

# The parasite assemblage of *Scomber japonicus* (Houttyun, 1782) off South Africa

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## ABSTRACT

In South Africa, knowledge of marine parasite diversity is lacking and is often ignored or underutilised. Parasitology has several potential applications in fisheries management, pollution monitoring, aquaculture and general community ecology. With increased knowledge and understanding, the role that parasites play in the marine ecosystems of South Africa is gradually being exposed. This study aimed to document the parasite assemblage of *Scomber japonicus* (commonly known as chub mackerel) off South Africa, and to determine which host characteristics (size, sex and region) influenced parasite infection indices. This species is a small to medium sized, pelagic fish, that has a cosmopolitan, anti-tropical distribution and with populations showing large-scale, environmentally dependant migratory behaviours. It is found off South Africa throughout the year but is most abundant between austral spring and summer. Thanks to their diverse diet and wide array of predators, *S. japonicus* is an ecologically important species, and although it was historically important in the South African purse-seine fishery with large catches taken in the 1960's and 1970's it is no longer, although small amounts are taken as bycatch.

A total of 152 fish ranging between 99 and 514 mm (FL) were sampled in this study and were found to host a total of 16 parasite taxa, 9 of which were identified to species level and 6 to genus level, as well as cysts that were not identified. The parasite assemblage was made up of two nematode species [*Anisakis simplex* (Rudolphi, 1809) and *Contracaecum* sp. (Railliet & Henry, 1912)], six digenean species [*Lecithocladium* sp. (Lühe, 1901), *Opechona bacillaris* (Molin, 1859), *Nematobothrium faciale* (Baylis, 1938), *Halvorsenius* sp. (Gibson, MacKenzie & Cottle, 1981), *Didymocystis* sp. (Ariola, 1902) and a metacercarian], three monogenean species [*Pseudokuhnia minor* (Goto, 1984), *Kuhnia* sp. (Sproston, 1945) and *Grubea cochlear* (Diesing, 1858)], one acanthocephalan species [*Rhadinorhynchus pristis* (Rudolphi, 1802)], one cestode species [*Tentacularia coryphaenae* (Bosc, 1802)], two myxozporan species [*Kudoa thyrsites* (Gilchrist, 1924) and *Ceratomyxa* sp. (Thélohan, 1892)], one copepod species [*Clavellisa scombri* (Kurz, 1877)] and one unidentified cyst species [Cyst 1]. Whilst no new host records

were recorded in this study, *N. faciale* and *Halvorsenius* sp. are new locality records. Generalized linear models were used to determine which host characteristics most influence the prevalence and infection intensity of the six most prevalent parasite taxa (*A. simplex*, *Lecithocladium* sp., *O. bacillaris*, *P. minor*, *R. pristis* and Cyst 1). All showed significant relationships between size and either prevalence or infection intensity or both. This was attributed to the fact that larger, older fish have had more opportunities to get infected than smaller, younger fish, as well as the different diets of adult and juvenile *S. japonicus* which, along with the fact that adults and juveniles tend to school separately, means that the level and diversity of parasites that they are exposed to are different. Three parasites, *A. simplex*, *P. minor* and *R. pristis* also showed significant spatial variation in either prevalence or infection intensity. The prevalence of *A. simplex* and the infection intensity of *P. minor* decreased with in an eastward direction, while the prevalence of *R. pristis* increased eastwards. The spatial trends in the prevalence of *A. simplex* and *R. pristis* were predicted to be driven by the diet of the fish, and the intensity spatial trend observed in *P. minor* infections was predicted to be driven by environmental factors. An analysis of the gill preference of *P. minor* revealed that the outermost gills, furthest from the spinal cord were favoured sites of infection. The driver behind this trend was not definitively identified, however space availability was removed through standardization and water flow is suspected to be the main factor affecting gill arch selection.

Using data from Oliva *et al.* (2008), the parasite assemblage of *S. japonicus* off South Africa was compared to the parasite assemblages of populations of this species in Brazil, Peru, Chile and Portugal using Nonmetric Multidimensional Scaling modelling (NMDS) and an ANOSIM. The NMDS plot showed that all populations were unique. The South African and Portuguese populations (R statistic = 0.28) as well as the Chilean and Peruvian populations (R statistic = 0.32) were the most similar, while the Brazilian population was the most dissimilar from the other populations analysed. The SIMPER analysis revealed that 16 parasite taxa account for 80% of the dissimilarity between the six populations

of *S. japonicus*. This result supports the conclusions made by Oliva *et al.* (2008) that extended separation is the main driver of interspecific differences in the parasite assemblages of this species. This study has increased our knowledge of South African marine biodiversity and of the ecology of South African chub mackerel, and further demonstrated how parasites can be used to elucidate the taxonomic status of their hosts.

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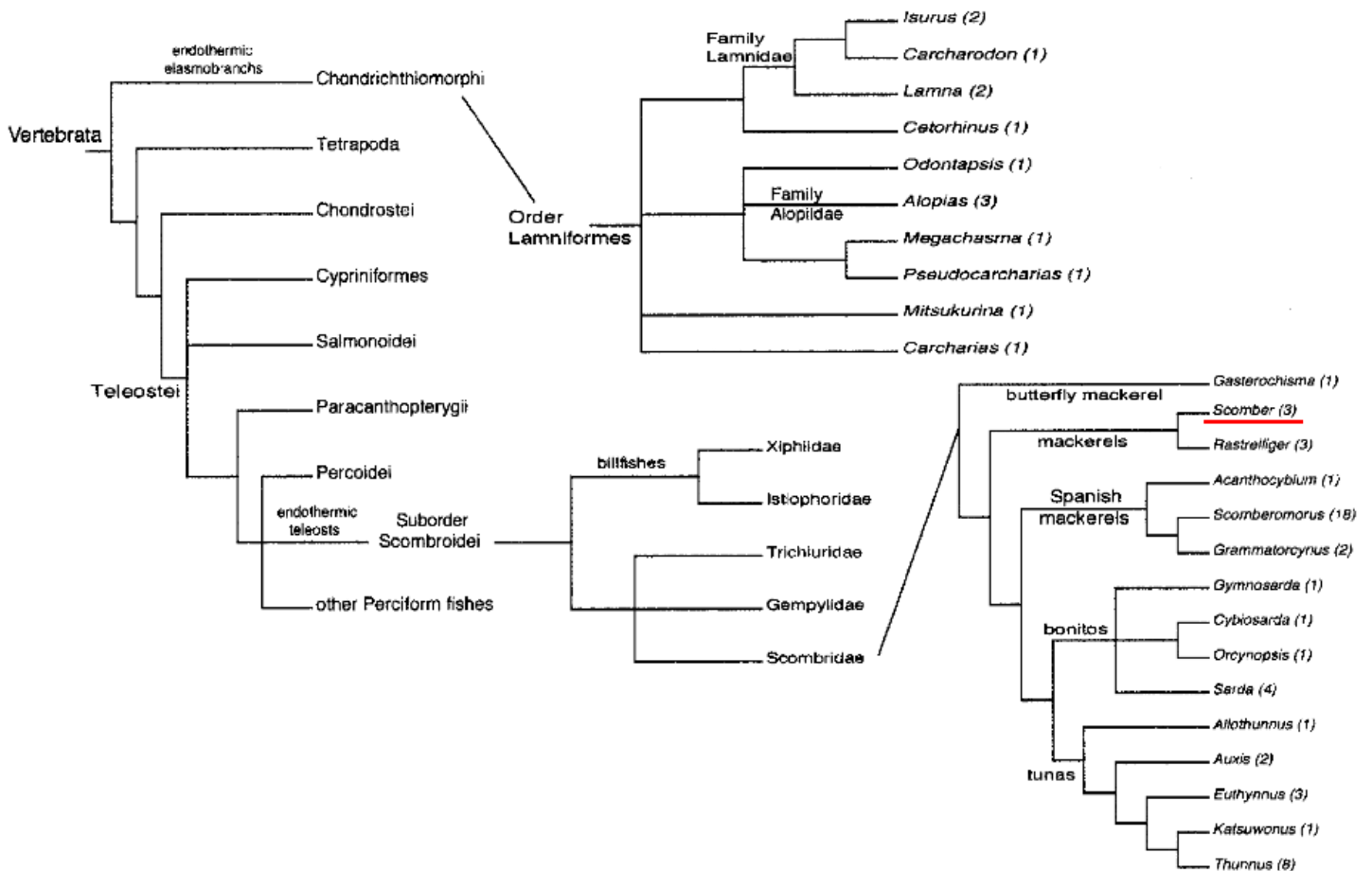
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# INTRODUCTION

## Literature review

### Introduction to *Scomber japonicus*

The family Scombridae includes all tunas, bonitos and ‘true-mackerels’ and can be divided into two subfamilies, the Gasterochismatinae and Scombrinae. The Gasterochismatinae contains just a single species while the Scombrinae contains four tribes including the Scombrini, which contains the genera *Rastelliger* and *Scomber* (Rivas, 1951; Hernández & Ortega, 2000;).



**Figure 1:** Diagram showing where the genus *Scomber* (red line) fits in the family Scombridae (Dickson & Graham, 2004)

There are four distinct species in the genus *Scomber*; *S. australasicus* (Cuvier, 1831), *S. colias* (Gmelin, 1789), *S. japonicus* (Houttuyn, 1782) and *S. scombrus* (Linneaus, 1758) (Hernández & Ortega, 2000).

These four species are both morphologically and genetically differentiated (Hernández & Ortega, 2000).

Classical taxonomy recognises only three species, *S. australasicus*, *S. japonicus* and *S. scombrus* based on different numbers of interneural bones however, both Infante *et al.* (2006) and Catanese *et al.* (2010) determined that there was a significant difference in mitochondrial DNA between *S. japonicus* and *S. colias* (previously thought to be a sub-species of *S. japonicus*). *Scomber indicus*, a potential addition to the genus *Scomber* (Abdussamad *et al.*, 2016), has been described but has not yet been added to electronic taxonomic databases such as FishBase or WoRMS.

