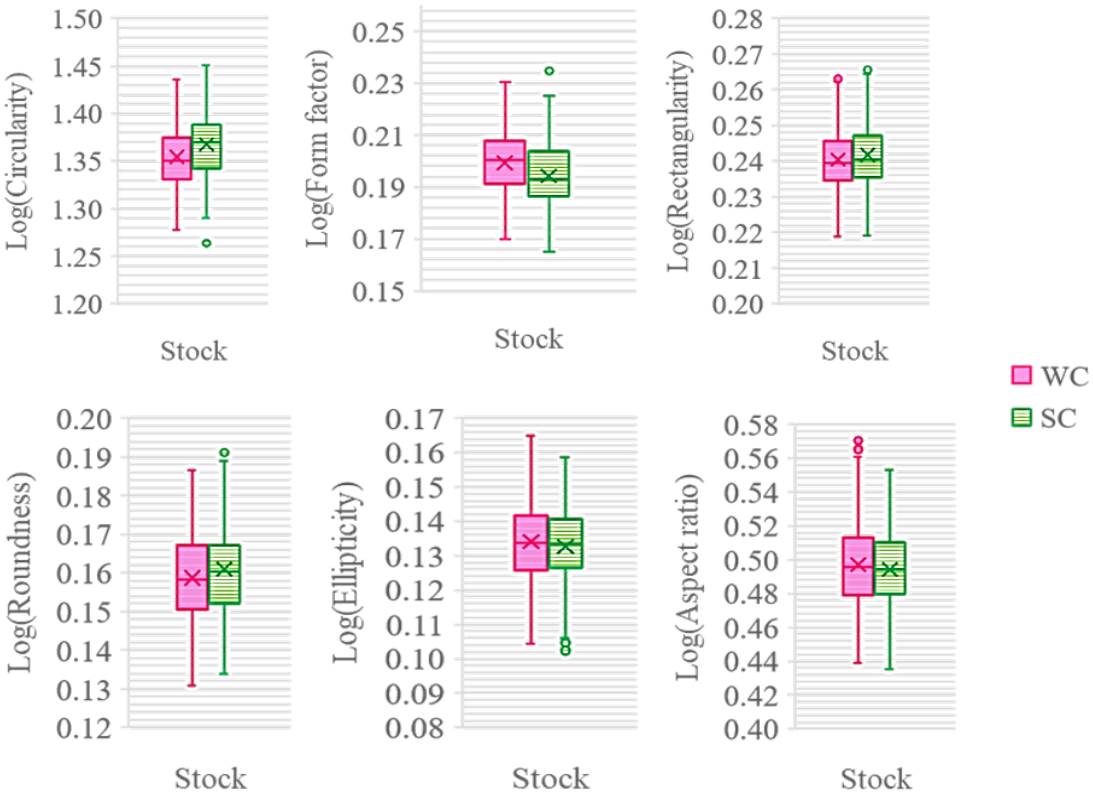


Figure 3.12. Frequency plots of the log-transformed circularity, form factor, rectangularity, roundness, ellipticity, and aspect ratio of *Genypterus capensis* otoliths from the putative west (WC) and south (SC) stocks off the coast of South Africa, for the years 2008, 2009, 2011, and 2014 - 2016.

ANOVA and Mann-Whitney U tests

An ANOVA revealed that for IMM samples, Log(CIRC) [($F_{(1,222)}$ = 5.018; p ≤0.000], Log(FF) [($F_{(1,222)}$ = 5.018; p ≤0.000], Log(ROUND) [($F_{(1,222)}$ = 5.018; p = 0.012], Log(ELL) [($F_{(1,222)}$ = 5.018; p = 0.001] and Log(AR) [($F_{(1,222)}$ = 5.018; p = 0.001], was found to have significant differences. For MAT samples, Log (ELL) [($F_{(1,346)}$ = 7.609; p = 0.006] showed significant differences. In Table 3.8, a Mann-Whitney U test revealed significant differences for Log(RECT)[U = 8142, p = 0.001], Log(ROUND)[U = 7842, p ≤0.000] and Log(AR)[U = 9138,5, p = 0.035] in MAT samples.

Table 3.7. ANOVA. p-values indicating differences in log-transformed otolith shape indices of all *Genypterus capensis* samples from the putative western (WC) and southern (SC) stocks off the South African coast for the years 2008, 2009, 2011, and 2014 - 2017.

Variable		p-value	
		Immature	**Mature**
Circularity	Log(CIRC)	**≤0.000**	0.745
Form Factor	Log(FF)	**≤0.000**	0.707
Roundness	Log(ROUND)	**0.012**	---
Ellipticity	Log(ELL)	**0.001**	**0.006**
Aspect Ratio	Log(AR)	**0.001**	---

Note*: Significant differences (p < 0.05) are highlighted in bold.*

Table 3.8. Mann-Whitney U test: p-values indicating differences in the log-transformed shape indices of *Genypterus capensis* otolith samples from the putative western and southern stocks off the South African coast, in the years 2008, 2009, 2011 and 2014 – 2017.

Variable		p-value	
		Immature	**Mature**
Rectangularity	Log(RECT)	0.259	**0.001**
Roundness	Log(ROUND)	---	**≤0.000**
Aspect Ratio	Log(AR)	---	**0.035**

Note*: Significant differences (p < 0.05) are highlighted in bold.*

3.3.3.4. Multivariate Analysis

Classification models

Five classification models [Random Forest (RF), Boosted Tree (BT), Artificial Neural Network (ANN), Support Vector Machine (SVM) and Linear Discriminant Analysis (LDA)] were used to determine the best variable combination of shape indices for discrimination of *Genypterus capensis* from the two putative stocks.

Predictor performance of variables was computed for each model, and a stepwise procedure was then executed by adding variables gradually in order of most important to least. The combinations of variables selected by each model based on the stepwise selection procedure and their respective overall classification (training and validation) was calculated. A Table showing all results is given in Appendix B:Tables B4 and B5. The variable combination showing the highest classification accuracy in the training set is show in Table 3.9.

The combinations of variables that gave the highest overall classification accuracy differed among the five classification models for both IMM and MAT sample sets.

In Table 3.9., for IMM samples, BT analysis showed the highest predictive power (= 0.71) for the validation set. When viewing the classification accuracy (Table 3.10) it can be seen that with BT analysis, showed a high, and relatively even, accuracy rate for IMM samples (overall = 73.21% ; WC = 73.39% ; SC = 73.0%).

As demonstrated in Tables 3.9 and 3.10, For MAT samples, BT analysis also showed the highest predictive power (= 0.795) for the validation set. However, when viewing the classification accuracy, a low percentage was shown by SC samples (overall = 81.03% ; WC = 97.75% ; SC = 25.93%). When using LDA however, even though a relatively low predictive power (= 0.641) was found, the accuracy rate was found to be a relatively better option when considering all cases (Overall = 62.90%; WC = 67.80%; SC = 46.90%).

Multidimensional scaling plots (MDS) were computed and are shown in Figure 3.13. For IMM samples, the variable combination determined by BT analysis showed a slight distinction between samples from the two putative stocks. For MAT samples, the variable combination determined by LDA showed extensive overlap between samples from the two putative stocks.

Table 3.9. Model selection of variables from *Genypterus capensis* from the putative western and southern stocks off the South African using a stepwise selection procedure

Sample set	Test	Variable combination (Log transformed values)	Accuracy	
			Training	Validation
Immature	**RF**	ROUND + CIR + FF + ELL	0.606	0.765
	BT	CIRC + FF + AR + ELL	0.710	**0.759**
	ANN	AR + FF + CIRC	0.688	0.688
	SVM	CIRC + FF + ROUND + ELL + AR	0.574	0.661
	LDA	CIRC + FF + ROUND	0.696	0.692
Mature	**RF**	RECT + ROUND	0.731	0.821
	BT	ROUND + ELL + AR + RECT	0.795	**0.831**
	ANN	RECT + AR + ROUND + ELL	0.776	0.816
	SVM	ROUND + ELL + RECT	0.759	0.766
	LDA	RECT + ROUND + ELL	0.641	0.629

Note: *CIRC – Circularity; FF – Form factor; RECT - Rectangularity; ROUND - Roundness; ELL – Ellipticity; AR – Aspect ratio; RF – Random Forest Analysis; BT – Boosted Tree Analysis; ANN – Artificial Neural Network; SVM -Support Vector Machine; LDA – Linear Discriminant Analysis; values in bold indicate the classification model with the highest predictive power*

Table 3.10. Comparison of the predictive performance of the five classification models based on variables obtained from *Genypterus capensis* from the putative western and southern stocks off South African coast. Cross-validation results are shown.

Sample set	Test	Classification accuracy (%)		
		OVERALL	WC	SC
Immature	RF	70.98	68.55	74.00
	BT	**73.21**	73.39	73.00
	ANN	66.10	59.40	73.30
	SVM	66.96	71.88	45.83
	LDA	69.20	72.60	65.00
Mature	RF	79.60	89.89	45.68
	BT	81.03	97.75	25.93
	ANN	74.30	97.40	13.80
	SVM	80.46	96.82	20.83
	LDA	**62.90**	67.80	46.90

Note: *RF – Random Forest Analysis; BT – Boosted Tree Analysis; ANN – Artificial Neural Network; SVM -Support Vector Machine; LDA – Linear Discriminant Analysis; WC – west putative stock; SC – south putative stock; values in bold indicate the classification model with the highest classification accuracy*

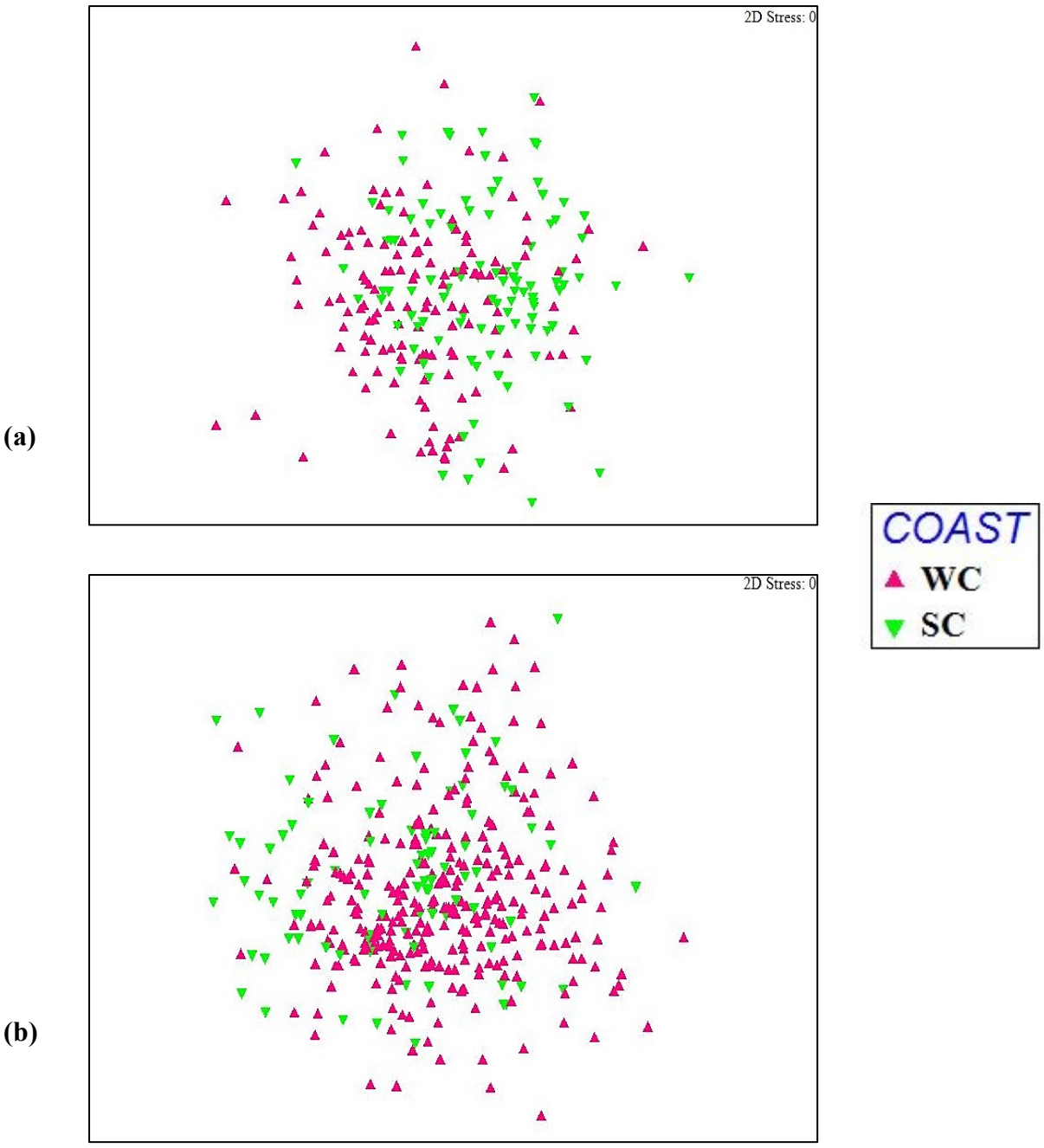


Figure 3.13. Multidimensional scaling plots (MDS) portraying differences between the putative western (WC) and southern (SC) stocks of *Genypterus capensis*, for the years 2008, 2009, 2011, and 2014 - 2017. **(a)** Immature samples: Variable combination determined by BT analysis **(b)** Mature samples: Variable combination determined by LDA**.**

Principal Component Analysis

PCAs were computed to indicate differences in log-transformed shape indices of *Genypterus capensis* caught off the west and south coast of South Africa. Analysis was applied to IMM and MAT sample sets. Tables 3.11 and 3.12, shows the eigenvalues, and correlation between the principal components; respectively. PCA plots are shown in Figure 3.14.

For IMM samples, the first two PCs showed eigenvalues greater than 1 and together (PC1 = 43.2%; PC2 = 33.2%) accounted for 76.4% of the total variation. In PC2, Log(ELL) [-0.610] and Log(AR) [-0.609] was shown to have a strong (> 0.5) correlation. In PC2, Log(CIRC) [-0.701] and Log(FF) [0.701] was shown to have a strong (> 0.5) correlation.

Together, the first two PCs (PC1 = 57.1%; PC2 = 28.8%) for the MAT samples had eigenvalues larger than 1, accounting for 85.9% of the variation in the sample. PC1 did not exhibit a significant correlation (<0.5) with any of the original variables. It was shown that PC2 had a significant (>0.5) correlation with Log(RECT) [-0.657].

For IMM samples, the PCA plot showed only partial overlap, implying a moderate distinction between the two putative stocks. In the PCA plots for MAT samples limited overlap is also observed between individuals from the two coasts indicating a marginal distinction between the two putative stocks.

Table 3.11. Eigenvalues of Principal Components and the % variation explained for log-transformed shape indices of *Genypterus capensis* samples from the putative west (WC) and south (SC) stocks, for the years 2008, 2009, 2011, and 2014 - 2017.

Sample set		PC1	PC2	PC3
Immature	Eigenvalues	**2.59**	**1.99**	**1.42**
	%Variation	43.20	33.20	23.60
	Cum.%Variation	43.20	76.40	100
Mature	Eigenvalues	**3.42**	**1.73**	0.85
	%Variation	57.10	28.90	14.10
	Cum.%Variation	57.10	85.90	100

Table 3.12. Correlation between Principal Components and original variables explained for log-transformed shape indices *Genypterus capensis* samples from the putative west (WC) and south (SC) stocks, for the years 2008, 2009, 2011, and 2014 - 2017.

Sample set	Variable	PC1	PC2	PC3
Immature	Log(CIRC)	0.094	**-0.701**	-0.011
	Log(FF)	-0.094	**0.701**	0.010
	Log(RECT)	-0.122	-0.004	**-0.824**
	Log(ROUND)	0.474	0.072	**-0.537**
	Log(ELL)	**-0.610**	-0.081	-0.125
	Log(AR)	**-0.609**	-0.079	-0.131
Mature	Log(CIRC)	-0.440	0.295	-0.468
	Log(FF)	0.441	-0.295	0.467
	Log(RECT)	0.100	**-0.657**	**-0.509**
	Log(ROUND)	0.468	-0.118	**-0.515**
	Log(ELL)	-0.439	-0.432	0.140
	Log(AR)	-0.436	-0.440	0.133

Note: *CIRC – Circularity; FF – Form factor; RECT - Rectangularity; ROUND - Roundness; ELL - Ellipticity and AR – Aspect ratio. Note: Variables shown to have a strong (> 0.5) correlation are highlighted in bold.*

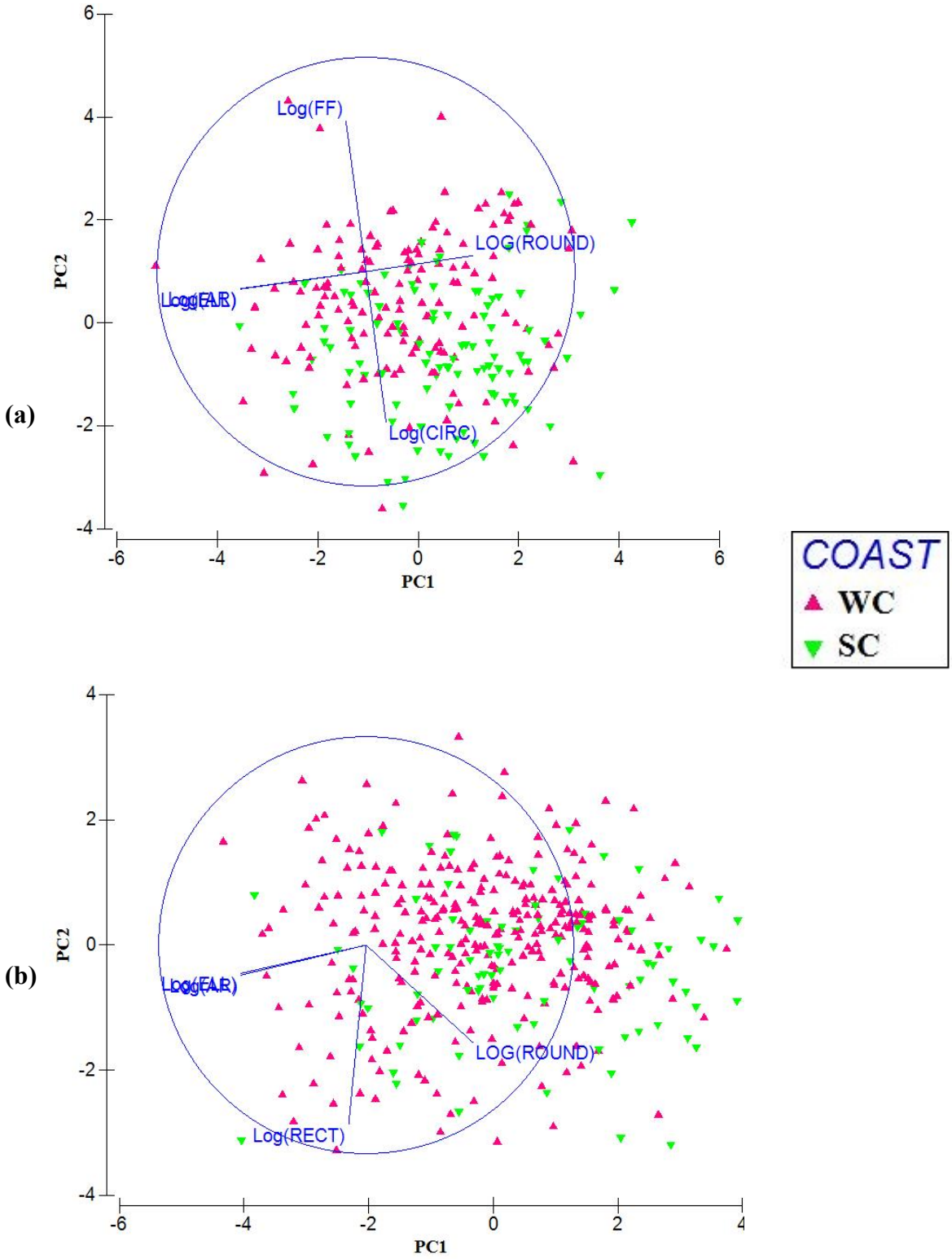


Figure 3.14. PCA plots using log-transformed shape indices for **(a)** immature and **(b)** mature *Genypterus capensis* otoliths from the putative west (WC) and south (SC) stocks, for the years 2008, 2009, 2011, and 2014 - 2017.

3.3.4. Elliptical Fourier Analysis

Of the 40 harmonics analysed for Fourier mean cumulative power ($FP_n\%_C$), the first seven represented 95%, the first ten represented 99%, and the first 36 represented 100% of the mean cumulative power (Figure 3.15). Thus, the first ten harmonics were used for PC analysis.

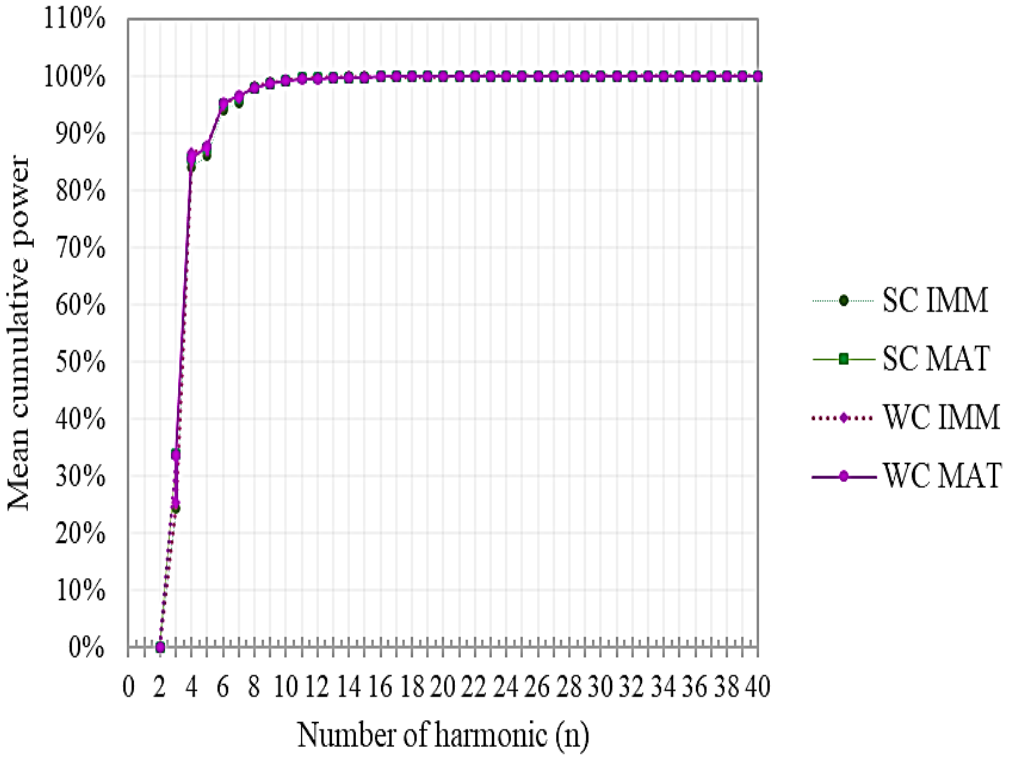


Figure 3.15. Fourier mean cumulative power (%) from harmonics analysed to study otolith shape of *Genypterus capensis* otoliths from the putative west (WC) and south (SC) stocks, for the years 2011, and 2014 -2016.

When considering the cumulated percentage of eigenvalues, the first 16 PC's represented 90%, the first 34 PC's represented 99%, and the first 43 PC's represented 100% of the evaluation for principal components for Elliptical Fourier Analysis (Figure 3.16).

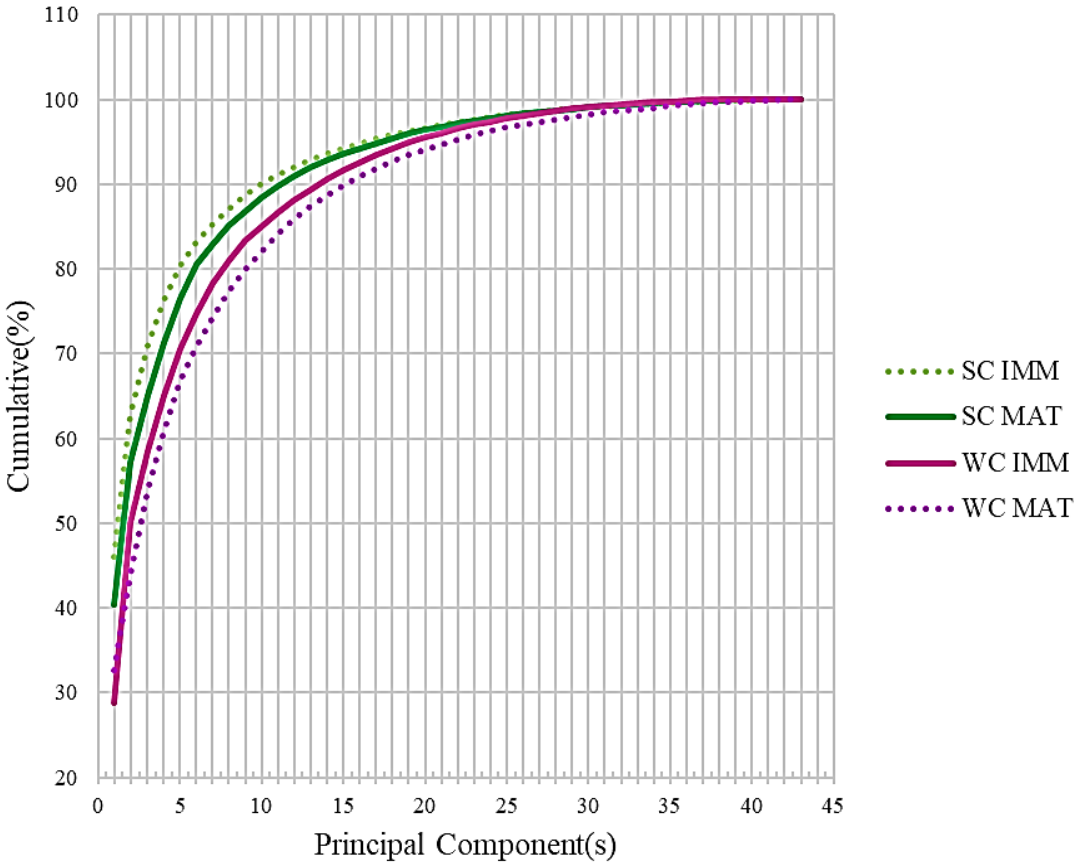


Figure 3.16. Cumulated percentage of eigenvalues describing principal component evaluation for Elliptical Fourier Analysis of *Genypterus capensis* otoliths from the putative west (WC) and south (SC) stocks, for the years 2011, and 2014 -2016.

An ANOVA comparing the Fourier power variables showed no significant changes ($p > 0.05$) between the mature and immature samples, as well as between the two hypothetical stocks (Table 3.13).

Table 3.13. ANOVA describing differences in Fourier power for harmonics analysed for otoliths of *Genypterus capensis* otoliths from the putative west and south stocks, for the years 2011, and 2014 -2016.

Variables		Variation source	SS	dF	MS	F	p-val.
IMM	WC *vs* SC	Between Groups	3.13E-09	1	3.13E-09	0.0160	0.899
		Within Groups	1.48E-05	76	1.95E-07		
		Total	1.48E-05	77			
MAT	WC *vs* SC	Between Groups	9.22E-11	1	9.22E-11	0.0004	0.983
		Within Groups	1.58E-05	76	2.08E-07		
		Total	1.58E-05	77			
SC	IMM *vs* MAT	Between Groups	6.36E-09	1	6.36E-09	0.0335	0.855
		Within Groups	1.44E-05	76	1.9E-07		
		Total	1.44E-05	77			
WC	IMM *vs* MAT	Between Groups	2.02E-10	1	2.02E-10	0.0009	0.976
		Within Groups	1.62E-05	76	2.14E-07		
		Total	1.62E-05	77			

Note*: IMM – Immature; MAT – Mature; WC – west putative stock; SC – south putative stock; SS = Sum-of-squares; dF = Degrees of freedom; MS =Mean Squares (MS); F = F-ratio; and p-val. = (significance).*

ANOVA and Mann-Whitney U tests

A summary of differences of *Genypterus capensis* otoliths from the two putative stocks, as shown by ANOVA and Mann-Whitney U tests is shown in Table 3.14. Differences were found for individual harmonics in IMM (a3, b3, c3, c10, c13, c33, c4, c5, d11, d5, d6) and MAT (a13, a28, a30, a31, a33, d3, d5, d36) and sample sets.

Table 3.14. ANOVA and Mann-Whitney U tests describing differences in Fourier power of individual harmonics analysed for otoliths of *Genypterus capensis* from the putative west and south stocks, for the years 2011, and 2014 -2016.

Immature			Mature		
Harmonic	**Test**	**p-value**	**Harmonic**	**Test**	**p-value**
a3	MWU	<0.001	**a13**	MWU	0.027
	ANOVA	<0.001		ANOVA	0.032
b3	MWU	0.002	**a28**	MWU	0.041
	ANOVA	0.023		ANOVA	0.035
c3	MWU	0.011	**a30**	ANOVA	0.037
	ANOVA	0.035	**a31**	MWU	0.034
c4	MWU	0.041	**a33**	ANOVA	0.032
c5	MWU	0.003	**d3**	MWU	0.003
	ANOVA	0.004		ANOVA	0.008
c10	MWU	0.026	**d5**	ANOVA	0.049
c13	ANOVA	0.025	**d36**	MWU	0.049
	ANOVA	0.028		ANOVA	0.016
c33	MWU	0.009			
	ANOVA	0.016			
d5	MWU	0.002			
	ANOVA	0.01			
d6	MWU	0.005			
	ANOVA	0.008			
d11	MWU	0.032			
	ANOVA	0.026			

Note*: MWU – Mann Whitney U test; ANOVA – Analysis of Variance.*

Multidimensional scaling (MDS) plots

Multidimensional scaling (MDS) plots using only the variables (harmonics) showing differences in univariate analysis for each otolith, was created, and is portrayed in Figure 3.17. For both IMM and MAT sample sets, MDS plot showed only restricted overlap between individuals from the two coasts indicating a relatively reasonable division between the two putative stocks.

Principal Component Analysis

Figure 3.17. MDS plots portraying significant harmonics shown by univariate analysis for *Genypterus capensis* samples from the western (WC) and southern (SC) putative stock , for the years 2011, and 2014 -2016. **(a)** Immature samples **(b)** Mature samples.

As shown in Table 3.15, when using IMM variables, the first two PCs accounted for 51.8% of the variation (PC1 = 30.5%; PC2 = 21.3%). PC1 showed a strong correlation to (> 0.5) harmonic b2 and c2, while PC2 was driven by a3. PC3 described 13.1% of the total variation, with a strong drive from harmonic d2. In PC4, describing 6.7% of the variation, harmonic a2 and b2 were the strongest drivers. The PCA plot (Figure 3.18a) showed only partial overlap between individuals from the two coasts indicating a modest division between the two putative stocks.

For MAT variables, the first two PCs accounted for 46.1% of the variation (PC1 = 34.7%; PC2 = 11.3%). However, PC2 did not show a strong

correlation to any of the variables. PC3 accounted for 11.1% of the total variation, and combined with PC1, described 45.9% of the total variation. PC1 showed a strong correlation to harmonic a2 while PC3 was driven by c2 and a2. These results are shown in Table 3.15. In PC4, describing 7.8% of the variation, harmonic b2 was the strongest driver. The PCA plot showed extensive overlap between individuals from the two coasts resulting in a negligible distinction between the two putative stocks (Figure 3.18b).

Table 3.15. Correlation between Principal Components and original variables (harmonics) explained for *Genypterus capensis* samples from the putative western and southern stocks, for the years 2011, and 2014 -2016.

Variable	Immature				Mature			
	PC1	**PC2**	**PC3**	**PC4**	**PC1**	**PC2**	**PC3**	**PC4**
a2	-0.376	0.321	0.231	**0.541**	**-0.501**	-0.159	0.076	0.148
a3	-0.093	**0.502**	-0.200	-0.207	-0.129	0.317	0.168	-0.302
a4	-0.168	0.002	-0.008	0.146	-0.199	-0.231	-0.097	0.041
a5	0.039	0.187	-0.156	0.006	-0.003	0.043	0.042	-0.146
a6	-0.056	-0.075	0.000	0.082	-0.049	-0.183	-0.063	0.023
a7	0.041	0.011	-0.053	-0.003	0.074	-0.002	0.069	-0.103
b2	**0.551**	0.162	-0.291	**0.646**	**0.536**	0.218	-0.034	0.280
b3	0.070	-0.431	0.196	0.204	0.175	-0.447	-0.156	0.143
b4	0.123	-0.237	-0.231	-0.036	0.343	0.032	-0.05	0.114
b5	0.030	-0.297	0.075	0.075	0.152	-0.296	-0.028	0.121
b6	0.058	-0.131	-0.111	-0.079	0.170	0.032	0.061	0.127
b7	0.048	-0.107	0.101	0.061	0.051	-0.067	0.054	0.108
c2	**-0.628**	-0.285	-0.402	0.240	-0.287	0.437	**-0.612**	0.458
c3	-0.113	0.235	-0.279	0.075	-0.128	0.131	0.140	-0.181
c4	-0.209	-0.027	-0.098	-0.043	-0.191	0.081	0.046	0.131
c5	-0.066	0.110	-0.129	-0.082	-0.029	0.201	0.050	0.006
c6	-0.015	0.140	0.191	0.002	-0.084	-0.022	0.102	0.078
c7	-0.001	0.089	-0.003	-0.018	-0.039	0.154	0.033	-0.045
d2	-0.138	0.107	**0.517**	0.087	-0.148	-0.025	**0.650**	**0.557**
d3	0.021	0.128	0.150	-0.224	0.150	0.366	0.269	0.062
d4	0.001	0.034	0.237	0.109	0.022	-0.005	0.021	0.313
d5	-0.030	-0.003	-0.154	-0.130	-0.024	0.072	-0.018	-0.038
d6	-0.054	-0.039	0.020	-0.047	-0.070	-0.152	-0.062	0.071
d7	0.084	0.110	0.043	0.015	-0.014	-0.031	0.029	-0.110

Note: *Variables shown to have a strong (> 0.5) correlation are highlighted in bold.*

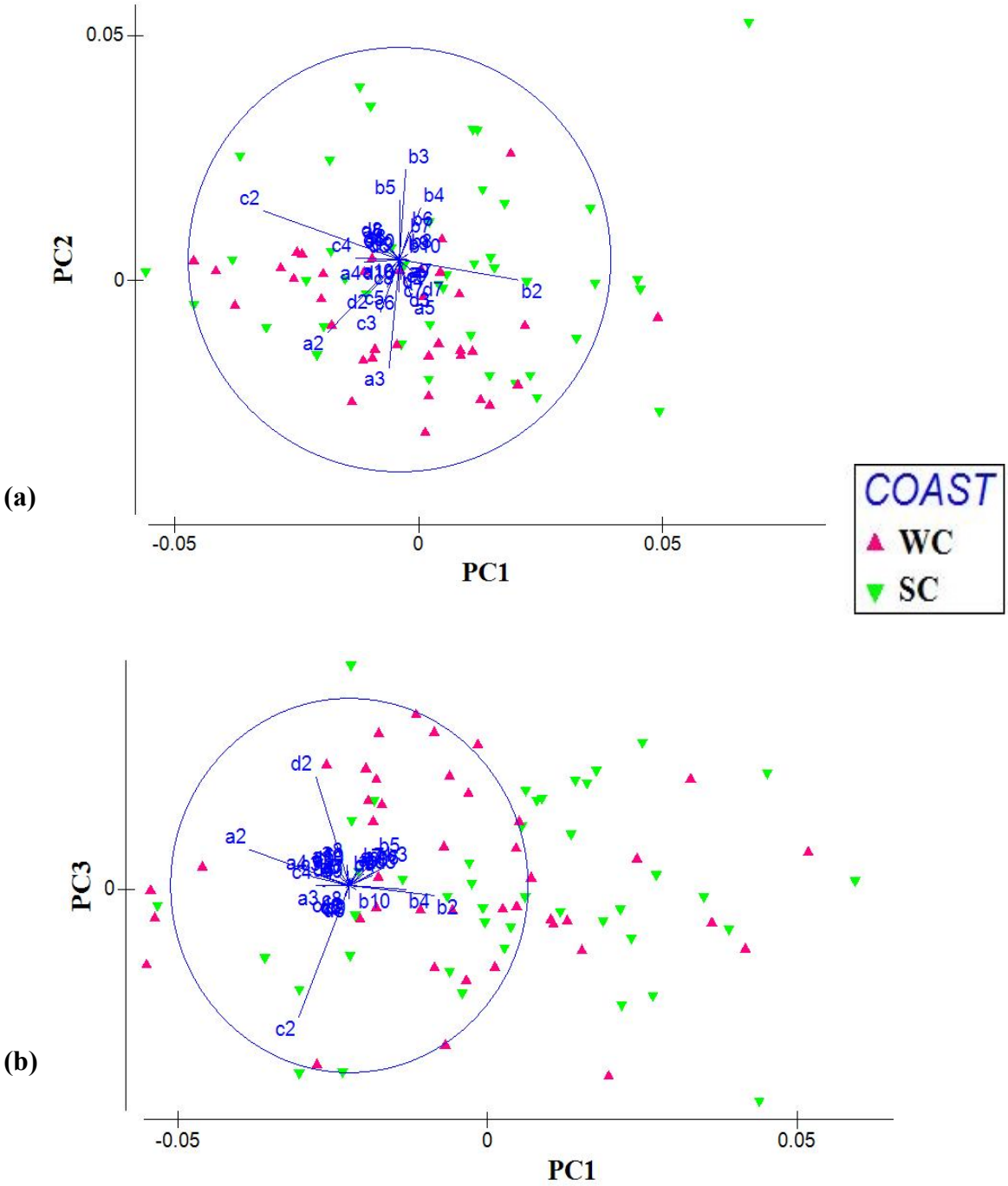
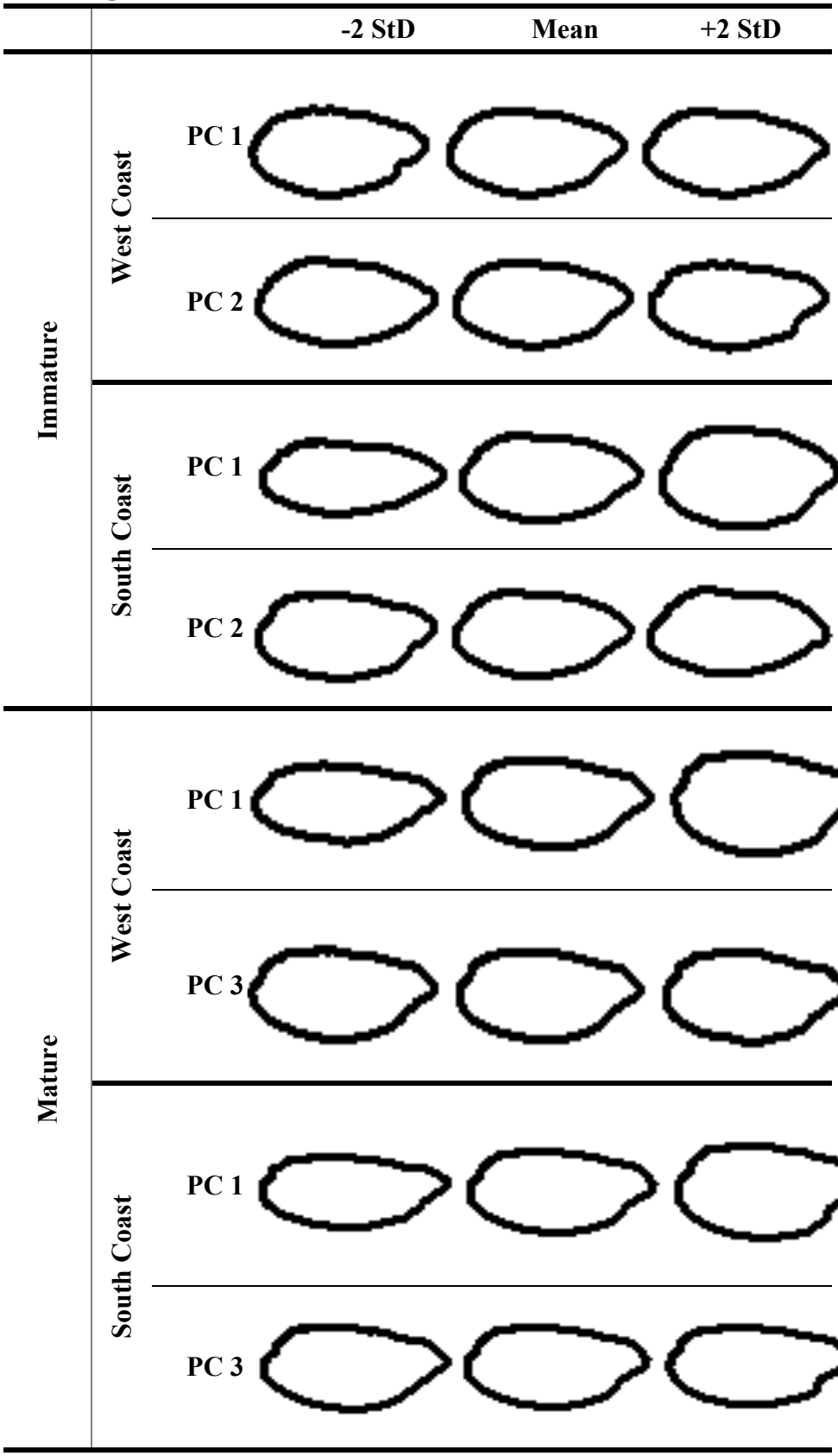


Figure 3.18. PCA plots using harmonics of Elliptical Fourier Analysis for **(a)** immature and **(b)** mature *Genypterus capensis* otoliths from the putative west (WC) and south (SC) stocks, for the years 2011, and 2014 -2016.