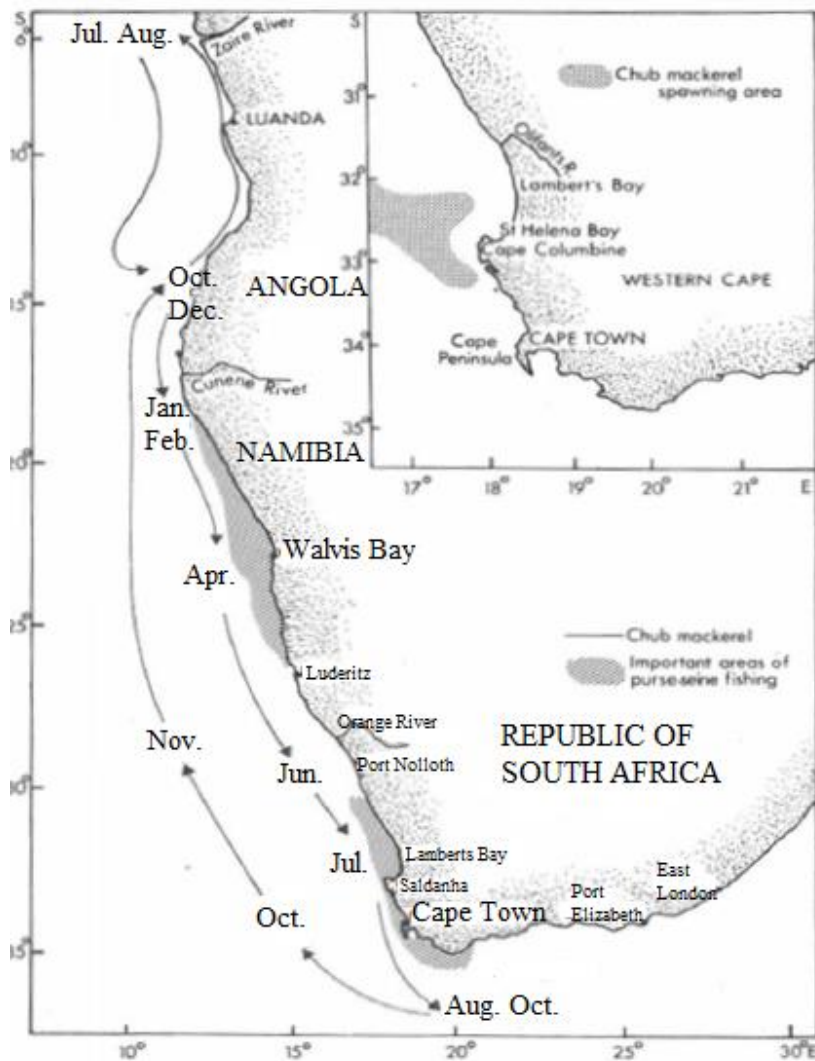
*Scomber japonicus*, like other species in the genus, is a small to medium, coastal pelagic fish species that can grow to around 50 cm (fork length; FL) and live to a maximum of 18 years (Hernández & Ortega, 2000). Although *Scomber* as a genus has a global, anti-tropical distribution, each species within the genus tends to occur in a particular range. *Scomber scombrus* and *S. colias* have similar ranges, concentrating in the North Atlantic, along the coasts of Europe and North America as well as the Mediterranean and Black seas (Hernández & Ortega, 2000). *Scomber australasicus* is found mainly in the Indo-Pacific and Pacific oceans (Hernández & Ortega, 2000).

As seen in other members of the genus, *S. japonicus* is a cosmopolitan species found in particularly high concentrations in the Atlantic and Pacific Oceans, along the coasts of Argentina and Chile in South America, California and Florida in North America, between Belgium and the British Isles and in the Mediterranean. It is also found in high concentrations along the east coast of Asia, particularly off Japan and Australia and is also abundant along the west coast of Africa between Morocco and South Africa (Hernández & Ortega, 2000; van der Lingen, 2016). It is sometimes found in the Indian Ocean, but typically not in large numbers.

*Scomber japonicus* has been of great economic importance since the late 1960's and remains an important food source for many countries around the world, especially in North Africa and East Asia (Hernández & Ortega, 2000).

## *Scomber japonicus* in Southern Africa

*Scomber japonicus*, known locally as chub mackerel, has a large geographical distribution around southern Africa extending from Angola to Kwazulu-Natal in South Africa (Crawford and de Villiers, 1984; Crawford, 1981). However, due to its large-scale seasonal migration patterns and environmental dependence, their distribution in their extensive range is very inconsistent (Crawford and de Villiers, 1984; Cury & Shannon, 2004). Crawford and de Villiers (1984) noted that *S. japonicus* occur in the Northern Benguela off Namibia between October and February and move southward to South Africa during April to August where they spawn off the south coast in August and October. After spawning they



return to the northern Benguela in the later part of the year. It is important to note that these patterns are not fixed and that there are many smaller groups that move in different areas to the main population. Their inconsistent movements are experienced in other parts of the world as well, and are attributed to environmental factors, food availability and the movements and migration patterns of other small pelagics that *S. japonicus* is known to school with (Cury & Shannon, 2004; Crawford and de Villiers, 1984; Villacastin-Herrero *et al.*, 1992).

**Figure 2:** Map showing the seasonal distribution of *S. japonicus* off the South-West coast of southern Africa (South-East Atlantic; from Crawford, 1995)

Economically, *S. japonicus* is an important fish species that is fished commercially by purse-seiners in many regions, most notably in the North-West Pacific, South-East Pacific and Central Atlantic, and it also supports several alternative and subsistence fisheries (David, 1987; Villacastin-Herrero *et al.*, 1992; Castro, 1993; Hernández & Ortega, 2000). Global annual catches reached a peak of 3.5 million tons in 1978 but their catches have decreased in recent years to between 1 and 2 million tons (van der Lingen, 2016).

In South Africa, *S. japonicus* was first caught by purse seiners in 1954 (Crawford and de Villiers, 1984) and their catches averaged around 30 000 tons annually between 1954 and 1966 (Hernández & Ortega, 2000). In 1967, there was an exceptionally high catch of 130 000 tons, the highest ever recorded in South Africa. Between 1965 and 1975, due to reduced *Sardinops sagax* catches and *S. japonicus* availability, the latter species comprised the majority of canned fish in South Africa (Sigam, 2016). Between 1976 and 1995, *S. japonicus* catches in South Africa were much smaller than previous years, with just 8 500 tons caught annually on average (Hernández & Ortega, 2000). Currently *S. japonicus* is not targeted off South Africa and is mainly caught as by-catch in several fisheries. In the pelagic and line fisheries, *S. japonicus* catches have increased in recent years and exploratory fisheries are being investigated to exploit *S. japonicus* between Mossel Bay and Port Alfred using jigging (van der Lingen, 2016). Demersal and mid-water trawl catches have decreased in the last five years, with landings of under 500 tons annually (van der Lingen, 2016).

*Scomber japonicus* is of great ecological importance and feeds on a wide variety of organisms at various stages of the life cycle. Baird (1978) and Sigam (2016) have described the diet of *S. japonicus* off South Africa and reported that immature individuals feed on smaller prey such as calanoid copepods and mysids, whereas adults, while still feeding on calanoid copepods and mysids, receive most of their dietary carbon from stomatopod larvae, squid and small fish. *Scomber japonicus* often school with other, smaller pelagic fish species such as sardine *S. sagax*, anchovy *Engraulis encrasicolus*, west coast round herring

*Etrumeus whiteheadii*, and Cape horse mackerel *Trachurus capensis*. As chub mackerel prey on these species, their movements and abundance patterns are often highly correlated (Castro, 1993; Hernández & Ortega, 2000; Yatsu *et al.*, 2005). *Scomber japonicus* are also preyed upon by many organisms, such as a variety of pinnipeds, cetaceans, sharks, tuna, and sea birds (David, 1987; Hernández & Ortega, 2000), with Ambrose *et al.* (2013) reporting that *S. japonicus* have become a major dietary component of common dolphin *Delphinus capensis* off the South African east coast in recent years. Cury *et al.* (2000) suggests that small pelagic species such as *S. japonicus* play a vital role in the health of upwelling ecosystems around South Africa, as their presence influences both predator and prey and can act as a trophic link between zooplankton, foraging fish and other pelagic predators.

### **Parasitism as a lifestyle**

It can be argued that all (or virtually all) living organisms are infected by some sort of parasite, and even some parasites themselves are infected by other parasites (Schmidt *et al.*, 1989; Poulin & Morand, 2000; Rohde, 2005; Melhorn, 2011). There are many definitions of parasitism, however in this thesis parasitism is defined as the close association of two organisms, where one organism (the parasite) derives some sort of benefit (usually food) from the other (the host). Due to this broad definition of parasitism, this includes commensal and phoretic associations, but excludes predation as this relationship is fundamentally different. Predators kill their prey, and while parasites derive a benefit from their host they do not necessarily adversely affect their host according to this definition.

Due to the vast diversity and number of parasites and their several different types of life cycles, many terms are used to describe parasites. There are parasites that infect numerous hosts (generalists) and parasites that are adapted and restricted to infecting a certain species or group of species (specialists) (Schmidt *et al.*, 1989; Rohde, 2005). Endoparasites infect the internal structures of organisms, and ectoparasites infect the external surface of their host (Rohde, 2005). Parasite can further be divided in those with simple or complex life cycles (Rhode, 2005). In those with simple life cycles the parasite

spends its entire life on one host, and requires physical contact between hosts or a free-living stage as a mode of infection for transmission. In those with complex life cycles, parasites will start their life on one host and the infection of another host is required for development or growth, which occurs through direct consumption of the original host by the second or by a free-living stage (Schmidt *et al.*, 1989; Rohde, 2005).

Parasites can also be divided into microparasites and macroparasites. Microparasites divide inside their host and are usually very small, including organisms such as viruses, bacteria, protozoans and small multicellular organisms <50 µm (e.g. myxozoans or plasmodia) (Marcogliese, 2004). Macroparasites are larger and include digeneans, monogeneans, cestodes, acanthocephalans, arthropods and many more (Marcogliese, 2004).

### **Parasite/human interactions**

Parasites are of great ecological and economic importance due to both their diversity and high abundance in the many ecosystems they impact (Bell & Burt, 1991; Lafferty *et al.*, 2006; Pedersen & Fenton, 2007; Kuris *et al.*, 2008). They interact with humans in several ways and can have numerous significant effects on our health, economy and the surrounding environments (Adams *et al.*, 1997; Shinn *et al.*, 2014; Timi & MacKenzie, 2014). Parasites can have positive impacts such as resulting in an improvement in an organism's immune response over time and preventing overcrowding, or they can have negative impacts such as hindering individual fitness or affecting the quality of aquaculture stocks (Adams *et al.*, 1997; Sheldon & Verhulst, 1996; Mourtisen & Poulin, 2005; Lafferty *et al.*, 2006).

### **Health impacts**

Parasites have been recorded in humans from ancient times and since then hundreds of parasites have been reported to infect humans (Adams *et al.*, 1997; Cox, 2002). Several marine parasites can infect humans, however these infections are typically accidental as those parasites usually have an aquatic

definitive host which is usually a marine mammal or bird (Rohde, 2005). Such parasites can sometimes pose serious health risks if not treated appropriately and can often have fatal consequences (Adams *et al.*, 1997; Rohde, 2005). There are several parasites that commonly infect humans, mainly nematodes from the family Anisakidae, *Diphyllobothrium* cestodes, and trematodes of the families Heterophyidae, Opisthorchiidae and Nanophyetidae (Adams *et al.*, 1997). These parasite infections are associated with the consumption of raw or undercooked seafood and can be very dangerous to humans. For example, Anisakid nematodes such as *Anisakis simplex* and *A. pegreffii*, the former which is regularly found in many marine organisms in South Africa (Reed, 2014; Smit & Hadfield, 2015), are both known to cause gastrointestinal discomfort and with prolonged exposure to sensitive individuals can cause fatal anaphylactic shock (Sakanari & McKerrow, 1989; Audicana *et al.*, 2002; Nieuwenhuizen *et al.*, 2006). Fisheries need to account for human health when exploiting fish populations, for example by avoiding areas known to contain fish with high parasite densities, catching fish of smaller size since bigger fish often have more parasites, and processing fish in specific ways to remove harmful parasites in order to minimize the risk of human infection (Adams *et al.*, 1997; Audicana *et al.*, 2002; Timi & MacKenzie, 2014, Shinn *et al.*, 2014). Cooking fish properly or freezing fish before consumption also kills all parasites that might pose a threat to human health (Adams *et al.*, 1997; Audicana *et al.*, 2002; Shinn *et al.*, 2014).

### **Economic significance**

Parasites play an integral role in the health of natural and even artificial systems, and due to the pressure fisheries put on natural fish populations and the increased reliance on aquaculture for marine resources, parasitology and its applications are crucial to the sustainable harvesting of marine resources (Pedersen & Fenton, 2007; Wood *et al.*, 2013; Shinn *et al.*, 2014; Timi & MacKenzie, 2014). Some fish parasites can greatly influence the value of fisheries products, for example in fish infected with myxozoan parasites from the genus *Kudoa*. Two species in this genus, *K. thrysites* and *K. paniformes* are known to infect

several fish species in South Africa, and can cause severe post mortem muscle degradation which makes the infected fish unmarketable (Langdon *et al.*, 1992; Henning *et al.*, 2013; Reed, 2015; Nunkoo *et al.*, 2016). Due to the health risks described above that accompany some parasite infections, fish that are caught in the wild or grown in mariculture facilities need to be properly processed to minimise the risk to human health (Adams *et al.*, 1997); these measures can be expensive and can influence the price and affordability of certain wild-caught or cultured marine products (Shinn *et al.*, 2014). The expansion of marine aquaculture since the late 1990's has also led to numerous parasitological challenges (Rohde, 2005; Timi & MacKenzie, 2014), since the practice of restricting large numbers of fish to a small volume results in increased packing density, which is beneficial to the transmission of many parasite species (Rohde, 2005). In the wild, fish parasites rarely have a major effect on the abundance of the host population, but parasites can have disastrous effects on captive fish populations because of the conditions in which they are kept (Rohde, 2005). Infection by fish lice *Lepeophtheirus salmonis* in the sea trout *Salmo trutta* farms in the North Atlantic causes skin disease and can also cause sea trout to return to the freshwater prematurely (Birkeland & Jakobsen, 1997), and myxosporean species that infect yellowtail *Seriola quinqueradiata* in Japan can cause scoliosis in fish when they infect the parts of the brain, which leads to increased mortality and decreased marketability (Rohde, 2005). These are just a few examples of how parasites can affect fish in mariculture, however these parasites not only endanger fish in captive populations but also affect wild populations as well, and since captive fish populations are often exposed to wild populations at some point in their life cycle this can lead to abnormal parasite loads in natural populations and can affect more than one ecosystem (Rohde, 2005).

### **Ecological significance**

Parasitology has traditionally been focused on the description of parasites with regards to their taxonomy and life cycles, partly in order to eliminate the risk they pose to humans (Timi & MacKenzie, 2014). Recently, due to the realisation of their importance to ecosystem functioning, parasitology has taken a

decisively ecological and epidemiological turn to study the extent of their influence on our surroundings (Poulin *et al.* 2014). Parasites are generally much smaller than their hosts, however despite their size they account for a significant amount of biomass in some ecosystems. Kuris *et al.* (2008) investigated the biomass contribution of parasites in three estuaries in California and found that the parasites contributed more biomass to the systems than the top predators. Some parasite taxa contributed more than others, with trematodes for example having especially high biomass levels akin to that of birds and fish (Kuris *et al.*, 2008). Parasites have also been observed to affect the behaviour of ecosystem engineers and in turn affect the surrounding ecosystem structure. In New Zealand, Mouritsen and Poulin (2005) studied the effects of the trematode *Curtuteria australis* on the cockle *Austrovenus stutchburyi*. They found that cockles infected with *C. australis* were more sessile and unable to successfully burrow, and that these behavioural effects resulted in higher species diversity in the system under intermediate infection intensities. Parasites can also reduce diversity in systems and can change entire community structures both directly and indirectly (Mouritsen & Poulin, 2005; Wood *et al.*, 2013). Parasites can also alter the complexity and structure of food webs, and although the addition of organisms into food webs generally alters the complexity and increases the density of connections, the inclusion of parasites can affect the patterns of resource flow and change the range of feeding links in food webs (Lafferty *et al.*, 2006; Dunne *et al.* 2013).

## **Parasite diversity**

### The transition to parasitism

It is widely accepted that parasites arose from free living ancestors, as the parasites that evolved required another organism to infect and parasites are closely related to many free-living species (Schmidt *et al.*, 1989; Poulin, 2000; Rohde, 2005). There are more than a few fossil records of parasites that date back to several million years ago, with Basset *et al.* (2004) finding evidence of parasitic infection by a vermiform metazoan in the shell of a brachiopod some 520 million years ago. In metazoan history, parasitism has

evolved approximately 60 times, excluding the parasitic arthropod lineages, with some parasitic lineages being more species diverse than their free-living relatives (Poulin, 2000). For a free-living organism to adopt a parasitic lifestyle, a degree of pre-adaptation is required (Poulin, 2011). Most importantly, the parasite precursor species would need a trait that would enable them to attach to the host and obtain a benefit that would increase their fitness and ability to outcompete their non-parasitic relatives, thus passing on these characteristics (Poulin, 2011). Subsequently, physiological dependence, transmission systems and reproductive adaptation would occur to make it a more effective and efficient parasite (Poulin, 2011). Houck (1994) states that many potential parasites and their hosts go through cycles of offence and defence, as the host attempts to protect itself from infection either morphologically, physiologically or through behavioural patterns. Eventually, often through a commensal or mutualistic mode, a parasitic relationship will occur if the parasite is able to overcome the defences of the host species.

#### Parasite diversity – How and why?

Parasitism is one of the most diverse lifestyles, encompassing organisms from an enormous range of taxa (Schmidt *et al.*, 1989; Poulin & Mourand, 2000; Rohde, 2005; Poulin, 2011). The reason for their success is not yet fully understood and would help taxonomists better understand species diversification, evolutionary history and its relationship to biodiversity (Poulin & Mouillot, 2004). Host diversity and parasite traits are a just two of the factors that researchers consider most integral to the high diversification of parasite lineages (Bell & Burt, 1991; Poulin, 2000; Poulin & Mouillot, 2004). Although it has been suggested that there may be no universal determinants of parasite diversity and that associations between parasite diversity and host features evolve independently in different host-parasite systems (Korallo *et al.*, 2007)

Host diversity is a crucial factor to the diversification of parasites because of the unique parasite lifestyle.

Parasites depend on their hosts for food, protection and sometimes reproduction hence a parasite host

can be considered their respective parasite's environment or island-like habitat (Schmidt *et al.*, 1989; Poulin, 2000; Poulin & Mouillot, 2004). According to island biogeography theory, hosts that have the optimal characteristics for colonization and transmission will yield a higher parasite diversity than hosts that lack such optimal characteristics (Kuris *et al.*, 1980). Host characteristics that promote speciation include a larger body size (which provides a higher capacity for parasite infection), a higher metabolic rate (which results in a higher rate of parasite consumption by host), and a large geographical range (which results in more numerous interactions with other parasite species) (Bell & Burt, 1991; Poulin, 2000; Poulin & Mouillot, 2004).

Epidemiology enables researchers to determine under what circumstances parasites are most successful, and in doing so determine what drives parasite diversification (Schmidt *et al.*, 1989; Poulin, 2000). Epidemiological models are extremely complex and use factors such as the rates of reproduction, mortality and recovery to calculate the invasiveness of parasites and determine which are the most influential characteristics in determining their success (Schmidt *et al.*, 1989; Poulin, 2000; Poulin & Mouillot, 2004).

Specific traits of parasites can also affect their ability to adapt and speciate, with size and life cycle complexity examples of parasite traits that can affect invasiveness. According to Fenchel (1993), small bodied organisms are more numerous than large bodied organisms, and whilst this pattern applies to some parasite taxa it does not apply to all: smaller endoparasitic organisms are generally more diverse than larger ones, but there is no trend in diversity and size for either parasitic arthropods or ectoparasites (Poulin & Morand, 1997).