Linear outlines of the: Mean (± 2 standard deviation), of otolith shapes for PC1 and PC3 of IMM samples and of the PC1 and PC2 of MAT samples are shown in Table 3.16.

Table 3.16. Mean ± 2 standard deviation of otolith shapes for principal components (PC) using immature and mature *Genypterus capensis* samples from the putative west and south coast stocks.



As can be seen in Table 3.17, for IMM specimens, the first ten harmonics, results showed an overall classification rate of over 65%, with BT analysis showing the highest rate at 85.54%. When using only the variables showing differences in univariate analysis, results for all analysis showed an overall classification accuracy of over 60%, with ANN showing the highest classification rate at 94.10%.For MAT specimens, the first ten harmonics, results showed an overall classification rate of over fifty percent, with BT analysis showing the highest rate at 86.21%. When using only the variables showing differences in univariate analysis, results for all analysis showed an overall classification accuracy of over seventy percent, with ANN showing the highest classification rate at 76.90%.

Table 3.17. Model selection of variables from *Genypterus capensis* from the putative western and southern stocks off the South African coast, for the years 2011, 2014 -2016.

Sample set	Test	Predictive performance				
		(%)			Accuracy	
		OVERALL	WC	SC	Training	Validation
Immature	**Harmonics: a2-a10,b2-b10,c2-c10,d2 -d10**					
	BT	**85.54**	84.62	86.36	0.885	**0.777**
	RF	84.34	84.09	84.62	0.869	0.722
	LDA	65.10	66.70	63.60	0.928	0.651
	ANN	71.40	42.90	57.90	0.823	0.714
	Harmonics: a3, b3, c3, c10, c13, c33, c4, c5, d11, d5, d6 (*as indicated by univariate analysis*)					
	BT	83.13	82.05	84.09	0.885	0.681
	RF	90.72	76.92	84.09	0.836	0.750
	LDA	68.70	71.80	65.90	0.771	0.687
	ANN	**94.10**	93.80	94.10	0.939	**0.941**
Mature	**Harmonics: a2-a10,b2-b10,c2-c10,d2 -d10**					
	BT	**86.21**	81.82	90.70	0.935	**0.682**
	RF	70.11	43.18	96.67	0.707	0.680
	LDA	59.00	51.30	65.90	0.855	0.590
	ANN	51.60	34.60	74.10	0.482	0.484
	Harmonics: a13, a28, a30, a31, a33, d3, d5, d36 (*as indicated by univariate analysis*)					
	BT	74.71	77.27	72.09	0.790	0.640
	RF	70.11	63.64	76.74	0.741	0.620
	LDA	73.60	74.40	68.20	0.736	0.713
	ANN	**76.90**	80.80	57.40	0.885	**0.769**

Note: *RF – Random Forest Analysis; BT – Boosted Tree Analysis; ANN – Artificial Neural Network; LDA – Linear Discriminant Analysis; WC – west putative stock; SC – south putative stock.*

3.4. DISCUSSION

The examination of the sagittal otolith shape and size has become a valuable tool for stock discrimination and species identification (He *et al.,* 2017; Ladroit *et al.,* 2017). However, the identification of specific factors leading to stock discrimination is difficult since shape analysis cannot specify whether variations are caused by the environment, genetics, or a combination thereof (Campana and Casselman, 1993; Lombarte and Lleonart, 1993; Torres *et al.,* 2000; Begg *et al.,* 2001; Cardinale *et al.* 2004; Renán *et al.,* 2016). According to various theories (Reznick *et al.,* 1989; Secor and Dean, 1989; Campana and Casselman, 1993; Cardinale *et al.*, 2004; Vignon and Morat, 2010; Renán *et al.,* 2016), environmental variations can change the otolith's growth rates, whilst genetic differences influences the form of the otolith.

3.4.1. The effect of sex, and right *vs* left otolith

Variations in otoliths are also found in individual fish of the same species and may be influenced by sex, age, year class, stock (Castonguay *et al.,* 1991; Begg and Brown, 2000; Monteiro *et al.,* 2005; Vignon and Morat, 2010), and environmental conditions like depth, water temperature and substrate type (Lombarte and Lleonart, 1993; Gagliano and McCormick, 2004; Mérigot *et al.,* 2007; Hüssy, 2008; Vignon and Morat, 2010).

Differences between the side of the head in which otoliths are extracted, has been identified as potential factor influencing otolith shape (Mille *et al.,* 2015). In this study, no significant differences were found between right and left otoliths of subsample of *Genypterus capensis* (Table 3.2).

Another influencing factor for otolith shape and length, is the sex of the fish (Mille *et al.,* 2015). Sexual dimorphism in otoliths are likely to result from differences in growth rate, physiology, and metabolism (Campana and Casselman, 1993; Begg and Brown, 2000; Simoneau *et al.,* 2000; Cardinale *et al.,* 2004; Mille *et al.,* 2015). For *G. capensis,* the drumming muscles in males are closely related to the olfactory properties of the otolith (Japp, 1989) and may enlarge and change the shape of the otolith at maturity (Japp, 1990).

In this study however, no significant differences were found between male and female otoliths (Table 3.3).

The impact of sex and variations between the right and left otoliths were disregarded since statistical analysis showed that there were no differences.

3.4.2. Otolith weight (OW) relative to otolith length (OL)

A linear growth in the otolith's weight infers an increase in fish age (Dehghani *et al.,* 2015). Research by Santana *et al.* (2018) indicated that OL and OW were sufficient measurements to differentiate *Prochilodus lineatus* sp. from three different locations (Paraná, Baía and Ivinhema rivers in Brazil). These characteristics explain differences in growth rates because of both environmental and anthropogenic pressures.

In the current study, the chemical elemental ratios and compositions contributing to the weight of *Genypterus capensis* otoliths is discussed comprehensively in Chapter 4. The otolith-weight-length relationship between the two stocks did not differ significantly in terms of otolith shape, as demonstrated by a t-test on linear regression trendlines (Figure 3.7) and an exponential curve visual comparison. As a result, this method proved insufficient for stock delineation..

3.4.3. Allometric relationships using otolith shape parameters

Allometric relationships refer to morphoanatomical and/or physiological occurring simultaneously with changes in body size. These changes may the compensatory effect required to maintain productivity in bodily functions. In fish species, the onset of maturity is one of the key triggers causing changes in growth patterns as the body energy is reallocated towards reproduction instead of growth (Medeiros *et al.,* 2021). In the current study, all the *Genypterus capensis* otolith shape parameters showed a strong positive relationship with the length of the fish (Table 3.4).

A linear increase in OL, and OB, develops with an increase in fish size (Dehghani *et al.,* 2015). Gauldie and Nelson (1990) reported that slow-growing fish (like *G. capensis*) produced short, compact crystals compared to

the longer, thinner ones found in fast-growing species, and this likely influences the overall shape of the developing otolith (Vignon and Morat, 2010).

Otolith dimensions: Stock differentiation

Regarding the overall mean ratios of TL:OL, and TL:OB, a student's t-test indicated significant differences for linear regression equations between the two putative stocks. The graphs (Figure 3.9) indicate that these differences occur at approximately TL = 60 cm, the length at which this species reaches maturity. From this it can be deducted that otolith growth occurs at different rates between the two stocks, with MAT SC specimens growing at a slower rate than MAT WC samples. This contrasts with results from Payne (1985) which indicated that SC samples grew faster compared to other areas in southern Africa. However, Payne (1985) also noted that the growth rate of the Cape Stock (synonymous with WC stock in this study) was increasing compared to prior studies, i.e.Wrzesinski (1975), Isarev (1976), Payne (1977), which was a likely result of overexploitation in the 1980s specifically on the West Coast. No significant differences for the OL:OB ($p = 0.525$) relationship was found between samples from the two coasts, indicating that in growth in this regard occurs at constant rate for the two putative stocks.

Otolith dimensions: Current study vs Payne (1977, 1985)

In this study, linear trendlines (Figure 3.9) showed that the TL:OL ratio increases positively with TL from ≈30:1 to ≈65:1, for young *vs* older *G. capensis*, which is similar to results from Payne (1977) in which the increase was 30:1 to 60:1. Payne (1977) also found that the TL:OB ratio relative to fish length (TL) increases from 80:1 to 100:1 as the fish ages and increases in length. Linear trendlines in this study showed positive relationship with TL, and supported a wider range compared to that of Payne (1985) with the TL:OB ratio increasing from 70:1 to 130:1, as the fish grew. Payne (1977) reported that the TL:OL relationship of *G. capensis* is relatively high compared to that of other fish species e.g., hake, *Merluccius capensis and M. paradoxus* (TL:OL = 25:1; Botha, 1971 in Payne 1977). A faster growth along

the OL (anterior-posterior) axis compared to along the OB (dorsal-ventral) axis results in otoliths becoming increasingly elongated as individuals mature (Galley *et al.,* 2006). As a result, otolith growth patterns show a negative allometric relationship regarding their length to width ratios (Simoneau *et al.,* 2000; Mille *et al.,* 2015). Payne (1977) found that the OL:OB ratio decreased with fish size, from 2.4:1 for otoliths from younger fish to 1.6:1 for otoliths of older *G. capensis* , and from this study showed OL:OB decreasing from 2.4:1 to 1.9:1, with increasing growth.

The WC stock in this study is synonymous to the Cape Stock in Payne (1985), whilst the SC stock is the same as his South-East stock. Significant differences for the OL:OB *vs* TL relation was found when a student's t-test was applied to regression curves comparing the two studies (Figure 3.10). Reasons for this is unknown, but may be because the TL-range in the current study was wider than that of Payne (1985). Another possibility is that these differences are a result of the resource being exploited in the 1980's. As explained in Section 1.2.3, Guerra (2019) indicated a reduction for females' age at 50% maturity when compared to Japp (1989), with the possibility that change may be a result of overexploitation of the resource via longline fishing in the 1980's. Research by Hollins *et al.* (2018) noted that the phenotypic composition of exploited fish stocks change, especially regarding life histories and reproduction. Fish physiology may also be influenced by environmental conditions (Dettleff, 2020).

3.4.4. Otolith shape indices

There is a clear correlation between somatic growth and otolith growth (Francis, 1990), even though the variables influencing otolith form not well understood (Burke *et al.,* 2008). The previous section explored the shape morphometric parameters that characterise otolith form as a size multivariate that has a strong association to fish dimensions. Shape indices are geometric variables that describe geometric complexity regardless of size (Cadrin and Friedland, 1999; Renan *et al.*, 2016).

Otolith growth patterns in inactive fish, such as *Genypterus capensis*, are less elongated and rectangular than those in active fish (Volpedo *et al.,* 2008; Tuset *et al.,* 2015; Madeiros, 2021). Moreover, otoliths from older fish are seen to be less elongate in terms of TL than those from younger fish (Payne, 1977). Differences in fish metabolism can also lead to variations in otolith size, with slower-growing fish typically having larger otoliths than fish growing quickly for the same size. Section 1.2.5 discusses the *G. capensis* life cycle, which increases in size with depth. In addition to the changes in the fishes reproductive biology, a change in habitat type can also have a direct influence on the shape of otoliths (Medeiros *et al.,* 2021).

In both the IMM (all variables except RECT) and MAT (all variables except CIRC and FF) sample sets, univariate analysis demonstrates a distinct differences between the two putative stocks.

For IMM samples, [CIRC + FF + ELL + AR] was found to be the best combination for stock discrimination using Boosted Analysis. In MAT samples, [RECT + ROUND + ELL + AR] was shown to best combination for stock discrimination using Linear Discriminant Analysis. These conclusions were further strengthened by the MDS (Figure 3.13) and PCA (Figure 3.14) plots, which, despite some overlap, demonstrated a distinct difference between the putative stocks.

A summary of the results for univariate, multivariate, and PCA analysis of log-

transformed shape indices otoliths used in this study is presented in Table 3.18.

Table 3.18. :Summary of results for univariate, multivariate and PCA analysis of log-transformed shape indices otoliths used in this study of *Genypterus capensis* from the putative western (WC) and southern (SC) stocks off the coast of South Africa2008, 2009, 2011 and 2014 – 2017.

Fish size	Analysis	Log (VARIABLE)	CIRC	FF	RECT	ROUND	ELL	AR	Refer to
Immature	Univariate		✓	✓		✓	✓	✓	Table 3.7 and 3.8
	Multivariate		✓	✓			✓	✓	Table 3.9 and 3.10
	PCA		✓	✓			✓	✓	Tables 3.11 and 3.12
Mature	Univariate				✓	✓	✓	✓	Table 3.7 and 3.8
	Multivariate				✓	✓	✓	✓	Table 3.9 and 3.10
	PCA				✓	✓			Tables 3.11 and 3.12

Note: *CIRC – Circularity; FF – Form factor; RECT - Rectangularity; ROUND - Roundness; ELL – Ellipticity; AR – Aspect ratio; MAT – Mature; IMM – immature; ✓- significant difference found.*

3.4.5. Elliptical Fourier analysis

Fourier analysis can be an efficient method for illustrating outline shapes and revealing differences. It does however not provide insight into the reasons for subtle shape differences (Cadrin and Fried-land 1999; Tuset *et al.,* 2006; Vignon and Morat, 2010). Thus, whilst factors determining the shape of otoliths are not well understood (Burke *et al.,* 2008), it has been noted that the anterior border of the *Genypterus capensis* otolith is found to be bluntly rounded, with the posterior end being sharper. The radial lobes of the species otoliths contribute to the surface area having an irregular appearance around the periphery of the otolith and the lobes become less noticeable as the fish ages (Payne, 1977).

Based on the findings of the univariate analysis (Table 3.13), the IMM

samples displayed variations in eleven different harmonics (a3, b3, c3, c10, c13, c33, c4, c5, d11, d5, d6), whereas the MAT samples displayed variations in eight different harmonics (a13, a28, a30, a31, a33, d3, d5, d36). A classification success rate of over 80% for three analytic approaches (BT, RF, and an ANN) in IMM samples, and over 70% in MAT samples, is achieved when using a combination of these variables, as illustrated in Table 3.16. Visualisations portrayed by MDS (Figure 3.17) and PCA plots (Figure 3.18) show limited overlap between individuals from the two coasts of IMM samples indicating partial delineation. For MAT specimens, prevalent overlap was shown in MDS and PCA plots indicating no delineation between stocks.

According to these results, otoliths of immature *G. capensis* samples may demonstrate stock differentiation through the application of Elliptical Fourier Analysis, however, this distinction is not evident in mature samples.

3.4.6. Intraspecific comparison

Regarding intraspecific comparison, when otolith length, and otolith breadth of *Genypterus capensis*, were independently measured as a percentage of the fish length, results were comparable to those found by Rossi-Wongtschowski *et al.* (2016) for *Genypterus brasiliensis* (Table 3.19).

As illustrated in Table 3.20., a subsample from the current study (WC: TL = 75.491 ± 3.909 cm; SC: TL = 75.285 ± 4.268 cm) with similar means as the fish length in specimens of Ladroit *et al.* (2017) for *Genypterus blacodes* (Sub-Antarctic: TL = 75.8 cm; South Sub-Antarctic: TL = 75.8 cm; North Chatham Rise TL = 75.8 cm), was analysed and results were found to be similar.

Table 3.19. Mean (± SD) and range of otolith length as a percentage of total length, otolith breadth as a percentage of otolith length, circularity, and rectangularity, for specimens of *Genypterus capensis* from the putative western (WC) and southern (SC) stocks, South Africa, and *Genypterus brasiliensis* from Brazil (as per Rossi-Wongtschowski *et al.*, 2016).

Species	Coast	Shape indices	(%)		CIRC	RECT
			OL / TL	OB / OL		
Genypterus capensis	WC	**Mean ± SD**	2.25 ±0.26	46.97 ± 4.17	21.56 ± 1.71	0.74 ± 0.04
		Range	1.10 - 3.76	34.32 - 60.21	16.06 - 26.22	0.64 - 0.92
	SC	**Mean ± SD**	2.39 ± 0.96	47.92 ± 5.85	22.48 ± 1.95	0.74 ± 0.04
		Range	0.62 - 6.55	37.01 - 109.38	17.37 - 30.05	0.66 - 0.83
Genypterus brasiliensis	Brazil	**Mean ± SD**	2.93 ± 0.21	48.84 ±3.03	21.84 ± 1.33	0.71 ± 0.02
		Range	2.57 - 3.11	44.43 - 54	19.29 - 23.68	0.68 - 0.74

Note: *OL – Otolith length; TL – Total length; OB – Otolith Breadth; CIRC – circularity; RECT – rectangularity.*

Table 3.20. Otolith shape parameters and indices recorded for *Genypterus blacodes* (Ladroit *et al.* 2017) compared to that of *Genypterus capensis* (TL = ±75 cm) in this study

Species	***Genypterus blacodes***			***Genypterus capensis***	
Location	**New Zealand**			**South Africa**	
	Sub-Antarctic		**Chatham Rise**	**West Coast**	**South Coast**
	South	**North**			
Fish length (cm)	75.8	75.6	75.5	75.491 ± 3.909	75.285 ± 4.268
Otolith weight (g)	0.295	0.296	0.306	0.329 ± 0.068	0.331 ± 0.059
Otolith length (mm)	15.785	15.829	15.657	15.967 ± 1.423	15.75 ± 1.22
Otolith width (mm)	7.612	7.593	7.823	7.593 ± 0.083	7.701 ± 0.091
Surface area (mm²)	89.075	88.94	90.05	90.571 ± 1.611	92.073 ± 1.55
Perimeter (mm)	42.885	43.044	43.793	44.041 ± 4.200	44.478 ± 3.798
Circularity	0.0054	0.0054	0.005	21.645 ± 1.967	21.835 ± 3.019
Form-factor	0.6086	0.6032	0.59	0.585 ± 0.05	0.585 ± 0.075
Roundness	0.488	0.484	0.504	0.451 ± 0.039	0.472 ± 0.053
Ellipticity	0.349	0.352	0.334	0.355 ± 0.036	0.344 ± 0.041
Aspect ratio	2.058	2.076	1.992	2.114 ± 0.177	2.063 ± 0.200

Note*: For South African data, mean ± standard deviation values are listed*

3.5. CONCLUSION

There were no discernible variations between male and female otoliths or between left and right otoliths in this investigation and the *Genypterus capensis* stocks on the South African coastline could not be distinguished using the otolith-weight-length method.

When examining allometric growth patterns, the mean ratios of TL:OL, TL:OB, and OL:OB, in relation to TL were comparable to that of Payne (1977). Significant differences were observed between stocks in the association between TL/OL vs TL and TL/OB vs TL, but not in the

relationship between OL/OB vs TL.

For otolith shape analysis, of the six shape indices tested, four (rectangularity, roundness, ellipticity, and aspect ratio) of the variables in mature specimens, showed significant differences between the putative stocks when using univariate and multivariate analysis. In immature samples, circularity, form factor, ellipticity, and aspect ratio, indicated significant differences when using multivariate analysis, and additionally roundness when using univariate analysis. Elliptical Fourier Analysis was applied utilising 36 harmonics, of which nine (a3, b3, c3, c4, c5, c10, c13, c33, and d5) in immature samples indicated significant differences in the stock structure between regions. However, in mature specimens, a clear distinction in mature specimens could not be determined using this technique.

In the study on *G. blacodes* La Droit *et al,* (2017) acknowledged that whilst otolith shape analysis may not be the 'silver bullet' for fish stock delineation, it is a very advantageous technique, and these results are echoed in this study. For the current analysis, when using otolith shape analysis, it may be concluded that *G. capensis* stocks demonstrate a higher differentiation in immature samples when compared to mature samples, which is indicative of the species' bentho-pelagic life cycle (See section 1.2.5).

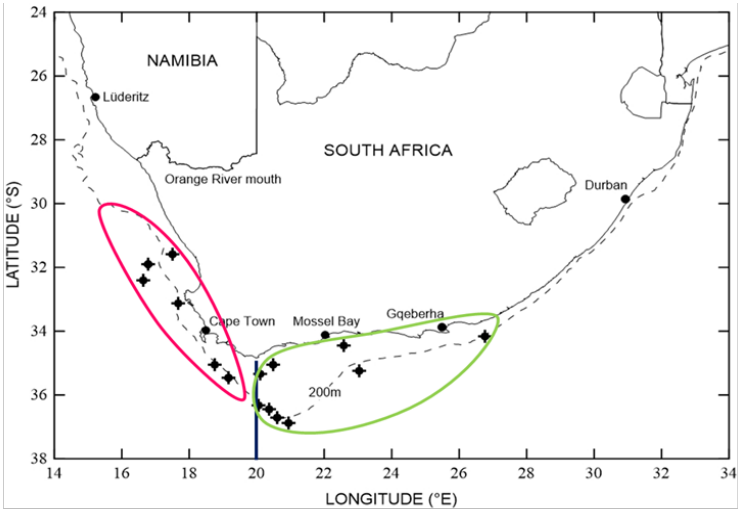
Chapter 4

Otolith Elemental Composition

"It is the sum of many parts which grow to be something great."

Oliver Bierhoff

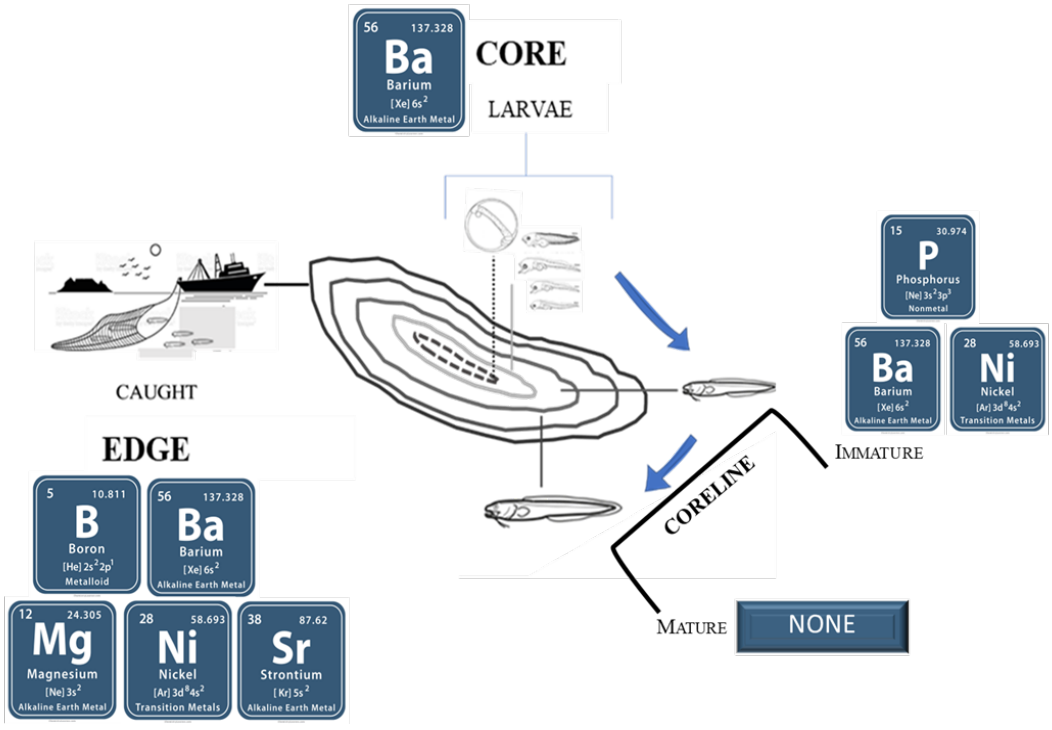
GRAPHICAL ABSTRACT



WC — *West coast putative stock*

SC — *South coast putative stock*

Elements showing significant differences between putative stocks at different sections of otolith



4.1. INTRODUCTION

As discussed in Chapter 3, the otoliths' primary function is to aid in hearing and balance of the fish (Tanner *et al.,* 2015). However, fish otoliths, which are commonly referred to as ear stones, are also known to be timekeepers (Campana, 1999). Pannela (1971, 1974, 1980) suggested that the otoliths of adult fish are composed of daily increments. A study by Morales-Nin (1987) indicated that, as with most teleost fish, the sagittal structure in *Genypterus capensis* otoliths displayed a periodic pattern of formation. The differences in otolith microchemical elemental composition amongst different groups of fish, has facilitated the use of otoliths as natural tags in stock discrimination studies (Tanner *et al.,* 2015).

4.1.1. Growth increments

Daily growth increments are formed around a larval nucleus (core). These circadian increments are bipartite with continuous zones emerging as ridges, and discontinuous zones as grooves (Campana and Nielson, 1985; Morales-Nin, 1987). The continuous incremental zone is chiefly composed of aragonite microcrystals (and on occasion vaterite) and appears wide and translucent. A second band appears to be a relatively narrow and opaque matrix of the protein otolin and forms a discontinuous unit of which organic material (characterised by a high abundance of acidic amino acids) is found to be the main constituent (Watabe *et al.,* 1982; Campana and Nielson, 1985; Morales-Nin, 1987). These increments can stipulate information for daily age determination and serve as markers for life history events e.g., hatching, settlement and metamorphosis (Lin *et al.,* 2012). Scanning electron microscope (SEM) photographs of growth rings are shown in Figure 4.1a, and the nuclei and primordial region, is shown in Figure 4.1b.

4.1.2. Otolith's incorporation of chemical elements

Otolith elemental composition is a result of the combined effects of external factors and endogenous processes (Morales-Nin, 2005). Abiotic influences include the temperature and elemental composition of seawater, and biotic factors include somatic growth and the ontogenetic stage (Campana, 2005;

Baumann *et al.,* 2015; Tanner *et al.,* 2015). Since newly deposited material is neither reworked nor reabsorbed after deposition, even during times of stress, the otolith is deemed as metabolically inert (Campana, 2005). Research by Morales-Nin (1987) noted that the main constituents of *G. capensis* otoliths were calcite (0,55 %) and a high proportion of protein (0,40 %).

The incorporation of trace elements into the calcium carbonate ($CaCo_3$) matrix (Figure 4.1c) is, at least in part, a function of crystallisation rate (Lowenstam and Wiener, 1989) and may take place by the following methods:

1. Substitution: cations which have a similar size and charge can replace one another (e.g., Sr can substitute Ca).
2. Co-precipitation: a solid is precipitated from a solution containing other ions, as may be the case with Mg, Li, and Ba.
3. Absorption: elements such as Na, Cl, Zn and K can be absorbed onto the crystal surface in the interstitial space (Morales-Nin *et al.,* 2005; Campana, 1999).

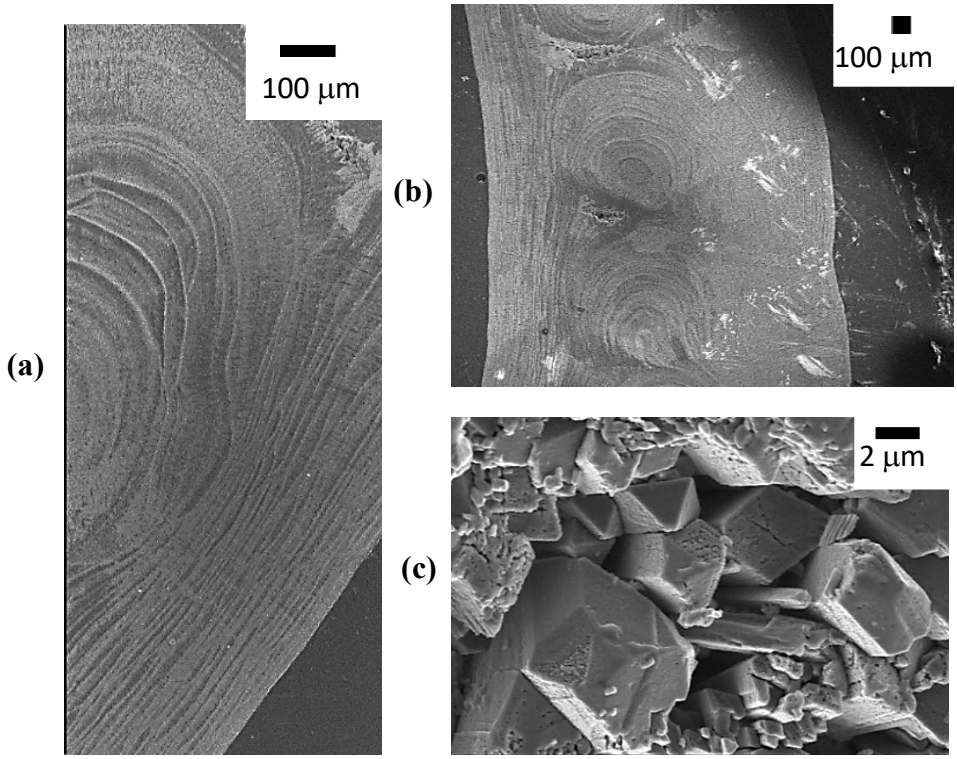


Figure 4.1. Scanning Electron Microscope (SEM) photographs showing the **(a)** growth rings **(b)** nuclei and primordial region **(c)** incorporation of elements in the otolith of *Genypterus capensis* [images courtesy of Riana Rossouw, Stellenbosch University]

Elements under weak internal controls are the most valuable in assessing fish location because they vary according to both ambient and physiological conditions (Mercier *et al.,* 2011). It is the trace elements (Concentrations < 0.1%) that have proven to serve as effective environmental markers (Campana *et al.,* 1997).

4.1.3. Otolith elemental composition for stock structure

Incremental growth accretions occur throughout the life of a fish (Ludsin *et al.,* 2006) and have been documented for both freshwater and marine taxa (Morales-Nin, 1987). This rhythmic incremental deposition could thus mark life events (Campana and Nielson, 1985; Morales-Nin, 1987) and a suite of valuable oceanographic and biological information can be derived from otoliths (Baumann *et al.,* 2015). The differences in otolith chemical composition amongst different groups of fish has facilitated the use of otoliths as natural tags in stock discrimination studies (Tanner *et al.,* 2015). The success of this application may be attributed to different geographic locations having different hydrographic characteristics (Campana *et al.,* 2005; Tanner *et al.,* 2015). It is however unknown whether variations in elemental composition of otoliths resulting from environmental exposure correspond to genetic differences in hosts (Campana, 1999).

Whilst the otolith shape (as discussed in Chapter 3) has a chiefly genetic basis that is vulnerable to local environments, otolith margin chemistry characterizes the current material, with results implying relatively recent geographic separation or mixing (Sturrock, 2012). The incorporation of any given element/ion from the environment into the otolith, follows a complicated multi-stage process with each stage separated by distinctively independent barriers. In view of that, specific elemental concentration, may at certain times reflect the same environmental condition at different points along the otolith transect.

4.1.4. Otolith microchemical analysis

As stated by Campana (2005), when using otolith microchemical analysis, the hypothesis being tested determines:

(i) the type of technique used,

(ii) the type of otolith used, and

(iii) the use of whole otoliths or only a portion thereof

A wide range of techniques are available as represented in Table 4.1. LA-ICPMS, is a beam-based variant of ICPMS which has the added advantage of targeting only a particular time scale of interest in the sectioned otolith (Campana, 2005a). In the current study, LA-ICPMS was conducted on *Genypterus capensis* sagittae otoliths as life cycle reconstruction, and variations between stocks at each life stage, was tested. Another advantage of this method is that it is qualitative to fully quantitative, and capable of analysing a range of major, minor, and trace elements (Abduriyim and Kitawaki, 2006).There are however disadvantages to this approach. To expose the growth sequence, the otolith must be sectioned. This procedure increases the risk of contamination and the possibility that some degree of beam penetration may infiltrate into underlying growth layers (Campana, 2005a). Even though LA-ICPMS (in addition to electron microprobe, proton induced X-ray emission) can deliver chemical signatures for various elements on a finer scale than whole otolith solution-based analysis, these methods are not feasible for classifying some trace elements e.g., some rare-earth elements (Smith and Jones, 2006).

Table 4.1. Techniques available for otolith microchemistry (Campana, 2005a)

Abbreviation	Technique
AAS	Atomic Absorption Spectrometry
ED-EM	Energy-Dispersive Electron Microprobes
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICPMS	Inductively Coupled Plasma Mass Spectrometry
ID-ICPMS	Isotope Dilution Inductively Coupled Plasma Mass Spectrometry
LA-ICPMS	Laser Ablation Inductively Coupled Plasma Mass Spectrometry
NAA	Neuron Activation Analysis
PIXE	Proton Induced X-Ray Emission
WD-EM	Wavelength Dispersive Electron Microprobes

Regarding the type of otolith used, it should be noted that the sagittae is not equal to lapilli for solution-based techniques. However, for elements that are abundant enough to be measured by LA-ICPMS, signatures of individual growth bands are likely to be similar for sagittae and lapilli otoliths (Smith and Jones, 2006). Whilst whole otolith analysis can be used as a natural tag, the examination of just the early life history is generally envisaged to be sufficient regarding stock identification studies (Campana, 1999).

4.1.4. The purpose of this study

Aim

The aim of this chapter is to assess the discriminating capability of the otolith elemental composition as a means of establishing stock delineation of *Genypterus capensis*. In this analysis, the hypothesis that discrepancies in the connectivity of populations brought about by oceanic borders correspond to distinct elemental fingerprints obtained from Laser Ablation Inductively Coupled Plasma Mass Spectrometry along the otolith transect, which in turn correspond to variances in the fish life cycle.

Objectives

1. Von Bertalanffy equations will be used to establish the age of the fish from which the otolith was extracted, as age and life history events may have an impact on element uptake and incorporation.
2. As part of the preliminary analysis, the number of ablated shots required to produce a reliable representative view of elemental concentrations will be determined.
3. Data obtained from Laser Ablation Inductively Coupled Plasma Mass Spectrometry will be separated using variables/elements for each section of the otolith. This will facilitate comparative analysis of life stages, as well as a comparative analysis of the two putative stocks.
4. For reconstruction of the life cycle of *Genypterus capensis* samples used in this study, an Independent-Samples Kruskal-Wallis Test will be executed and subsequently, pairwise comparisons of variables showing significant differences will be employed. Furthermore, 3D graphs and PCA will be performed to illustrate differences.
5. The elements exhibiting significant differences between the two potential stocks will be computed using non-parametric tests, and classification models utilizing a stepwise selection process will be employed to identify the best combination of variables for stock delineation. Thereafter, MDS and PCA will be computed to illustrate the variances.

4.2. MATERIALS AND METHODS

4.2.1. Sample locations

All specimens of *Genypterus capensis* used in this method of study, were collected during research surveys conducted by the Department of Forestry, Fisheries, and the Environment (DFFE) over a period of four years: 2014 – 2017 (as described in Section 2.2.1). Figure 4.2 illustrates the position of the otolith specimens from which the host fish was captured for otolith microchemistry investigation.