The focus of biogeography is to describe the spatial and temporal patterns of biodiversity, and this is an extensively studied field of science due to the substantial amounts of information that the biogeography of organisms reveals about how events in the past have affected their evolutionary history (Rohde, 2005;

Luque & Poulin, 2007; Poulin *et al.*, 2011). Due to the reasons explained above it is important to identify areas of high and low parasite diversity and potential threats to these ecosystems (Rohde, 2005; Luque & Poulin, 2007). There are two main theories on the biogeography of parasites, each of which describes the patterns of parasite diversity with regards to longitude and latitude (Rohde, 2005). There are two regions where parasite diversity is concentrated, namely the Indo-Pacific and Atlantic oceans, with the Indo-Pacific being slightly more speciose than the Atlantic (Rohde, 2005). Generally, there is higher species diversity at lower latitudes and this pattern is observed in some parasites as well (Poulin, 2000; Rohde, 2005). Ectoparasite species richness increases at a higher rate towards the equator (Poulin, 2000; Rohde, 2005), but when corrected for host species richness endoparasites show no trend, while ectoparasites still tend to be more diverse at lower latitudes (Poulin, 2000; Rohde, 2005). The occurrence of this trend is mostly attributed to the temperature gradient between latitudes, however, more research is needed as no concrete evidence has been found to support this (Poulin, 2000; Rohde, 2005).

#### Parasitology and its uses

Parasitology has traditionally been dedicated to the description, management and ultimate elimination of parasites, due to their close association with negative health effects on humans and their neighbouring organisms (Timi & MacKenzie, 2014). Due to the complexity of many parasite life cycles, and their high degree of influence on the natural systems surrounding them, the field of applied parasitology has evolved dramatically over the past several decades (Melhorn, 2011; Shinn *et al.*, 2014; Timi & MacKenzie, 2014). Biological control, environmental sciences and fisheries management are a few of the more prominent sectors that have recognised the usefulness of parasitology in their research (Timi & MacKenzie, 2014). As stated above, aquaculture has grown in prominence and fisheries around the world are relying on mariculture to supplement the increasing demand for marine resources (Timi & MacKenzie, 2014). The rapid development of mariculture to supplement the increasing demand for marine resources means that there will also be a rise in the impacts associated with this activity such as

increased parasite infections (Timi & MacKenzie, 2014). This demonstrates the need for some measure of parasite management to reduce the risk that harmful parasite outbreaks in captive populations wouldn't infect natural populations and affect nearby systems (Adams *et al.*, 1997; Hayward *et al.*, 2007).

Environmental science has become an important research topic in the face of global change (Landsberg, 1998; Sures, 1999). Pollution has been identified as key contributor to the degradation many of the world's natural systems, which has caused it to be a central topic to many research fields, including parasitology (Landsberg, 1998; Sures, 1999). Environmental parasitology is the term used to describe the study of the relationships that parasites have with pollutants in their environment (Rohde, 2005). Pollutants can have various effects on parasite populations and can either increase or decrease parasite abundance (Rohde, 2005). Endoparasite abundance usually decreases under increased pollution whereas ectoparasite abundance tends to increase in high pollution environments (Rohde, 2005). A parasite's relationship with environmental pollution is complex due to the many aspects that contribute to their response to pollution, therefore more research is needed to better understand and ultimately predict the relationship that parasites have with environmental pollution (Landsberg, 1998; Sures, 1999; Rohde, 2005). An area which has had much success in recent decades is the use of parasites to monitor heavy metal accumulation in aquatic environments (Landsberg, 1998; Sures, 1999; Rohde, 2005). Heavy metals can have morphological, behavioural and physiological effects on organisms when present at high concentrations therefore it is important that they are monitored in all areas, especially in regions which are particularly vulnerable to pollution (Bryan *et al.*, 1971). Some parasite taxa such as acanthocephalans and cestodes can accumulate heavy metals in much higher concentrations than their hosts, and therefore are valuable as they can be used as bio-indicators to monitor the chemical state of the environment in which they live (Rainbow & Philips, 1993; Sures, 1999; Rohde, 2005).

Herrington *et al.* (1939) were the first to consider using parasites as a tool to investigate the stock structure of fish populations in the North-West Atlantic and the parasite bio-tag method has since been

developed into a respected tool that many fisheries rely on to determine population connectivity and to identify stocks (Rohde, 2005; Catalano *et al.*, 2013; Timi & MacKenzie, 2014). Increased knowledge of both parasite and fish ecology, has enabled the development of several guidelines to properly identify effective and appropriate parasite biological tags (Mackenzie and Abaunza, 1998; Rohde, 2005; Catalano *et al.*, 2013; MacKenzie and Abaunza, 2013). The following parasite characteristics are considered the most important for choosing a suitable bio-tag:

- The parasite needs to have significantly different levels of infection in the different regions of the host's distribution range. Infection level can be measured in several ways, and prevalence, abundance and infection intensity have been used in numerous studies to describe the level of infection (Reed *et al.*, 2012; Timi & MacKenzie, 2014; Nunkoo *et al.*, 2016; van der Lingen *et al.*, 2015)
- The parasite should have a simple life cycle, and preferably only infect a single host species. This reduces the need for research into the various biotic and abiotic factors that affect the transmission and relationship between hosts and their parasites. However, with increased knowledge and research being conducted in parasitology and improved understanding of parasite life cycles this requirement will likely be negated (Rohde, 2005).
- The parasite should not affect the fitness of the host in any way. However, some parasites are known to affect, either directly or indirectly, both host behaviour and fitness. High infection intensities of coccidian *Eimeria sardinae* (Thélohan, 1820) are associated with substantial reductions in testes size of *Sardinops sagax* (Jenyns, 1841) off the coast of South Africa and may lead to parasitic castration and hence affect the host's reproductive ability (Ssempe, 2013) therefore *E. sardinae* is an unsuitable parasite for use as a biological tag. Species from the

superorder Rhizocephala are also known to affect host behavior: these parasitic barnacles infect decapod crustaceans and grow in the same place as their egg sacs, and as they infect both males and females they cause both sexes to protect their “clutch” of parasites as if they were eggs (Høeg, 1995). Parasites that can be fatal to their hosts are also not suitable as bio-tags.

- The parasite needs to be easily identifiable and detectable. Parasites that require little processing or dissection are ideal as they eliminate the probability of errors and are also less expensive and faster to research.
- The parasite should preferably have very little inter-annual variability in infection levels, although analysing data across multiple year classes can alleviate this problem.
- The parasite needs to be able to survive inside the host for at least the duration of the study. A life span of one year is preferable, however, shorter survival times are acceptable under certain study conditions, for example when studying a seasonal migration.

Both Mackenzie and Abaunza (1998) and Rohde (2005) acknowledge that finding a single parasite species that fulfils all these criteria is extremely difficult, therefore allowances are made and some criteria can take preference over others under circumstances where they are not as important or have been compensated for in some way. Often a range of parasites are selected and used together as bio-tags to ensure all the criteria are met (Mackenzie & Abaunza, 1998; Rohde, 2005; Catalano *et al.*, 2014). There are two strategies that can be used when conducting a biological tag study. The first involves describing the entire parasite assemblage of the target host and then using the subset method described above to select one or a small number of biotags that satisfy all or most criteria. Having one or a few biotags

requires a large sample size and is ideal for small fish that are easy to obtain, have less diverse parasite assemblages, and low levels of infection. The second method involves using the entire parasite assemblage of the fish as bio-tags and uses complex multivariate statistical analyses. It is ideal for small sample sizes (for example if the target host is difficult to obtain) of large target hosts with diverse parasite assemblages and elevated levels of infection (Mackenzie & Abaunza, 1998; Rohde, 2005, Catalano *et al.*, 2014). There are many challenges that need to be overcome in applied parasitology, however, increased research focused on fisheries management and environmental sciences can eliminate many issues that hinder progress (Rohde, 2005).

### **Marine parasitology in South Africa**

South African marine parasitology started 200 years ago, when William Elford Leach described an ectoparasitic isopod *Anilocra capensis* in 1818 (Smit & Hadfield, 2015). Since then, many more marine parasite species have been described by various local and international parasitologists. In its infancy, South African marine parasitology was led by scientist such as Dr. Keppel Barnard, Reverend Thomas Stebbing, Harold Fantham and Dr. Brian Kensley (among others) and focused on taxonomic research with the aim of describing various parasites from South African waters. More recently, while still consisting of taxonomic research to a large degree marine parasitology in South Africa has also developed applied and ecological aspects (Smit & Hadfield, 2015). There have been several parasitological studies conducted on species such as the Cape hakes *Merluccius capensis* and *M. paradoxus* (Botha, 1986), kingklip *Genypterus capensis* (Payne, 1986), sardine *Sardinops sagax* (Reed *et al.* 2012; van der Lingen *et al.*, 2015; Weston *et al.*, 2015), snoek *Thryxites atun* (Gilchrist, 1924; Nunkoo *et al.*, 2016), oilfish *Ruvettus pretiosus* (Nunkoo *et al.*, 2017), and angelfish *Brama brama* (Mackintosh *et al.*, 2018). Several applied parasitological studies focusing on stock structure (Reed *et al.*, 2012; van der Lingen *et al.*, 2015; Weston *et al.*, 2015), heavy metal pollution (Morris *et al.*, 2016) and trophic interactions (Weston, 2018) have also recently been conducted. In South Africa, the parasites of commercially

important fish species are becoming increasingly well documented, however there is little known about the parasites of other fish species (Reed, 2015), and species of ecological importance should also be studied.