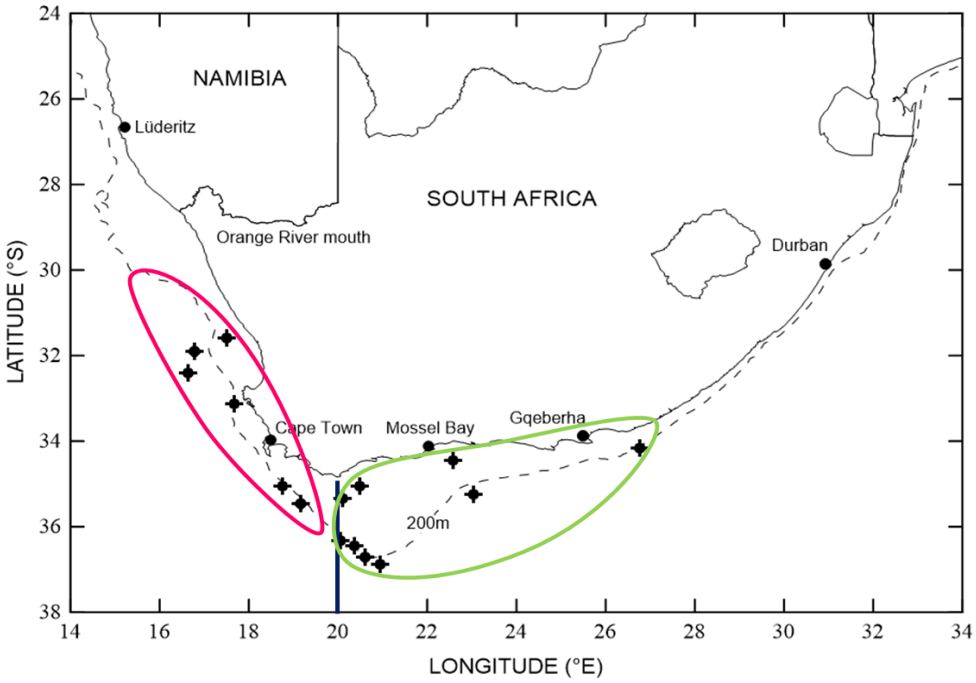


Figure 4.2. Locations where *Genypterus capensis* samples were collected during research surveys conducted by the Department of Forestry, Fisheries, and the Environment (DFFE) from 2014 to 2017. The 200m depth isobath is shown with the black dashed line. West coast (WC) samples are outlined in pink, and South coast (SC) samples are outlined in green. The boundary at Cape Agulhas (34.8163° S, 20.0152° E) is shown with a solid black line.

The sagittae otoliths had already been removed, cleaned, dried, and used for shape analysis before being preserved in vials that were labelled with the specimen number (as described in Section 3.2).

4.2.2. Determining the age of the otolith

Fish show consistent changes in otolith chemistry throughout their life history in terms of both age and reproductive cycles (Tanner *et al.,* 2012). Physiological changes which occur because of age and growth, as well as otolith precipitation rates resulting from age, and life history events (e.g., reproduction, gonad development), could influence element uptake and incorporation (Sturrok *et al.,* 2012). Tanner *et al.* (2012) recommended targeting individuals from the same year class.

Compared to the other approaches used in this dissertation, LA-ICPMS is a relatively expensive technique. Only a subset of samples could therefore be used for this analysis. However, prior to selecting the sample set, the age of

the fish from which the otoliths were obtained needed to be determined. This is to ensure a comprehensive output of data for analysis and so that otoliths from each stock could be paired to achieve a relatively similar spread. The procedure often employed when using otoliths for ageing is the bake-and-embed method (Hanchet and Uozumi, 1996) after which the otolith is cross sectioned and saw-cut (e.g., Japp 1990; Horn, 2021). For the current study, this method was not an option since the otolith would be used for LA-ICPMS which is an invasive method (the other otolith was kept in archive). Statistical analysis was therefor used for age-determination of the fish/otolith.

A study by Japp (1990) made use of whole and sectioned otoliths to determine the age of *Genypterus capensis,* and the three parameter Von Bertalanffy model to describe and compare growth was reviewed. The Von Bertalanffy equations describing the combined (male and female) growth was used to determine the otolith age in this study. Separate equations were given for each coast:

$$\mathbf{WC : L_{(t)} = 1291{,}5(1 - e^{-0.1414(t+0.32)})}$$

$$\mathbf{SC : L_{(t)} = 1360.2(1 - e^{-0.1423(t-0.22)})}$$

Von Bertalanffy equations describing the combined (male and female) growth on the WC and SC, where L(t) = total length at age t, L∞ refers to the theoretical maximum size the fish would reach if it were to grow indefinitely in the manner described by the model, K is the intrinsic rate of decrease in growth and t0 is the age when L(t) =0.

An exponential graph was created and used as a guideline to determine the approximate age of the fish based on its TL (Figure 4.3). For *G. capensis,* the length at maturity is reached at TL ≈ 60 cm (See Section 1.2.3). From the graph it can be determined that a TL of approximately 60 cm (WC: TL = 59.04 cm; SC: TL = 56.59 cm) correlates with an age of four years. As discussed in Section 1.2.5.3, different spatial distributions have been noted for *G. capensis* aged 0 – 3 years (IMM - immature) , compared to those aged 4 years and older (MAT – mature) and this fact was considered when

interpreting coreline data.

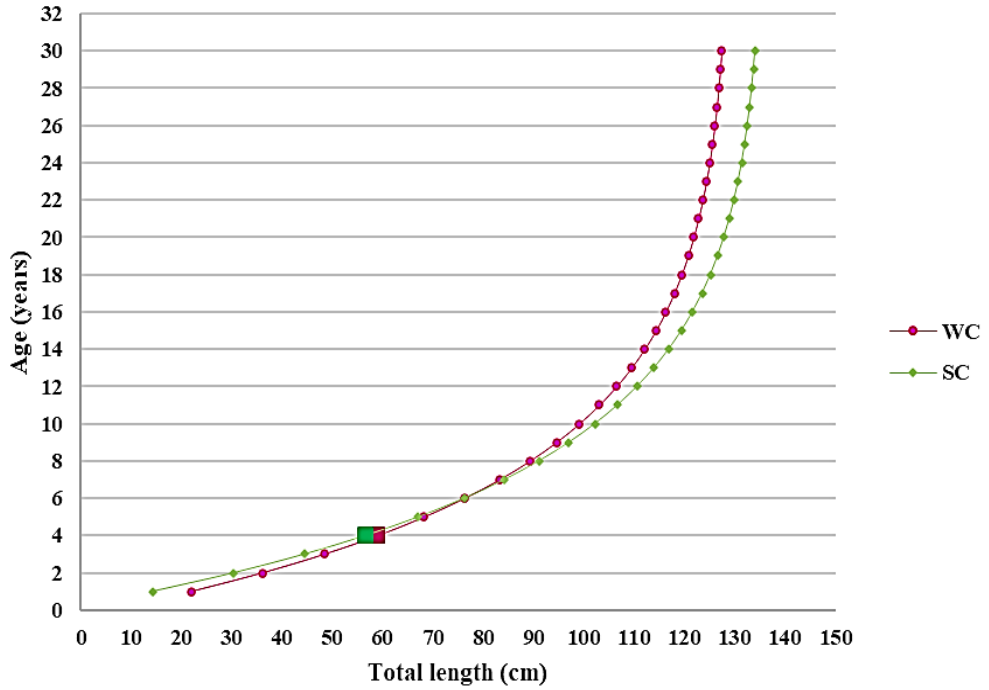


Figure 4.3. Exponential linear graph showing approximate age of *Genypterus capensis*, with corresponding maximum total length (TL in cm) of specimens from the putative west coast (WC) and south coast (SC) stocks for the years 2014 - 2016. **Note:** data calculated from Von Bertalanffy equations by Japp (1990).

To cover a comparable range for each coast, otoliths were paired by selecting an otolith from each putative stock, with similar ages. In total, 25 five otolith pairs (n = 50; WC: n =25; SC: n= 25) were selected, and details are listed in Table 4.2. The otoliths were transported to the University of Stellenbosch Geology Department where a Resolution M-50-LR Excimer laser ablation system is connected to an Agilent 7700 ICP-MS for trace element analysis.

4.2.3. Laser Ablation Inductively Coupled Plasma Mass Spectrometry

Otoliths were placed ventral side up on the perimeter of a thermoplastic resin ring. The rings were filled with a resin-base comprised of Specifix-40 curing agent and Specifix resin (Figure 4.4 a – d). These were placed in an oven until the otoliths were embedded in the resin base (Figure 4.6 e and f), and then mounted and polished (Figure 4.4. g and h). This was done to ensure that the

surface area was smooth and levelled, and to expose the core of the otolith (Figure 4.4 i). Otoliths were then placed into the laser ablation inductively coupled plasma mass spectrometer to be ablated (Figure 4.4. j - l). This system is made up of a resolution M-50-LR Excimer laser ablation system that is connected to an Agilent 7700 ICP-MS. Glass was treated as a reference for quantification (NIST - National Institute of Standards and Technology standards), using standard bracketing.

Table 4.2. The year, otolith number, total length, and approximate age of fish from which paired otoliths were obtained from *Genypterus capensis* specimens caught off the putative west (WC) and south coast (SC).

Year	Otolith number		Total length (mm)		Approximate age (years)		Pair
	SC	**WC**	**SC**	**WC**	**SC**	**WC**	
2014	52	8	566	566	4	3	A
	56	81	632	633	4	4	B
	48	3	652	654	4	4	C
	55	137	682	682	5	4	D
	53	39	700	700	5	5	E
	51	59	729	726	5	5	F
	65	10	737	738	5	5	G
	49	105	741	741	5	5	H
	64	36	742	742	5	5	I
	47	6	756	755	5	5	J
	54	19	758	758	5	5	K
	50	31	866	860	7	7	L
	57	115	962	958	8	9	M
2015	158	263	487	496	3	3	N
	253	257	510	511	3	3	O
	252	259	540	534	3	3	P
	157	260	595	594	4	4	Q
	265	266	852	866	7	7	R
2016	358	218	229	229	1	1	S
	362	208	265	262	1	1	T
	353	209	340	341	2	1	U
	234	197	590	591	4	4	V
	232	161	645	652	4	4	W
	212	310	295	295	1	1	X
	241	160	705	789	5	6	Y

4.2.4. Determining the number of laser ablated points required

A preliminary study was done on a single otolith from a WC sample (Otolith #160) and one from a SC sample (Otolith #241).

Otoliths #160 and #241 had a final total of n = 193 and n = 182 laser ablated points on each, respectively. Results were obtained for 17 elements: Boron (B), Barium (Ba), Calcium (two isotopes Ca^{43} and Ca^{44}), Cobalt (Co), Copper (Cu), Iron (Fe), Potassium (K), Lithium (Li), Magnesium (Mg), Manganese (Mn), Sodium (Na), Nickel (Ni), Lead (Pb), Rubidium (Rb), Tin (Sn), Strontium (three isotopes Sr^{86}, Sr^{87}, Sr^{88}) and Zinc (Zn). Data was expressed as molar ratios to calcium (Ca^{44}) and aberrant measures (negative measures, calibration errors) were removed and concentrations below the limits of detection was set to zero.

More than 99% of the otolith is composed of calcium carbonate ($CaCO_3$) in an organic matrix (Degens *et al.,*1969; Campana *et al.,*1997). The remaining 1% comprises abundant minor elements which are subject to strong physiological regulation (Kalish, 1989; Thresher *et al.,* 1994; Campana *et al.,* 1997; Morales-Nin *et al.,* 2005). The carbonate molar ratios of the elements tested in this study was found in different concentrations, varying from relatively high (range 0.1 – 1), e.g., Sr and Na, to relatively low (0.000000 - 0.000001), e.g., Co, Pb, Sn. The range of elemental ratios of all seventeen elements (expressed as molar ratios to calcium Ca^{44}) for this study, is presented in Table 4.3.

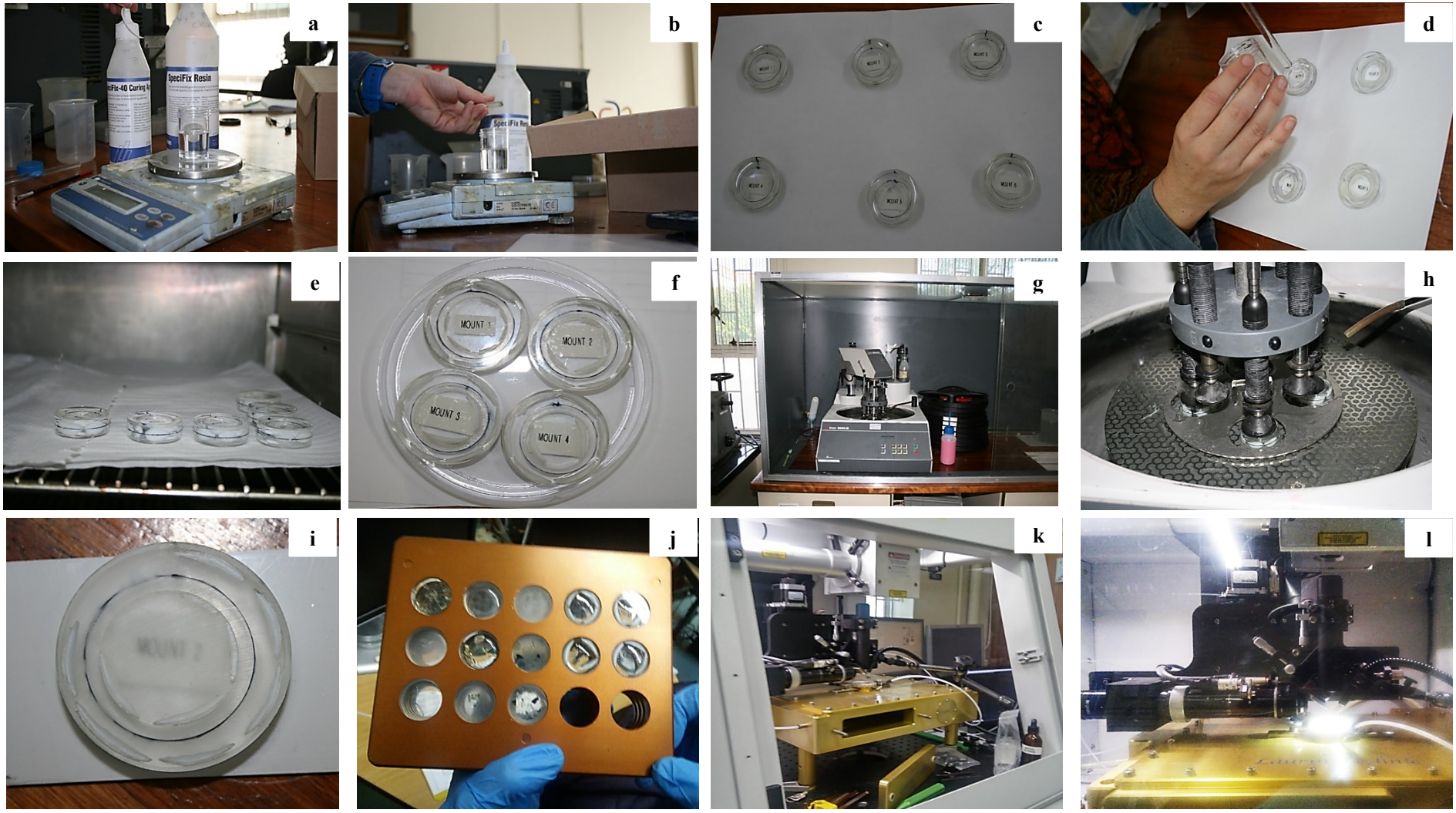


Figure 4.4. Specifix-40 curing agent and Specifix resin blended **(a and b)** otoliths were mounted in resin base **(c and d)**, these were placed in an oven **(e and f)** embedded otolith polished **(g and h)**. After being polished **(i)** mounts **(j)** were placed into the laser ablation inductively coupled plasma mass spectrometer **(k and l).**

Table 4.3. The range of the molar ratios concentrations of elements relative to Calcium (Ca^{44}) measured in the ablated otoliths shots of *Genypterus capensis*.

Range	Element [/Ca]
0.1 - 1	**Sr**, Na
0.01 - 0.1	K
0.001 - 0.01	**Mg**
0.0001 - 0.001	**Mn**, **Fe**, **Ba,** P, B
0.00001 - 0.0001	Rb
0.000001 - 0.00001	**Ni**, **Cu, Li,** Zn
0.000000 - 0.000001	**Pb**, Sn, Co

Note*: Elements most likely to serve as environmentally influenced stock indicators as per Campana (2005a), are highlighted in bold.*

Two dimensional plots were created to visualise the carbonate molar elemental concentrations for whole-ablated otoliths of fish from the west (otolith #160), and south putative stocks (otolith #241). Figure 4.5 portrays results for B, Ba, Mg, Ni, P and Sr. Figures portraying the remaining elements tested are shown in Appendix C: Figure C1. To then determine the number of ablated shots required for a representative view of elemental concentrations, the *mean* and *standard error* for each element was calculated using the equations:

$$X_{\text{element}} = \frac{\Sigma x}{n}$$

where $X_{element}$ is the mean value, Σx is the sum of the elemental concentrations and n is the total number of samples

$$\sigma_M = \frac{\sigma}{n}$$

where σ_M is the standard error value; (σ) is the standard deviation of the carbonate referenced elemental and n = number of laser shots

It was determined that a total of 31 shots per otolith was enough to determine stock differentiation. It was decided that forty shots (n = 40) measuring all seventeen elements (expressed as molar ratios of Ca^{44}) used in this study would be measured for further analysis. Greater spatial resolution is found in the early years of a fish's life, compared to later years (Fablet, 2007). Fifteen (n = 15) shots were taken from the otolith core to determine the larval stage composition, with the remaining shots transecting along the coreline and including the edge of the otolith.

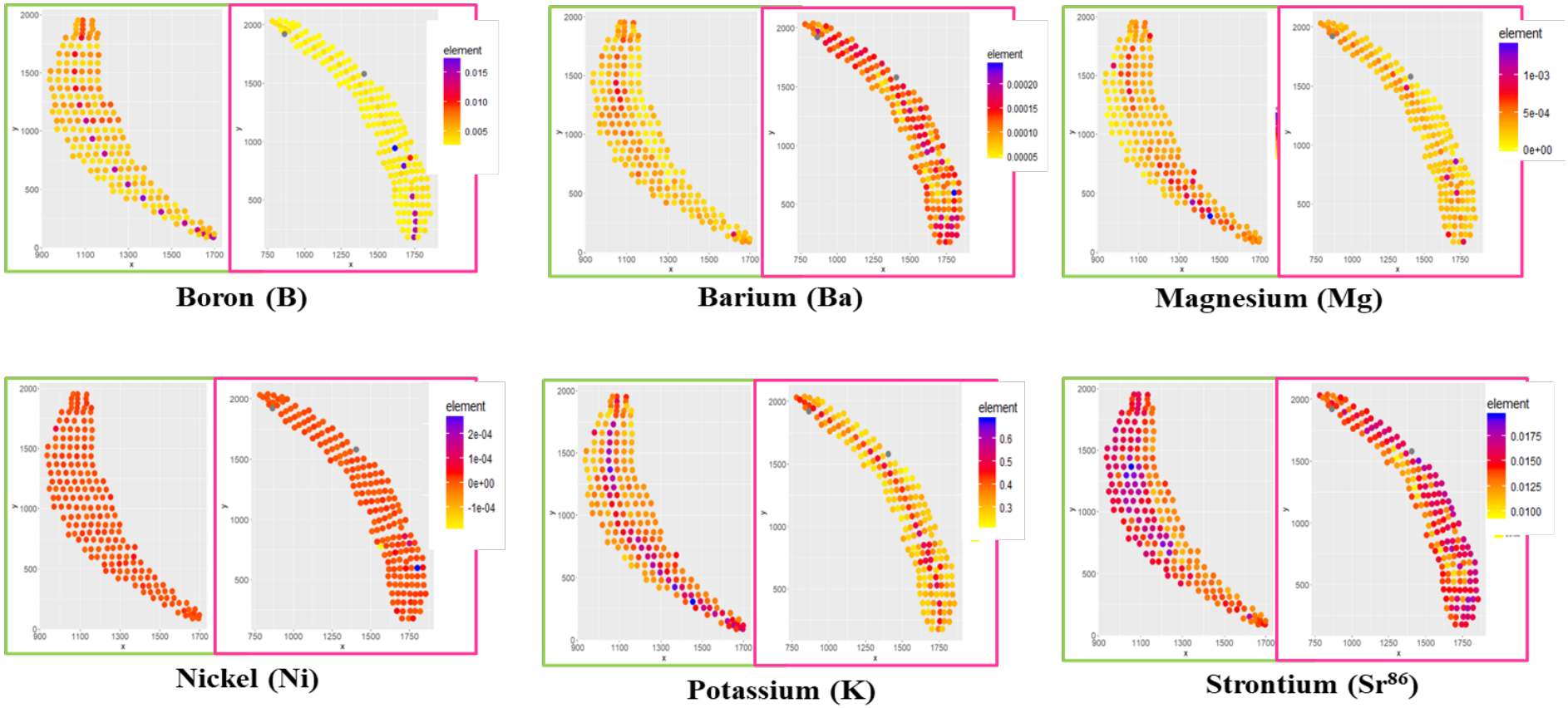


Figure 4.5. Two dimensional plots for visual portrayal of molar ratios concentrations of elements relative to Calcium (Ca^{44}): boron, barium, magnesium, nickel, potassium, and strontium, at laser ablated points of an otolith of a *Genypterus capensis* specimen from the west putative stock (otolith #160 ; right pink border), and the putative south stock (otolith #241; green border, left).

4.2.5. Selecting, recording, and assigning ablation points

Ablation points were manually selected, ensuring sufficient data would be obtained for the: core, central core line and edges. For identification, each ablated point was numbered, and the ablated otolith was photographed and saved to file with a label.

According to Morales-Nin (1987), the otolith nucleus encompasses the area on the otolith formed from birth to the first slow growth ring and characterizes the first six to nine months of the *G. capensis* life. It is characterized by thick, clearly distinguishable increments with a density that is visibly higher than that of the rest of the otolith. In this study, the otolith core was visually recognized and spots were ablated inside the core area, in three parallel lines.

After formation of the core increments, otolith growth progressed at a similar rate, repeating the characteristic oval shape of the otoliths of this species. Increments had widths of approximately 10µm (Morales-Nin, 1987). In this study, for coreline data, a radius from core to the edge of the otolith was ascertained, and a series of spots were ablated in three parallel lines.

The edge consisted of at least three representative spots, depending on the size of the otolith. Pooled (core + coreline + edge) data was labelled as 'All'. The beam points were separated at distance-intervals of 60 - 250µm, depending on the length of the otolith. Data was then processed using Glitter Software and Microsoft Excel.

4.2.6. Statistical methods

An explanation of the Shapiro-Wilk test, ANOVA's, Mann-Whitney U tests, Random Forest, Boosted Tree, Multidimensional (MDS) plots, and Principal component analysis (PCA), is provided in Section 2.2.4. These methods are used in the current chapter, and additional assessment methods used are explained below:

4.2.6.1. Wald-Wolfowitz Runs Test

The Wald-Wolfowitz runs test is a nonparametric alternative to the t-test for independent samples. Data is arranged in the same way as for the t-test for independent samples, having an independent variable, with at least two distinct codes, that uniquely identifies the group membership of each case in the data file. Several runs are computed to assess that the variables differ not just with respect to mean, but also the general shape of the distribution. The null hypothesis assumes that samples are drawn from the same population (TIBCO, 2021). Significant differences are displayed at a significance level of 0.05 in this study.

4.2.6.2. The Kruskal-Wallis H test

The Kruskal-Wallis H test is a rank-based nonparametric test (also referred to as the "one-way ANOVA on ranks"). It can be used to determine if there are statistically significant differences exist between two (or more) groups of an independent variables and a continuous (or ordinal) dependent variable (TIBCO, 2021). Significant differences are shown at a significance level of 0.05 in this study.

4.2.6.3. Pairwise Comparisons

Pairwise comparisons are used to determine which group differences are statistically significant. Each row tests the null hypothesis that the individual samples (WC samples and SC samples) distributions are the same (TIBCO, 2021). Asymptotic significances (2-sided tests) are displayed at a significance level of 0.05 in this study.

4.2.7. Statistical analysis

When using elemental fingerprints as stock discriminators, genetic differences are not implied (Campana, 2005a), but results do promote a high spatio-temporal resolution of the life of the fish from which it was obtained (Tanner *et al.,* 2015). Using the LA-ICPMS technique, only particular time scales of interest were targeted, with:

- otolith core (+ near core) ablated shots → representing the larvae phase,
- coreline ablated shots → representing the life of the fish, and
- edge ablated shots represent → representing the time just before the fish was captured

Data was separated using variables/elements for each section of the otolith. Coreline results were further separated into IMM and MAT subsets. The data was then analysed in two ways:

1. Comparative analysis of life stages

   To determine differences between life stages (i.e., larvae *vs* time-of capture, immature *vs* mature) within each stock, as well as between fish from the two putative stocks, data was compared at each life stage:

   - The Kruskal-Wallis H test and post-hoc Pairwise comparisons were used to determine differences along the coreline, as well as between core and edge data amongst stocks.

2. Comparative analysis of two putative stocks

   To determine differences between fish from the two putative stocks, data was compared at each life stage i.e., core, coreline, and edge:

   - Non-parametric tests were employed for univariate analysis, and included ANOVA's, Mann-Whitney U tests, and the Wald-Wolfowitz Runs Tests.
   - Non-parametric tests for multivariate analysis included machine learning techniques: Boosted Tree (BT) and Random Forest (RF) analysis, as well as PCA's. The best variable combination from BT and RF results were visually portrayed on an MDS plot, and PCA plots were also created.

4.3. RESULTS

4.3.1. Comparative analysis of life stages

For reconstruction of the life cycle of *Genypterus capensis* samples used in this study, core *vs* edge data (portraying larvae *vs* time-of capture), and coreline data (portraying immature *vs* mature) from ablated shots was

examined, in- and between- stocks.

4.3.1.1. Core vs Edge

4.3.1.1.1. Independent Kruskal-Wallis Test

An Independent-Samples Kruskal-Wallis Test indicating differences in elemental composition of core (CR) and edge (ED) ablated shots of *G. capensis* otoliths from the putative western (WC) and southern (SC) stocks off the South African coast was performed. All 17 elements were tested and results showing significant differences ($p < 0.05$) are presented in Table 4.4: Mn/Ca ($H_{(3)} = 31.847$, $p \leq 0.000$), Li/Ca($H_{(3)} = 16.437$, $p = 0.001$), Fe/Ca($H_{(3)} = 17.472$, $p = 0.001$). and Ni/Ca($H_{(3)} = 13.075$, $p = 0.004$).

Table 4.4. Independent-Samples Kruskal -Wallis Test results for the years 2014 to 2016 showing variations in the elemental composition of the core and edge of *Genypterus capensis* otoliths from the putative western and southern stocks off the coast of South Africa.

Variable	Total (n)	Test Statistic	Degrees of Freedom	p-value
Mn/Ca	96	31.847[a]	3	**≤0.000**
Li/Ca	96	16.437[a]	3	**0.001**
Fe/Ca	96	17.472[a]	3	**0.001**
Ni/Ca	96	13.075[a]	3	**0.004**

Note: *a -The test statistic is adjusted for ties)*

4.3.1.1.2. Pairwise Comparisons

Pairwise comparisons using results from the Independent-Samples Kruskal-Wallis Test was performed and the p-values obtained are shown in Table 4.5. A complete Table including test statistics and standard error, is shown in Appendix C: Table C2.

For WC samples, significant differences were found in the elemental composition of the core *vs* edge ablated shots for: Mn/Ca, Fe/Ca, and Ni/Ca ($p = 0.001$). For SC specimens, Mn/Ca ($p = 0.021$) and Li/Ca ($p = 0.0005$) showed significant differences. For ED data, results showed marginal significant difference ($p = 0.052$) when comparing coasts (WC ED *vs* SC ED). Between coasts, no significant differences were found for CR data. Three-

dimensional bar graphs visually portraying results is shown in Figure 4.6.

Table 4.5. Pairwise Comparisons: P-values indicating differences in elemental composition of core and edge ablated shots of *Genypterus capensis* otoliths from the putative west and south coast stocks

Sample1 - Sample 2	WCED - WCCR	SCED - SCCR	WCED - SCED	WCCR - SCCR
	p-value			
Mn/Ca	**3.86E-06**	**0.021**	0.083	0.563
Li/Ca	0.083	**0.0005**	0.988	0.563
Fe/Ca	**0.0005**	0.248	0.083	0.563
Ni/Ca	**0.001**	0.286	0.052	0.809

Note: *WC – west coast stock; SC – south coast stock, CR - core; ED – edge*

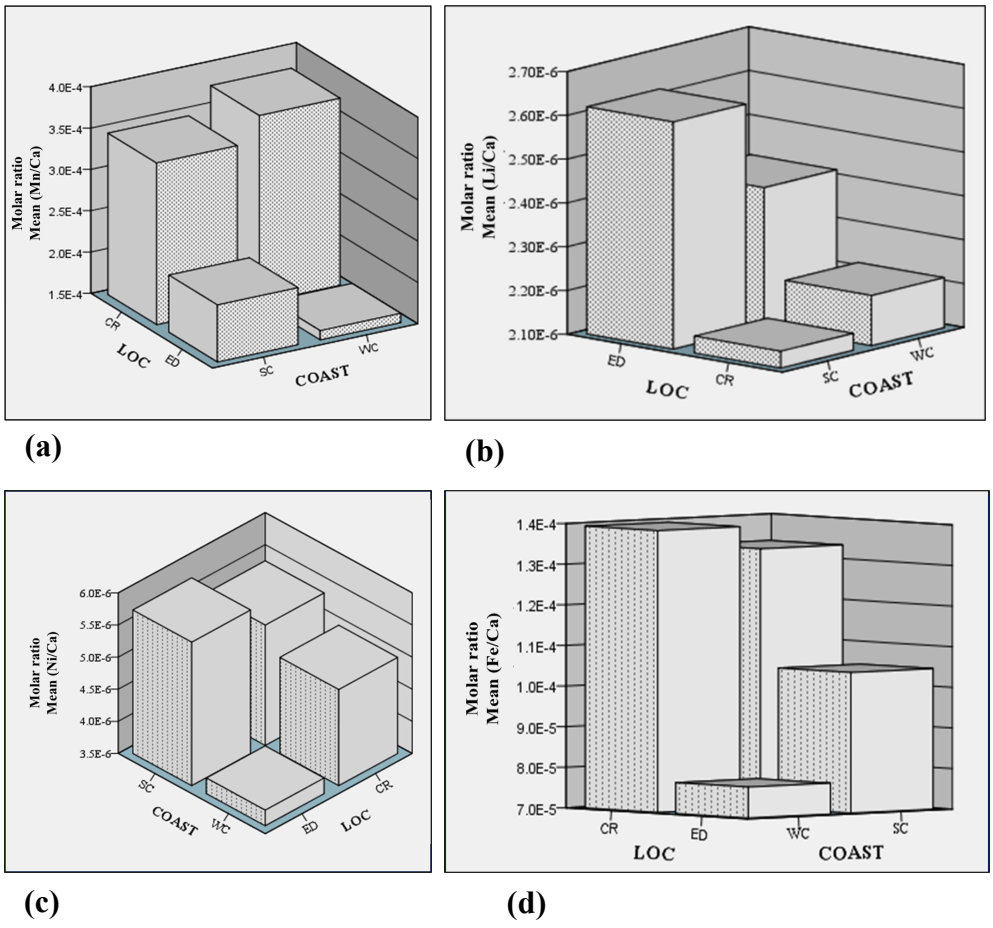


Figure 4.6. Three-dimensional bar graphs showing the mean elemental molar ratios of (a) Mn/Ca (b) Li/Ca (c) Ni/Ca and (d) Fe/Ca on the ED (edge) and CR (core) locations (LOC) of *Genypterus capensis* otoliths from the putative west coast (WC) and south coast (SC) stocks for the years 2014 – 2016.

4.3.1.2. Coreline: Immature vs mature

Kruskal-Wallis Test Summary

An Independent-Samples Kruskal-Wallis Test indicating differences in elemental composition for coreline ablated shots of immature (IMM) and mature (MAT) *G. capensis* samples from the putative western (WC) and southern (SC) stocks off the South African coast was performed. All 17 elements were tested and results showing significant differences ($p < 0.05$) are shown in Table 4.4: Rb/Ca, Ni/Ca, Cu/Ca, and Co/Ca.

Table 4.6. Independent-Samples Kruskal-Wallis Test indicating differences in elemental composition of immature and mature specimens using coreline-ablated shots of *Genypterus capensis* otoliths from the putative western (WC) and southern (SC) stocks off the South African coast.

Variable	Total N	Test Statistic	dF	p-value
Rb/Ca	47	16.796[a]	3	**0.001**
Ni/Ca	48	14.569[a]	3	**0.002**
Cu/Ca	48	11.166[a]	3	**0.011**

Note: *a -The test statistic is adjusted for ties; elemental composition = molar ratios concentrations of elements relative to Calcium (Ca^{44})*

Pairwise Comparisons

Pairwise comparisons using results from the Independent-Samples Kruskal-Wallis Test was performed and the p-values obtained are shown in Table 4.7. A complete Table including test statistics and standard error, is shown in Appendix C: Table C2.

For WC specimens, significant differences for Rb/Ca ($p = 0.0008$) and Ni/Ca ($p = 0.033$) were found. For SC specimens, significant differences were found for Rb/Ca ($p = 0.002$), Ni/Ca ($p = 0.013$) and Cu/Ca ($p = 0.039$) when comparing the two age groups. For IMM specimens, results showed and marginal significant difference ($p = 0.05$) when comparing coasts (WC IMM *vs* SC IMM). Between coasts, no significant differences were found between for MAT sample sets.

Three-dimensional bar graphs showing the mean elemental composition, hereby visually portraying results, is shown in Figure 4.7.

Table 4.7. Pairwise Comparisons: p-values indicating differences in elemental composition of the coreline of IMM and MAT *Genypterus capensis* otoliths for fish from the putative western and southern stocks off the South African coast

Sample 1 - Sample 2	WC MAT - WC IMM	SC MAT - SC IMM	WC IMM - SC IMM	WC MAT - SC MAT
		p-value		
Rb/Ca	**0.008**	**0.002**	0.262	0.545
Ni/Ca	**0.033**	**0.013**	0.05	0.082
Cu/Ca	0.138	**0.039**	0.064	0.167

Note: *Significant differences ($p < 0.05$) are highlighted in bold*

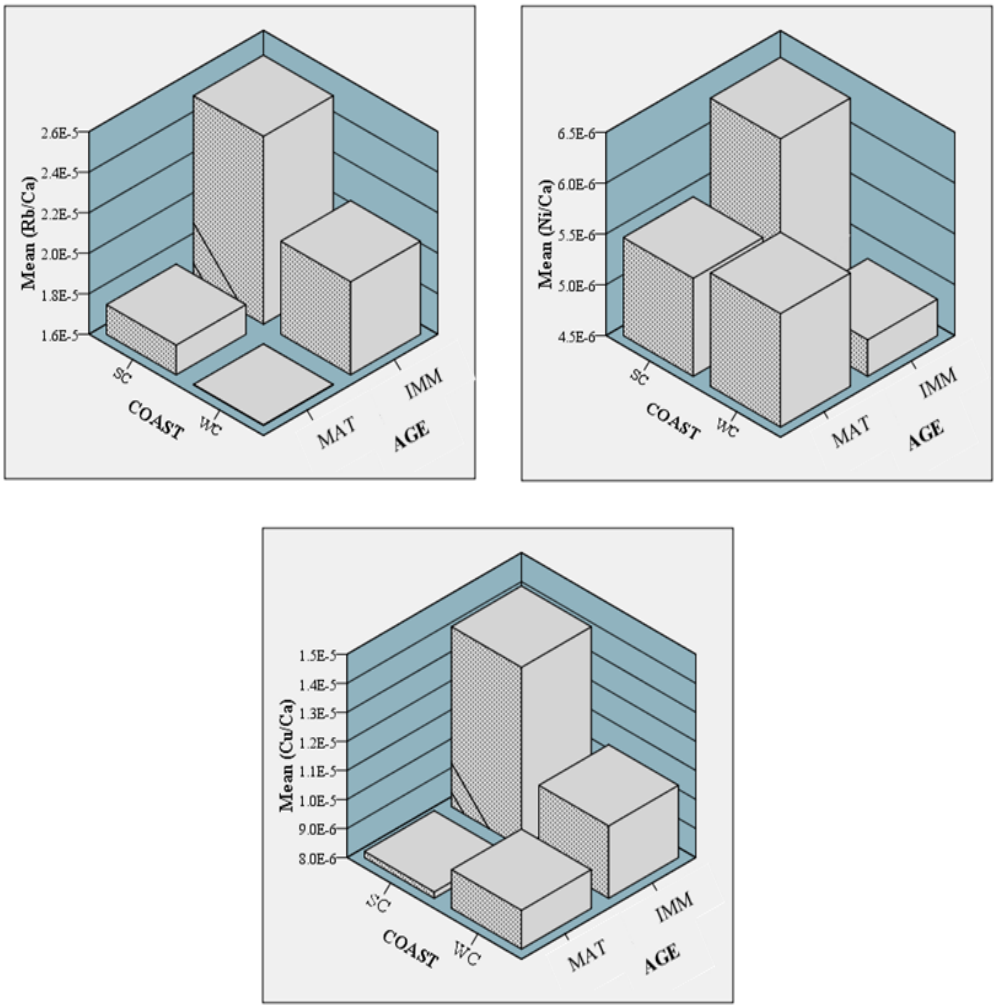


Figure 4.7. Three-dimensional bar graphs showing the mean elemental composition of **(a)** Rb/Ca **(b)** Ni/Ca and **(c)** Cu/Ca of *Genypterus capensis* otolith for mature (MAT) and immature (IMM) specimens from the putative west coast (WC) and south coast (SC) stocks for the years 2014 – 2016.

4.3.1.3. Principal component analysis

PC analysis was carried out on core *vs* edge, and immature *vs* mature datasets. The eigenvalues of principal components is shown in Table 4.8, and the correlation between the PC's and original variables is shown in Table 4.9.

Core vs edge

For WC samples, the first two PC's showed an eigenvalue greater than 1. Cumulatively, PC1 (50%) and PC 2 (35.5%) showed 84.5% of the total variation. In these results, PC 1 showed a strong correlation with both Mn/Ca (-0.719) and Fe/Ca (-0.5) and PC2 showed a strong correlation with Fe/Ca (0.697) and Ni/Ca (-0.717).

For the southern putative stock, the first PC showed an eigenvalue greater than 1. Cumulatively, PC1 (68.9%) and PC2 (31.1%) accounted for 100% of the total variation. In these results, the first component showed a strong correlation with Mn/Ca (0.707) and Li/Ca (0.707).

PCA plots showed marginal overlap between the two groups indicating differences in elemental composition between the early life stage (CR) and time of capture (ED) for both WC samples (Figure 4.8a) and SC samples (Figure 4.8b).

Coreline: Immature vs mature

For west coast samples, the first PC showed an eigenvalue greater than 1. Cumulatively, PC1 (68.9%) and PC 2 (31.1%) showed 100% of the total variation. In these results, the first two components showed a strong correlation with both Rb/Ca and Ni/Ca as variables in both PC1 and PC2.

For the southern putative stock, the first two PC's showed an eigenvalue greater than 1. Cumulatively, PC1 (50.8%) and PC2 (33.3%) accounted for 84.1% of the total variation. In these results, the first component showed a strong correlation with Ni/Ca (0.699) and Cu/Ca (0.707) and the second component showed a strong correlation with Ni/Ca (0.988).

PCA plots showed marginal overlap between the two groups indicating differences in elemental composition between the IMM and MAT life stages for both WC samples (Figure 3.8c) and SC samples (Figure 3.8d).

Table 4.8. Eigenvalues of Principal Components and the % variation explained for original otolith elemental characters of *Genypterus capensis* samples from the putative west (WC) and south (SC) stocks.

Otolith section	Coast		PC1	PC2	PC3
Core *vs* Edge	WC	Eigenvalues	**1.5**	**1.04**	0.465
		%Variation	50	34.5	15.5
		Cum.%Variation	50	84.5	100
	SC	Eigenvalues	**1.05**	0.95	
		%Variation	52.4	47.6	
		Cum.%Variation	52.4	100	
Coreline: Immature *vs* Mature	WC	Eigenvalues	**1.38**	0.62	
		%Variation	68.9	31.1	
		Cum.%Variation	68.9	100	
	SC	Eigenvalues	**1.52**	**1.00**	0.477
		%Variation	50.8	33.3	15.9
		Cum.%Variation	50.8	84.1	100

Table 4.9. Correlation between principal components and original variables explained for otolith elemental characters of *Genypterus capensis* samples from the putative western (WC) and southern (SC) stocks.

Otolith section	Coast	Variable	PC1	PC2	PC3
Core *vs* Edge	WC	Mn/Ca	**-0.719**	-0.002	**-0.695**
		Fe/Ca	**-0.500**	**0.697**	**0.515**
		Ni/Ca	-0.483	-0.717	**0.502**
	SC	Mn/Ca	**0.707**	**-0.707**	
		Li/Ca	**0.707**	**0.707**	
Coreline: Immature *vs* Mature	WC	Rb/Ca	**-0.707**	**0.707**	
		Ni/Ca	**0.707**	**0.707**	
	SC	Rb/Ca	-0.110	**0.988**	0.107
		Ni/Ca	**0.699**	0.154	**-0.699**
		Cu/Ca	**0.707**	0.002	**0.707**

Note: *Significant drivers (> 0.5) are highlighted in bold*

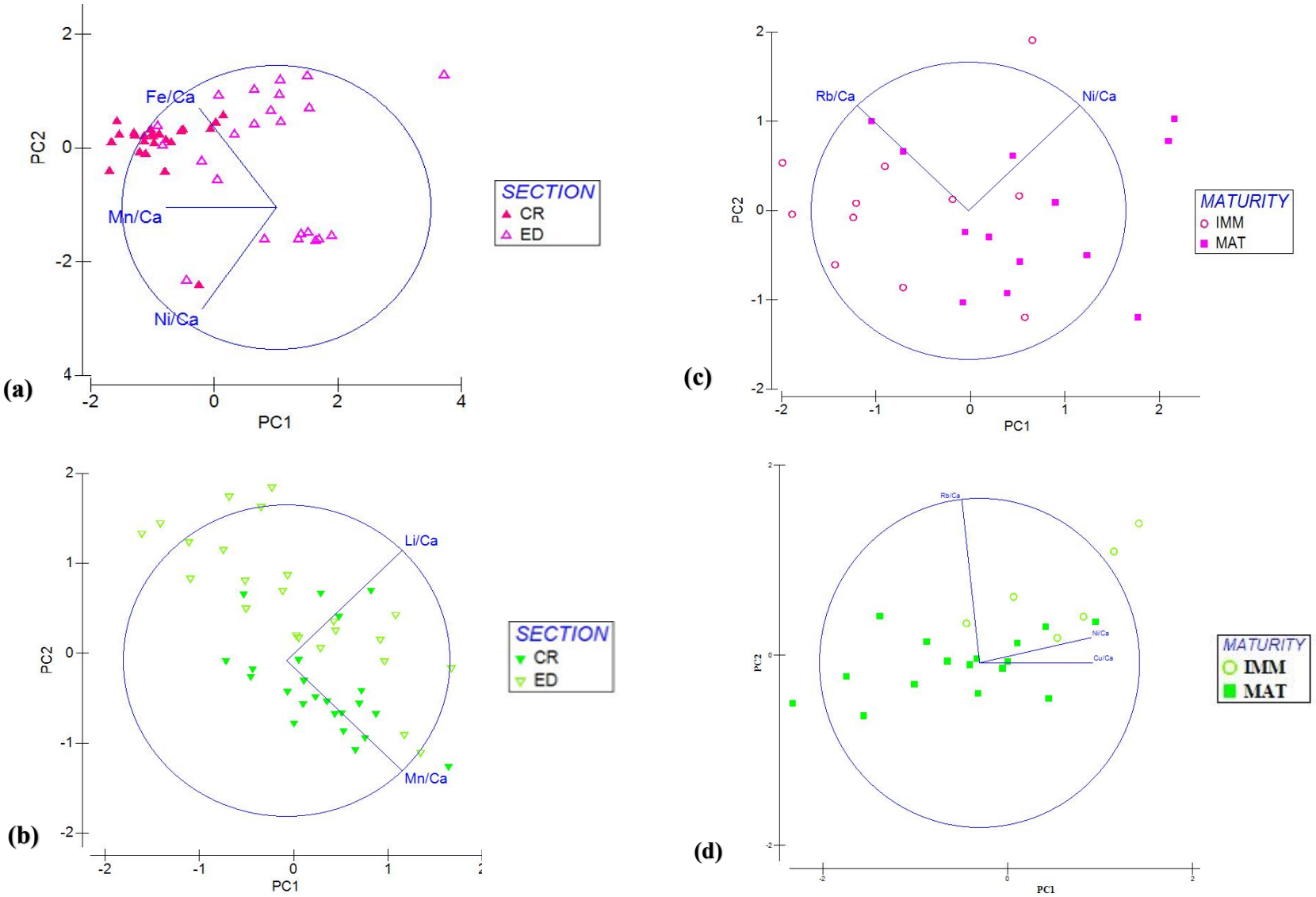


Figure 4.8. PCA plots using elemental ratios obtained from laser-ablated shots by LA_ICPMS, for core (CR), edge (ED), immature (IMM) and mature (MAT) *Genypterus capensis* otoliths of fish from the putative western (WC) and southern (SC) stocks off the South African coast stocks for the years 2014 – 2016.

4.3.2. Comparative analysis of two putative stocks

In Section 4.3.1., differences between the otolith elemental composition at different stages of the fish's life was examined. In this section, each life stage represented by the different portions of the otolith was compared to test for differences in stock structure.

4.3.2.1. Test for Normality

The Shapiro-Wilk test was used to test for normality was applied to: all, core, coreline (immature and mature), and edge data, with results shown in Table 4.10. As most samples showed a non-normal distribution ($p < 0.05$), non-parametric tests were employed for further analysis.

4.3.2.2. Univariate Analysis

Non-parametric tests

A summary of the results from the non-parametric tests used to test differences in molar ratios of elements is shown in Table 4.11. A complete Table is shown in Appendix C: Tables C3 – C5.

When all-ablated shots were tested, no significant ($p > 0.05$) were found. For core-ablated shots, the Wald-Wolfowitz Test showed differences in Ba/Ca ($p = 0.028$). For ablated shots along the coreline of immature fish, Ni/Ca showed significant differences in an ANOVA ($F_{(1,17)} = 15.155$, $p = 0.0011$), Mann-Whitney U ($U = 8$, $p = 0.003$) and a Wald-Wolfowitz Test ($p = 0.034$). A Mann-Whitney U test showed differences in P/Ca ($U = 13$, $p = 0.02$) and a Wald-Wolfowitz Test showed significant results for Ba/Ca ($p < 0.05$). For ablated shots along the coreline of mature specimens, no significant differences ($p > 0.05$) were found. For edge-ablated shots, a Mann-Whitney U test showed significant results for Ni/Ca ($U = 148$, $p – 0.003$) and a Wald-Wolfowitz showed significant differences for Sr/Ca ($p = 0.003$), Mg/Ca ($p = 0.028$), B/Ca ($p = 0.028$) and Ba/Ca ($p = 0.001$).

Boxplots showing the mean values of elements showing significant differences in univariate analysis, is portrayed in Figure 4.11.

Table 4.10. Tests of Normality: The Shapiro-Wilk test assessing elemental ratios from all ablated shots, and those from the core, core line (mature and immature specimens), and edge, of *Genypterus capensis* otolith samples from the putative west coast (WC) and south coast (SC).

[Element/Ca]	Coast	All	Core	Coreline		Edge
				IMM	MAT	
		p-value				
Boron (B)	SC	**0.006**	**0.012**	0.991	**0.005**	0.122
	WC	**0.002**	**0.000**	0.065	0.296	**0.022**
Barium (Ba)	SC	0.730	0.215	0.596	0.888	0.620
	WC	0.949	0.777	0.890	0.984	**0.026**
Cobalt (Co)	SC	0.196	0.185	0.648	0.614	0.849
	WC	0.368	0.106	0.394	0.396	0.467
Copper (Cu)	SC	**0.001**	**0.000**	0.586	0.405	0.062
	WC	0.402	**0.049**	0.257	0.460	**0.016**
Iron (Fe)	SC	0.323	0.457	0.766	0.698	**0.000**
	WC	0.419	0.858	0.462	0.321	**0.000**
Potassium (K)	SC	0.372	0.448	0.322	0.118	0.309
	WC	0.065	0.839	0.922	0.267	0.711
Lithium (Li)	SC	**0.013**	**0.010**	**0.020**	0.096	0.155
	WC	0.251	0.265	0.135	0.192	0.463
Magnesium (Mg)	SC	0.535	0.152	0.780	0.157	0.625
	WC	**0.001**	0.648	**0.018**	**0.043**	0.474
Manganese (Mn)	SC	0.998	0.279	0.482	0.323	**0.017**
	WC	0.448	0.321	0.298	0.394	**0.000**

[Element/Ca]	Coast	All	Core	Coreline		Edge
				IMM	MAT	
		p-value				
Sodium (Na)	SC	**0.004**	0.060	0.156	**0.017**	0.050
	WC	**0.000**	**0.033**	**0.002**	**0.005**	**0.047**
Nickel (Ni)	SC	0. 159	0.977	**0.048**	0.134	0.290
	WC	0.076	**0.034**	0.514	**0.025**	0.610
Phosphorus (P)	SC	0.117	**0.020**	**0.045**	0.957	0.189
	WC	**0.000**	**0.001**	**0.006**	0.398	0.184
Lead (Pb)	SC	0.626	**0.000**	0.467	0.499	**0.021**
	WC	**0.003**	**0.000**	0.050	0.905	**0.012**
Rubidium (Rb)	SC	**0.007**	**0.012**	0.331	0.368	0.267
	WC	0.316	**0.050**	0.972	0.405	0.209
Tin (Sn)	SC	0.128	**0.025**	0.820	**0.020**	**0.030**
	WC	**0.034**	**0.002**	**0.036**	0.079	**0.006**
Strontium (Sr)	WC	0.056	0.377	0.427	0.211	0.678
	SC	0.248	0.093	0.430	0.209	0.002
Zinc (Zn)	SC	**0.012**	**0.028**	0.997	0.122	0.325
	WC	0.175	0.382	0.541	0.244	0.715

Note: *IMM – otoliths from immature fish; MAT – otoliths mature fish; all elements are listed as molar ratios to Ca (Calcium)*

Table 4.11. Summary of significant results shown by non-parametric tests, indicating differences in elemental means for all, core, coreline (immature and mature) and edge data of *Genypterus capensis* otolith samples from the putative western and southern stocks off the coast of South Africa.

Otolith Section		[Element /Ca]	Test used	p-value
All		---	---	---
Core		Ba	Wald-Wolfowitz Test	0.028
Coreline	**Immature**	Ba	Wald-Wolfowitz Test	0.028
		P	Mann-Whitney U	0.021
		Ni	ANOVA	0.001
			Mann-Whitney U	0.003
			Wald-Wolfowitz Test	0.034
	Mature	---	---	---
Edge		B	Wald--Wolfowitz	0.003
		Ba		0.001
		Mg		0.028
		Sr		0.028
		Ni	Mann-Whitney U	0.004

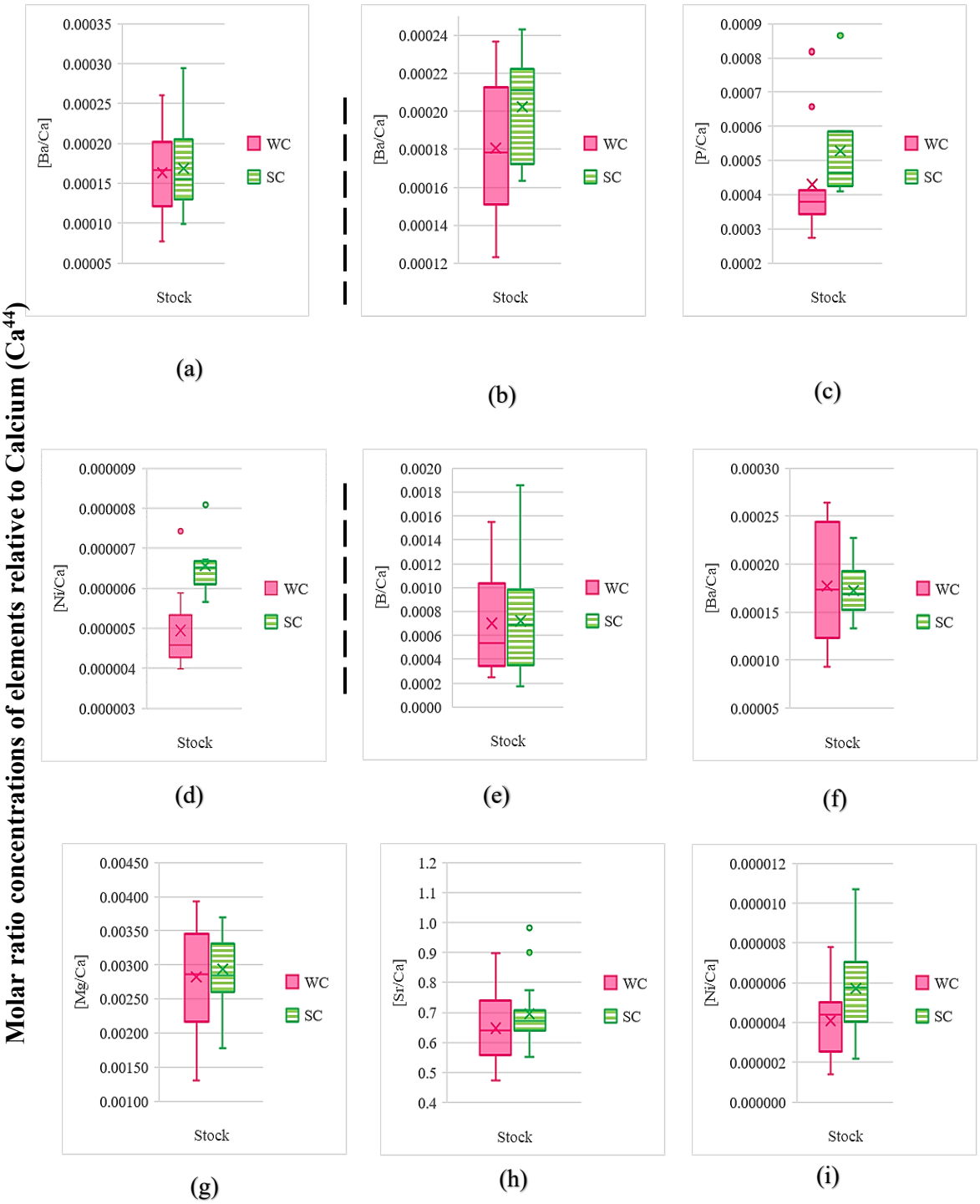


Figure 4.9. Boxplots illustrating molar ratios concentrations of elements relative to Calcium (Ca^{44}); showing differences in **(a)** core **(b – d)** coreline and **(e – i)** edge data obtained for *Genypterus capensis* otoliths using Laser Ablation Inductively Coupled Plasma Mass Spectrometry.

Note: *Ba – Barium, P – Phosphorus, Ni – Nickel, Sr – Strontium, Mg – Magnesium, B – Boron and Ca – Calcium; x represent mean and whiskers represent upper and lower quartiles*

4.3.2.3. Multivariate analysis

4.3.2.3.1. Boosted Tree (BT) and Random Forest (RF) analysis

To determine the best variable combination of elements required for the discrimination of *Genypterus capensis* from the two putative stocks, two classification models - Random Forest (RF) and Boosted Tree (BT) - were used on all, core, coreline and edge data.

Predictor performance of variables was computed for each model, and a stepwise procedure was then executed by adding variables gradually in order of most important to least. The combinations of variables selected by each model based on the stepwise selection procedure and their respective overall classification (training and validation) was calculated. A Table showing all results is given in Appendix C: Table C6. The selection of variables showing the highest classification accuracy in the training set is show in Table 4.12. The combinations of variables that gave the highest overall classification accuracy is shown in Table 4.13. It must be noted that coreline data could not be split into mature and immature counterparts, as independently, the groups had insufficient sample sizes.

Regarding the best variable combinations results differed between models' with BT requiring less variables (Table 4.12) and portraying a greater accuracy (Table 4.13) relative to RF. Even so, when using only these variable combinations in MDS plots, extensive overlap was visually apparent, as can be seen in Figure 4.10. An absolute select combination of variables could thus not be conclusively determined for stock delineation in core, coreline and edge data.

Table 4.12. Accuracy of training and test sets using model selection of variables of *Genypterus capensis* from the putative western and southern stocks off the South African coast, based on a stepwise selection procedure.

Otolith section	Test	Variable combination [/Ca]	Accuracy	
			Train	Test
All	BT	Sr+K	0.88	0.5
	RF	Ni+Cu+Rb+Co+Mg	0.79	0.31
Core	BT	Rb+Ni	0.85	0.73
	RF	Co+Cu+Pb+Na+Ni+Sr	0.65	0.53
Coreline	BT	Li+K+Pb+Mg+Sr+Rb+Mn+Zn	0.98	0.71
	RF	P+Na+Rb+Fe+Mg+B+Ba+Zn+Pb+Mn+Cu+Sr+K+Sn+Li	0.69	0.68
Edge	BT	Ni+Mg	0.85	0.87
	RF	Ni	0.74	0.71

Note: *RT – Random Forest Analysis; BT – Boosted Tree Analysis*

Table 4.13. Correct classification percentage for model selection of variables of *Genypterus capensis* from the putative western and southern stocks off the South African coast based on a stepwise selection procedure.

Otolith section	Test	(%)		
		Overall	WC	SC
All	BT	75.51	62.5	88.00
	RF	63.27	91.76	36.00
Core	BT	81.25	91.67	70.83
	RF	60.42	95.83	25.00
Coreline	BT	85.42	87.5	83.33
	RF	66.67	91.67	41.67
Edge	BT	85.42	91.67	79.17
	RF	72.92	87.5	58.33

Note: *RT – Random Forest Analysis; BT – Boosted Tree Analysis; WC = West Coast; SC = South Coast*

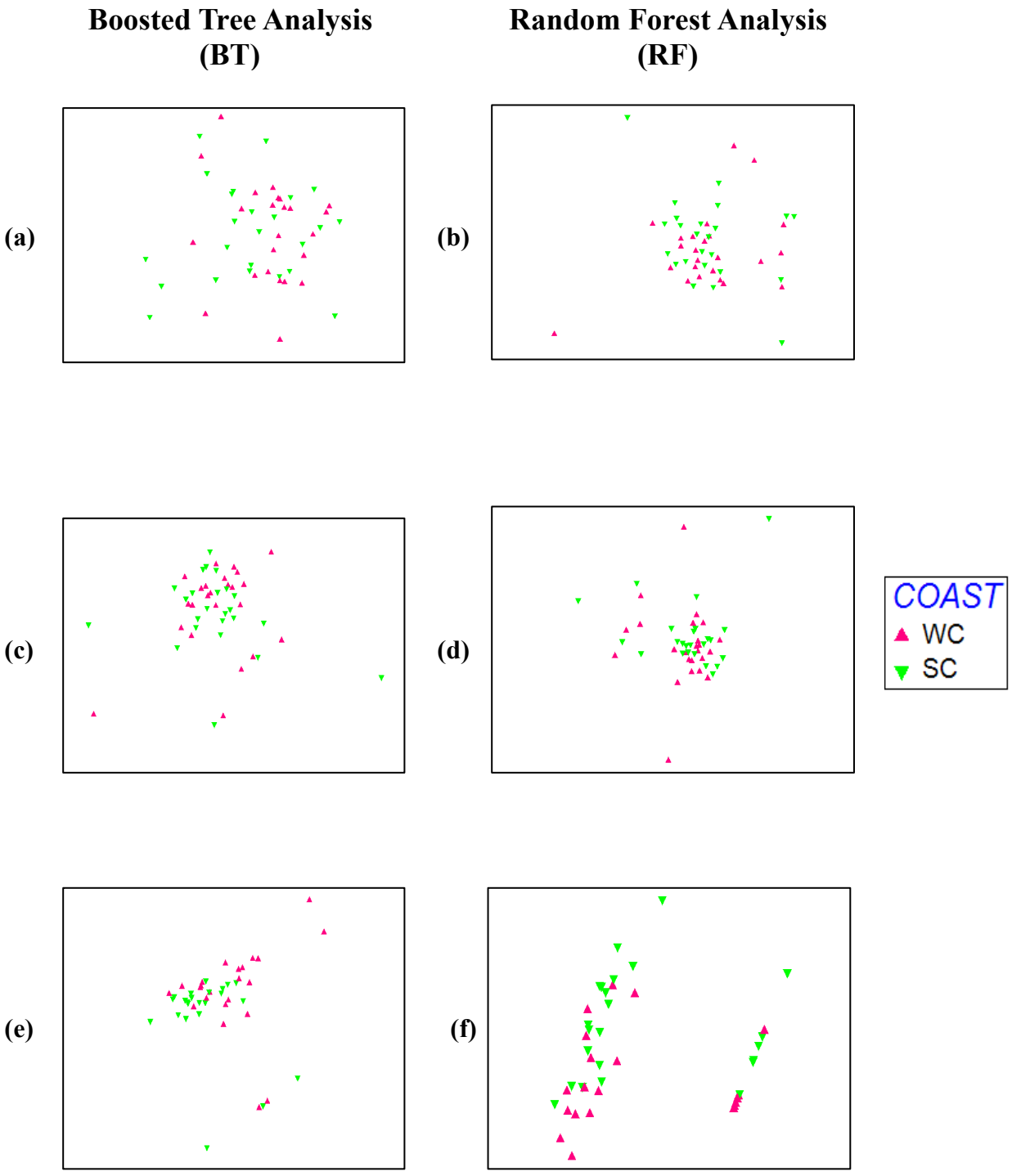


Figure 4.10. Multidimensional scaling plots (MDS) portraying differences when using boosted tree **(left)** and random forest **(right)** analysis for: **(a and b)** core, **(c and d)** coreline and **(e and f)** edge data, of laser ablated *Genypterus capensis* otoliths.

4.3.2.3.2. Principal component analysis (PCA)

For multivariate analysis, PCA plots depicting differences in otolith elemental composition between stocks, are shown. Tables containing the related eigenvalues, and correlations between PC's are listed in Appendix C: Tables C7 an C8.

When using all elements from all ablated shots, results from the first five principal components were have eigenvalues greater than one and cumulatively explained 77% of the total variation.

In Figure 4.11, PC1 (30.4%) and PC2 (18.9%) explained 49.3% of the total variation. Correlation between principal components indicated that PC1, PC2, PC3 and PC5 were not strongly correlated to any of the original variables (< 0.5). In PC4, one element: P/Ca (-0.696) was shown to have a strong negative correlation. The PCA plot showed an extensive overlap between individuals from the two coasts indicating that there is no distinction between the two putative stocks.

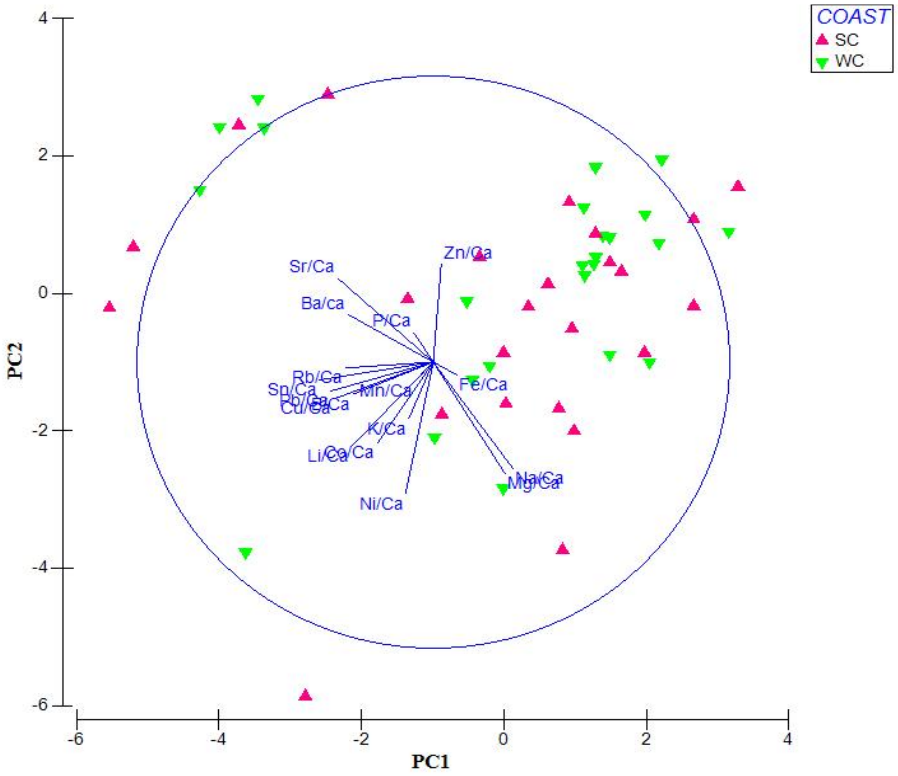


Figure 4.11. PCA plots using elemental ratios of all ablated shots of *Genypterus capensis* otoliths from the putative western (WC) and southern (SC) stocks off the South African for the years 2014 – 2016.

For core-ablated shots, the first five PCs showed an eigenvalue greater than 1 and explained 71.6% of the total variation. In Figure 4.12a, PC1 (23.9%) and PC2(16.1%) explained 40% of the total variation. In PC1, none of the variables showed a strong positive relationship (< 0.5). In PC2, only one variable showed a strong correlation: Ni/Ca (-0.52). In PC4, Co/Ca (0.661) showed a strong correlation and in PC5 Mn/Ca (-0.536) showed a strong correlation.

In Figure 4.12b, the ablated shots along the coreline portraying IMM fish is shown. Here, the first two PCs showed an eigenvalue greater than 1 and accounted for 80% of the total variation. Cumulatively, PC1 (41.1%) and PC2 (19.9%) showed 61% of the total variation. In these results, the first two components showed a weak correlation (< 0.5) with all the original variables. In PC3, P/Ca (0.606) and Fe/Ca (0.514) showed a strong correlation. PC4 showed a strong relationship with Rb/Ca (-0.55) and PC5 showed a strong correlation with K/Ca (-0.518).

In Figure 4.12c, the ablated shots along the coreline for MAT fish is shown. The first five components showed an eigenvalue greater than 1 and accounted for 77.7% of the total variation. Cumulatively, PC1 (29.4%) and PC2 (17.6%) showed 46.9% of the total variation. In these results, the first four components showed a weak correlation (< 0.5) with all the original variables. In PC5, a strong correlation was found with Mn/Ca (-0.628).

For edge-ablated shots, in Figure 4.12d, the first five PCs showed an eigenvalue greater than 1 and accounted for 59.8% of the total variation. The first two PCs (PC1 = 17.9% and PC2 = 12.7%) accounted for 30.5% of the total variation and is portrayed in Figure 4.16. Results show that PC1, PC2, PC3 and PC5 was not strongly correlated to any of the original variables (< 0.5). In PC4, one element: Pb/Ca (-0.552) was shown to have a strong correlation.

The PCA plots showed extensive overlap between individuals from the two coasts visually portraying no distinction between the two putative stocks.

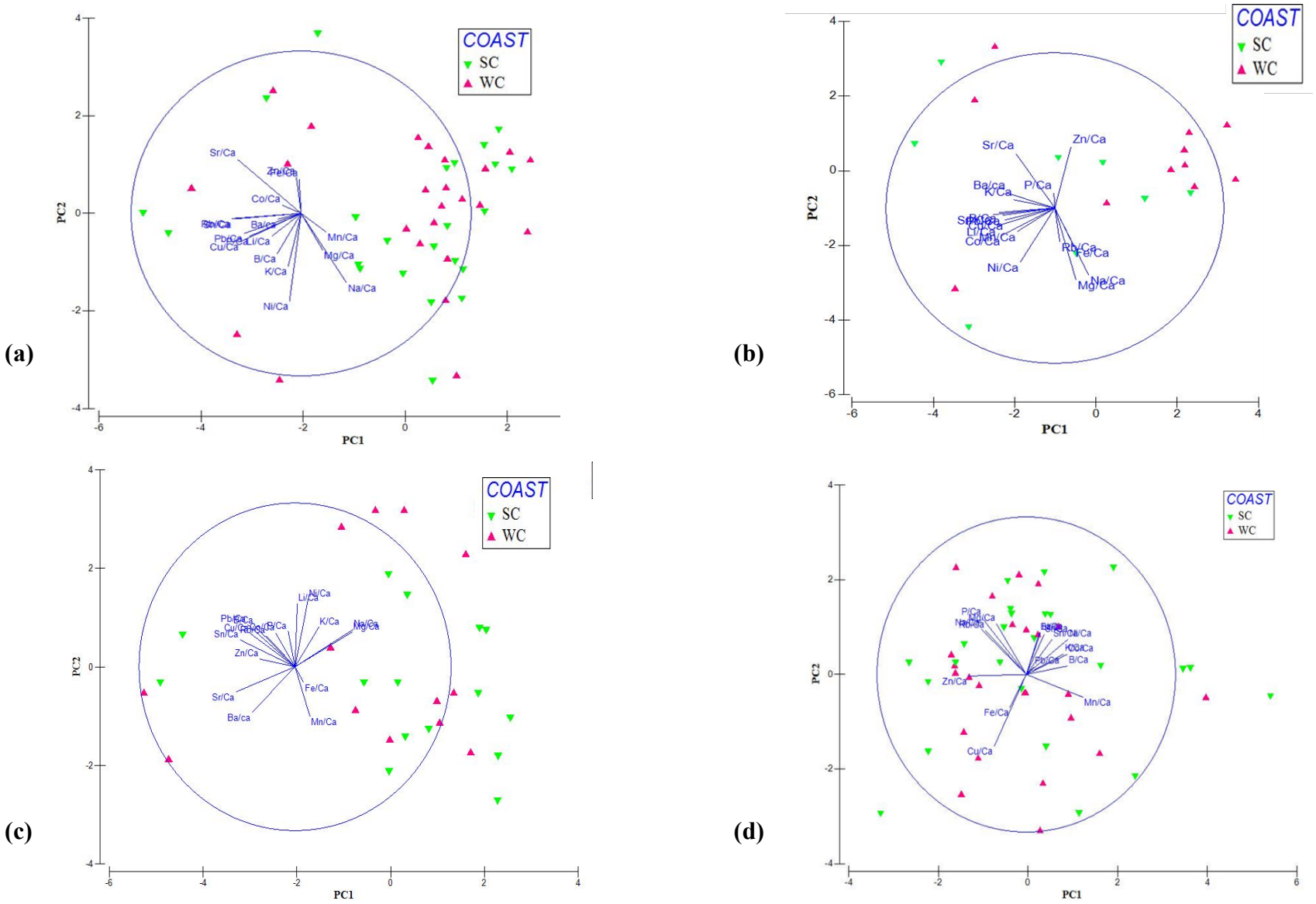


Figure 4.12. PCA plots using elemental ratios obtained from (a) core (b) coreline: immature (c) coreline: mature (d) edge, laser-ablated shots by LA_ICPMS, for. *Genypterus capensis* otoliths of fish from the putative western (WC) and southern (SC) stocks off the South African coast stocks for the years 2014 – 2016.

4.4. DISCUSSION

Elements face three major boundaries before entering the otolith, moving from:

water (or food) → plasma → endolymph → otolith

Once reached, elements can be integrated into the otolith by being randomly trapped in the crystal lattice, getting substituted for Ca on the growing crystal surface, or by getting attached to organic matrix constituents (Kalish, 1989; Campana, 1999; Hussy *et al.,* 2021). The chemical composition of *Genypterus capensis* otoliths can be influenced by both endogenous (diet, ontogeny, genetics) and exogenous (natural and anthropogenically influenced differences in temperature, pH, salinity, geology, amongst others) factors, all of which are thoroughly discussed in Chapter 1 and cited in Table 4.14.

Table 4.14. Reference to information in this thesis describing factors that may contribute to the chemical composition of *Genypterus capensis* otoliths.

Process	Characteristic	Refer to
Endogenous	Diet	Section 1.2.4
	Ontogeny	Section 1.2.4
	Genetics	Table 1.8
Exogenous	Environmental influences	Section 1.1.

A brief overview highlighting significant points is discussed:

4.4.1. Endogenous processes

4.4.1.1. Diet

Morales-Nin (1987) noted that sub-daily components were not apparent in the increments of *G. capensis* otoliths, and the feeding regularity of this species could be a contributing factor to this. There is noticeable change in diet with increasing depth and size (Macpherson 1983, Nyegaard *et al.* 2004, Dunn *et al.* 2010, Mitchell 2010). However, only slight differences were found in the diet between fish from the two coasts, when comparing fish of similar ages

(Guerra, 2019).

From this, it can be inferred that the influence of diet on otolith elemental uptake may be apparent when comparing fish of different ages but limited when assessing differences between coasts.

4.4.1.2. Ontogeny

This species follows a migratory life cycle (Putman *et al.,* 2019). Differences between coasts is documented for larval distribution and spawning seasonality (Olivar and Sabatés (1989). Different spatial distributions are found for immature (fish aged 0 – 3 years) compared to mature (fish aged 4 and older) fish. With increasing age, the fish move to increasing depths, and a horizontal depth distribution related to size was also noted for this species (Badenhorst and Smale, 1991; Payne, 1985; Botha, 1985). The depth of the foraging grounds which adults inhabit have relatively stable conditions (Japp and Hecht, 1990). The sub-daily components (discussed in the previous Section 4.4.1.1.) could further be explained by the behavioural nature in which mature individuals of this species remains close to the bottom, hereby experiencing the daily stability offered by the benthic environment.

Considering that otolith growth is affected by external factors such as the environmental conditions of the areas the fish inhabits throughout its lifetime (Campana and Neilson 1985; Gutierrez and Morales-Nin 1986; Morales-Nin, 1987; Ider *et al.,* 2017) ontogeny should be considered as a potential influence when interpreting results of *G. capensis* samples.

4.4.1.3. Genetics

Otolith core results may be a portrayal of reproductive isolation being affected by natal spawning site and/or local adaptation (e.g., *Macruronus magellanicus*; McKeown *et al.,* 2015).

The degree of genetic influences on elemental uptake in *G. capensis* otoliths is not known. However, even with a multiple stock hypothesis, low genetic divergence suggests there is extensive gene flow between populations (Henriques *et al.,* 2016), inferring genetics has a limited influence on otolith

elemental uptake in this species.

4.4.2. Exogenous processes

Differences in the marine environment along the Southern African coastline, is exhaustively discussed in Chapter 1. Anthropogenic influence includes the discharge of wastewater such as domestic sewage, industrial waste, and storm flow water. Effluent is discharged from over 60 licenced pipelines are present along the coastline of South Africa. Additionally, there are hundreds of stormwater outlets. The runoff includes heavy metals, oil residues, plastics, nutrients, and pathogenic microorganisms (Breetzke *et al.,* 2016). Differences in salinity and pH, is largely a result of anthropogenic sources (RSA DEA, 2018).

4.4.2.1. Life history comparison evaluation

Fish show consistent changes in otolith chemistry throughout their life history in terms of both age and reproductive cycles. Physiological changes which occur because of age and growth, as well as otolith precipitation rates resulting from age, and life history events (e.g., reproduction), could influences element uptake and incorporation (Tanner *et al.,* 2012). A summary of results found in this study, when comparing the otolith elemental composition at different life stages for *Genypterus capensis,* is shown in Table 4.15.