### **Aims and objectives**

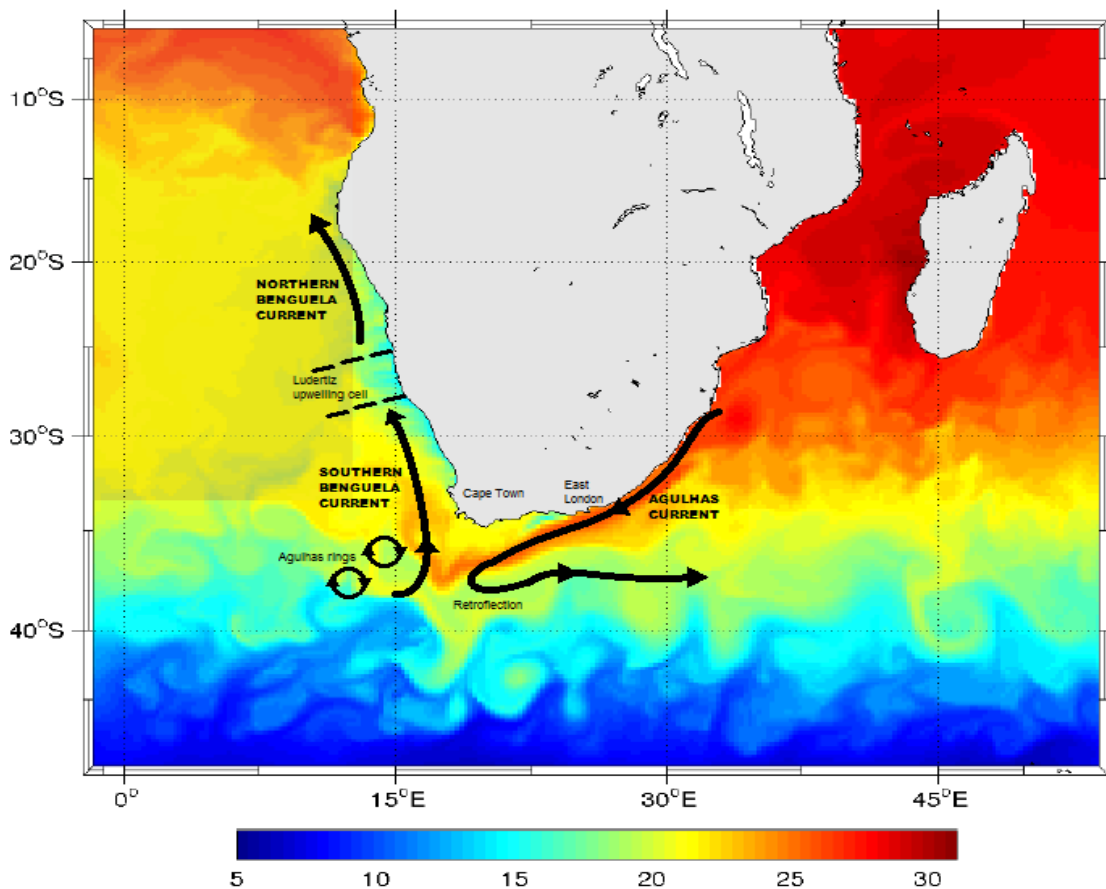
The intention of this research is to fully describe the parasite assemblage of *Scomber japonicus* in South Africa and to identify possible drivers of parasite infection levels. To fulfil this goal, the following objectives are targeted:

- Identify the parasitic species that infect *Scomber japonicus* in South African waters.
- Identify any relationships that parasite species have with host characteristics to determine drivers of infection.
- Compare the parasite assemblage of *S. japonicus* in South Africa to the parasite assemblage of *S. japonicus* populations elsewhere in the world

# METHODOLOGY

## Study site

South Africa is home to a highly diverse marine environment, with over 12 000 species currently identified from its 3 650 km of coastline and associated 1 million km<sup>2</sup> of Exclusive Economic Zone (EEZ) (Griffiths *et al.*, 2010). Substantial differences in environmental parameters such as temperature, salinity and productivity around the coast are considered by many to be the primary contributing factor towards the high degree of diversity and complexity observed in South African marine ecosystems (Griffiths *et al.*, 2010). Two major currents exist around the coasts of South Africa, each with very different characteristics providing organisms with multiple potential niches to exploit (Chang, 2008; Griffiths *et al.* 2010; Beal *et al.*, 2011; Kirkman *et al.* 2016)



**Figure 3:** Map showing the mean sea surface temperatures off South Africa over a 2-day summer period. The Benguela and Agulhas currents are indicated with arrows. The Agulhas Current's point of retroflexion is also indicated as well as Agulhas rings (Chang, 2008)

The Agulhas Current flows inshore down the east coast of South Africa and, after East London, leaves the coastline and flows along the continental shelf break to the tip of the Agulhas bank (Beal *et al.* 2011). At around 40°S the current retroflects back towards the Indian Ocean and releases eddies termed ‘Agulhas rings’ into the Atlantic Ocean that carry warm and nutrient-deficient water from the Indian Ocean into the Benguela Current ecosystem on the west coast (Beal *et al.* 2011). The Agulhas Current, being a typical eastern boundary current, is warm, narrow and fast flowing (2 m.s<sup>-1</sup>) (Beal *et al.* 2011). The Agulhas Bank, inshore of the Agulhas Current, is a temperate shelf system with shelf-edging and coastal upwelling that supports several important fishery sectors (Kirkman *et al.*, 2016)

The Benguela Current is one of the world’s major eastern boundary currents (Hutchings *et al.*, 2009; Girffiths *et al.*, 2010) and supports the Benguela Current upwelling ecosystem, which is separated into northern and southern sub-regions by the Lüderitz upwelling cell off the coast of Namibia (Hutchings *et al.*, 2009). Benguela current is a cold, broad and slow flowing current (0.1 – 0.3 m. s<sup>-1</sup>) and the Southern Benguela features several inshore upwelling cells (Hutchings *et al.*, 2009, Kirkman *et al.*, 2016). Large scale mixing occurs on the south coast between Cape Agulhas and Cape Point, a result of the Agulhas and Benguela currents intersecting one another (Hutchings *et al.*, 2009). The strong upwelling observed on the west coast results in immense productivity which supports high abundances of organisms at several trophic levels (Hutchings *et al.*, 2009, Kirkman *et al.* 2016). This elevated biomass has meant that commercial fisheries have concentrated on the west and southwest coasts of South Africa (Hutchings *et al.*, 2009, Kirkman *et al.*, 2016).

### **Sampling**

Samples of *Scomber japonicus* were collected from the west (west of 20°E), south (20 to 27°E) and east (east of 27°E) coasts of South Africa (Figure 4); however the vast majority of these came from the south coast between Cape Agulhas and Port Alfred, whereas samples from the west and east coasts were limited

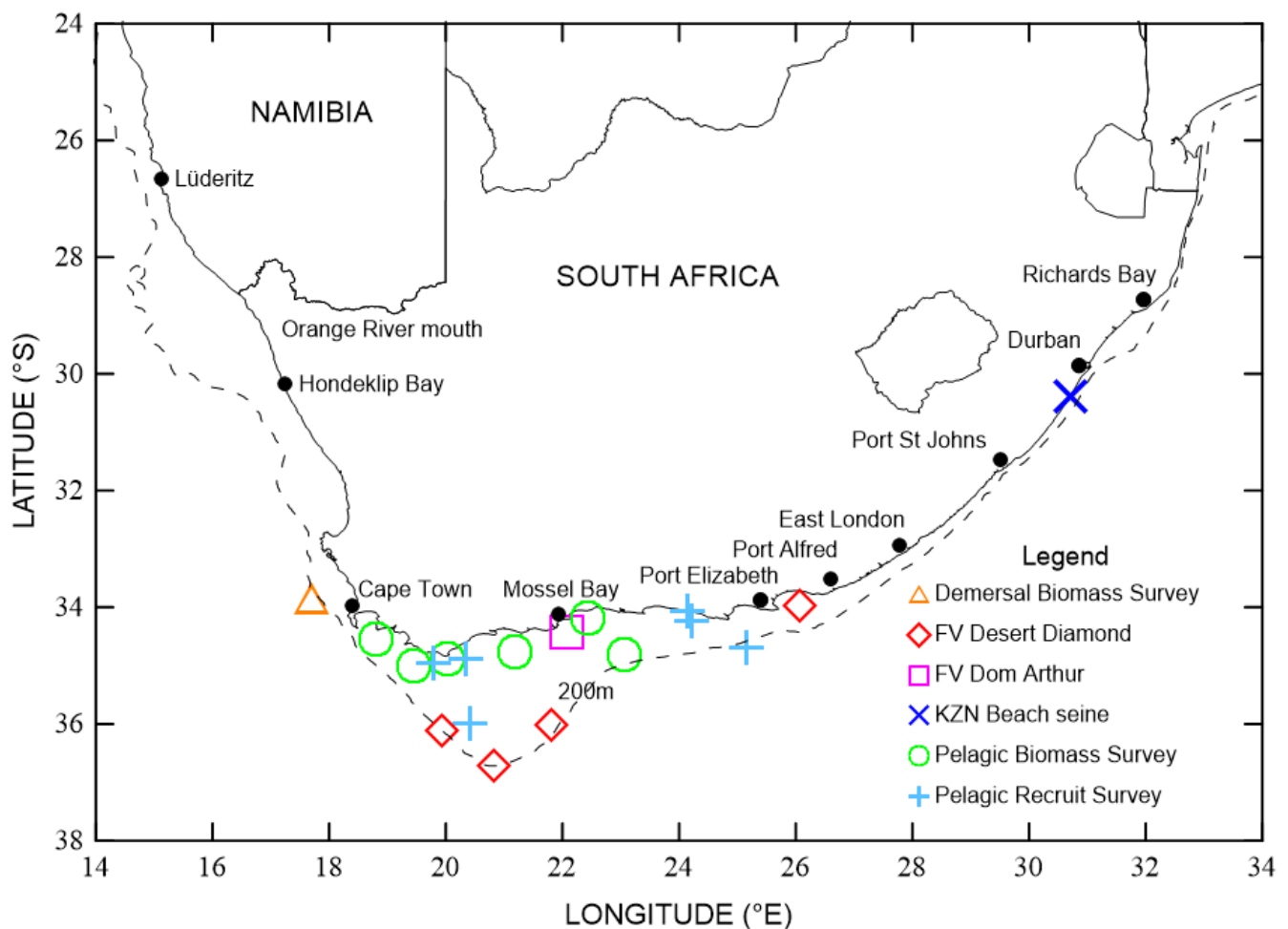
and concentrated in areas around Cape Town and Durban respectively. West and south coast samples were caught during research surveys for pelagic and demersal species conducted by the Department of Agriculture, Forestry and Fisheries (DAFF), and from observer-collected samples from commercial purse-seine and midwater fishing vessels, between November 2016 and August 2017. Samples from the east coast were collected in July 2017 from the beach-seine fishery for sardine off the Kwa-Zulu Natal coast. Samples were placed in plastic bags, labelled with catch and location data before being and frozen and subsequently transported to the Biological Sciences Department, University of Cape Town, where they were kept frozen at -20°C until processing.