Regarding core *vs* edge data, for otolith elemental uptake, a relationship between otolith iron (Fe) and temperature was established (Gauldie *et al.,*1978). This could explain differences in Fe-concentrations between core and edge data since the life cycle occurs in different environments. Dissolved manganese (Mn) concentrations in water appears to decrease with depth, and distance from coastlines (Sturrock *et al.,* 2012). However, the higher Mn concentrations in the otolith core was also suggested to be a result of maternal transmission. (Brophy *et al.,* 2004; Ruttenberg *et al.,* 2005). Morales-Nin (1986) found *Merluccius capensis* otoliths have abundant protein concentrations in the nuclear region of the otolith, and it is possible that the protein matrix has an affinity for elements such as Mn. This is the likely

explanation for the increased Mn-concentrations in the core, relative to edge.

Observations on Rb suggests that this element is randomly absorbed into the crystal lattice as it only appears in the endolymph and the carbonate fraction of the otolith, with no enrichment (Thomas *et al.,* 2017). Rubidium (in addition to Mg, Li, K, Rb, Al, Pb, Cd, K, Li, Fe, Cu, and Zn) may display environmental sensitivity, hereby serving as a useful geographic marker (Miller *et al.,* 2006; Izzo *et al.,* 2016; Thomas *et al.,* 2017). Differences in Rb-concentrations could therefore be explained by the different environments which immature fish inhabit, relative to mature fish. Nickel (in addition to Co, Zn, and Pb) incorporation into the otolith is primarily facilitated by physiological roles as biomolecule co-factors, rather than as a result of the environment (Hussy *et al.,* 2021). Copper enters marine environment through geological processes such as weathering, erosion, deposition, and volcanic eruptions. Anthropogenic influences include copper mining, its use in the agricultural sector (pesticides), and metal and electrical manufacturing which all accelerate rates of absorption into the marine environment (RSA DEA, 2018). Peaks of Cu may be attributed to industrial or geological influences, whilst rubidium (Rb) usually presents a negative relationship with salinity, and these are potential reasons for the differences in coreline data for immature *vs* mature samples.

Using multivariate analysis, PCA plots showed marginal overlap between CR and ED, as well as coreline (IMM vs MAT) data. As discussed in Section 1.2.5, the species follows a trend in which an increase in age leads to an increase in depth. It is therefore plausible that differences are found between the different life stages.

4.4.2.1. Evaluation of differences in stocks

A summary of univariate results found in this study when comparing otolith elemental composition of *Genypterus capensis* samples between the putative western and south stocks is shown in Table 4.16.

Evidence on matrix-bound elements, including Cu, Zn Fe, Mn, Co, Ni and P, in the otolith, is somewhat limited (Izzo *et al.,* 2016; Thomas *et al.,* 2017;

Miller *et al.,* 2006 in Hussy *et al.,* 2021). The elements that prove dominant as tracers of environmental history, are those that substitute Ca during crystal growth, or those that get randomly trapped inside the crystal lattice. Barium (Ba) and strontium (Sr) directly replace calcium (Ca) molecules in the $CaCO_3$ matrix facilitating higher abundances in otoliths. Ba-concentration considered a nearly complete reflection of ambient concentrations since the bulk of this element originates from surrounding waters (Hussy *et al.,* 2021). Sr variations in otoliths were initially thought to be related to temperature fluctuations but have been found to be linked to metabolic and ontogenetic shifts. (Both Sr and Ca are conserved with salinity. In water, [Sr/Ca] ratios increase sharply at salinities from 0 to ~8 psu but tend to remain relatively stable at above 8 psu. Movements between areas of differing Sr water concentrations (caused by variation in bedrock geology and salinity-related environmental concentrations) are the most likely drivers of patterns in otolith Sr-concentration (Morales-Nin *et al.,* 2005).

In this study, univariate analysis showed differences for Ba-concentrations, with higher mean concentrations in SC stocks for core, coreline (immature specimens) and on the edge. These differences infer that the specimens from each of these two areas, inhabit different spaces during various parts of their lifetimes.

For coreline (immature) ablated shots, univariate analysis also indicated differences in phosphorus and nickel. In South Africa, economically significant phosphate deposits are restricted to igneous and marine sedimentary geological environments. Offshore deposits can be found on the continental shelf at Agulhas Bank, extending from Cape Town eastwards as far as Gqeberha (Roux *et al.*, 1989). Phosphorite pellets and phosphate rocks are located on the western continental margin of South Africa (Birch, 1979). It would therefore be expected that higher P-concentrations would be found in WC samples, however, a higher mean was found in SC samples. A likely explanation is that upwelling waters on the SC bring nutrients such as phosphorus to the surface (RSA DEA, 2018). This explanation is further backed by higher mean concentration of Ba in SC samples, as this element is

also associated to exposure to upwelled water (Baumann *et al.,* 2015).

According to Hussy *et al.* (2021), elements such as Sr, Ba, Mg (and Mn) are assumed to be incorporated into the otolith lattice in equilibrium with environmental concentration, and without effects of temperature, salinity, feeding rate, ontogeny, growth, and maturation. For edge-ablated shots, univariate analysis indicated differences in B, Ba, Mg, Sr, and Ni, which may be explained by biomineralization mechanisms.

Using multivariate analysis, a distinct variable combination when using both BT and RF analysis, together, proved inconclusive. This is since different elemental combination were suggested by each, and MDS plots showed spatial commonality for both. Furthermore, PCA's showed extensive overlap. Even so, lifecycle reconstruction for *G.capensis* using univariate and comparative analysis, facilitates the interpretation of some differences in the behaviour and environment of the two putative stocks.

Table 4.15. Summary of results, with reference to information in this chapter , for comparative analysis of life stages using otolith elemental composition for *Genypterus capensis* in this study.

Variable [Element/Ca]	Otolith Section	Stock		Details	Please see
		WC	SC		
Core *vs* Edge (CR vs ED)					
Mn	CR	↑		[Mn/Ca] higher on WC core	Table 4.5 Table 4.6 Figure 4.6
	ED		↑	[Mn/Ca] higher on SC edge	
Li	CR	↑		[Li/Ca] higher on WC core	
	ED		↑	[Li/Ca] higher on SC edge	
Fe	CR	↑		[Fe/Ca] higher on WC core	
	ED		↑	[Fe/Ca] higher on SC edge	
Na	CR		↑	[Na/Ca] higher on SC core	
	ED		↑	[Na/Ca] higher on SC edge	
Coreline: Immature *vs* Mature (IMM vs MAT)					
Rb	IMM		↑	[Rb/Ca] higher in SC immature	Table 4.6 Table 4.7 Figure 4.7
	MAT		↑	[Rb/Ca] higher in SC mature	
Cu	IMM		↑	[Cu/Ca] higher in SC immature	
	MAT	↑		[Cu/Ca] lower in WC mature	
Ni	IMM		↑	[Ni/Ca] higher in SC immature	
	MAT	↑		[Ni/Ca] lower in WC mature	

Table 4.16. Summary of results from this study, with **r**eference to information in this chapter, for comparative analysis of otolith elemental composition at different otolith sections for *Genypterus capensis* samples from the putative west (WC) and south (SC) coast stocks.

Otolith Section		[Element /Ca]	Mean concentration		Details	Please see
			WC	SC		
All		---	---	---		
Core		Ba		↑	[Ba/Ca] higher in SC stock	Table 4.11 Figure 4.9a
Coreline	**Immature**	Ba		↑	[Ba/Ca] higher in SC stock	Table 4.11 Figure 4.9b
		P		↑	[P/Ca] higher in SC stock	Table 4.11 Figure 4.9c
		Ni		↑	[Ni/Ca] higher in SC stock	Table 4.11 Figure 4.9d
	Mature	---	---	---		
Edge		B		↑	[B/Ca] higher in SC stock	Table 4.11 Figure 4.9e
		Ba		↑	[Ba/Ca] higher in SC stock	Table 4.11 Figure 4.9f
		Mg		↑	[Mg/Ca] higher in SC stock	Table 4.11 Figure 4.9g
		Sr	↑		[Sr/Ca] lower in SC stock	Table 4.11 Figure 4.9h
		Ni		↑	[Ni/Ca] higher in SC stock	Table 4.11 Figure 4.9i

4.5. CONCLUSION

For otolith elemental composition, 17 elements were tested. Barium, phosphorus, and nickel indicated significant differences in immature fish between the putative stocks. No significant differences were found in elemental composition between mature fish otolith samples. The differences between the two life stages are representative of the bentho-pelagic life cycle of this fish (See Section 1.2.5).

Campana (1999) envisaged that even though whole otolith analysis can be used as a natural tag, the examination of just the early life history is sufficient for stock identification studies (Campana, 1999). On this basis, in the current study, barium, phosphorus, and nickel may serve as elemental tags for the composition of otoliths in stock delineation studies of *G. capensis.* Further studies with a larger sample size focusing only on early otolith growth increments could prove useful in determining a more concrete elemental fingerprint for the subject species.

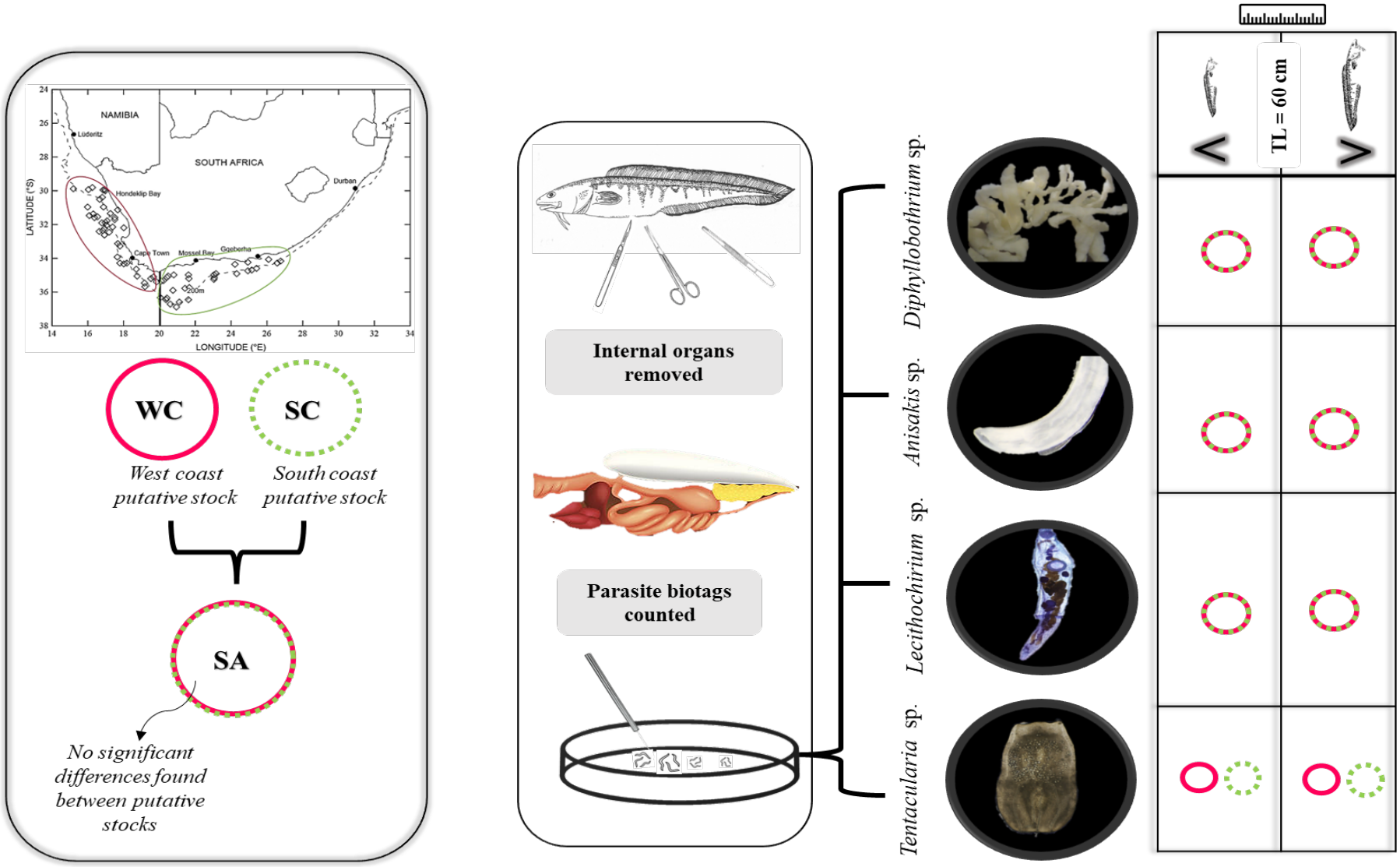
Chapter 5

PARASITES AS BIOTAGS

"Those who exploit the suffering of others are called parasites. Those who exploit their own suffering are called artists."

— Marty Rubin

GRAPHICAL ABSTRACT



5.1. INTRODUCTION

5.1.1. The parasite-host relationship

A parasite may be defined as "an organism living in, on, or with another organism in order to obtain nutrients, grow, or multiply, often in a state that directly or indirectly harms the host" (Merriam-Webster, 2021). Parasites are naturally acquired by the host, and infection can occur indirectly when the host ingests contaminated prey, or directly when the parasite attaches or penetrates the host (Baldwin *et al.,* 2012).

The kind of parasites present is determined by the diet and habitat of the host. Post-infection, parasites can remain in (or on) the host for prolonged periods of time, depending on longevity of the parasite species and other aspects of the host-parasite association (Rohde, 1984). Parasites can provide ecological information of its host e.g., the hosts diet and feeding behaviour, movements and ranges, the connectivity of stocks, recruitment patterns of juveniles, as well as the hosts evolutionary history (Catalano *et al.,* 2014). Parasites are therefore able to provide information which may prove useful for stock discrimination. Biological tagging involves using parasites as tags to infer information about the host populations thereby potentially discriminating between different stocks within a range (Mosquera *et al.,* 2003).

5.1.2. Parasitology in stock structure studies

Biological tagging functions on the principle that fish can become infected by a parasite only when that fish has been within the endemic area of that parasite (Mackenzie and Abaunza, 1998; Lester and MacKenzie, 2009; Mattiucci *et al.,* 2015). An endemic area for a parasite is defined by a territory in which conditions are suitable for the transmission of the parasite. Even when the host (fish) is outside of this area, it can be inferred that the fish was at some point within the area that the parasite is located in. The parasites age/life-cycle stage can then further aid in determining when the fish was infected as it was at that point present in the parasites endemic area by construing the maximum time since the fish could have been infected (Mackenzie and Abaunza, 1998).

Some of the earliest research on parasites as indicators of different intraspecific population of aquatic hosts, includes: Dogiel and Bychocsky (1939), and Herrington *et al.* (1939). Since then, research by Sindermann (1961), Kabata (1963), and Margolis (1965) has facilitated the widespread acceptance of this technique (Lester and MacKenzie, 2009), and the use of parasites as biological tags for population structure studies has expanded. This method of study now includes a wide range of fish species and geographical localities, with research being primarily focused on (but not limited to) economically significant fish species (Catalano *et al.,* 2014). Some of the more recent research on the use of parasites as biotags includes: de Moor *et al.* (2017), Gonzalez-Poblete *et al.* (2022), Irigoitia *et al.* (2022), Klapper *et al.* (2016), López-Moreno *et al.* (2023), Ogbon *et al.* (2023), and Porter *et al.* (2023).

A deeper comprehension of parasite assemblages and infection models can improve management choices for commercially important fish species. The efficacy of this technique is demonstrated by the management of the sardine (*Sardinops sagax*) fishery in South Africa, which currently employ stock-specific sardine evaluation models that incorporates parasite data (De Moor *et al.,* 2017). The decision was partially based on parasitological studies conducted by MacKenzie and Abaunza (2005), Reed *et al.* (2012), and van der Lingen *et al.* (2015). Moreover, substantial evidence for several sardine stocks was supplied by a digenean parasite biotag (van der Lingen *et al.*, 2015; Weston *et al.*, 2015).

Using parasites as biotags for stock discrimination has been shown to be reliable with an overall probability of 71% success in fish stock delineation (Poulin and Kamiya, 2013). Caution should, however, be exercised when using this method (Lester and Mackenzie, 2009): a holistic approach should be implemented (Sindermann, 1983; Mackenzie and Abaunza, 1998), and the effect of age influencing the intensity of infection should be eliminated by only comparing fish from similar age classes (MacKenzie and Abaunza, 1998).

5.1.3. Parasites as biotag

Multiple explanations could be offered to support the findings of a biological tag research study (MacKenzie and Abaunza, 1998). Some general points to take into consideration regarding the host-parasite relationship, are listed below:

1. Marine organisms commonly host generalist parasites. In marine food webs, generalist parasites can infect a wide variety of host species, spread through several paths, and are often found even in cases where host distribution is irregular (Marcogliese, 1995, 2002).
2. Even if limited knowledge of the life cycle of a particular species of tag parasite is known, information can be inferred from related species as it is likely to be broadly similar (Mackenzie and Abaunza, 1998).
3. According to MacKenzie and Abaunza (1998), a decrease or increase in the level of infection in a particular area could be explained by the reasons listed in Table 5.1.

Table 5.1. Reasons for decrease or increase in the level of infection by a parasite on a host in a particular area (**Source**: adapted from MacKenzie and Abaunza, 1998)

Decrease	Increase
Selective mortality of heavily infected hosts	Immigration of another host population with a higher level of infection
Immigration of another host population with a lower level of infection	Emigration of the least infected component of the original population
Emigration of the most heavily infected component of the original population	
A combination of two or more of these	

5.1.4. Characteristics of an ideal biotag

The characteristics of an ideal biotag include (Sinderman, 1961; Kabata 1963; Sinderman, 1983; Leaman and Kabata, 1987; MacKenzie, 1987; Mackenzie & Abaunza, 1998):

- ✓ Parasites should be long-lived in and have a minimum effect on the host.
- ✓ Parasites should be easily identifiable and preferably site-specific.
- ✓ Endoparasites should be used since ectoparasites may fall off during capture.
- ✓ Parasite life cycle should be broadly understood.
- ✓ Temporal variability in infection patterns should be understood and accounted for

Significantly different levels of infection should be shown by the subject host in different parts of the study area. Differences are gauged using prevalence and/or mean intensity of infection between samples (Mackenzie and Abaunza, 1998). Some of the advantages and disadvantages of this method relative to artificial tagging, is summarised in Table 5. 2.

Table 5.2. Advantages and disadvantages regarding the use of parasites as biotags (**Source:** Mackenzie and Abaunza, 1998).

Advantages	Disadvantages
This method is more appropriate for use in deep-water species in which artificial tagging may prove difficult.	Inadequate information on the parasite and their ecology.
It is cost effective, compared to artificial tagging.	Marine parasites in many parts of the world are largely an understudied group, making identification difficult.
Minimal doubts concerning the possible abnormal behaviour of artificially tagged hosts.	Linking the age of the host and parasite is important but may not be possible since aging techniques of some invertebrates and fish have not been validated.

5.1.5. The purpose of this study

A table listing parasite species found in fish from the genus *Genypterus,* was

provided by Dr Ken Mackenzie, and can be found in Appendix D: Table D1. For the subject species, *Genypterus capensis,* Sibanda (2015) conducted a preliminary survey of the parasite assemblage and found 20 parasites. Of these, three potentially useful parasite biotags were identified by a discriminant function analysis, even though differences were not statistically significant enough to support a two-stock hypothesis for the fish species. The three parasites identified were: *Anisakis* sp., *Tentacularia* plerocercoid, and *Diphyllobothrium* sp. Additionally, *Lecithochirium* sp., a fourth parasite, was also considered as a potential biotag in the current study based on the qualities of an ideal biotag (Section 5.1.3). This endoparasite is site specific (found in stomach or intestine), easily identifiable, and its lifecycle is understood (Manter, 1954; Bray, 1991).

Aim

The current study aims to expand on the work of Sibanda (2015) by investigating the possibility of two stock structures in South African waters for *Genypterus capensis*, based on four potential parasite biotags, namely: *Diphyllobothrium* sp., *Tentacularia* sp. plerocercoid, and *Anisakis* sp., as well as *Lecithochirium* sp.

Objectives

- Determine whether there is a correlation between the level of parasite infestation with the total length, and sex, of the host fish.
- Assessing the three primary quantitative descriptors, namely prevalence, abundance, and mean intensity of parasites, for mature and immature fish samples from each of the two putative stocks.
- Utilizing univariate analysis to evaluate whether there are differences between the two hypothetical stocks for immature (Kruskal-Wallis) and mature (Mann-Whitney U) subsamples, for each of the four selected parasite biotags.
- using PCA as a multivariate technique to determine whether stock differences exist between WC and SC samples for immature and mature sample sets.

5.1.6. Parasite biotags used in this study

Images of the parasites examined, are shown in Figure 5.1., and classification up to genus level (Worms Editorial Board, 2021) of the potential biotags, as well as the site of infection in the host fish, are listed in Table 5.3.

Table 5.3. Classification up to genus level (Worms Editorial Board, 2021) of potential biotags for stock delineation of *Genypterus capensis*, along the WC and SC of South Africa. Site of infection is also included

Kingdom	Phylum	Class	Order	Family	Genus	Site of infection
Animalia	Nematoda	Chromadorea	Rhabditida	Anisakidae	*Anisakis* Dujardin, 1845	Visceral cavity
	Platyhelminthes	Trematoda	Plagiorchiida	Hemiuridae	*Lecithochirium genypteri* Manter,	Stomach, intestine
		Cestoda	Diphyllobothriidea	Diphyllobothriidae	*Diphyllobothrium* Cobbold, 1858	Stomach, intestine
			Trypanorhyncha	Tentaculariidae	*Tentacularia* Bosc, 1797	Stomach lining (Plerocercoids)

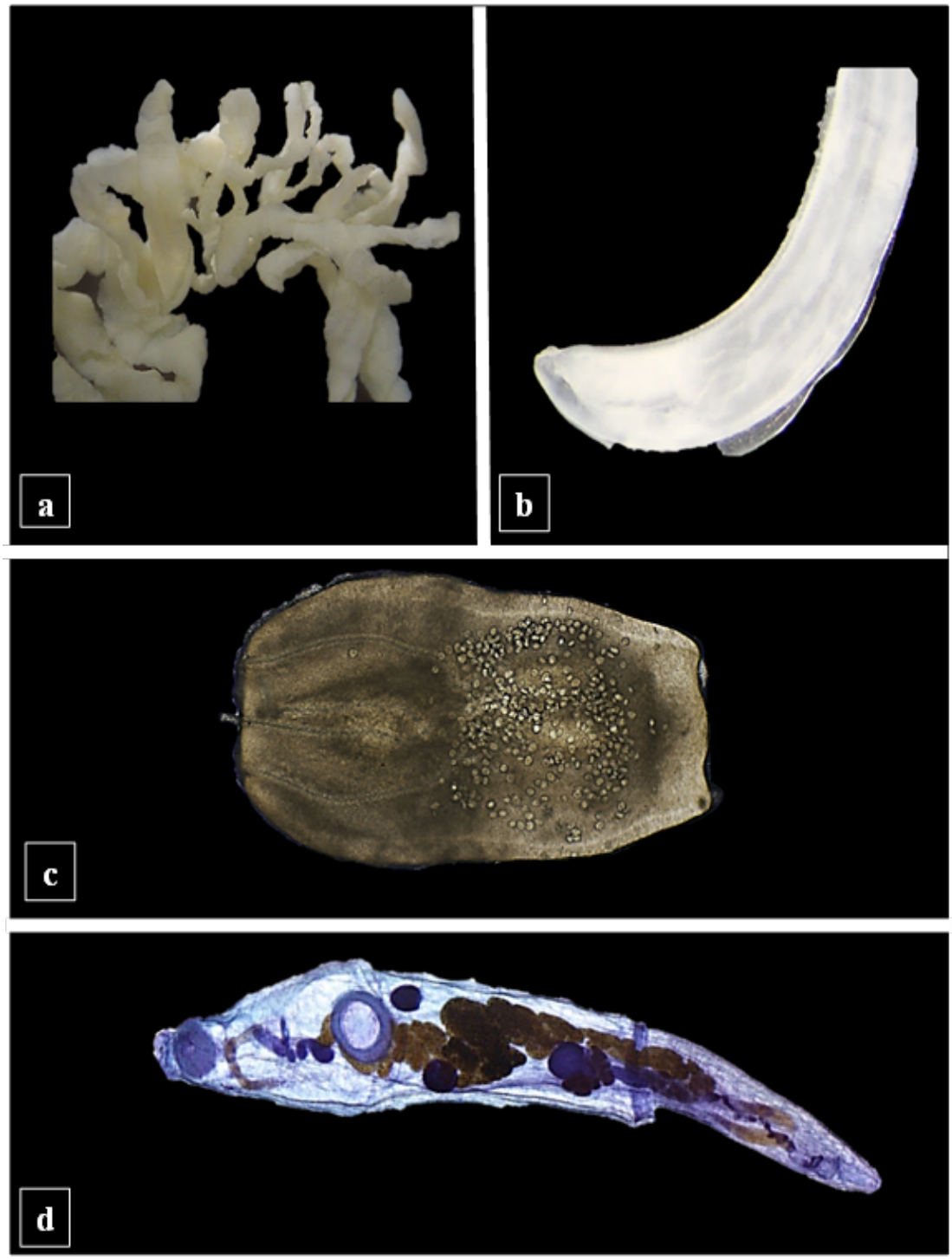


Figure 5.1. Images of parasites used in this study as bioindicators for the stock delineation of *Genypterus capensis*: **(a)** *Diphyllobothrium* sp., under the microscope (20 x magnification Leica DM750) **(b)** *Anisakis* sp. tail under the microscope (20 x magnification Leica DM750) **(c)** *Tentacularia* plerocercoid (40 x magnification Leica DM750); and **(d)** *Lecithochirium* sp.

Source: Images courtesy of Sizakele Sibanda and Dr Piet King.

5.2. MATERIALS AND METHODS

5.2.1. Sample location

All *Genypterus capensis* samples were collected during research surveys conducted by the Department of Forestry, Fisheries, and the Environment (DFFE) over a period of four years: 2014 – 2017 (as described in Chapter 2). The locations at which samples were caught are portrayed Figure 5.2.

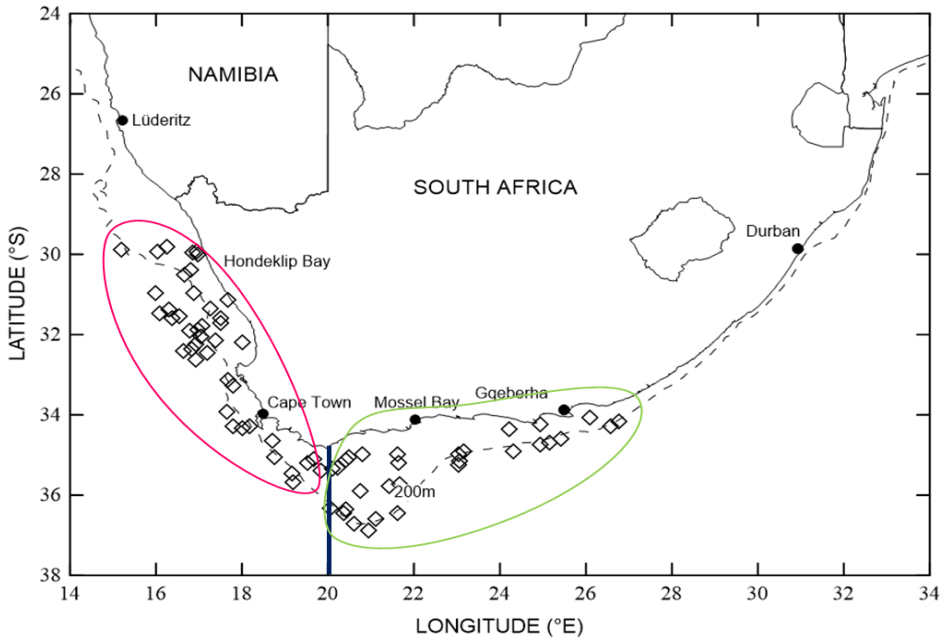


Figure 5.2. Locations where *Genypterus capensis* samples were collected during research surveys conducted by the Department of Forestry, Fisheries, and the Environment (DFFE) from 2014 to 2017. The 200m depth isobath is shown with the black dashed line. West coast (WC) samples are outlined in pink, and South coast (SC) samples are outlined in green. The boundary at Cape Agulhas (34.8163° S, 20.0152° E) is shown with a solid black line.

5.2.2. Extraction, collection, and identification of parasite biotags from *Genypterus capensis* specimens

Prior to dissection, each fish was thawed, measured, and weighed. After morphometric measurements, and otolith removal were completed (as per Chapters 2 and 3), the fish was cut longitudinally along the ventral end. The visceral area was scanned and probed to locate parasites, and the internal organs were removed. Each of these organs were placed into separate petri dishes. These organs were cut lengthwise with a dissecting scissor or blade, and contents were transferred into another petri dish. When necessary,

contents were gently scraped off the organ using a dissection blade and reverse osmosis water was used to dilute the innards, if necessary, for easier inspection.

The parasites were identified based on diagnostic characteristics typical of recognized parasite groups that infect fish belonging to the *Genypterus* genus. Using Sibanda (2015) as a guide, the identification process was carried out in conjunction prior literature available for *Genypterus* species which includes works by Payne (1986) for *G. capensis,* George-Nascimento *et al.* (2004) for *G. blacodes*, Alves *et al.* (2002) for *G. brasiliensis* and Minaya-Angoma *et al.* (2020) for *G. maculatus.* When additional support was needed for identification, the parasite sample was preserved in 70% ethanol and Dr Ken Mackenzie and/or Dr Piet King were consulted.

Parasites were found using the naked eye and by observing diagnostic characteristics of the parasite under Leica EZ34 dissecting microscope and/or Leica DM750 compound microscope (when necessary). *Tentacularia* sp. plerocercoids were found embedded in the fillets of the abdominal musculature. The entire tissue was removed and used in counts. *Diphyllobothrium* sp. and *Lecithochirium* sp. counts were discovered by looking at the stomach and intestinal contents in the Petri dish. A dissecting needle was used to investigate the tiny, tightly coiled, brownish cysts that were discovered clinging to the abdominal cavity's mesenteries or entrenched in the liver or gonad tissue in the visceral cavity. Payne (1986) had recognized these cysts as *Anisakis* sp.

5.2.3. Statistical analysis

Microsoft Excel Office[16] was used to create bar graphs and calculate mean, range, standard deviation, correlation coefficients, prevalence, abundance, and intensity (Bush *et al.,* 1997). IBM SPSS[26] was used to create frequency plots, and compute Mann-Whitney U tests and the Kruskal-Wallis test. PRIMER[6] was used to perform Principal Component Analysis (Statistical methods described in Section 2.2.4).

5.2.3.1. Quantitative descriptors of parasite populations

According to Baldwin *et al.* (2011), the following methods: (1) Community diversity - the total variation in the number of parasite species recovered (2) Prevalence - the percentage of fish infected (3) Abundance - the number of parasites per fish host, and (4) Intensity - the number of parasites per infected fish host (Bush *et al.,* 1997), have been used to discriminate between fish stocks. By examining parasite communities, host populations could be further subdivided over several spatial and time scales (MacKenzie and Abaunza, 1998; Lester, 1990; Nadler, 1995; Marcogliese *et al.,* 2003; McClelland *et al.,* 2005; Criscione *et al.,* 2006; Baldwin *et al.* 2012; Reed *et al.,* 2012; and van der Lingen *et al.,* 2015; Weston *et al.*, 2015).

The three main quantitative descriptors of parasite populations as described by Bush *et al.* (1997), was calculated:

1. **Prevalence** measures the number of hosts infected by one or more individuals of a parasite species or taxonomic group divided by the number of host individuals examined and is expressed as a percentage.

$$\textbf{\textit{Prevalence}} = \frac{[\textbf{\textit{No.of hosts infected}}]}{[\textbf{\textit{Total number.of hosts}}]} \; \textbf{\textit{x}}\; \mathbf{100}$$

   These statistics show the presence and/or absence of a parasite in the host.

2. **Mean intensity** measures the number of parasites of a species infecting a population of a host species.

$$\textbf{\textit{Mean intensity}} = \frac{[\textbf{\textit{Total No.of parasites species}}]}{[\textbf{\textit{Number of infected host population}}]}$$

3. **Mean abundance** measures the total number of individuals of a particular parasite species in a sample of the total number of the host species sample under study.

$$\textbf{\textit{Mean abundance}} = \frac{[\textbf{\textit{Total No.of parasites species}}]}{[\textbf{\textit{Total number of host ndividuals (infected+uninfected)}}]}$$

5.3. RESULTS

5.3.1. Effects of host total length (TL) on parasite assemblage

The number of fish from each coast at 10cm TL-intervals is shown in Figure 5.4.

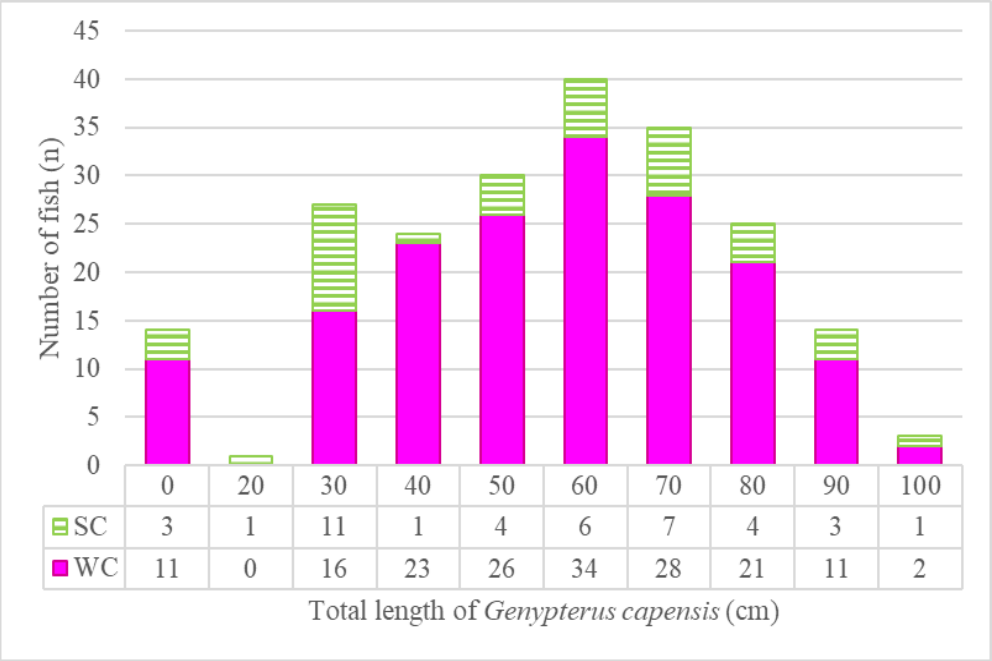


Figure 5.3. The total number (n) of *Genypterus capensis* samples per 10cm-TL intervals, used for this study, from the putative west coast (WC) and south coast (SC) stocks of South Africa, for the years 2014 – 2017.

To investigate the relationship between each parasite biotag and the TL of the host fish, *Genypterus capensis,* a correlation test was computed, and results are displayed in Table 5.4. With values closer to zero, there appears to be weak correlation between *Diphyllobothrium* sp., *Lecithochirium* sp. and *Tentacularia* sp. with the TL of the host, whilst a moderate correlation was portrayed by for *Anisakis* sp.

Table 5.4. Correlation test: association between the number of parasites, *Anisakis* sp., *Diphyllobothrium* sp., *Lecithochirium* sp., and *Tentacularia* sp., and the total length (TL) of the host fish, *Genypterus capensis*, from the putative west (WC) and south coast (SC) stocks, during the years 2014–2017.

Parasite	**ALL**	**WC**	**SC**
		Correlation coefficient (r)	
Anisakis sp.	0.486	0.471	0.586
Diphyllobothrium sp.	0.239	0.282	0.233
Lecithochirium sp.	0.024	0.070	0.068
Tentacularia sp.	0.164	---	0.303

Note: *(---) = no data available*

The average number of parasites (n) on fish at different host TL-intervals is shown in Figure 5.4. In Figure 5.4a, *Anisakis* sp. showed that when the TL of the fish host grew, the average number of infestations increased. On both coasts, the rate increases progressively with fish lengths between 60 and 100 cm with the average number being higher in SC hosts. In Figure 5.4b, the quantity of *Diphyllobothrium* sp. varied by coast for fish of varying lengths. For SC specimens, this parasite was not found in fish with a total length less than 50cm (TL < 50 cm). Comparing SC specimens to other length intervals, the average was greater at the 80cm length interval, whereas WC specimens were more numerous at the other length intervals. Fish reaching 90 cm exhibited the highest concentration of *Tentacularia* sp., which were exclusively found in SC samples (Figure 5.4c). *Lecithochirium* sp. showed having varying counts for fish of different lengths (Figure 5.4d).