### **Fish and parasite processing**

#### **Fish processing**

Fish were processed regularly between February 2017 and June 2018. Samples were thawed at room temperature before being dissected. After thawing sufficiently, the fish were measured (caudal length (CL), fork length (FL) and total length (TL) were recorded to the nearest mm) and weighed (wet body weight (WBW) was recorded to the nearest 0.5 g). Fish were assigned a sexual maturity based on Baird (1977) who reported *Scomber japonicus* begin maturing at 330 mm (standard length); this value was converted to fork length using the equation provided by Baird (1977) with a resulting value of 351 mm. Fish larger than this size were regarded as mature and those smaller than this length immature, although it must be pointed out that this may be an overestimate of the true size at maturity given lower values for this species elsewhere reported by other authors (Hernandez & Ortega, 2000 and Cerna & Plaza, 2014). The external surface of fish, including skin, fins, mouth and nares were examined for ectoparasites. The opercula, gill arches and eyes were removed and examined in petri dishes using a Leica EZ4 dissecting microscope between 8X and 35X magnification. Body cavities were opened, mature fish were sexed and their gonads were removed, weighed (gonad weight (GW) recorded to the nearest 0.5 g) and staged according to the seven-stage classification system outlined by Baird (1977). The visceral organs were

removed and both the body cavity itself and the external surfaces of organs were examined for parasites under the same dissecting microscope. The stomach, pyloric caeca and intestines were opened, and the lining and contents were examined in a petri dish under the same dissecting microscope. Temporary wet mounts were prepared from muscle, kidney, liver, heart, gonads and gall bladder (when found) tissues and examined using a Leica DM500 compound light microscope between 40X and 400X magnification. Tissue samples were taken from the same location in/on the fish or organ in all samples. All macroparasites, along with the number of individuals of each taxon and site/s of infection were recorded,



**Figure 4:** Map showing the locations of *Scomber japonicus* samples collected off South Africa (2016 - 2017) and the number of individuals in a single field of view (FOV) under 400X magnification of the various tissue samples was recorded for all microscopic parasites.

## **Parasite processing**

All macroparasites encountered (except for the most abundant – see below) while processing fish samples were collected and preserved. Each parasite was first brushed or shaken in a vial with tap water to remove all debris, before being preserved in 90% ethanol. Due to the considerable number of *Pseudokuhnia minor* individuals that were found, only those from the 4<sup>th</sup> left gill arch were preserved. Acanthocephalans and cestodes were first soaked in fresh water to allow their proboscis to extend from their bodies. This was not always successful, however all acanthocephalans and cestodes were still preserved. Digenean and monogenean individuals were first flattened before being preserved in the ethanol, which was done in order to make their internal organs easier to see and identify. Randomly selected individuals from each parasite taxon were used to formally identify that species, with those individuals stained in Mexican red for between two and three hours before being dehydrated with a series of ethanol-water baths (starting at 50% then increasing by 10% Ethanol at each stage till 90% ethanol). After dehydration, the parasites were cleared using clove oil and then mounted on a permanent slide using a DPX resin. Literature, in the form of identification keys and images (when available) were used to identify the collected parasites to the lowest taxonomic level. Identifications were based on morphological characteristics. The opinions and expertise of several skilled parasitologists, including Mr. Irfan Nunkoo, Dr. Cecile Reed, Prof. Ken MacKenzie, Emeritus Prof. Klaus Rohde and Dr. Marcelo Oliva were used in the identifications.

**Table 1:** Table showing the season and number of individual *Scomber japonicus* processed for parasites from each sampling location from South Africa (2016 to 2017)

<b>Station</b>	<b>Sample source</b>	<b>Date landed</b>	<b>Autumn/Winter (86)</b>	<b>Spring/Summer (66)</b>
A32922	Pelagic Biomass Survey	November 2016		4
A32942	Pelagic Biomass Survey	November 2016		5
A32961	Pelagic Biomass Survey	November 2016		7
A32993	Pelagic Biomass Survey	December 2016		20
A33015	Pelagic Biomass Survey	December 2016		16
A33027	Pelagic Biomass Survey	December 2016		10
A33093	Demersal Biomass Survey	January 2017		4
5331	FV Dom Arthur	May 2017	4	
5610	FV Desert Diamond	August 2017	4	
Trawl 19	FV Desert Diamond	June 2017	7	
Trawl 50	FV Desert Diamond	June 2017	13	
Trawl 50	FV Desert Diamond	August 2017	13	
Pennington	KZN beach seine	July 2017	11	
A33250	Pelagic Recruit Survey	June 2017	4	
A33258	Pelagic Recruit Survey	June 2017	4	
A32966	Pelagic Recruit Survey	July 2017	10	
A33297	Pelagic Recruit Survey	July 2017	4	
A33298	Pelagic Recruit Survey	July 2017	8	
A33299	Pelagic Recruit Survey	July 2017	4	

## Host biological factor analysis

There are several factors that are known to influence the prevalence and extent of parasite infections in a host which can be roughly grouped into biotic and abiotic factors. Examining both of these groups is essential to understanding the influence that parasites have on their hosts, both biologically and ecologically, as well as understanding what makes host more susceptible or resilient to an infection (Bauer, 1959; Iyaji, 2009).

Fish size, sex, condition factor, gonado-somatic index, location and season of collection were selected as host biological characteristics for comparison with parasite infection indices in order to better understand the drivers of the parasite assemblages of *S. japonicus*. Buchmann (1989) and Cable & Van Oosterhout (2007) both report that parasite prevalence and size can be positively correlated with host size and there have also been numerous studies that have associated variation in parasite load with seasonality of host ranges. Nunkoo *et al.* (2016) found that both condition and gonad development were among the strongest predictor variables for the parasites of *Thrysites atun*. It is important to note that the effects of the factors on the different species of a host's parasite assemblage is not consistent and that factor effects can differ greatly.

The condition of the fish was calculated according to the method outlined by van der Lingen *et al.* (2006). A condition factor (CF) was calculated for each fish sampled according to the following calculation:

$$\text{Condition factor} = \frac{\text{Observed total mass}}{\text{Expected total mass}}$$

The expected WBW was calculated by fitting a power regression to the WBW and FL data and determining the relationship according to the following equation:

$$\text{Expected mass} = m \times \text{length}^c$$

Two one-way ANOVA's were conducted, one to determine whether there was difference in condition between males, females and immature fish, and a Mann-Whitney U test was done to determine whether there was seasonal variation in condition factor. Condition factor was also examined using a box plot, separating fish by both sex and season.

The gonad development of each fish was measured using the gonado-somatic index (GSI), calculated according to the following equation used by Griffiths (2002):

$$\text{Gonado - somatic index} = \frac{\text{Gonad mass}}{\text{Observed total mass} - \text{gonad mass}} \times 100$$

The GSI was examined using a box plot separating the fish by season and sex. A Mann-Whitney U test was also conducted, comparing the GSI of each season in order to test any trends observed in the box plot.

## **Statistical analysis**

### **Exploratory Data Analysis**

Developed by John Tukey in the late 1960's, exploratory data analysis (EDA) is a critical step in the analysis of data (Behrens, 1997). The main aims of the EDA are to check that assumptions are met, expose outliers, and reveal trends in the data (Behrens, 1997, Bolker, 2008). There is no set protocol for carrying out an EDA, however generally the data is looked at graphically, and these graphics are supplemented by quantitative statistics (DuToit *et al.*, 2012, Behrens, 1997, Zuur *et al.*, 2010, Bolker, 2008). Assumptions such as normality, heteroscedasticity and independence are crucial to test for, as if they are not met then the data need to be either transformed in order to use parametric tests for analysis, or non-parametric tests and generalized linear models (GLM's) need to be used for analysis (Bolker, 2008). The EDA procedures outlined by Bolker (2008) and Zuur *et al.* (2010) were employed as

guidelines to analyse the data collected in this study. Bolker (2008) describes an EDA for application of a general linear mixed model (GLMM), however the principles are still relevant to this study.

Continuous variables such as fork length (FL), mass, gonado-somatic index (GSI), longitude and condition factor (CF) were examined for normality using histograms and tested for normality and heteroscedasticity using the Shapiro-Wilks test. Outliers in the continuous variables were identified using boxplots. Scatterplots, as well as the Pearson correlation test were used to detect collinearity between continuous variables. Chi-squared tests were used to determine whether the categorical variables (season and sex) were independent or not.

The exploratory data analysis showed that the FL and WBW of *S. japonicus* were significantly correlated via a power relationship, therefore only FL was used as an index of host size for analyses as any pattern observed in one of the factors would be reflected in the other. A Kruskal Wallis test and *post hoc* comparison was employed to determine whether there was a difference in FL between the three sexes (male, female and immature fish) and a Mann-Whitney U test was used to assess differences in FL with regards to seasonality.

## Determinants of infection

The EDA established that the host data does not fit the assumptions required for parametric testing. A generalized linear model (GLM) was therefore selected to predict the host characteristics most likely responsible for variability in the prevalence and infection intensity of the various parasite taxa. A GLM is a linear regression that tolerates variables that are not normally distributed, which is preferred as the data do not have to be transformed and instead a link function that relates the mean of the response variable to the linear form of the predictor variables is used (McCulloch, 1997). There are three elements that make up a GLM, namely the response variable, the predictor variables and a link function (McCulloch, 1997; Chatfield *et al.*, 2010). The GLM's in this study were used to identify significant variables for predicting the prevalence and infection intensity of the various parasite taxa found infecting *Scomber japonicus* off South Africa.