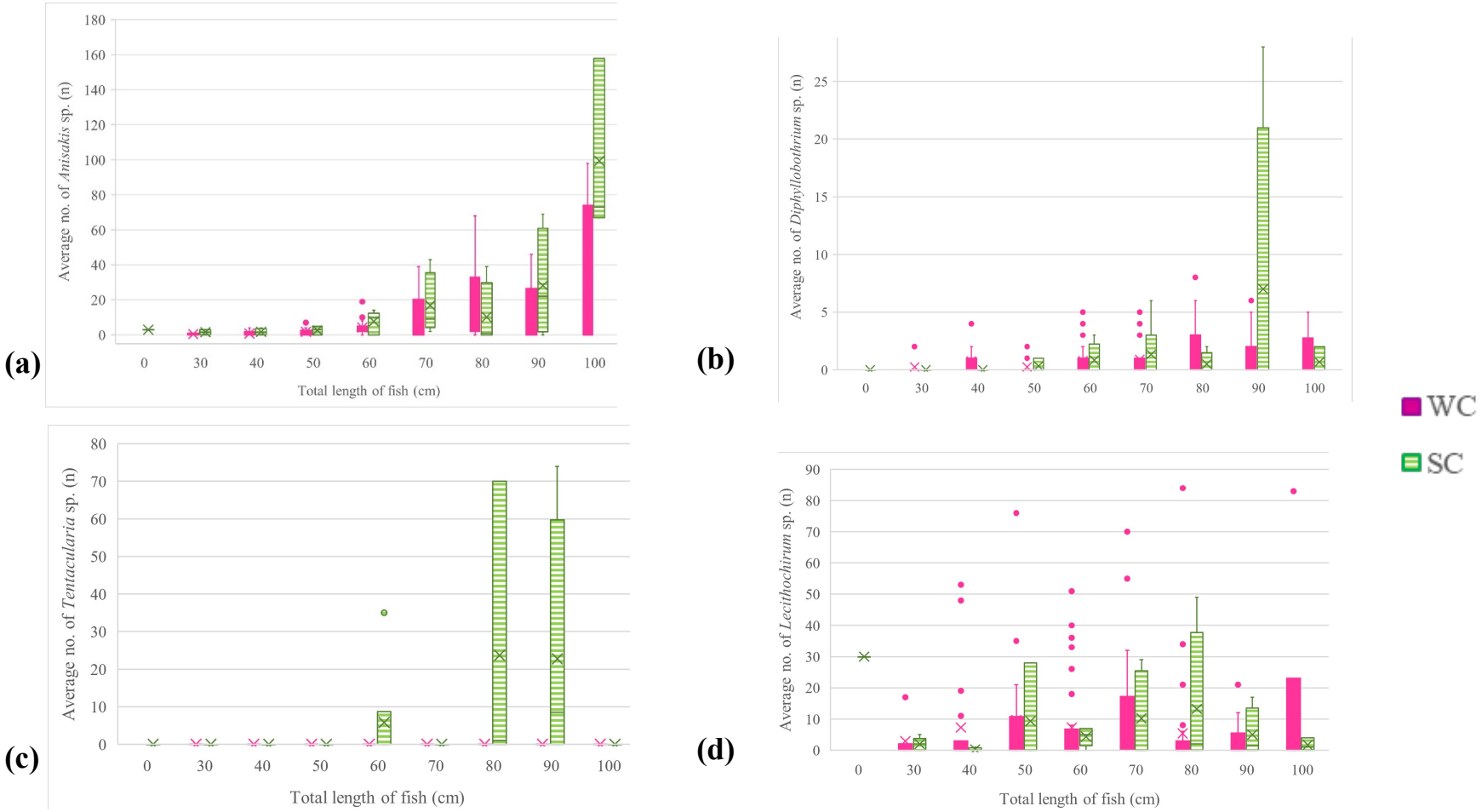


Figure 5.4. Bar graphs portraying the average ± SD number of parasite **(a)** *Anisakis* sp. **(b)** *Diphyllobothrium* sp. **(c)** *Tentacularia* sp. **(d)** *Lecithochirium* sp., at different total lengths (TL) of the host *Genypterus capensis*, along the west (WC) and south coast (SC) of South Africa, for the years 2014 – 2017.

5.3.1. Effects of sex on parasite assemblage

The total number of males, female and juvenile fish used in this study is shown in Figure 5.5.

Figure 5.5. The total number (n) of male, female, and juvenile *Genypterus capensis* samples used in this study, from the putative west coast (WC) and south coast (SC) stocks of South Africa, for the years 2014 – 2017.

A correlation test was conducted to assess the association between each biotag with the sex of the host fish. The results indicate a weak relationship, as displayed in Table 5.5. Additionally, an ANOVA was performed to assess variations in parasite counts between the sexes of the host species. Table 5.6 displays a summary of the p-values that were obtained. The complete Table showing results is included in Appendix D: Table D2.

Table 5.5. Correlation test coefficients (r): relationship between parasite biotags with the sex of the host fish, *Genypterus capensis*, from the putative west (WC) and south coast (SC) stocks, during the years 2014–2017.

Parasite biotag	**ALL**	**WC**	**SC**
		Correlation coefficient (r)	
Anisakis sp.	0.112	0.095	0.078
Diphyllobothrium sp.	0.019	-0.119	0.210
Lecithochirium sp.	0.025	0.097	-0.195
Tentacularia sp.	-0.06	---	-0.244

Note: *(---) = no data available*

Table 5.6. ANOVA: p-values indicating differences for parasite biotags in male and female *Genypterus capensis* hosts from the putative western and southern coast of South Africa, for the years 2014 – 2017.

	COAST	COMBINED	WC	SC
Parasite biotag			**p-value**	
Anisakis sp.		0.068	0.268	0.445
Diphyllobothrium sp.		0.086	0.167	0.318
Lecithochirium sp.		0.785	0.260	0.277
Tentacularia sp.		0.424	---	0.176

As no significant differences ($p > 0.05$) were found between sexes, per coast, males and females were pooled together for further analysis.

5.3.2. Quantitative descriptors of parasite populations

The prevalence (Prev %), mean intensity (MI), and mean abundance (Abund.) of the four selected parasite biotags for IMM (Table 5.7) and MAT (Table 5.8) *Genypterus capensis* was computed.

Overall, *Anisakis* sp. was the most prevalent, and had the highest mean intensity in both MAT and IMM samples from both coasts. *Lecithochirium* sp. was the most abundant species in in MAT (16.851) samples and IMM (32.823) fish samples was, although *Anisakis* sp. (32.529) showed a nearly equal abundance in IMM samples.

Table 5.7. Table showing the prevalence, mean intensity, and abundance of *Anisakis* sp., *Diphyllobothrium* sp., *Lecithochirium* sp. and *Tentacularia* sp., recorded for immature samples of *Genypterus capensis* (n = 117) from the putative west (WC) And south (SC) coast stocks Of South Africa for the years 2014 2017.

	WC (n = 94)			**SC (n = 23)**		
	Prev (%)	**MI**	**Abund.**	**Prev (%)**	**MI**	**Abund.**
Anisakis sp.	69.14	3.446	16.244	78.26	13.00	32.529
Diphyllobothrium sp.	34.04	1.750	1.449	8.69	2.500	2.353
Lecithochirium sp.	46.80	36.000	10.436	56.52	7.769	32.824
Tentacularia sp.	---	---	---	4.34	35.000	10.125

Note: *Prev (%) = percentage prevalence of infection; MI = mean intensity of infection; Abund. = Abundance*

TABLE 5.8. Table showing the prevalence, mean intensity, and abundance of *Anisakis* sp., *Diphyllobothrium* sp., *Lecithochirium* sp. and *Tentacularia* sp., recorded for mature samples of *Genypterus capensis* (n = 95) from the putative west (WC) and south coast (SC) stocks of South African for the years 2014 2017.

	WC (n = 45)			SC (n = 17)		
	Prev (%)	**MI**	**Abund.**	**Prev (%)**	**MI**	**Abund.**
Anisakis sp.	70.51	23.036	2.383	82.35	39.500	10.174
Diphyllobothrium sp.	47.43	3.054	0.596	29.41	8.000	0.217
Lecithochirium sp.	52.56	19.854	16.851	52.94	62.000	4.391
Tentacularia sp.	---	---	---	25.00	40.500	1.522

Note: *Prev (%) = percentage prevalence of infection; MI = mean intensity of infection; Abund. = Abundance*

5.3.3. Univariate analysis

To indicate differences in the selected parastite biotags of *Genypterus capensis* for the two putative stocks, a Mann-Whitney U test was employed for MAT samples, and, due to small sample size, an Independent-data Kruskal-Wallis Test Summary was employed for IMM data. A summary of p-values from these tests is shown in Table 5.9. The complete tables are listed in Appendix D: Tables D3 – D4.

The Independent-Samples Kruskal-Wallis Test Summary indicated no significant differences for: *Anisakis* sp. ($H_{(1)}$ = 2.369, p = 0.124), *Diphyllobothrium* sp. ($H_{(1)}$ = 3.688, p = 0.055), and *Lecithochirium* sp. ($H_{(1)}$ = 0.019, p = 0.891). Significant differences were found in *Tentacularia* sp. ($H_{(1)}$ = 3.792, p < 0.000).

For MAT specimens, the results of the Mann-Whitney U test revealed no significant differences (p > 0.05) for *Anisakis* sp. (U = 502.5, p = 0.115) and *Diphyllopbothrium* sp. *(U* = 764.5*, p* = 0.277) *and Lecithochirium* sp. (U = 638.0, p = 0.797). Significant differences were found in *Tentacularia* sp. (U = 330.0, p < 0.000).

Table 5.9. Summary of p-values from tests used to indicate differences shown by potential parasite biotags of *Genypterus capensis* specimens from the putative western (WC) and southern (SC) stocks off the coast of South Africa for the years 2014 – 2017.

Sample set	**Immature**	**Mature**
Test used	**Kruskal-Wallis**	**Mann-Whitney U**
Parasite	**p-value**	
Anisakis sp.	0.124	0.115
Diphyllobothrium sp.	0.055	0.277
Lecithochirium sp.	0.891	0.797
Tentacularia sp.	**≤0.000**	**≤0.000**

5.3.4. Multivariate analysis

For multivariate analysis, PCA's were computed for IMM and MAT *Genypterus capensis* samples sets.

In the case of IMM samples, it was shown that the first two principal components (PC1 = 29.6%, PC2 = 26.1%) accounted for 55.7% of the total variation and had eigenvalues greater than 1. The first two principal components of MAT samples were found to have eigenvalues larger than 1, and together (PC1 = 33.1%, PC2 = 25.3%) they accounted 58.4% of the variation in the sample. These results are shown in Table 5.10.

Table 5.10. Eigenvalues of Principal Components and the percentage variation explained for potential parasite biotags of *Genypterus capensis* samples from the putative west coast and south coast stocks along the South African coast, for the years 2014 - 2017.

Sample set		**PC1**	**PC2**
Immature	Eigenvalues	**1.18**	**1.05**
	%Variation	29.6	26.1
	Cum.%Variation	29.6	55.7
Mature	Eigenvalues	**1.32**	**1.01**
	%Variation	33.1	25.3
	Cum.%Variation	33.1	58.4

For IMM samples, when looking at the correlation between these PC's and

the original variables, PC1 was found to be strongly correlated with three of the original variables, namely *Diphyllobothrium* sp. (-0.65), *Lecithochirium* sp. (0.51) and *Tentacularia* sp. (-0.52). *Anisakis* sp. (0.805) was found to have a strong correlation in PC2. This indicates that these variables vary together. In MAT samples, when looking at the correlation between these PC's and the original variables, PC1 was found to be strongly correlated with two of the original variables namely: *Anisakis* sp. (-0.508) and *Lecithochirium* sp. (-0.594). PC2 was found to be strongly correlated with *Diphyllobothrium* sp. (0.621) and *Tentacularia* sp. (-0.568). This indicates that these variables vary together. These results are shown in Table 5.11.

The PCA plots (Figure 5.8) for both IMM and MAT sample sets showed extensive overlap between individuals from the two coasts indicating that there is no distinction between the two putative stocks.

Table 5.11. Correlation between Principal Components and original variables explained for potential parasite biotags of *Genypterus capensis* samples from the putative west coast and south coast stocks along the South African coast, for the years 2014 - 2017.

Sample set	Variable	PC1	PC2
Immature	*Anisakis* sp.	0.23	**0.805**
	Diphyllobothrium sp.	**-0.65**	0.409
	Lecithochirium sp.	**0.51**	-0.217
	Tentacularia sp.	**-0.52**	-0.371
Mature	*Anisakis* sp.	**-0.508**	0.45
	Diphyllobothrium sp.	0.463	**0.621**
	Lecithochirium sp.	**-0.594**	-0.299
	Tentacularia sp.	0.417	**-0.568**

Note: *Variables shown to have a strong (> 0.5) correlation are highlighted in bold.*

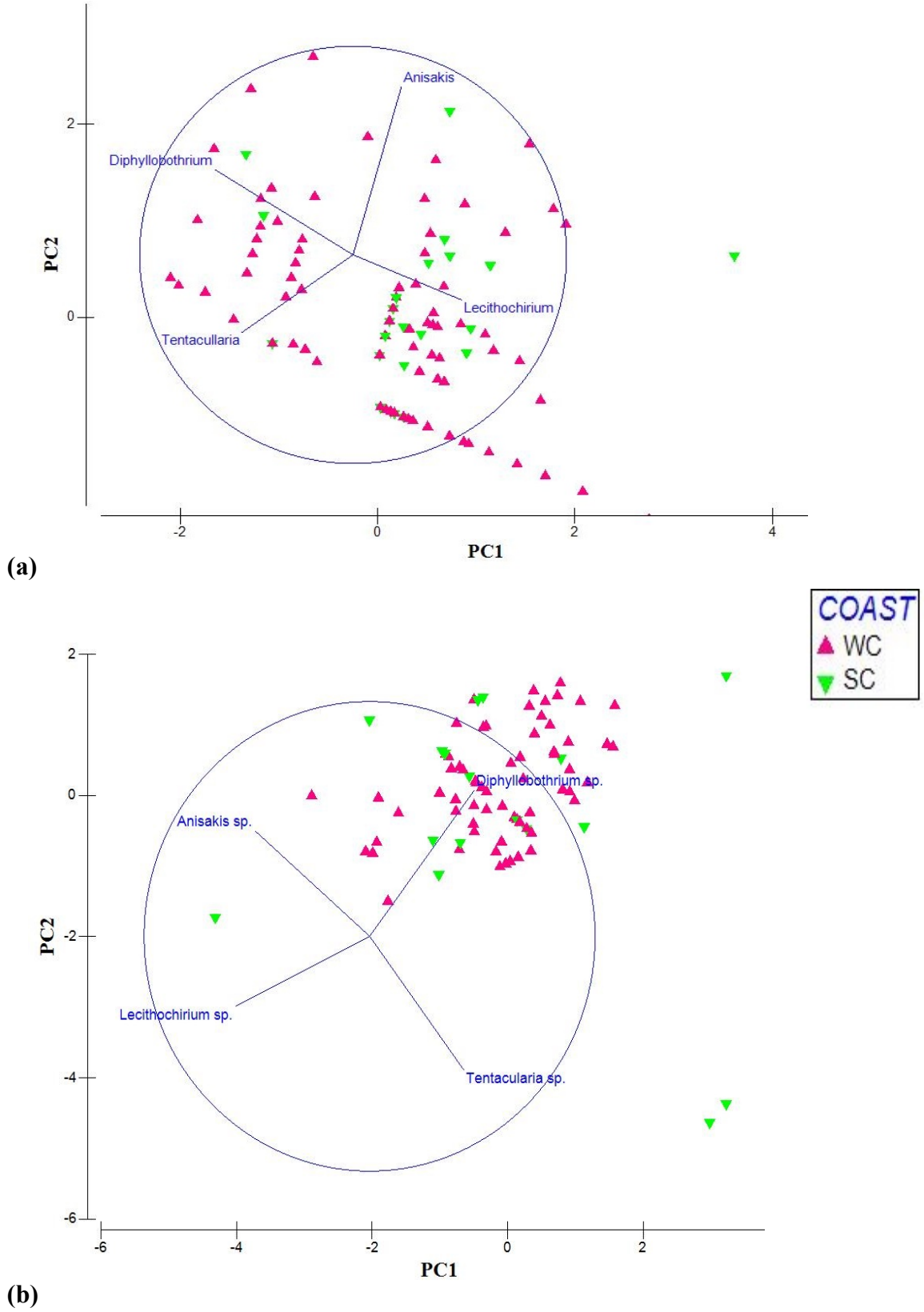


Figure 5.6. PCA plot depicting differences shown by potential parasite biotags (*Anisakis* sp., *Diphyllobothrium* sp., *Lecithochirium* sp. and *Tentacularia* sp.) of **(a)** immature and **(b)** mature *Genypterus capensis* samples from the putative west coast (WC) and south coast (SC) stocks along the South African coast, for the years 2014 – 2017.

5.4. DISCUSSION

Because there is a chance that sex-related variations in infection levels exist, Mackenzie and Abaunza (1998) recommended that differences between male and female fish be considered. As was the case with *Genypterus blacodes* (Alves *et al.,* 2002), where the host sex did not alter parasite prevalence or mean abundance, no significant variations in parasite accumulation were found between male and female *G. capensis* in the current study.

It has been found that the level of infection of parasites generally changes with the hosts age (Rhode, 1984), and correlation between host length and the occurrence of parasites has been extensively noted in marine and freshwater fish (Luque *et al.,* 1996; Alves and Luque, 2001; Al-Zubaidy, 2007).

In the current study, *Anisakis* sp. was the only one of the four chosen biotags to exhibit a moderate correlation with the host fish's TL (Table 5.4) and to show an increase in the average number of infestations as the TL of the fish host increases (Figure 5.4). These findings are consistent with those reported by Payne (1986). *Anisakis simplex sensu stricto* was promoted by Cross *et al.* (2007) as a useful biological tag. Since they are one of the most prevalent and widespread parasites of teleost fish (Mackenzie and Abaunza, 1998), larval anisakid nematodes are commonly used as biotags. One of the parasite's beneficial characteristics is its year-round temporal availability, which allows it to survive for extended periods of time in a particular region.

According to MacKenzie and Abaunza (1998), observed parasite frequency distributions can reveal details about the dynamics of the parasite population and offer evidence for the varying degrees of infection in various host age groups. *Anisakis* sp. was found in ±70% of WC samples and ±80% of SC, with a mean intensity higher on the SC, but not enough to suggest scientifically significant differences in univariate analysis (Tables 5.7 – 5.9).Even though *Diphyllobothrium* sp. exhibited a high average in the 90 – 100 cm class length, for SC specimens, this did not translate into a significant correlation (Figure 5.3; Table 5.4). Additionally, univariate analysis revealed

that there were no significant differences for immature samples ($p = 0.055$), even though WC samples had a five-fold higher prevalence.

Fish gut parasite communities are largely influenced by the diet of the fish. (Dogiel, 1961). Based on research by Beukes (2020), IMM *G. capensis* (size class 0 – 50cm) consisted of Callionymiformes on the WC, and additionally Gadiformes and Pleuronectiformes on the SC. The same prey, which includes *Pterygosquilla armata capensis*, *Paracallionymus costatus*, *Cynoglossus zanzibarensis*, and Gadiformes, was found in MAT (size class 51cm and higher) for fish from both coasts. This may help to clarify why dietary choices had a limited effect on the results in which there was a lack of statistically significant variations in the quantification of the chosen parasite biotags.

Beukes (2020) also pointed out that the majority of *G. capensis* prey consisted of chordates, or fish, and arthropods, or crustaceans, which were found to be the most significant phyla consumed. It is likely that the prevalence of the parasites utilized as biotags in the current investigation is probably influenced by this.

Fish and decapod prey consumption was found to be the primary cause of *Anisakis* sp. in *G. capensis* by Payne (1986). Additionally, Ángeles-Hernández (2020) noted that cephalopods, shrimp, crabs, and marine fish are examples of intermediate hosts for *Anisakis* sp. For *Diphyllobothrium* sp., marine fish (in addition to freshwater and anadromous fish) serve as the second intermediate host, whilst copepod species, or crustaceans, serve as the first intermediate host (Wittner *et al.*, 2011). Regarding *Tentacularia* sp., Dupouy-Camet and Peduzzi (2014) observed that when carnivorous fish consume planktonic crustaceans, procercoids transform into plerocercoids. Even though parasite specimens have not been identified to species level in the current study, worldwide, *T. coryphaenae* has been reported as a plerocercoid from many species of teleost fish (Palm, 2004). A plerocercoid refers to the last larval form, the infective form, that is found in the second intermediate host of many Cestoda with aquatic life cycles (Combes, 2005). After migrating to the host's muscles or viscera, the plerocercoid can remain dormant for several years and continually excyst and re-encyst if an infected

fish is eaten by other predatory fish (paratenic hosts).

Digenean trematodes are transmitted to their final host through the predator-prey relationship and the diet of the host species appears to be the main factor affecting the parasite transmission (Al-Zubaidy, 2010). Digeneans (Hemiuridae) are commonly found inside the digestive tract of marine fish and *Lecithochirium* is the most common genus of this family (Shih *et al.,* 2004).

In the current study, *Tentacularia* sp. plerocercoid was found only in fish from the putative SC stock. Out of forty SC fish, five (or 12.5%) were found to be infected; four of these were mature individuals and one was an immature specimen that measured 55.5 cm. This resulted in a prevalence of 25% for MAT, and 4.34% for IMM samples. It could be inferred that this parasite species is exclusive to SC samples when based solely on data from the current study. Sibanda's (2015) investigation did, however, show that this parasite was present in WC samples, at a low prevalence (0.059%). The reason for the overdispersal of *Tentacularia* sp. plerocercoid in only some host fish but not others is unknown. One explanation for the biological role of parasite aggregation could be that it stabilizes the host-parasite relationship, among other things (May 1977). The parasite's life cycle would end upon the host's death but the host population would not necessarily be significantly impacted. Kennedy (1975). Parasite aggregation is considered one of the “laws” in parasite ecology whereby a few hosts harbouring many parasites, in contrast to most hosts having a low number (Rabajante *et al.,* 2019).

5.5. CONCLUSION

A study by Sibanda (2015) led to the selection of three parasites (*Anisakis* sp., *Diphyllobothrium* sp., *Tentacularia* sp.) as potential biotags for the stock delineation of *Genypterus capensis* off the coast of South Africa. The current study formed an extension of that and further included *Lecithochirium* sp. as a fourth biotag as it embodies the characteristics of an ideal biotag.

For *Anisakis* sp., *Diphyllobothrium* sp., and *Lecithochirium* sp., in both immature and mature sample sets, differences in prevalence, abundance, and mean intensity were found, but not enough to support statistical discrimination. The prevalence of *Tentacularia* sp. was low, hence the results should be interpreted cautiously. Furthermore, a large amount of overlap in PCA plots suggests the absence of stock differentiation.

The current study's use of the selected biotags did not allow for a reliable conclusion. It is advised that more research be done and that a bigger sample size, including fresh samples, be obtained seasonally so that biotags can be re-evaluated.

Chapter 6

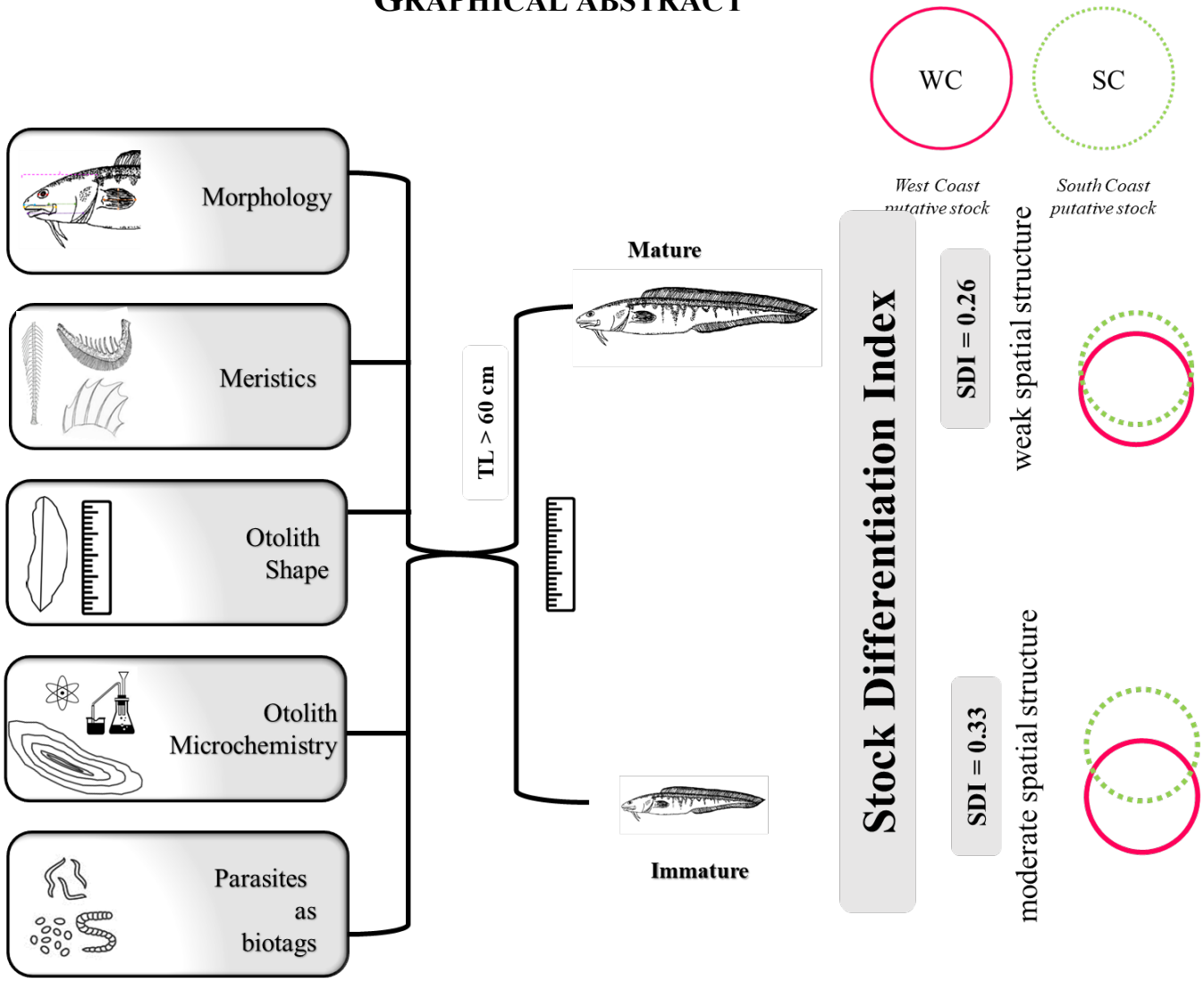
SYNTHESIS

"Individually, we are one drop.

Together, we are an ocean"

- Ryunosuke Satoro

GRAPHICAL ABSTRACT



6.1. INTRODUCTION

6.1.1. Overview

According to FAO (2023), roughly one-third of the world's assessed fisheries are under strain and globally, the number of overfished stocks has tripled. In fisheries management, policy frameworks depend on defined spatial/stock units to prevent overfishing of resources (Secor, 2014) and effective management systems can enhance sustainable utilization of renewable resources such as fisheries (Melnychuk, 2022). However, ineffective management and practices could lead to subunits or stocks with lower productivity being overharvested and may even lead to their collapse (e.g., Wooster, 1992; Hutchings, 2005; Cadrin, 2020). Furthermore, defining stock structure can be difficult (Cadrin, 2020, See Section 1.4.2) and the risks of unsustainable exploitation of marine resources are amplified by incongruities between populations and stocks (Fu and Fanning, 2004; Cadrin and Secor, 2009; Ying *et al.,* 2011; Goethel and Berger, 2017).

Secor (2014) and Punt (2019) stated that fish stocks are not necessarily genetically distinct as low levels of genetic mixing (through one or several past generations) are usually enough to obscure population structure, but with limited demographic consequences (Lowe and Allendorf, 2010; Marengo *et al.,* 2017; Punt, 2019). Historically, applying either genetic or non-genetic techniques in isolation formed the foundation of stock structure studies. This simple type of approach imposes substantial risk since as non-genetic techniques may not accurately reflect actual stock diversity, however, independent genetic techniques could produce conservative results. Hence, a combination of these techniques could be used to validate each other (Daley *et al.,* 2000).

Genetics is generally considered to be the more suitable technique to discriminate populations (Ward, 2000). Begg & Waldman (1999) stated that genetic differentiation should ideally form the basis for stock distinction, however, in some cases, low levels of gene flow between stocks may prevent the detection of differences in essentially discrete stocks. In this case, non-

genetic means of differentiation could then play a vital role in identifying populations.

Congruency between genetic and non-genetic techniques could facilitate increased confidence regarding stock structure, as was the case for *Genypterus blacodes* in Australian waters (Daley *et al.,* 2000). However, results could be divergent, as was the case for Eastern Pink Ling (*G. blacodes*) where commonwealth commercial catch rates had indicated the existence of different stocks east and west of South Cape (Tasmania), but no genetic differences between the areas were observed (Ward *et al.* 2001; Patterson *et al.* 2019; Chick and Lowry, 2020). For *G. blacodes* in New Zealand waters, life history parameters showed phenotypic stock differences, but lacked genetic differentiation (Horn, 2005), and a lack of congruency was also found between genetic and morphological indicators of stock structure for monkfish *Lophius vomerinus* (Leslie and Grant, 1990) off South Africa.

When this lack of congruency between methods is found, results from different stock identification methods must be compared and combined (e.g., Abaunza *et al.,* 2008; Tanner *et al.,* 2014; Reis-Santos *et al.,* 2015; Izzo *et al.,* 2017; Ulrich *et al.,* 2017; Reis-Santos *et al.,* 2018) and a precautionary management strategy should be advised. An understanding of the population structure of a fish species, the distribution of fishing effort and the fishing mortality rate, are all essential prerequisites for effective fishery management (Grimes *et al.,* 1987).

6.1.2. Overview of the stock structure of *Genypterus capensis*

South Africa has a wide range of marine fisheries and commercially, the demersal fishery is the most valuable. This fishery is mainly focused on *Merluccius capensis* and *M. paradoxus* (MSC, 2022), with additional catches of *Genypterus capensis, Lophius vomerinus, Austroglossus pectoralis,* and adult *Trachurus trachurus* (Griffiths *et al.,* 2010).

Historically, there was disagreement regarding the stock structure of *G. capensis* (see details in Table 1.8). Morphological, catch history and spawning data indicated both single- (Badenhorst, 1988; Japp, 1989), and

multi-stock structure (Isarev, 1976; Payne, 1977; Payne, 1984; Payne, 1985; Olivar and Sabates, 1989) depending on the study. For genetic data, research by Grant and Leslie (2005) using allozyme markers concluded the presence of a single genetic population of *G. capensis*. However, research by Henriquez *et al.* (2016) using mtDNA and microsatellites indicated the existence of at least two genetic units, and the study by Schulze *et al.* (2020) made use of the most up-to-date genetic markers (SNPs) and detected the presence of two separate stocks of *G. capensis* (West Coast and South Coast) with some degree of gene flow between the two components. An overview of genetic and genomic analysis is discussed in Section 1.4.3.2. of this thesis. The degree of demographic separation, however, remains undetermined, and this denotes that there is extensive connectivity across the range of the species, potentially facilitating a dynamic, panmictic system that is composed of mixed stocks (Schulze *et al.,* 2020; DEFF, 2020). Taking these results into account, this study aimed to resolve the existence of one or multiple stocks using a multi-method phenotypic approach.

As discussed in Section 1.4.3.5, a holistic approach is considered to be the best procedure for assessing the spatial structure of fish populations (Begg and Waldman, 1999; Cadrin *et al.,* 2014; Kerr *et al.,* 2017). However, a combination of tracers having various spatiotemporal resolutions does prove challenging (Tanner *et al.,* 2016). As portrayed in Figure 6.1., whereas otolith shape and otolith microchemistry show a degree of spatial (over 100's of kilometres) and temporal (portraying the individuals' lifespan) overlap these two methods do not overlap with genetic analysis in a temporal setting (Tanner *et al.,* 2015). The influence of genetics on the phenotypic characteristics examined in this study are listed in Table 6.1.

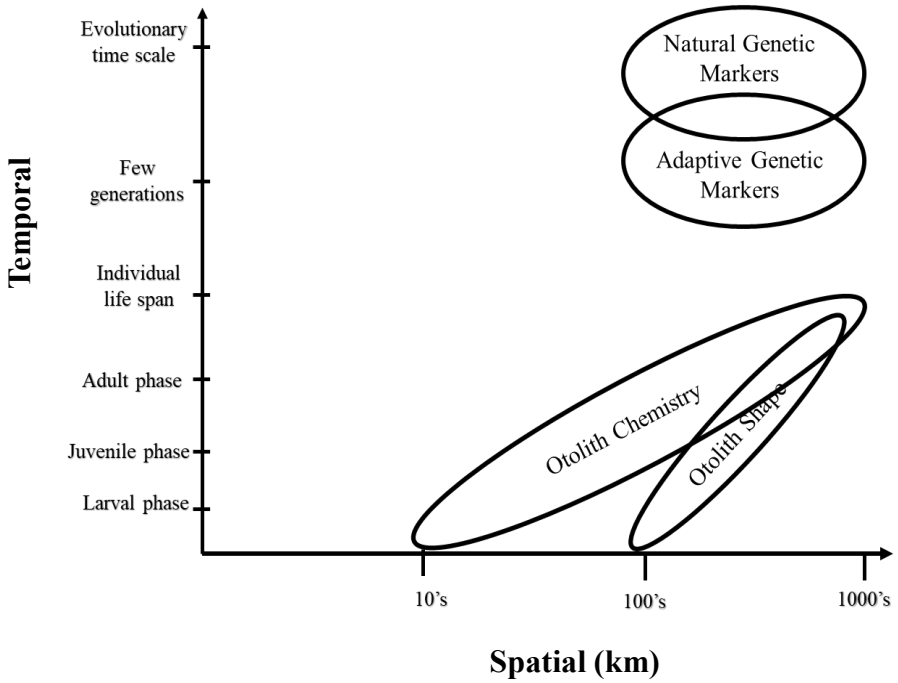


Figure 6.1. Graph summarizing spatio-temporal scope of different markers applicable in stock delineation studies, where the X-axis = spatial scale and the Y-axis = temporal scale ranging from individual life history stages to evolutionary time scales (**Source:** Adapted from Tanner *et al.,* 2015).

Table 6.1. The role of genetics in the phenotypic approaches used in this study

Approach	Influence	Reference
Morphology	Variations have a genetic basis determined by individuals during the post recruitment stages	Smith *et al.,* 2005
Meristics	Variations have a genetic basis determined during the larval and early juvenile stages	Smith *et al.,* 2005
Otolith shape analysis	An interplay of environmental, ontogenetic, and genetic influences, all have an influence on the resulting shape of the otolith	Ladroit *et al.,* 2017; Hussey *et al.,* 2016; Cardinale *et al.,* 2004
Otolith microchemistry	Unknown whether variations in elemental composition of otoliths resulting from environmental exposure correspond to genetic differences in hosts	Campana, 1999
Parasites as biotags	Mainly focused on the environmental influence regarding stock structure: Represent the residence and movements of fish in different environments throughout its lifetime	Welsch *et al.,* 2015

To determine the success of this phenotypic approach, the Stock Differentiation Index (SDI; Welch *et al.,* 2015; Izzo *et al.,* 2017), was employed. The output of the SDI, a discussion, as well as suggested directions for future research is deliberated upon.

6.2. MATERIALS AND METHODS

6.2.1. Applying the Stock Differentiation Index calculation (SDI)

The Stock Differentiation Index (SDI; Welch *et al.,* 2015; Izzo *et al.,* 2017) has been developed to easily combine tracers for stock structure with various spatiotemporal resolutions into a single framework. It is a semi-quantitative method used to delineate between putative stocks or different regions (Welch *et al.,* 2015).

Using univariate analysis from all the techniques employed in this study, a variable showing significant differences ($p < 0.05$) was assigned a value: DV = 1. On the contrary, a variable that did not show significant differences, was assigned a value of DV = 0. The pairwise SDI was then calculated as:

$$\mathbf{SDI = \Sigma\ DV\ /\ Count\ DV}$$

Where Σ DV corresponds to the sum of DVs for one pair of subunits and Count DV is the total number of tracers used.

The SDI ranges between 0 and 1 and measures the relative differences among subunits. A value of SDI = 0, indicates no spatial structure and indicates that the null hypothesis (H_0 = single stock) of a single stock must be retained (even if there is no clear evidence of a single stock). Conversely, a value of SDI = 1, indicates maximal spatial differences between subunits (Welch *et al.,* 2015). Expanding on this valuation, thresholds have been defined by Izzo *et al.* (2017) whereby the strength of the spatial segregation is assessed (Table 6. 2).

Table 6.2. Thresholds denoting spatial differences shown by the Stock Differentiation Index (**Source**: Interpreted from text in Izzo *et al.*, 2017)

Stock Differentiation Index **(SDI)**	**Evidence of spatial structure**	
0.99	**Strong**	**> 0.66** indicates strong evidence of stock spatial structure
0.91		
0.82		
0.74		
0.66	**Moderate**	**0.33 – 0.66** indicates moderate spatial structure
0.57		
0.49		
0.41		
0.33		
0.24	**Weak**	**> 0.33** indicates weak evidence of stock spatial structure
0.16		
0.07		
0.00		

6.3. RESULTS

6.3.1. Stock Differentiation Index

A moderate spatial structure was found for immature samples (SDI = 0.33), whilst mature samples showed a weak spatial structure (SDI = 0.26).

Table 6.3. Stock Differentiation Index (SDI) values for immature and mature samples of *Genypterus capensis* using a multi-method approach

Variable			Immature	Mature
Morphological measurements	Cephalic	PREO	0	0
		POSTO	0	0
		UJ	1	0
		LJ	1	0
		HL	0	0
		OD	1	0
		IOD	1	0
	Pectoral fin ray	PFL	0	0
		PFW	0	0
Meristic counts	Fin ray	FR	0	0
	Vertebral	V	0	0
	Total Gill Raker	GR	0	0
Otolith shape indices	Circularity	CIRC	1	0
	Form factor	FF	1	0
	Rectangularity	RECT	0	1
	Roundness	ROUND	1	1
	Ellipticity	ELL	1	1
	Aspect ratio	AR	1	1
Otolith Elemental Composition	Strontium	Sr/Ca	0	1
	Sodium	Na/Ca	0	0
	Potassium	K/Ca	0	0
	Magnesium	Mg/Ca	0	1
	Phosphorus	P/Ca	1	0
	Boron	B/Ca	0	1
	Manganese	Mn/Ca	0	0
	Iron	Fe/Ca	0	0
	Barium	Ba/Ca	1	1
	Rubidium	Rb/Ca	0	0
	Nickel	Ni/Ca	1	1
	Zinc	Zn/Ca	0	0
	Copper	Cu/Ca	0	0
	Lithium	Li/Ca	0	0
	Cobalt	Co/Ca	0	0
	Lead	Pb/Ca	0	0
	Tin	Sn/Ca	0	0
Parasite biotag counts	*Anisakis* sp.		0	0
	Diphyllobothrium sp.		0	0
	Lecithochirium sp.		0	0
	Tentacularia sp.		1	1
Stock Differentiation Index		***SDI***	**0.33**	**0.26**

6.4. DISCUSSION

These findings suggest that while some stock demarcation is seen in immature samples, the genetic differentiation of *Genypterus capensis* off the coast of South Africa does not transfer into significant and regionally varied phenotypic variation in mature specimens. This supports the theory that the two genomically distinct *G. capensis* stocks have distinct and localized spawning sites off the western and southern coastlines of South Africa. It also implies that mixing between the two stocks is lowest for juvenile fish and increases with age. The results of this outcome are reviewed and guidelines and recommendations for further research are discussed.