**Table 2:** Table showing the predictor variables chosen for the GLM's of prevalence and infection intensity

Predictor variable	Description
FL	Size of host (Fork length (mm))
Sex	Sex of host (male, female, immature)
Season	Season in which fish was caught
Longitude	Location of fish when caught
Sex * FL	Interaction between the host sex and host size

The logit link function was used in the GLM for prevalence, as prevalence is a binary response and therefore, has a binomial distribution. For infection intensity the logarithmic link function was used as the infection intensity displayed a Poisson distribution and therefore required a link function that could explain the high degree of dispersion.

The general equation for each of the GLM's were:

$$\text{Logit}_{prevalence} = FL + Sex + Season + Longitude + Sex * FL + \varepsilon_i$$

$$\text{Log}_{infection\ intensity} = FL + Sex + Season + Longitude + Sex * FL + \varepsilon_i$$

Only parasites with a prevalence between 20% and 90% were modelled, as a low prevalence makes it difficult for the model to detect trends in the data with a high degree of spread, and if the prevalence is too high, then the model will not be able to explain enough of the variation in the data. The infection intensity model was only applied to parasites that had a prevalence of over 30%, as a prevalence lower than this would also not provide the model with enough data to detect a trend.

These general models, including all the predictor variables, were used to analyse the influencers of prevalence and infection intensity of all parasite taxa that fulfilled the above criteria. An ANOVA was used to evaluate the significance of each predictor variable in the model, insignificant variables were dropped from the GLM in order of magnitude and the model was rerun until only significant variables were retained in the model. Akaike's information criterion (AIC) was used as the method of model selection; this estimates the amount of information that would be lost in the prediction of a model by balancing the complexity and the goodness of fit of that model (Symonds & Moussalli, 2010). Therefore, the model with the lowest AIC and lowest amount of deviance was considered the most optimal and accurate. Quasi-AIC (QAIC) was used in place of AIC when dealing with the infection intensity model, due to the over dispersion of the infection intensity data. An ANOVA was used to analyse and compare the deviance explained by each model. Once the optimal model was selected, the goodness of fit was measured using the following equation used by (Weston, 2013):

$$\text{Goodness of fit} = 1 - \frac{\text{Residual deviance}}{\text{Null deviance}}$$

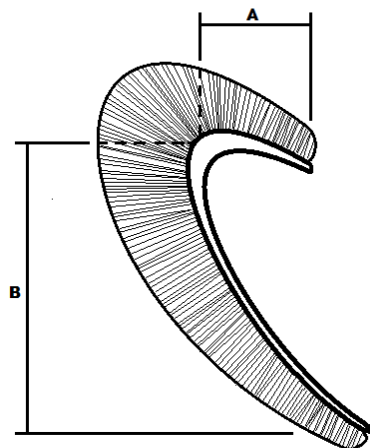
## **Parasite community analysis**

A species accumulation curve was plotted to estimate the adequacy of this study in fully describing the parasite assemblage of *Scomber japonicus* off South Africa. Non-metric Multidimensional Scaling (NMDS) was used to examine the seasonal and sex differences in the parasite community of *S. japonicus* from South Africa. NDMS was also used to compare the parasite assemblage of *S. japonicus* off South Africa to the parasite assemblages of this species elsewhere. NDMS is a type of ordination that is based on ecological distance (dissimilarity matrix) and is a rank-based approach that is able to deal with a wider range of data types with unknown distributions. Raw data describing the parasite assemblages of *S. japonicus* off Chile, Brazil, Peru and Portugal collected between 2002 and 2003 were kindly obtained from Marcelo Oliva from the University of Antofagasta, Chile (Oliva *et al.*, 2008) and combined with the parasite data from this study. All NMDS ordinations were done using abundance data as both prevalence and abundance produced similar results.

Analysis of Similarity (ANOSIM) was used to assess the significance of the difference between groupings in the NMDS plots. ANOSIM is a test that is aligned with NMDS in that it also uses ranked dissimilarities and returns an R value which is based on the difference of the mean ranks between groups and within groups. The R value can range between -1 and 1; values closer to 1 indicate that the dissimilarity between groups is greater than the dissimilarity within the groups (suggesting a high degree of separation between groupings), values closer to 0 that the high and low ranks are evenly distributed both between and within groups (suggesting little separation between groupings) and values closer to -1 indicating that dissimilarity is greater within groups than between groups suggesting problematic sampling or unidentified ecological processes. A Similarity Percentage (SIMPER) test was used to identify the species which contributed most significantly to the overall variation in community structure among groupings. Parasite taxa which were not identified to species level were included, using the suspected but unconfirmed identification.

## Gill parasite analysis

Numerous studies have described the many facets of the biology and ecology of fish gill parasites (Paling, 1965; Geets *et al.*, 1997; Gutierrez and Mortonelli, 1999; Lo & Morand, 2001). The gills of a fish can be considered a microhabitat where many niches can be fulfilled and therefore many gill parasites have become highly specialized and show strong microhabitat preferences (Rohde, 1979). Gill restrictions, parasite asymmetry, water flow and spatial availability are among the drivers of gill parasite preferences (Rohde, 1979). In order to better understand the microhabitat ecology of gill parasites of *S. japonicus* in South Africa, the number and position (whether on first, second, third or fourth gill arch) of each gill parasite encountered during this study was recorded for each sampled host. As the most prevalent parasite, the infection level of *P. minor* was selected as a proxy parasite load. The gill arches of *S. japonicus* are not of a uniform size, therefore the abundance of the parasites infecting each gill arch required standardization for the size difference in order to be comparable. This was done by measuring the FL and the gill arch length (GAL, measured to the nearest mm) from the tip of the upper gill arch to the tip of the lower gill arch (A + B in figure 5) of each of the four gill arches from the left and right sides of 40 *S. japonicus*



**Figure 5:** Diagram showing the dimensions used to measure gill arch length. Upper gill arch length (A) and lower gill arch length (B).

Scatterplots of the GAL and fork lengths were then plotted separately for each gill arch and the relationships between these variables determined using linear regression. The length of each gill arch (GAL) of each host sampled for parasites was then calculated using the equations shown in Table 3:

**Table 3:** Equations used to predict gill arch length (GAL) from FL for each of the four gill arches on the left and right-hand sides of *S. japonicus* from South Africa. Gill arches are numbered from 1 (innermost gill arch) to 4 (outermost gill arch).

Gill arch	Left	Right
1	$GAL = 0.0143(FL) - 0.5105$	$GAL = 0.0130(FL) + 0.0375$
2	$GAL = 0.0181(FL) - 0.7034$	$GAL = 0.0178(FL) - 0.3295$
3	$GAL = 0.0195(FL) - 0.2001$	$GAL = 0.0170(FL) + 0.4308$
4	$GAL = 0.0206(FL) + 0.0408$	$GAL = 0.0189(FL) + 0.4675$

A gill arch infection factor (GAIF) was then calculated for each gill arch of each fish according to the following equation:

$$GAIF = \frac{\text{Number of } P. \textit{minor} \text{ individuals present on gill arch}}{\text{Predicted GAL (mm)}}$$

A series of student t-tests were used to determine if there were differences between the mean GAIF on the left and right gill arches for each gill arch. An ANOVA and *post hoc* Tukey test, and a series of student t-tests were used to determine if there were significant differences between the mean GAIF on the left and right gill arches for each gill arch.

# RESULTS

## Exploratory Data Analysis

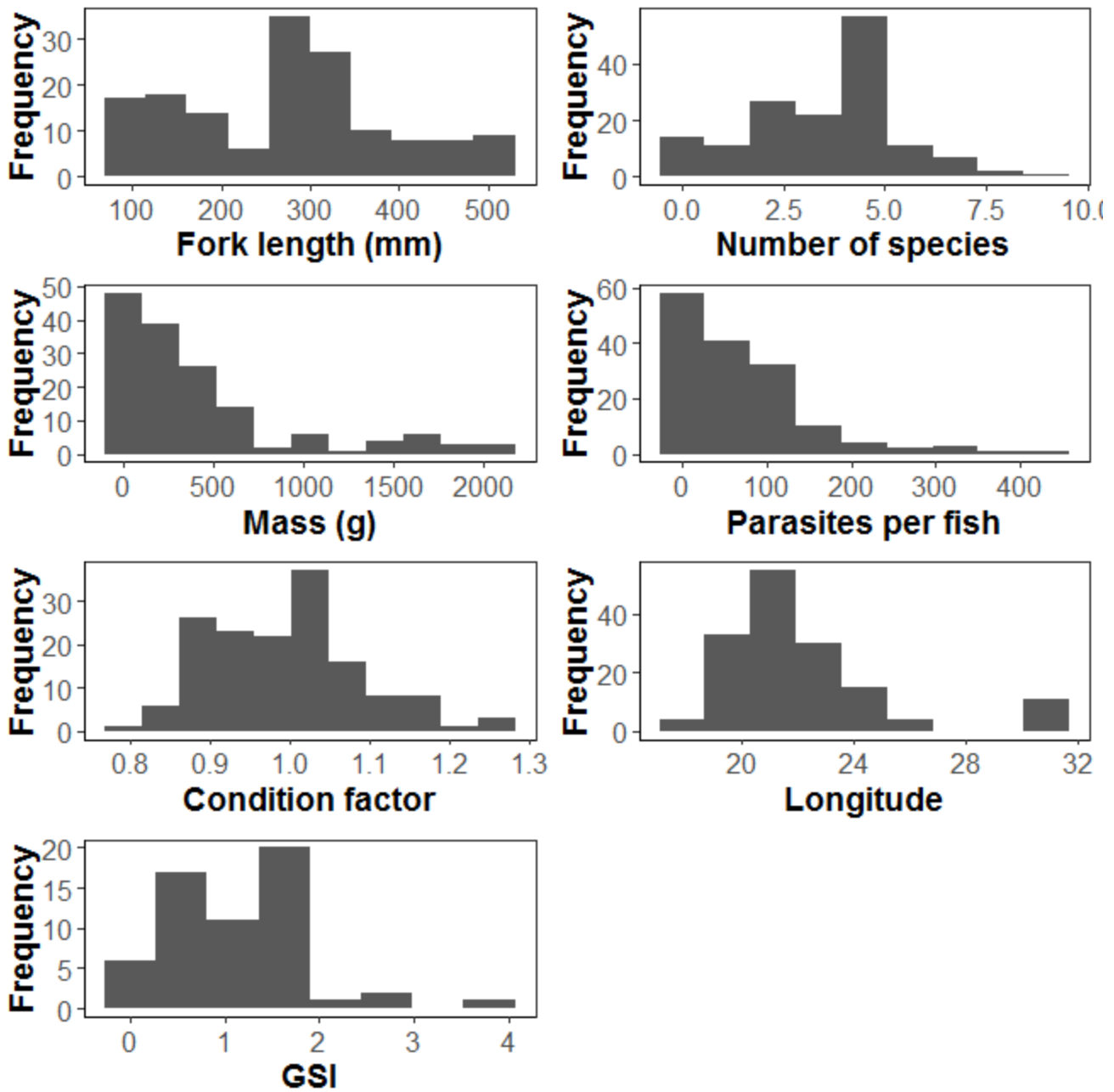
A total of 152 *Scomber japonicus* were collected from around South Africa between November 2016 and August 2017 and processed between February 2017 and June 2018. Fork lengths of the sampled fish ranged between 99 mm and 514 mm, and their weight ranged between 8.5 g and 2082 g. Of the 152 individuals, 16 were male, 17 were female and 119 were immature.