6.4.1. Review of information

Spatial structure and connectivity may play a critical role in in population- and metapopulation-level developments. It is therefore vital to develop tools to incorporate these phenomena into models, leading to better understanding of the local and regional population dynamics, and improved management decisions (Kerr, 2010).

In scientific studies, larval dispersal has been considered the chief driver in shaping the connectivity in marine species for a long time (Cowen and Sponaugle, 2009). This notion was however reassessed, and attention was drawn to the significance of adult-mediated connectivity (Frisk *et al.,* 2014). It is possible that connectivity occurs through entrainment during the juvenile/adult stage, in which individuals from one population move to another during periods of spatial overlap (McQuinn, 1997; Corten, 2002; Kerr, 2010). Instead of larval activity, the new paradigm focuses on population connectivity in which connectivity along the species entire life cycle is evaluated (Secor, 2015).

The bentho-pelagic life cycle (as discussed by Marshall and Morgan, 2011, Bierne *et al.,* 2016, and Putman *et al.,* 2019) is characteristic of this *Genypterus capensis*. As discussed in Section 1.2.5, this species has planktonic larval phase, moving to increasing depths with size before entering a benthic adult phase (Japp, 1989; Olivar and Sabatés, 1989; Japp and Hecht, 1990; Badenhorst and Smale, 1990; Japp, 1990; Shin *et al.,* 2004).

Using morphological characters, an allometric growth pattern was shown for the species (Table 2.3). Variables showing significant differences between stocks by immature specimens (PREO, LJ, OD, and IOD), differed to those shown in mature specimens (PFL and PFW). Allometric differences were also revealed by otolith shape analysis. A student's t-test indicated significant differences between stocks for the overall mean ratios of TL:OL, and TL:OB. However, no significant differences was found for the OL:OB relationship, indicating growth occurs at constant rate for each putative stocks (Section 3.4.3). Differences in otolith elemental composition was found for core *vs* edge and coreline (immature *vs* mature) segments of the otolith (Section 4.3.1).

Numerous dynamic processes operate in nearshore waters which as a result have more complex flows when compared to the coastal and deep ocean (Pineda *et al.,* 2007). A depiction of the South African coastline is described in Section 1.1., describing the presence of currents, a thermal and a saline front, and upwelling systems. When compared to the pelagic environment, the benthic area at depths of 300 – 550-m (at which mature *G. capensis* reside), is relatively stable. Differences in stock structure were more apparent in immature sample sets compared to mature sample sets. The relationship between genetics and the phenotypic approaches used in this study, is listed in Table 6.1. It appears that the volatile differences in pelagic environments, compared to steady conditions in benthic environments, allows genetic differences to be more apparent in immature samples when compared to mature samples. Japp and Hecht (1990) noted that early life history stages are likely to be significantly affected by changes in surface water compared to adult fish.

6.5. DIFFERENCES IN LENGTH-AT-MATURITY:

The otolith shape indices had the strongest discriminatory power of all characteristics examined in this study, and immature specimens showed more marked differences than mature ones. However, there have been incongruencies regarding the estimated length-at-maturity of *Genypterus capensis* (See Section 1.2.3). Based on research by Payne (1985), Japp (1989) and Japp and Hecht (1990) an average length-at-maturity of TL = 60 cm used for this study. However, one needs to take into consideration that the *G.capensis* resource was largely overexploited in the 1980's by longline fishing (see Section 1.3.1). When reviewing long term changes to demersal fish assemblages inshore on the Agulhas Bank, Currie *et al.* (2020) found that the state of these assemblages may have been affected by anthropological impacts e.g., fisheries, climate change, pollution, and river flow reduction. From 1986 to 2003, it appeared as if the Agulhas Bank demersal community showed an increase in diversity and evenness, but a decrease in fish size indices (Yemane *et al.,* 2008; Yemane *et al.,* 2010; Currie, 2020). Furthermore, Guerra (2019) indicated a reduction in the L_{50} values of *G. capensis* females compared to Japp (1989) and it was assumed this change was caused by fishing exploitation.

To assess the possible effects of a reduced L_{50}, two different length-at-maturity measurements was assumed (TL = 50 cm, and TL = 40 cm), and ANOVA, Mann-Whitney U test, and PCA were used to analyse shape indices data for these size classes. Results of the ANOVAs for L_{50} values of TL = 50 cm and TL = 40 cm showed significant differences for five (circularity, form factor, roundness, ellipticity, and aspect ratio) of the six shape indices tested (Table 6.4). These results are the same as when a L_{50} value of TL = 60 cm was used, and the stronger results (lower p-values) in the ANOVA shown for TL = 50 cm (compared to TL = 40) could be attributed to a larger sample size of the former.

Table 6.4. ANOVA and Mann-Whitney U test indicating differences in log-transformed shape indices of immature *Genypterus capensis* samples at different length-at-maturity values

Length at maturity		**50 cm**		**40 cm**	
Variable	**Test**	**p-value**			
		ANOVA	**MWU**	**ANOVA**	**MWU**
Circularity	Log(CIRC)	**<0.001**	**<0.001**	**0.003**	**0.003**
Form factor	Log(FF)	**<0.001**	**<0.001**	**0.003**	**0.003**
Rectangularity	Log(RECT)	0.932	0.834	0.741	0.634
Roundness	Log(ROUND)	**0.002**	**0.002**	**0.049**	**0.038**
Ellipticity	Log(ELL)	**<0.001**	**<0.001**	**0.021**	**0.029**
Aspect ratio	Log(AR)	**<0.001**	**<0.001**	**0.022**	**0.029**

Note: *ANOVA – Analysis of Variance, MWU – Mann-Whitney U test*

If TL = 50 cm is considered the length-at-maturity, the first three PC's showed eigenvalues greater than 1. The first two PC's (PC1 = 46.6% and PC2 = 32.1%) accounted for 78.7% of the total variation (Table 6.5). As can be seen in Table 6.6, PC1 was found to be strongly correlated to roundness, ellipticity and aspect ratio [Log(ROUND) = 0.516; Log(ELL) = -0.558; Log(AR) = -0.557] and PC2 showed a strong correlation to circularity and form factor [Log(CIRC) = -0.654 and Log (FF) = 0.653].

If TL = 40 cm is considered the length-at-maturity, the first three PC's again showed eigenvalues greater than 1. The first two PC's (PC1 = 47.5% and PC2 = 32.5%) accounted for 80% of the total variation (Table 6.5). As can be seen in Table 6.6, PC1 was found to be strongly correlated to roundness, ellipticity and aspect ratio [Log(ROUND) = 0.511; Log(ELL) = -0.545; Log(AR) = -0.545] and PC2 showed a strong correlation to circularity and form factor [Log(CIRC) = -0.612 and Log (FF) = 0.612]. Relatively low overlap between individuals from the two coasts on the PCA plots (Figure 6.2) provides evidence of stock delineation.

Table 6.5. Eigenvalues of Principal Components and the % variation explained for log-transformed shape indices of *Genypterus capensis* samples from the putative west (WC) and south (SC) stocks, for the years 2008, 2009, 2011, and 2014 – 2017, at different potential length-at-maturity values.

Assumed length-at-maturity		PC1	PC2	PC3
TL = 50 cm	Eigenvalues	**2.79**	**1.92**	**1.28**
	%Variation	46.6	32.1	21.3
	Cum.%Variation	46.6	78.7	100
TL = 40 cm	Eigenvalues	**2.85**	**1.95**	**1.2**
	%Variation	47.5	32.5	20
	Cum.%Variation	47.5	80	100

Table 6.6. Correlation between Principal Components and original variables explained for log-transformed shape indices of *Genypterus capensis* samples from the putative west and south stocks, for the years 2008, 2009, 2011, and 2014 – 2017, at different potential length-at-maturity values.

Assumed length-at-maturity	Variable	Log (Variable)	PC1	PC2	PC3
Total length (TL) = 50 cm	Circularity	CIRC	0.235	**-0.654**	0.131
	Form factor	FF	-0.237	**0.653**	-0.131
	Rectangularity	RECT	0.006	-0.170	**-0.859**
	Roundness	ROUND	**0.516**	0.101	-0.429
	Ellipticity	ELL	**-0.558**	-0.231	-0.144
	Aspect ratio	AR	**-0.557**	-0.232	-0.151
Total length (TL) = 40 cm	Circularity	CIRC	0.268	**-0.612**	0.231
	Form factor	FF	-0.269	**0.612**	-0.228
	Rectangularity	RECT	-0.017	-0.314	**-0.820**
	Roundness	ROUND	**0.511**	0.053	-0.455
	Ellipticity	ELL	**-0.545**	-0.273	**-0.084**
	Aspect ratio	AR	**-0.545**	-0.272	-0.091

Note: *Variables shown to have a strong (> 0.5) correlation are highlighted in bold.*

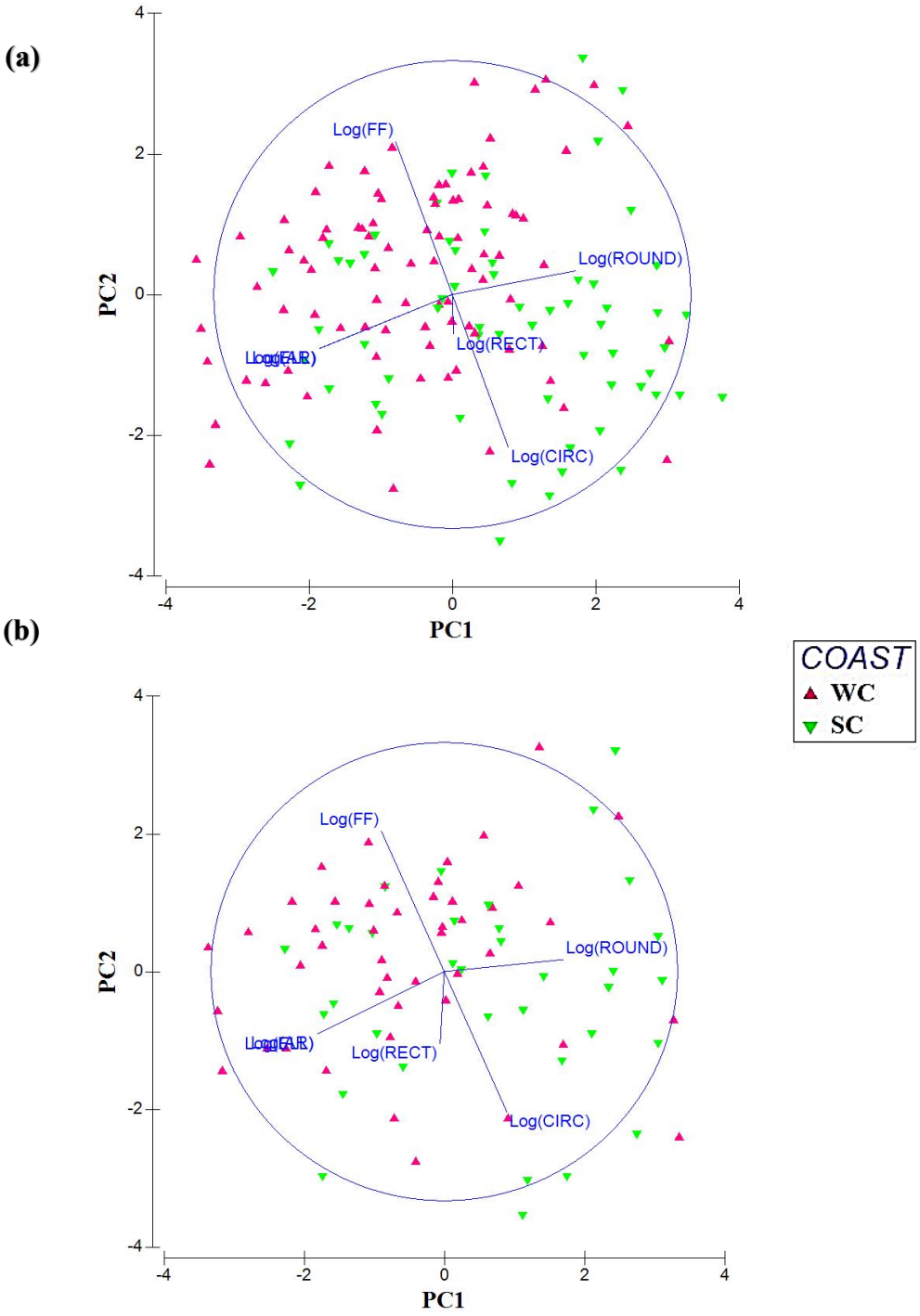


Figure 6.2. PCA plots using log-transformed shape indices for **(a)** Total length (TL) = 40 cm **(b)** Total length (TL) = 40 cm, for immature *Genypterus capensis* otoliths from the putative west (WC) and south (SC) stocks, for the years 2008, 2009, 2011, and 2014 - 2017.

6.6. RECOMMENDATIONS FOR FUTURE RESEARCH:

6.6.1. Evaluation of otolith annuli for stock discrimination

Given the ability of otolith shape indices to differentiate fish from the putative west and south coast stocks, analyses of otolith shape at an early stage inferred via measurement of the shape of the first otolith annulus (laid down when a fish is 1-year old) may be a useful phenotypic characteristic for stock identification. The clarity and precision of annual increments of otoliths have been observed in both freshwater (*Aplodinotus grunniens*; Pereira *et al.,* 1995), and marine (e.g., *Pogonias cromis*; Campana and Jones, 1998) fish. In some cases, based on otolith annual rings, age approximations of over 100 years have been documented for many deep-water fishes (Campana and Thorrold, 1998).

In fish having distinct otolith annuli, a trace of the area bounded by the first annual ring would match the shape of the otolith when annulus formation is initiated. As a result, shape parameters produced from this internal trace could be used in intraspecific analyses to examine population structure at a very early age, and size, which is not commonly collected from either commercial or research trawls. This is since, as illustrated in Figure 6.3, environmental dynamics e.g., sea temperature and food availability, has been linked to spatial differences in fish growth rates. Since there is a proportional relationship between otolith growth and somatic growth, variations in growth rates will result in a corresponding variation in otolith shape and microstructure (Gauldie and Nelson, 1990; Munk *et al.,* 1991; Campana and Casselman, 1993; Gallego *et al.,* 1999; Bailey and Heath, 2001; Fox *et al.,* 2003; and Campana and Neilson, 1985 in Burke and King, 2007).

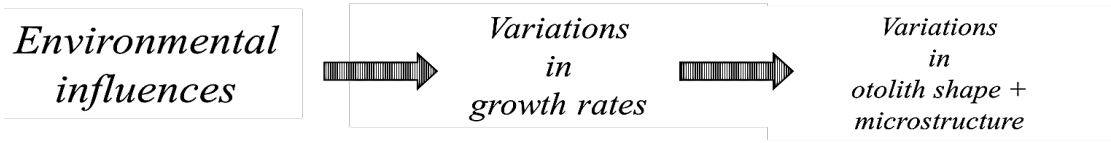


Figure 6.3. Environmental influences on otolith structure (**Source**: interpreted from text in Burke and King, 2007)

Thus, assemblages of fish that sustain distinct distributions for either a portion, or their entire lives, can be distinguished by:

- assessing the microstructure analysis of daily increments,
- evaluating the internal distances between the nuclei and annual rings and/or
- estimating the shape of the otolith at an early age using the 1st annulus (Turan, 2000; Begg *et al.,* 2001; DeVries *et al.,* 2002; Cardinale *et al.,* 2004; Berg *et al.,* 2005; Stransky and MacLellan, 2005; and Suthers and Sundby, 1993 in Burke *et al.,* 2007)

Using this approach, Burke *et al.* (2007) were able to successfully distinguish between the migrant and resident components of the Celtic Sea winter-spawned herring (*Clupea harengus*) populations. Their accuracy success of 97% is proof that internal measurements of otoliths can provide convincing results and suggest that this technique for stock discrimination should be tested for *G, capensis*.

6.6.2. Larger study area

South Africa's Exclusive Economic Zone (EEZ) serves as both the outer limit and seaward boundary of all Marine Planning Areas. The four minor bio-geographic zones that make up South Africa's marine space are the Western Marine Planning Area, Southern Marine Planning Area, Eastern Marine Planning Area, and Prince Edward Islands (Government Gazette, RSA, 2019). In view of *Genypterus capensis* as a marine fishery resource, the challenge posed by DFFE (2016) was whether distinct stocks could be found on South Africa's west and south coasts, which are delimited by Cape Agulhas (34.8163° S, 20.0152° E). For the current study, this boundary line served as the split between the two stocks.

Phylogeographic breaks around Cape Point and Cape Agulhas on the south-west coast have been documented, coinciding with the biogeographic disjunction between cool-temperate and warm-temperate biota. The area bounded by these two locations is occasionally referred to as a transition zone.

Considering that the genetics study by Schulze *et al.* (2020) indicates that the 'degree of demographic separation is unknown', methods from this study, with genetic analysis (on the same specimens), could be expanded to a larger distribution to reflect a greater area of habitat occupied by *G. capensis* around the South African coast. Numerous species exhibit phylogeographic splits at both locations, exhibiting unique lineages that are exclusive to this transition zone. To determine where the spatial structure boundaries lie, the area will have to be appropriately delineated into smaller sub-regions for future research. Additionally, further studies could include comparisons with fish from the east coast of South Africa, as well as specimens from Namibia

6.6.3. Inclusion of fresh samples and seasonal variability

Sampling is often opportunistic since the species is (aptly) caught as incidental bycatch. Future studies should be done on a seasonal basis to include temporal variability, especially regarding parasitological analysis as the parasite assemblage may differ seasonally (Espínola-Novelo and Oliva, 2021). Fresh samples (that have not been frozen) should also be analysed to ensure a complete parasite collection is obtained. The parasite biotags used in this study was based on the parasite assemblage documented by Sibanda (2015) in which parasites were not classified to species level. Individual parasite species may appear morphologically identical, but are genetically distinct (Baldwin *et al.,* 2012), and future studies should therefore include both morphological and molecular characteristics for parasite identification and discrimination. Additionally, as suggested by Daley *et al.* (2000) for the con-generic *G. blacodes,* close involvement in the sampling process is an integral part of any stock structure study. The precise (not approximated) locality information must be recorded, and stomach contents must be obtained in good condition. It should be ensured that specimens are frozen and not chilled on ice as stomach contents my rot very rapidly if not frozen immediately (Daley *et al.* 2000).

The interannual division of the sample (n = 384) resulted in relatively small sample sizes, which hindered conclusions drawn from multivariate statistical

analysis. Lack of data is a common problem in fish morphometric investigations when using multivariate analyses. According to Kocovsky *et al.* (2009), researchers that conducted hypothetical assessments using PCA and DFA recommended a ratio of at least 3:3.5 between the number of organisms observed (n) and the variables included in the analysis. According to McGarrigal *et al.* (2000), using inadequate n values to calculate covariance can result in incorrect conclusions about group differences. It is therefore recommended to use a larger sample set in future for interannual and seasonal variation.

6.6.4. Comparison of otolith elemental fingerprints with those of other fish in trawl nets

The intraspecific incorporation of elements into the otolith is often a result of physiological factors whilst interspecific patterns are influenced by the environment. Interspecific differences in otolith elemental incorporation have been observed, which could be expected considering the extensive variation in physiology, biochemistry, and otolith morphology among fish species (Sturrock *et al.,* 2012). A comparative study with other species (e.g., *Merluccius* sp.*, Lophius vomerinus*) caught in the same trawls could provide a better understanding and reasoning for the results obtained from the elemental composition of *G.capensis* otoliths in this study. A study which combines otolith microchemistry with fatty acid profiles from fish has not yet been done (Tanner *et al.,* 2012) and this should be investigated. Possible expansion of this study could include this method of comparison.

6.7. CONSIDERATIONS ON THE EFFECTS OF CLIMATE CHANGE

Fish physiology is influenced by environmental factors, especially those related to water quality. Among these, temperature, pH, and dissolved oxygen (DO) are essential for maintaining fish homeostasis in marine environments, and variations in these parameters may cause the species to become stressed (Dettleff *et al.,* 2020).

In a study published in 2022, Dettleff *et al.* assessed many physiological and molecular biomarkers to examine the impact of heat stress in a *Genypterus* species for the first time. Ultimately, the findings demonstrated that heat stress produced a pertinent stress response in the fish, resulting in significant adverse impacts on the skeletal muscle. These included the development of molecular atrophy and oxidative damage to the red cusk-eel's DNA and lipids. The effects of climate change on marine species has also previously been reported for G*adus morhua* L. (Aursnes, 2011), *Horabagrus brachysoma* (Dalvi *et al.*, 2012), *Sardinops sagax* (van der Lingen *et al.,* 2016).

Angola, Namibia, and South Africa's socioeconomic development heavily depends on the fisheries resources of the Benguela Current Large Marine Ecosystem (BCLME). Their high level of environmental vulnerability highlights the significance of preserving an ecosystem that is resilient by making it more resistant to the effects of both natural and human-induced pressures (Kainge *et al.,* 2020). Given the average ten-year rise in sea temperature, it is imperative to comprehend how fish, respond to varying water temperatures in the real world of climate change (Dettleff *et al.,* 2020).

6.8. CONCLUDING REMARKS

These results indicate that the genomic differentiation of *Genypterus capensis* off the South African coast does not translate into substantial and spatially differentiated phenotypic variation in mature specimens, however moderate stock delineation is observed in immature samples. This supports the theory that the two genomically distinct *G. capensis* stocks have distinct and localised spawning sites off the West and South coastlines of South Africa and implies that mixing between the two stocks is lowest for juvenile fish and increases with age.

Thus, the results of this study suggest that there are two subpopulations, and it is advised that the one-stock management approach be reassessed in light of *G. capensis* commercial management in South African marine environments. In the future, it will be critical to coordinate this expanding knowledge of the species biological features to ensure that management units accurately represent population structure. It is recommended that fisheries management strategies should be adjusted to achieve optimal sustainable production from each stock, and to prevent declines in genetic diversity by using a precautionary approach and treating the two areas as separate stocks.

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APPENDIX A

Table A1. Latitude and longitude and number of Genypterus capensis (n) captured from stations in the years 2015 – 2017.

YEAR	STA	LAT	LONG	n	YEAR	STA	LAT	LONG	n
2015	*D00609*	*-36.342*	*20.409*	2	2016	*D00621*	*-34.64*	*18.714*	1
	D0519	*-35.865*	*21.228*	4		*D00783*	*-34.913*	*23.17*	1
	D00786	*-34.917*	*24.312*	2		*D00605*	*-35.051*	*18.761*	1
	D0494	*-36.328*	*20.066*	1		*D00806*	*-34.227*	*24.937*	2
	D0512	*-36.447*	*20.367*	1		*D00727*	*-33.132*	*17.673*	5
	D0557	*-34.073*	*26.097*	12		*D00762*	*-36.586*	*21.103*	2
	C0428	*-31.893*	*16.783*	9		*D00766*	*-35.734*	*21.666*	1
	D0543	*-35.256*	*23.036*	1		*D00782*	*-34.982*	*23.041*	1
	C0375	*-36.886*	*20.943*	1		*D00609*	*-36.342*	*20.409*	2
	D0572	*-34.745*	*24.941*	1		*D00849*	*-34.979*	*20.796*	1
	D0570	*-34.683*	*25.153*	2		*D00753*	*-36.724*	*20.613*	9
	D0488	*-35.061*	*20.499*	1		*D00792*	*-34.595*	*25.417*	1
	D00637	*-32.448*	*17.196*	5		*D00822*	*-35.159*	*23.052*	1
	C0400	*-33.918*	*17.654*	4		*D00826*	*-36.451*	*21.618*	1
	D00776	*-34.894*	*22.216*	5		*D00800*	*-34.274*	*26.569*	1
	D00725	*-32.133*	*17.382*	9		*D00830*	*-35.787*	*21.435*	2
	D00679	*-32.133*	*17.382*	1		*D0491*	*-35.905*	*20.763*	1
	D0565	*-32.133*	*17.382*	5		*D00643*	*-31.549*	*16.543*	3
	D00635	*-32.408*	*16.648*	3		*D00845*	*-34.982*	*21.617*	3
	D00640	*-32.408*	*16.648*	1		*D00850*	*-35.061*	*20.499*	2
	D00627	*-34.325*	*18.018*	1		*D00851*	*-35.327*	*20.215*	2
	D00725	*-32.133*	*17.382*	6		*D00852*	*-35.334*	*20.114*	3
	D00641	*-32.133*	*17.382*	3		*D00839*	*-34.464*	*22.593*	5
2016	*D00642*	*-31.6*	*16.38*	1	2017	*A33075*	*-32.23*	*16.936*	14
	D00663	*-32.202*	*18.014*	4		*A33127*	*-32.634*	*16.921*	9
	D00626	*-34.288*	*18.18*	2		*A33119*	*-33.282*	*17.803*	11
	D00678	*-29.921*	*16.946*	4		*A33107*	*-35.399*	*19.836*	5
	D00636	*-32.349*	*16.828*	2		*A33109*	*-35.092*	*19.665*	6
	D00647	*-30.959*	*15.999*	1		*A33143*	*-29.875*	*15.191*	1
	D00608	*-35.457*	*19.158*	4		*A33065*	*-30.517*	*16.671*	6
	D00811	*-34.363*	*24.206*	3		*A33066*	*-30.378*	*16.803*	5
	D00654	*-31.127*	*17.682*	3		*A33069*	*-29.946*	*16.864*	5
	D00768	*-35.187*	*21.655*	3		*A33108*	*-35.19*	*19.534*	2
	D00608	*-35.457*	*19.158*	4		*A33076*	*-31.872*	*16.973*	13
	D00658	*-31.589*	*17.505*	1		*A33138*	*-29.8*	*16.25*	7

NOTE: STA – station; LAT – latitude; LONG - longitude

Table A2. *ANOVA. Differences in morphological characters of immature Genypterus capensis from the putative western (WC) and southern (SC) stocks*

		SS	df	MS	F	p-value
UJ	Between Groups	8.643	1	8.643	19.573	**<0.001**
	Within Groups	82.138	186	0.442		
	Total	90.781	187			
LJ	Between Groups	10.726	1	10.726	10.663	**0.001**
	Within Groups	194.142	193	1.006		
	Total	204.867	194			
OD	Between Groups	1.087	1	1.087	7.021	**0.009**
	Within Groups	28.938	187	0.155		
	Total	30.025	188			

Note: SS = Sum-of-squares, dF = Degrees of freedom, MS =Mean Squares (MS), F =F-ratio and p-values (significance); UJ - upper jaw; LJ - lower jaw; OD - orbital diameter

Table A3. *Mann-Whitney U. Differences in meristic characters of immature Genypterus capensis from the putative western (WC) and southern (SC) stocks*

	UJ	LJ	OD	IOD
Mann-Whitney U	1860	2544.5	2313.5	2183
Wilcoxon W	13336	14172.5	13044.5	10568
Z	-3.147	-2.214	-2.618	-2.088
p-value	**0.002**	**0.027**	**0.009**	**0.037**

Note: UJ - upper jaw; LJ - lower jaw; OD - orbital diameter; IOD - interorbital distance

Table A4. *Model selection and predictive performances of variables from Genypterus capensis from the putative western (WC) and southern (SC) stocks off the South African coast, based on stepwise selection procedure.*

	Training	Validation	OVERALL	SC	WC
Immature					
Boosted Tree Analysis (BT)					
IOD	0.860	0.654	68.72	25.58	80.92
IOD+PFW	0.844	0.708	65.64	23.26	77.63
IOD+PFW+POSTO	0.905	0.813	71.79	55.81	76.32
IOD+PFW+POSTO+UJ	0.912	0.766	69.74	46.51	76.32
IOD+PFW+POSTO+UJ+HL	0.872	0.761	65.64	37.21	73.68
IOD+PFW+POSTO+UJ+HL+LJ	0.913	0.783	67.69	41.86	75
IOD+PFW+POSTO+UJ+HL+LJ+PREO	0.879	0.761	67.18	44.19	73.68
IOD+PFW+POSTO+UJ+HL+LJ+PREO+OD	0.893	0.804	67.69	39.53	75.66
IOD+PFW+POSTO+UJ+HL+LJ+PREO+OD+PFL	0.940	0.804	67.69	39.53	75.66
Random Forest Analysis (RF)					
LJ	0.659	0.483	60.51	67.44	58.55
LJ+UJ	0.810	0.690	67.69	58.14	78.95
LJ+UJ+POSTO	0.796	0.672	72.82	74.42	72.37
LJ+UJ+POSTO+IOD	0.788	0.714	65.64	69.77	64.47
LJ+UJ+POSTO+IOD+HL	0.789	0.771	66.15	58.14	68.42
LJ+UJ+POSTO+IOD+HL+OD	0.772	0.761	63.08	62.79	63.16
LJ+UJ+POSTO+IOD+HL+OD+PREO	0.797	0.830	74.36	48.84	73.03
LJ+UJ+POSTO+IOD+HL+OD+PREO+PFL	0.839	0.804	67.69	62.79	69.08
LJ+UJ+POSTO+IOD+HL+OD+PREO+PFL+PFW	0.867	0.778	65.64	62.79	66.45

	Training	Validation	OVERALL	SC	WC
Mature					
Boosted Tree Analysis (BT)					
POSTO	0.829	0.762	70.75	41.03	81.48
POSTO+PREO	0.819	0.786	70.75	30.77	85.19
POSTO+PREO+IOD	0.861	0.641	64.63	56.41	67.59
POSTO+PREO+IOD+LJ	0.852	0.564	61.2	38.46	69.44
POSTO+PREO+IOD+LJ+PFL	0.832	0.735	60.54	33.33	70.37
POSTO+PREO+IOD+LJ+PFL+UJ	0.876	0.735	63.27	41.03	71.3
POSTO+PREO+IOD+LJ+PFL+UJ+PFW	0.876	0.735	63.27	41.03	71.3
POSTO+PREO+IOD+LJ+PFL+UJ+PFW+OD	0.876	0.676	61.9	48.72	66.67
POSTO+PREO+IOD+LJ+PFL+UJ+PFW+OD+HL	0.841	0.647	59.18	30.77	53.85
Random Forest Analysis (RF)					
POSTO	0.626	0.600	61.9	58.97	41.03
POSTO+HL	0.630	0.641	62.59	51.28	66.67
POSTO+HL+IOD	0.587	0.500	52.38	53.85	51.85
POSTO+HL+IOD+OD	0.578	0.447	49.66	58.97	46.3
POSTO+HL+IOD+OD+LJ	0.636	0.432	54.42	56.41	53.7
POSTO+HL+IOD+OD+LJ+UJ	0.667	0.528	54.42	56.41	53.7
POSTO+HL+IOD+OD+LJ+UJ+PREO	0.704	0.531	53.74	53.85	53.7
POSTO+HL+IOD+OD+LJ+UJ+PREO+PFW	0.643	0.406	49.98	61.54	44.44
POSTO+HL+IOD+OD+LJ+UJ+PREO+PFW+PFL	0.719	0.576	55.78	64.1	52.78

***Table A5**. Model selection and predictive performances of variables from Genypterus capensis from the putative western (WC) and southern stocks (SC) off the South African coast based on stepwise selection procedure.*

Variable combination	Training	Validation	OVERALL	WC	SC
Boosted Tree Analysis (BT)					
FR	0.718	0.603	61.21	87.5	7.14
FR + GR	0.750	0.603	63.55	86.11	17.14
FR + GR + V	0.744	0.569	62.15%	84.03	17.14
Random Forest Analysis (RF)					
FR	0.711	0.597	60.28	62.5	55.71
FR + GR	0.748	0.508	60.75	61.11	60
FR + GR + V	0.773	0.547	63.08	68.06	52.86

Note: FR - Fin ray count; V - Vertebral count; GR - Gill raker count

APPENDIX B

Table B1. Differences in linear regression slopes for scatterplots of variables for Genypterus capensis otoliths, from the putative western (WC) and southern (SC) stocks for the years 2008-2011 and 2014-2017.

Calculation	OL vs TL		OL:OB vs TL		TL:OL vs TL		TL:OB vs TL	
	WC	**SC**	**WC**	**SC**	**WC**	**SC**	**WC**	**SC**
Slope	0.013	0.014	-0.004	-0.004	0.311	0.264	0.497	0.389
n	510	235	510	235	510	235	510	235
SE(reg)	0.193	0.178	0.178	0.176	5.724	6.360	14.603	15.70
SE(slope)	0.001	0.00	0.00	0.00	0.013	0.016	0.033	0.040
Difference	0.001		0.00		0.047		0.107	
SE (diff)	0.001		0.001		0.021		0.052	
t-stat	1.329		0.636		2.280		2.085	
df	741		741		741		741	
p	0.184		0.525		**0.023**		**0.037**	

Note: OL – Otolith length; TL – Total length; OB – Otolith Breadth; OW - otolith weight; SE - standard error, t-stat - test statistic, df = degrees of freedom

Table B2. ANOVA indicating differences in log-transformed otolith shape parameters of all Genypterus capensis samples from the putative western (WC) and southern (SC) stocks off the South African coast for the years 2008, 2009, 2011, and 2014 - 2017.

Log(Variable)			SS	df	MS	F	p
Immature	CIRC	Between Groups	0.003	1	0.003	37.136	0.000
		Within Groups	0.015	222	0		
		Total	0.018	223			
	FF	Between Groups	0.018	1	0.018	36.953	0.000
		Within Groups	0.106	222	0		
		Total	0.124	223			
	ROUND	Between Groups	0.005	1	0.005	6.425	0.012
		Within Groups	0.157	222	0.001		
		Total	0.162	223			
	ELL	Between Groups	0.01	1	0.01	11.194	0.001
		Within Groups	0.194	222	0.001		
		Total	0.203	223			
	AR	Between Groups	0.004	1	0.004	11.221	0.001
		Within Groups	0.072	222	0		
Mature	CIRC	Between Groups	0	1	0	0.106	0.745
		Within Groups	0.042	346	0		
		Total	0.042	347			
	FF	Between Groups	0	1	0	0.142	0.707
		Within Groups	0.288	346	0.001		
		Total	0.288	347			
	ROUND	Between Groups	0.016	1	0.016	19.162	**0.000**
		Within Groups	0.292	346	0.001		
		Total	0.308	347			
	ELL	Between Groups	0.01	1	0.01	7.609	**0.006**
		Within Groups	0.449	346	0.001		
		Total	0.459	347			

Note: SS - Sum-of-squares, dF - Degrees of freedom, MS - Mean Squares

Table B3. Mann-Whitney U test. Differences in the log-transformed shape indices for Genypterus capensis otolith samples from the putative western (WC) and southern (SC) stocks off the South African coast, in the years 2008, 2009, 2011 and 2014 – 2017.

	Ranks			Test Statistic			
COAST	N	Mean Rank	Sum of Ranks	MWU	WW	Z	p-value
Immature							
WC	100	107.06	10706	5656	10706	-1.128	0.259
SC	124	116.89	14494				
Total	224						
Mature							
WC	81	207.48	16806	8142	43920	-3.368	**0.001**
SC	267	164.49	43920				
Total	348						
WC	81	211.19	17106	7842	43620	-3.747	**0.000**
SC	267	163.37	43620				
Total	348						
WC	81	153.82	12459.5	9138.5	12459.5	-2.112	**0.035**
SC	267	180.77	48266.5				
Total	348						

Note: RECT - Rectangularity, ROUND – Roundness, AR – Aspect ratio, MWU - Mann-Whitney U, WW- Wilcoxon W

Table B4. Model selection of variables for immature Genypterus capensis samples from the putative western and southern stocks off the South African using a stepwise selection procedure

Test	Variable combination (Log transformed values)	Accuracy	
		Training	Validation
RF	ROUND	0.458	0.625
	ROUND + CIR	0.639	0.717
	ROUND + CIR + FF	**0.694**	0.691
	ROUND + CIR + FF + ELL	0.606	0.765
	ROUND + CIR + FF + ELL + AR	0.676	0.719
BT	CIRC	0.645	0.728
	CIRC + FF	0.645	0.728
	CIRC + FF + AR	0.677	0.735
	CIRC + FF + AR + ELL	**0.710**	0.759
	CIRC + FF + AR + ELL + ROUND	0.677	0.704
ANN	AR	0.521	0.629
	AR + FF	**0.694**	0.685
	AR + FF + CIRC	0.688	0.688
	AR + FF + CIRC + ELL	0.676	0.687
	AR + FF + CIRC + ELL + ROUND	0.657	0.714
SVM	CIRC	0.571	0.696
	CIRC + FF	0.482	0.637
	CIRC + FF + ROUND	0.571	0.720
	CIRC + FF + ROUND + ELL	0.518	0.565
	CIRC + FF + ROUND + ELL + AR	**0.574**	0.661
LDA	CIRC	0.652	0.652
	CIRC + FF	0.652	0.652
	CIRC + FF + ROUND	**0.696**	0.692
	CIRC + FF + ROUND + ELL	0.688	0.683
	CIRC + FF + ROUND + ELL + AR	0.688	0.688

Note: CIRC – Circularity, FF – Form factor, RECT - Rectangularity, ROUND - Roundness, ELL - Ellipticity and AR – Aspect ratio.

Table B5. Model selection of variables for mature Genypterus capensis samples from the putative western and southern stocks off the South African using a stepwise selection procedure

Test	Variable combination (Log transformed values)	Accuracy	
		Training	Validation
RF	RECT	0.472	0.529
	RECT + ROUND	**0.731**	0.821
	RECT + ROUND + AR	0.710	0.797
	RECT + ROUND + AR + ELL	0.701	0.788
BT	ROUND	**0.803**	0.801
	ROUND + ELL	0.795	0.818
	ROUND + ELL + AR	0.795	0.818
	ROUND + ELL + AR + RECT	0.795	0.831
ANN	RECT	0.767	0.767
	RECT + AR	0.727	0.787
	RECT + AR + ROUND	**0.816**	0.776
	RECT + AR + ROUND + ELL	0.776	0.816
SVM	ROUND	0.655	0.609
	ROUND + ELL	0.690	0.693
	ROUND + ELL + RECT	**0.759**	0.766
	ROUND + ELL + RECT + AR	0.713	0.743
LDA	RECT	0.615	0.615
	RECT + ROUND	0.609	0.606
	RECT + ROUND + ELL	**0.641**	0.629
	RECT + ROUND + ELL + AR	0.641	0.629

APPENDIX C

Table C1. Pairwise Comparisons: Differences in elemental composition of core (CR) and edge (ED) ablated shots of G. capensis otoliths from the putative western (WC) and southern (SC) stocks off the South African coast.

Element	Sample 1 - Sample 2	WCED - SCED	WCED - SCCR	WCED - WCCR	SCED - SCCR	SCED - WCCR	SCCR - WCCR
Mn/Ca	Test Statistic	8.667	32.792	38.375	24.125	-29.708	-5.583
	Std. Error	8.042	8.042	8.042	8.042	8.042	8.042
	Std. Test Statistic	1.078	4.078	4.772	3.000	-3.694	-0.694
	Sig.	0.281	0.000	0.000	0.003	0.000	0.487
	Adj. Sig.	1.000	0.000	0.000	0.016	0.001	1.000
Fe/Ca	Test Statistic	14.729	27.167	29.938	12.438	-15.208	-2.771
	Std. Error	8.015	8.015	8.015	8.015	8.015	8.015
	Std. Test Statistic	1.838	3.389	3.735	1.552	-1.897	-0.346
	Sig.	0.066	0.001	0.000	0.121	0.058	0.730
	Adj. Sig.	0.397	0.004	0.001	0.724	0.347	1.000
Ni/Ca	Test Statistic	15.563	24.125	26.063	8.563	10.500	-1.938
	Std. Error	8.041	8.041	8.041	8.041	8.041	8.041
	Std. Test Statistic	1.935	3.000	3.241	1.065	1.306	-0.241
	Sig.	0.053	0.003	0.001	0.287	0.192	0.810
	Adj. Sig.	0.318	0.016	0.007	1.000	1.000	1.000
Li/Ca	Test Statistic	-1.188	-19.688	-26.542	-18.500	25.354	6.854
	Std. Error	8.041	8.041	8.041	8.041	8.041	8.041
	Std. Test Statistic	-0.148	-2.448	-3.301	-2.301	3.153	0.852
	Sig.	0.883	0.014	0.001	0.021	0.002	0.394
	Adj. Sig.	1.000	0.086	0.006	0.128	0.010	1.000

Table C2. Pairwise Comparisons: Differences in elemental composition of Immature vs Mature specimens coreline-ablated shots of Genypterus capensis otoliths from the putative western (WC) and southern (SC) stocks off the South African coast.

Element	Sample 1 - Sample 2	WC MAT - WC IMM	SC MAT - SC IMM	WC MAT - SC MAT	WC IMM - SC IMM	WC MAT - SC IMM	SC MAT - WC IMM
Rb/Ca	Test Statistic	14.846	19.179	3.096	7.429	22.275	-11.750
	Std. Error	5.617	6.213	5.120	6.629	6.428	5.370
	Std. Test Statistic	2.643	3.087	0.605	1.121	3.465	-2.188
	Sig.	0.008	0.002	0.545	0.262	0.001	0.029
	Adj. Sig.[a]	0.049	0.012	1.000	1.000	0.003	0.172
Ni/Ca	Test Statistic	-12.245	15.063	9.528	12.346	24.591	-2.716
	Std. Error	5.735	6.062	5.483	6.291	6.505	5.228
	Std. Test Statistic	-2.135	2.485	1.738	1.963	3.780	-0.520
	Sig.	0.033	0.013	0.082	0.050	0.000	0.603
	Adj. Sig.[a]	0.197	0.078	0.494	0.298	0.001	1.000
Cu/Ca	Test Statistic	-8.142	12.962	-7.226	12.045	20.188	0.916
	Std. Error	5.483	6.291	5.228	6.505	6.062	5.735
	Std. Test Statistic	-1.485	2.060	-1.382	1.852	3.330	0.160
	Sig.	0.138	0.039	0.167	0.064	0.001	0.873
	Adj. Sig.[a]	0.826	0.236	1.000	0.384	0.005	1.000
Co/Ca	Test Statistic	9.709	-8.029	8.034	-6.354	-16.063	-1.675
	Std. Error	5.443	6.161	6.371	5.196	5.617	6.003
	Std. Test Statistic	1.784	-1.303	1.261	-1.223	-2.860	-0.279
	Sig.	0.074	0.193	0.207	0.221	0.004	0.780
	Adj. Sig.[a]	0.447	1.000	1.000	1.000	0.025	1.000

Table C3. Wald-Wolfowitz Test showing elements that indicated differences in elemental means for core, coreline (immature) and edge of Genypterus capensis otolith samples from the putative west (WC) and south (SC) coast stocks

	Element [/Ca]		No. of runs	Z	p-value
Core	Ba	Exact Number of Runs	18	-1.8967	0.028935473
Coreline: Immature	Ba	Exact Number of Runs	12	1.08472	0.861752798
	Ni	Exact Number of Runs	6	-1.8249	0.034055728
Edge	Sr	Minimum Possible	15	-2.7721	0.002785118
		Maximum Possible	16	-2.4803	0.006564214
	Mg	Minimum Possible	18	-1.8967	0.028935473
		Maximum Possible	23	-0.4377	0.330804039
	B	Minimum Possible	18	-1.8967	0.028935473
		Maximum Possible	21	-1.0213	0.153559448
	Fe	Minimum Possible	18	-1.8967	0.028935473
		Maximum Possible	29	1.31308	0.905422369
	Ba	Minimum Possible	14	-3.0639	0.001092512
		Maximum Possible	18	-1.8967	0.028935473

Table C4. ANOVA showing elements that indicated differences in elemental means in coreline (immature) of Genypterus capensis otolith samples from the putative west (WC) and south (SC) coast stocks

Element [/Ca]			**Sum of Squares**	**df**	**Mean Square**	**F**	**p-value**
Immature	Ni	Between Groups	1.216E-11	1	1.216E-11	15.155	0.0011
		Within Groups	1.36E-11	17	8.027E-13		
		Total	2.581E-11	18			

Table C5. Mann-Whitney U Test showing elements that indicated differences in elemental means for coreline (immature) and edge of Genypterus capensis otolith samples from the putative west (WC) and south (SC) coast stocks

	Element [/Ca]	**Mann-Whitney U**	**Wilcoxon W**	**Z**	**p-value**
Immature	P	13	79	-2.3095	0.0209
	Ni	8	74	-2.9726	0.0029
Edge	Ni	148	448	-2.8886	0.0038

Table C6. *Model selection of variables of Genypterus capensis from the putative western and southern stocks off the South African coast based on a stepwise selection procedure Accuracy of training and test sets and correct classification percentage.*

			Train	Test	Overall	WC	SC
All	Boosted Tree	Sr	0.76	0.56	69.39	41.7	96.0
		Sr+K	0.88	0.50	75.51	62.5	88.0
		Sr+K+Li	0.82	0.44	67.35	62.5	72.0
		Sr+K+Li+Rb	0.82	0.44	67.35	58.3	76.0
		Sr+K+Li+Rb+Mn	0.82	0.44	67.35	58.3	76.0
		Sr+K+Li+Rb+Mn+Pb	0.85	0.38	67.35	50.0	84.0
		Sr+K+Li+Rb+Mn+Pb+Na	0.85	0.38	67.35	50.0	84.0
		Sr+K+Li+Rb+Mn+Pb+Na+Cu	0.85	0.38	67.35	50.0	84.0
		Sr+K+Li+Rb+Mn+Pb+Na+Cu+Mg	0.82	0.25	63.27	45.8	80.0
		Sr+K+Li+Rb+Mn+Pb+Na+Cu+Mg+P	0.71	0.67	65.31	83.3	48.0
		Sr+K+Li+Rb+Mn+Pb+Na+Cu+Mg+P+Ba	0.71	0.67	65.31	83.3	48.0
		Sr+K+Li+Rb+Mn+Pb+Na+Cu+Mg+P+Ba+Fe	0.82	0.40	63.27	50.0	76.0
		Sr+K+Li+Rb+Mn+Pb+Na+Cu+Mg+P+Ba+Fe+B	0.91	0.40	69.39	58.3	80.0
		Sr+K+Li+Rb+Mn+Pb+Na+Cu+Mg+P+Ba+Fe+B+Co	0.82	0.27	59.18	45.8	72.0
		Sr+K+Li+Rb+Mn+Pb+Na+Cu+Mg+P+Ba+Fe+B+Co+Zn	0.79	0.47	63.27	45.8	80.0
		Sr+K+Li+Rb+Mn+Pb+Na+Cu+Mg+P+Ba+Fe+B+Co+Zn+Ni	0.82	0.47	65.31	50.0	80.0
		Sr+K+Li+Rb+Mn+Pb+Na+Cu+Mg+P+Ba+Fe+B+Co+Zn+Ni+Sn	0.82	0.47	65.31	50.0	80.0
	Random Forest	Ni	0.63	0.59	61.00	87.5	36.0
		Ni+Cu	0.56	0.35	48.98	100.0	0.0
		Ni+Cu+Rb	0.56	0.35	48.98	100.0	0.0
		Ni+Cu+Rb+Co	0.59	0.41	53.06	100.0	8.0
		Ni+Cu+Rb+Co+Mg	0.79	0.31	63.27	91.8	36.0
		Ni+Cu+Rb+Co+Mg+Sn	0.66	0.41	57.14	100.0	16.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe	0.56	0.35	46.94	95.8	0.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe+Mn	0.61	0.44	53.06	95.8	12.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe+Mn+P	0.50	0.47	46.94	95.8	0.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe+Mn+P+Sr	0.63	0.53	55.10	91.7	20.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe+Mn+P+Sr+K	0.50	0.53	48.98	95.8	4.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe+Mn+P+Sr+K+Ba	0.43	0.58	46.94	95.8	0.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe+Mn+P+Sr+K+Ba+Na	0.40	0.63	46.94	95.8	0.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe+Mn+P+Sr+K+Ba+Na+B	0.43	0.58	46.94	95.8	0.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe+Mn+P+Sr+K+Ba+Na+B+Zn	0.47	0.53	44.90	87.5	4.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe+Mn+P+Sr+K+Ba+Na+B+Zn+Pb	0.45	0.56	44.90	91.7	0.0
		Ni+Cu+Rb+Co+Mg+Sn+Fe+Mn+P+Sr+K+Ba+Na+B+Zn+Pb+Li	0.53	0.58	51.02	91.7	12.0
Core	Boosted Tree	Rb	0.88	0.33	70.83	87.5	54.2
		Rb+Ni	0.85	0.73	81.25	91.7	70.8
		Rb+Ni+B	0.73	0.67	70.83	70.8	70.8
		Rb+Ni+B+Cu	0.67	0.53	62.50	66.7	58.3
		Rb+Ni+B+Cu+Sn	0.70	0.53	64.58	62.5	66.7
		Rb+Ni+B+Cu+Sn+Co	0.73	0.67	70.83	70.8	70.8
		Rb+Ni+B+Cu+Sn+Co+Sr	0.67	0.60	64.58	70.8	58.3
		Rb+Ni+B+Cu+Sn+Co+Sr+Na	0.88	0.53	77.08	87.5	66.7
		Rb+Ni+B+Cu+Sn+Co+Sr+Na+Fe	0.67	0.53	62.50	66.7	58.3
		Rb+Ni+B+Cu+Sn+Co+Sr+Na+Fe+Ba	0.88	0.47	60.42	62.5	58.3
		Rb+Ni+B+Cu+Sn+Co+Sr+Na+Fe+Ba+Mg	0.79	0.53	70.83	75.0	66.7
		Rb+Ni+B+Cu+Sn+Co+Sr+Na+Fe+Ba+Mg+Zn	0.85	0.47	72.92	83.3	62.5
		Rb+Ni+B+Cu+Sn+Co+Sr+Na+Fe+Ba+Mg+Zn+K	0.70	0.67	67.85	62.5	75.0
		Rb+Ni+B+Cu+Sn+Co+Sr+Na+Fe+Ba+Mg+Zn+K+P	0.82	0.47	70.83	79.2	62.5
		Rb+Ni+B+Cu+Sn+Co+Sr+Na+Fe+Ba+Mg+Zn+K+P+Mn	0.91	0.53	79.17	91.7	66.7
		Rb+Ni+B+Cu+Sn+Co+Sr+Na+Fe+Ba+Mg+Zn+K+P+Mn+Pb	0.76	0.60	70.83	75.0	66.7
		Rb+Ni+B+Cu+Sn+Co+Sr+Na+Fe+Ba+Mg+Zn+K+P+Mn+Pb+Li	0.76	0.73	75.00	75.0	75.0

			Train	Test	Overall	WC	SC
	Random Forest	Co	0.48	0.53	50.00	100.0	0.0
		Co+Cu	0.45	0.59	50.00	100.0	0.0
		Co+Cu+Pb	0.58	0.47	50.00	100.0	0.0
		Co+Cu+Pb+Na	0.52	0.47	50.00	100.0	0.0
		Co+Cu+Pb+Na+Ni	0.59	0.44	54.17	95.8	12.5
		Co+Cu+Pb+Na+Ni+Sr	0.65	0.53	60.42	95.8	25.0
		Co+Cu+Pb+Na+Ni+Sr+Sn	0.67	0.39	56.25	91.7	20.8
		Co+Cu+Pb+Na+Ni+Sr+Sn+K	0.66	0.47	58.33	95.8	20.8
		Co+Cu+Pb+Na+Ni+Sr+Sn+K+Ba	0.79	0.32	60.42	62.5	58.3
		Co+Cu+Pb+Na+Ni+Sr+Sn+K+Ba+Mn	0.45	0.58	50.00	100.0	0.0
		Co+Cu+Pb+Na+Ni+Sr+Sn+K+Ba+Mn+Li	0.45	0.58	50.00	100.0	0.0
		Co+Cu+Pb+Na+Ni+Sr+Sn+K+Ba+Mn+Li+Zn	0.64	0.45	56.25	95.8	16.7
		Co+Cu+Pb+Na+Ni+Sr+Sn+K+Ba+Mn+Li+Zn+P	0.50	0.50	50.00	100.0	0.0
		Co+Cu+Pb+Na+Ni+Sr+Sn+K+Ba+Mn+Li+Zn+P+Fe	0.50	0.50	50.00	100.0	0.0
		Co+Cu+Pb+Na+Ni+Sr+Sn+K+Ba+Mn+Li+Zn+P+Fe+Rb	0.54	0.45	50.00	100.0	0.0
		Co+Cu+Pb+Na+Ni+Sr+Sn+K+Ba+Mn+Li+Zn+P+Fe+Rb+Mg	0.52	0.47	50.00	100.0	0.0
		Co+Cu+Pb+Na+Ni+Sr+Sn+K+Ba+Mn+Li+Zn+P+Fe+Rb+Mg+B	0.59	0.37	50.00	100.0	0.0
Coreline	Boosted Tree	Li	0.82	0.73	79.17	83.3	75.0
		Li+K	0.79	0.47	68.75	54.2	83.3
		Li+K+Pb	0.85	0.47	79.92	58.3	87.5
		Li+K+Pb+Mg	0.85	0.53	75.00	70.8	79.2
		Li+K+Pb+Mg+Sr	0.91	0.53	79.17	75.0	83.3
		Li+K+Pb+Mg+Sr+Rb	0.94	0.40	75.00	70.8	79.2
		Li+K+Pb+Mg+Sr+Rb+Mn	0.82	0.47	68.75	58.3	79.2
		Li+K+Pb+Mg+Sr+Rb+Mn+Zn	1.00	0.71	85.42	87.5	83.3
		Li+K+Pb+Mg+Sr+Rb+Mn+Zn+Sn	1.00	0.57	81.25	79.2	83.3
		Li+K+Pb+Mg+Sr+Rb+Mn+Zn+Sn+Ba	0.97	0.71	83.33	83.3	83.3
		Li+K+Pb+Mg+Sr+Rb+Mn+Zn+Sn+Ba+Ni	0.97	0.57	79.17	75.0	83.3
		Li+K+Pb+Mg+Sr+Rb+Mn+Zn+Sn+Ba+Ni+Cu	1.00	0.71	85.42	87.5	83.3
		Li+K+Pb+Mg+Sr+Rb+Mn+Zn+Sn+Ba+Ni+Cu+B	0.85	0.43	66.67	66.7	66.7
		Li+K+Pb+Mg+Sr+Rb+Mn+Zn+Sn+Ba+Ni+Cu+B+P	0.88	0.43	68.75	66.7	70.8
		Li+K+Pb+Mg+Sr+Rb+Mn+Zn+Sn+Ba+Ni+Cu+B+P+Co	0.97	0.43	75.00	66.7	83.3
		Li+K+Pb+Mg+Sr+Rb+Mn+Zn+Sn+Ba+Ni+Cu+B+P+Co+Na	0.91	0.29	77.08	70.8	83.3
		Li+K+Pb+Mg+Sr+Rb+Mn+Zn+Sn+Ba+Ni+Cu+B+P+Co+Na+Fe	0.94	0.21	66.67	58.3	75.0
	Random Forest	P	0.58	0.35	50.00	100.0	0.0
		P+Na	0.65	0.47	58.33	91.7	25.0
		P+Na+Rb	0.55	0.41	50.00	100.0	0.0
		P+Na+Rb+Fe	0.61	0.29	50.00	100.0	0.0
		P+Na+Rb+Fe+Mg	0.66	0.19	50.00	100.0	0.0
		P+Na+Rb+Fe+Mg+B	0.59	0.38	52.08	100.0	4.2
		P+Na+Rb+Fe+Mg+B+Ba	0.53	0.44	50.00	100.0	0.0
		P+Na+Rb+Fe+Mg+B+Ba+Zn	0.53	0.44	47.92	95.8	0.0
		P+Na+Rb+Fe+Mg+B+Ba+Zn+Pb	0.48	0.53	47.92	95.8	0.0
		P+Na+Rb+Fe+Mg+B+Ba+Zn+Pb+Mn	0.67	0.44	52.08	66.7	37.5
		P+Na+Rb+Fe+Mg+B+Ba+Zn+Pb+Mn+Cu	0.52	0.47	47.92	95.8	0.0
		P+Na+Rb+Fe+Mg+B+Ba+Zn+Pb+Mn+Cu+Sr	0.48	0.53	47.92	95.8	0.0
		P+Na+Rb+Fe+Mg+B+Ba+Zn+Pb+Mn+Cu+Sr+K	0.38	0.68	47.92	95.8	0.0
		P+Na+Rb+Fe+Mg+B+Ba+Zn+Pb+Mn+Cu+Sr+K+Sn	0.70	0.56	62.50	91.7	33.3
		P+Na+Rb+Fe+Mg+B+Ba+Zn+Pb+Mn+Cu+Sr+K+Sn+Li	0.69	0.68	66.67	91.7	41.7
		P+Na+Rb+Fe+Mg+B+Ba+Zn+Pb+Mn+Cu+Sr+K+Sn+Li+Ni	0.43	0.61	47.92	95.8	0.0
		P+Na+Rb+Fe+Mg+B+Ba+Zn+Pb+Mn+Cu+Sr+K+Sn+Li+Ni+Co	0.52	0.53	50.00	91.7	8.3

			Train	Test	Overall	WC	SC
Edge	Boosted Tree	Ni	0.76	0.73	75.00	87.5	62.5
		Ni+Mg	0.85	0.87	85.42	91.7	79.2
		Ni+Mg+Zn	0.73	0.73	72.92	83.3	62.5
		Ni+Mg+Zn+K	0.79	0.73	77.08	91.7	62.5
		Ni+Mg+Zn+K+Na	0.73	0.53	66.67	95.8	37.5
		Ni+Mg+Zn+K+Na+P	0.73	0.53	66.67	95.8	37.5
		Ni+Mg+Zn+K+Na+P+Pb	0.73	0.53	66.67	95.8	37.5
		Ni+Mg+Zn+K+Na+P+Pb+Fe	0.73	0.53	66.67	95.8	37.5
		Ni+Mg+Zn+K+Na+P+Pb+Fe+Sn	0.73	0.53	66.67	95.8	37.5
		Ni+Mg+Zn+K+Na+P+Pb+Fe+Sn+B	0.79	0.60	72.92	91.7	54.2
		Ni+Mg+Zn+K+Na+P+Pb+Fe+Sn+B+Ba	0.88	0.67	81.25	83.3	79.2
		Ni+Mg+Zn+K+Na+P+Pb+Fe+Sn+B+Ba+Cu	0.85	0.60	77.08	75.0	79.2
		Ni+Mg+Zn+K+Na+P+Pb+Fe+Sn+B+Ba+Cu+Rb	0.82	0.67	77.08	87.5	66.7
		Ni+Mg+Zn+K+Na+P+Pb+Fe+Sn+B+Ba+Cu+Rb+Sr	0.73	0.53	66.67	95.8	37.5
		Ni+Mg+Zn+K+Na+P+Pb+Fe+Sn+B+Ba+Cu+Rb+Sr+Co	0.88	0.47	75.00	83.3	66.7
		Ni+Mg+Zn+K+Na+P+Pb+Fe+Sn+B+Ba+Cu+Rb+Sr+Co+Mn	0.73	0.53	66.67	95.8	37.5
		Ni+Mg+Zn+K+Na+P+Pb+Fe+Sn+B+Ba+Cu+Rb+Sr+Co+Mn+Li	0.76	0.53	68.75	95.8	41.7
	Random Forest	Ni	0.74	0.71	72.92	87.5	58.3
		Ni+Fe	0.81	0.59	72.92	87.5	58.3
		Ni+Fe+K	0.48	0.53	50.00	100.0	0.0
		Ni+Fe+K+Sr	0.71	0.71	70.83	95.8	45.8
		Ni+Fe+K+Sr+Na	0.59	0.38	52.08	100.0	4.2
		Ni+Fe+K+Sr+Na+P	0.52	0.47	50.00	100.0	0.0
		Ni+Fe+K+Sr+Na+P+Mg	0.53	0.44	50.00	100.0	0.0
		Ni+Fe+K+Sr+Na+P+Mg+Mn	0.52	0.47	50.00	100.0	0.0
		Ni+Fe+K+Sr+Na+P+Mg+Mn+Pb	0.59	0.58	58.33	100.0	16.7
		Ni+Fe+K+Sr+Na+P+Mg+Mn+Pb+Li	0.59	0.53	56.25	95.8	16.7
		Ni+Fe+K+Sr+Na+P+Mg+Mn+Pb+Li+Sn	0.55	0.53	54.17	95.8	12.5
		Ni+Fe+K+Sr+Na+P+Mg+Mn+Pb+Li+Sn+Ba	0.68	0.50	60.42	100.0	20.8
		Ni+Fe+K+Sr+Na+P+Mg+Mn+Pb+Li+Sn+Ba+B	0.50	0.50	50.00	100.0	0.0
		Ni+Fe+K+Sr+Na+P+Mg+Mn+Pb+Li+Sn+Ba+B+Zn	0.54	0.50	52.08	100.0	4.2
		Ni+Fe+K+Sr+Na+P+Mg+Mn+Pb+Li+Sn+Ba+B+Zn+Cu	0.57	0.45	52.08	100.0	4.2
		Ni+Fe+K+Sr+Na+P+Mg+Mn+Pb+Li+Sn+Ba+B+Zn+Cu+Co	0.52	0.47	50.00	100.0	0.0
		Ni+Fe+K+Sr+Na+P+Mg+Mn+Pb+Li+Sn+Ba+B+Zn+Cu+Co+Rb	0.90	0.53	75.00	95.8	54.2

Table C7. Eigenvalues of Principal Components and the % variation explained for original otolith elemental characters of Genypterus capensis samples from the putative west (WC) and south (SC) stocks.

		PC1	PC2	PC3	PC4	PC5
All	Eigenvalues	**5.17**	**3.21**	**2.28**	**1.38**	**1.06**
	%Variation	30.4	18.9	13.4	8.1	6.2
	Cum.%Variation	30.4	49.3	62.6	70.8	77
Core	Eigenvalues	**3.47**	**2.33**	**2.14**	**1.37**	**1.07**
	%Variation	23.9	16.1	14.8	9.4	7.4
	Cum.%Variation	23.9	40	54.8	64.3	71.6
Coreline	Eigenvalues	**6.99**	**3.39**	**2.03**	**1.19**	0.874
	%Variation	41.1	19.9	12	7	5.1
	Cum.%Variation	41.1	61	73	80	85.2
Coreline	Eigenvalues	**5**	**2.98**	**2.35**	**1.53**	**1.34**
	%Variation	29.4	17.6	13.8	9	7.9
	Cum.%Variation	29.4	46.9	60.8	69.8	77.7
Edge	Eigenvalues	**3.04**	**2.16**	**1.87**	**1.75**	**1.35**
	%Variation	17.9	12.7	11	10.3	8
	Cum.%Variation	17.9	30.5	41.6	51.9	59.8

Table C8. Correlation between principal components and original variables explained for otolith e putative western (WC) and southern (SC) stocks

Otolith Section	PC	[Element/Ca]									
		Sr	**Na**	**K**	**Mg**	**P**	**B**	**Mn**	**Fe**	**Ba**	**Rb**
ALL	PC1	-0.321	0.27	-0.083	0.242	-0.066	-0.272	-0.063	0.08	-0.286	-0.295
	PC2	0.292	-0.373	-0.199	-0.389	0.104	-0.113	-0.066	-0.045	0.166	-0.02
	PC3	-0.116	-0.184	-0.201	-0.185	-0.112	0.466	-0.295	-0.199	-0.255	-0.357
	PC4	0.062	-0.078	-0.37	-0.016	**-0.696**	0.044	0.195	0.095	0.228	-0.03
	PC5	0.07	0.002	0.492	0.058	-0.283	0.031	-0.465	-0.458	0.024	0.291
CORE	PC1	-0.374	0.267	-0.076	0.128	-0.304	-0.14	0.146	-0.012	-0.138	-0.409
	PC2	0.332	-0.427	-0.325	-0.228	-0.141	-0.247	-0.114	0.208	-0.036	-0.032
	PC3	0.043	0.227	0.43	0.059	0.041	-0.428	0.26	0.341	0.491	0.24
	PC4	-0.2	0.017	-0.084	0.022	0.019	0.031	0.105	0.461	-0.319	-0.16
	PC5	-0.021	0.252	-0.378	0.088	0.231	-0.37	**-0.536**	0.241	-0.042	0.03
CORELINE (IMMATURE)	PC1	-0.231	0.199	-0.242	0.124	-0.006	-0.333	-0.221	0.114	-0.281	0.028
	PC2	0.35	-0.429	0.052	-0.459	0.095	-0.029	-0.15	-0.251	0.095	-0.215
	PC3	-0.096	-0.017	0.158	-0.094	**0.606**	-0.189	0.192	**0.514**	-0.222	-0.142
	PC4	0.082	0.166	0.327	0.06	-0.113	-0.079	-0.354	-0.023	-0.055	**-0.732**
	PC5	-0.18	-0.192	**-0.518**	-0.357	-0.091	0.291	-0.052	0.134	-0.354	-0.309
CORELINE (MATURE)	PC1	-0.377	0.371	0.153	0.367	-0.044	-0.259	0.095	0.055	-0.274	-0.183
	PC2	-0.153	0.227	0.243	0.212	0.217	0.25	-0.302	-0.096	-0.277	0.189
	PC3	-0.153	-0.108	-0.282	-0.172	-0.399	0.342	-0.062	-0.229	-0.247	-0.422
	PC4	-0.022	0.217	-0.482	0.185	-0.022	-0.165	0.055	0.344	-0.053	-0.26
	PC5	-0.136	-0.012	-0.096	0.046	0.344	-0.013	**-0.628**	-0.229	-0.143	-0.064
EDGE (pooled)	PC1	0.118	-0.302	0.251	-0.199	-0.294	0.275	0.382	-0.11	0.089	-0.272
	PC2	0.251	0.288	0.129	0.32	0.357	0.054	-0.145	-0.209	0.266	0.278
	PC3	0.442	-0.279	-0.272	-0.262	0.079	-0.22	-0.023	-0.28	0.477	-0.133
	PC4	-0.026	-0.157	-0.023	-0.327	0.072	-0.141	-0.035	0.315	0.192	0.321
	PC5	0.074	0.247	-0.003	0.334	-0.253	-0.47	0.062	-0.033	0.021	-0.201

APPENDIX D

***Table** D2. Parasite species found in Genypterus species. Site of infection and references included* (**Source**: Courtesy of Dr Ken McKenzie).

Group	Parasite species	Site of infection	*Genypterus* sp.	Reference
Myxosporea	*Ceratomyxa laxa*	Gall bladder	*blacodes, chilensis, maculatus*	George-Nascimento *et al.,* 2004
	C. hokarari	Gall bladder	*blacodes, chilensis, maculatus*	Meglitsch, 1960; George-Nascimento *et al.,* 2004
	C. inversa	Gall bladder	*blacodes, chilensis, maculatus*	Meglitsch, 1960; George-Nascimento *et al.,* 2004
	C. lepidopusi	Gall bladder	*blacodes, chilensis, maculatus*	George-Nascimento *et al.,* 2004
	Myxobolus sp.	Gills	*brasiliensis*	Sardella *et al.,* 1998
Digenea	*Tormopsolus brasiliensis*	Intestine	*brasiliensis*	Knoff *et al.,* 2010
	Aporocotyle mariachristinae, A. ymakara	Bulbus arteriosus, aorta	*blacodes*	Hernandez-Orts *et al.,* 2012
	Aporocotyle keli		*chilensis*	Kohn *et al.,* 2007
	Aporocotyle kuri		*maculatus*	Munoz & George-Nascimento 2008
	Aporocotyle smithi		*capensis*	Parukhin & Tkachuk, 1980
	Lecithochirium genypteri		*blacodes, brasiliensis, maculatus*	Sardella *et al.,* 1998; Munoz & George-Nascimento 2008
	Acanthocolpus brasiliensis		*brasiliensis*	Alves *et al.,* 2002
	Zoogonidae gen. sp.	Intestine	*maculatus*	Munoz & George-Nascimento 2008
	Derogenes varicus	Stomach	*blacodes*	Kohn *et al.,* 2007
	Pseudolepidapedon brasiliensis	Intestine	*brasiliensis*	Kohn *et al.,* 2007
	Helicometrina nimia	Intestine	*maculatus*	Kohn *et al.,* 2007

Monogenea	*Pseudempleurosoma gibsoni*		*brasiliensis*	Alves *et al.,* 2002
Nematoda	*Philometra chilensis*	Ovary	*chilensis*	Moravec *et al.,* 2011
	Anisakis larvae	Visceral cavity, muscle	*capensis, blacodes, brasiliensis, maculatus*	Payne, 1986; Sardella *et al.,* 1998; Knoff *et al.,* 2007
	Pseudoterranova larvae	Mesenteries, muscle	*blacodes, brasiliensis*	Sardella *et al.,* 1998; Alves *et al.,* 2002
	Hysterothylacium larvae	Mesenteries	*brasiliensis*	Alves *et al.,* 2002
	Hysterothylacium aduncum	Stomach, intestine	*blacodes, brasiliensis*	Sardella *et al.,* 1998
	Raphidascaris larvae			Alves *et al.,* 2002
	Contracaecum larvae	Mesenteries	*blacodes, brasiliensis, maculatus*	Sardella *et al.,* 1998; Alves *et al.,* 2002; Munoz &George-Nascimento 2008
	Terranova larvae			Alves *et al.,* 2002
	Cucullanus genypteri	Intestine	*brasiliensis, blacodes, maculatus*	Sardella *et al.,* 1997, 1998; Alves *et al.,* 2002; Munoz & Gearge-Nascimento, 2008
	Pseudascarophis genypteri	Intestine	*chilensis*	Munoz & George-Nascimento, 2001
	Procamallanus sp.	Intestine	*brasiliensis*	Alves *et al.,* 2002
Cestoda	*Anonchocephalus chilensis*	Intestine	*blacodes, brasiliensis, maculatus*	Sardella *et al.,* 1998; Pereira, 2000; Munoz & George-Nascimento, 2008
	Diphyllobothrium sp. larvae			Knoff *et al.,* 2008
	Tentacularia coryphaenae larvae	Body (embedded)	*capensis, blacodes*	Clemente *et al.,* 2004

	Nybelinia sp. *larvae*	Visceral cavity	*brasiliensis*	
	Heteronybelinia sp, larvae	Muscle	*brasiliensis*	
	Hepatoxylon trichiuri larvae		*capensis, brasiliensis, maculatus*	Payne, 1986; Clemente *et al.,* 2004; Munoz & George-Nascimento, 2008
	Otobothrium cysticum larvae		*brasiliensis*	
	Progrillotia dollfusi larvae		*brasiliensis*	
	Grillotia heptanchi larvae	Muscle	*chilensis*	Carvajal & Campbell, 1979
	Callitetrarhynchus gracilis larvae	Visceral cavity, muscle	*brasiliensis*	
	Scolex pleuronectis	Intestine	*blacodes, brasiliensis, maculatus*	Sardella *et al.,* 1998; Munoz & George-Nascimento, 2008
	Lacistorhynchus sp. larvae	Mesenteries	*brasiliensis*	Alves *et al.,* 2002
Acanthocephala	*Corynosoma* spp. juv.		*blacodes, brasiliensis*	Sardella *et al.,* 1998; Munoz & George-Nascimento 2008
	Corynosoma australis juv.	Mesenteries	*brasiliensis*	Alves *et al.,* 2002
	Bolbosoma sp. juv.			Munoz & George-Nascimento, 2008
	Polymorphus sp. juv.	Mesenteries	*brasiliensis*	Alves *et al.,* 2002
	Echinorhynchus sp.	Intestine	*blacodes*	Sardella *et al.,* 1998
Copepoda	*Sphyrion laevigatum*	Body (embedded)	*brasiliensis, capensis, blacodes*	Payne, 1986; Sardella *et al.,* 1998; Brickle *et al.,* 2003
	Pseudolernentoma brasiliensis	Oral cavity	*brasiliensis*	Luque & Alves, 2003
	Argentinochondria patagonensis	Inner operculum	*brasiliensis*	Etchegoin *et al.,* 2003

	Lepeophtheirus yañezi	Body surface	*maculatus*	Munoz & George-Nascimento, 2008
	Chondracanthus genypteri	Gills	*blacodes*	Sardella *et al.,* 1998
Hirudinea	*Piscicolidae* gen. sp.	Gills	*brasiliensis, maculatus*	Alves *et al.,* 2002; Munoz & Gearge-Nascimento, 2008

Table D2. ***ANOVA*** *indicating differences shown by potential parasite biotags (Anisakis sp., Diphyllobothrium sp., Lecithochirium sp. and Tentacularia sp.) of mature Genypterus capensis specimens, from the putative western (WC) and southern (SC) stocks off the coast of South Africa, for the years 2014 - 2017.*

			SS	df	MS	F	p-value
Combined	***Anisakis* sp.**	Between Groups	1733.081	1	1733.081	3.385	0.068
		Within Groups	83954.29	164	511.916		
		Total	85687.37	165			
	***Diphyllobothrium* sp.**	Between Groups	0.208	1	0.208	0.029	0.865
		Within Groups	1168.876	164	7.127		
		Total	1169.084	165			
	***Lecithochorium* sp.**	Between Groups	215.928	1	215.928	0.075	0.785
		Within Groups	472608.5	164	2881.759		
		Total	472824.5	165			
	***Tentacularia* sp.**	Between Groups	54.164	1	54.164	0.642	0.424
		Within Groups	11557.64	137	84.362		
		Total	11611.8	138			
West Coast	***Anisakis* sp.**	Between Groups	344.312	1	344.312	1.239	0.268
		Within Groups	37234.09	134	277.866		
		Total	37578.4	135			
	***Diphyllobothrium* sp.**	Between Groups	5.516	1	5.516	1.934	0.167
		Within Groups	382.219	134	2.852		
		Total	387.735	135			
	***Lecithochorium* sp.**	Between Groups	2795.478	1	2795.478	1.278	0.26
		Within Groups	293074.8	134	2187.125		
		Total	295870.2	135			
	***Tentacularia* sp.**	Between Groups	0	1	0	.	.
		Within Groups	0	108	0		
		Total	0	109			
South Coast	***Anisakis* sp.**	Between Groups	884.533	1	884.533	0.6	0.445
		Within Groups	41268.97	28	1473.892		
		Total	42153.5	29			
	***Diphyllobothrium* sp.**	Between Groups	27.601	1	27.601	1.033	0.318
		Within Groups	747.866	28	26.71		
		Total	775.467	29			
	***Lecithochorium* sp.**	Between Groups	7440.016	1	7440.016	1.231	0.277
		Within Groups	169234.7	28	6044.095		
		Total	176674.7	29			
	***Tentacularia* sp.**	Between Groups	702.905	1	702.905	1.927	0.176
		Within Groups	9849.854	27	364.809		
		Total	10552.76	28			

Table D3. Mann-Whitney U Test indicating differences shown by potential parasite biotags (Anisakis sp., Diphyllobothrium sp., Lecithochirium sp. and Tentacularia sp.) of mature Genypterus capensis specimens, from the putative western (WC) and southern (SC) stocks off the coast of South Africa, for the years 2014 - 2017.

	Total (N)	Mann-Whitney U	Wilcoxon W	Standard Error	Standardized Test Statistic	p-value
Anisakis sp.	95	502.5	3583.5	101.899	-1.575	0.115
Diphyllobothrium sp.	95	764.5	3845.5	93.422	1.086	0.277
Lecithochirium sp.	95	638.0	3719.0	97.313	-0.257	0.797
Tentacularia sp.	71	330.0	1870.0	29.037	-3.788	**0.000**

Table D4 Independent-Samples Kruskal-Wallis Test Summary: p-values indicating differences shown by potential parasite biotags (Anisakis sp., Diphyllobothrium sp., Lecithochirium sp. and Tentacularia sp.) of immature Genypterus capensis specimens, from the putative western (WC) and southern (SC) stocks off the coast of South Africa, for the years 2014 - 2017.

	Total N	Test Statistic	Degree of Freedom	p-value
Anisakis sp.	118	2.369[a,b]	1	0.124
Diphyllobothrium sp.	118	3.688[a,b]	1	0.055
Lecithochirium sp.	118	0.019[a,b]	1	0.891
Tentacularia sp.	115	3.792[a,b]	1	**0.000**

a. The test statistic is adjusted for ties.
b. Multiple comparisons are not performed because the overall test does not show significant differences across samples.