Histograms of FL, WBW, GSI and longitude revealed that these variables were not normally distributed, which was confirmed with the Shapiro Wilks test ( $p < 0.05$ ). The histogram for CF displayed a normal distribution with a slight skewness to the left, however the Shapiro Wilks test indicated condition factor was in fact non-normal ( $W = 0.97$ ,  $p - \text{value} = 0.005$ ). All continuous variables were found to be significantly correlated, except for FL and CF which showed no relationship ( $p - \text{value} > 0.05$ ).

**Table 4:** Table showing the correlation results for each of the continuous variables for *Scomber japonicus* collected off South Africa (2016 and 2017)

Correlation	R <sup>2</sup>	df	p-value
FL-WBW	0.89	150	1.17 X10 <sup>-55</sup>
FL-GSI	0.54	56	1.36 X10 <sup>-05</sup>
FL-CF*	0.09	149*	2.85 X10 <sup>-01</sup>
WBW-GSI	0.56	56	6.00 X10 <sup>-06</sup>
WBW-Condition factor*	0.18	149*	3.04 X10 <sup>-02</sup>

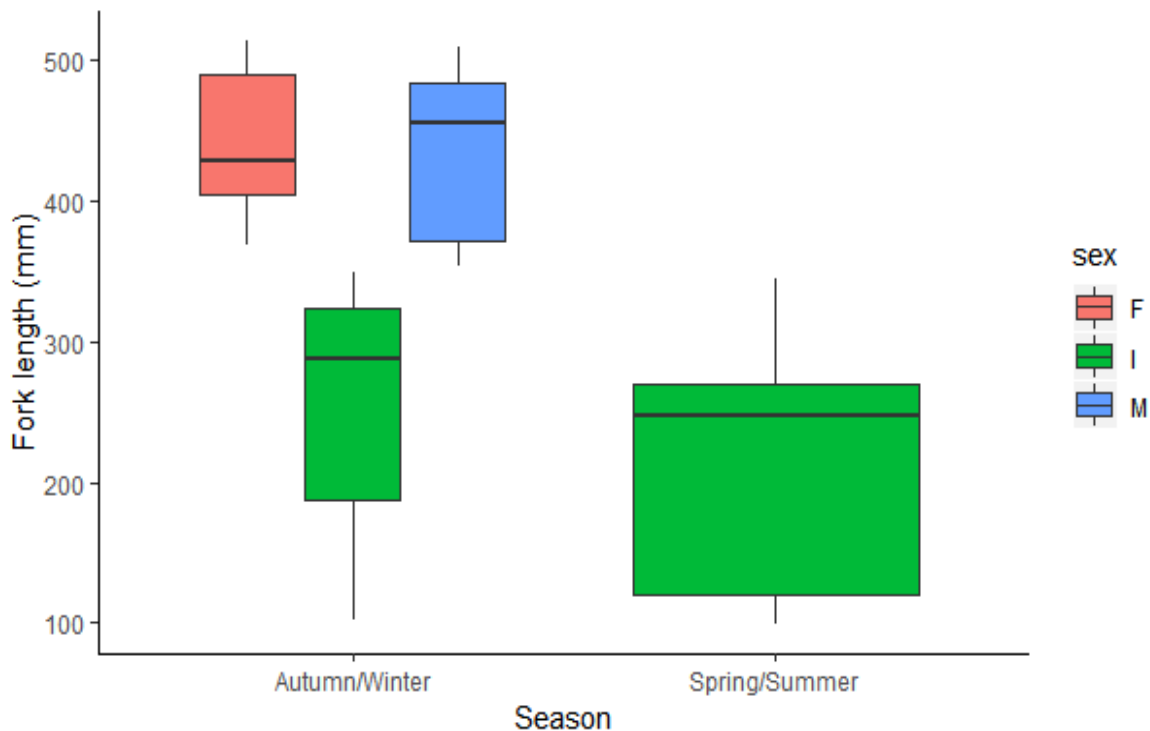
\* Outlier removed



**Figure 6:** Frequency distribution histograms of the biological characteristics of *Scomber japonicus* processed for parasite analysis, including fork length, wet body weight (mass), condition factor, gonado-somatic index, number of parasite taxa per host, total number of individual macroparasites per host and catch location.

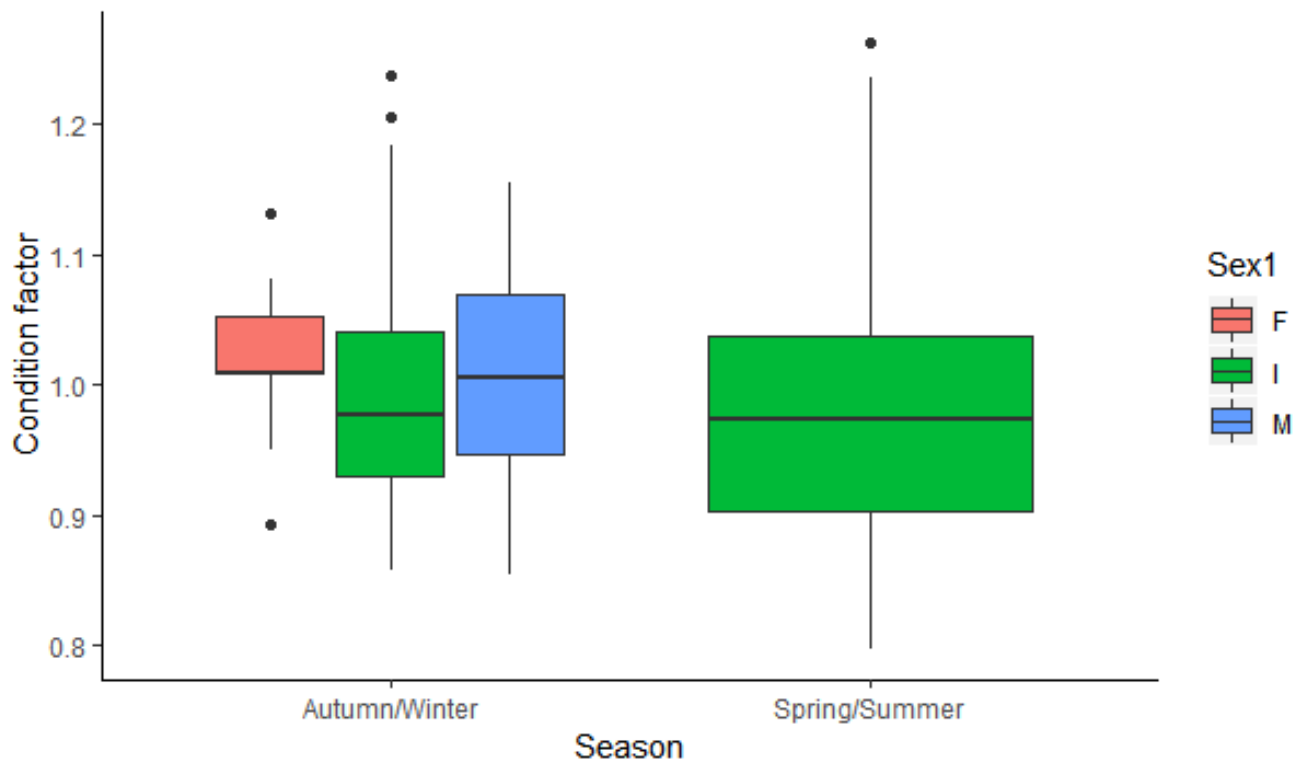
### Host biological factor analysis

The sample of 16 males, 17 females and 119 immature fish, skewed the sex ratio significantly towards immature fish ( $\chi^2 = 138.25$ ,  $df = 2$ ,  $p - \text{value} = 2.2 \times 10^{-16}$ ). Males had a mean FL of 435.6 mm (SD = 56.2 mm), females a mean FL of 441.2 mm (SD = 51.7 mm) and immatures a mean FL of 228.9 mm (SD = 82.4 mm), and whilst FL differed significantly between sexes ( $\chi^2 = 77.017$ ,  $df = 2$ ,  $p - \text{value} = 2.2 \times 10^{-16}$ ) males and females were not significantly different ( $p - \text{value} > 0.05$ ) but both differed significantly from immature fish ( $p - \text{value} > 0.05$ ). Only one male and no females were caught in the spring/summer so no seasonal differences in the mean size of adult fish could be tested for, but a Mann-Whitney U test showed that immature fish displayed significant seasonal variation ( $U = 2626.5$ ,  $p - \text{value} = 2.175 \times 10^{-6}$ ) in mean FL



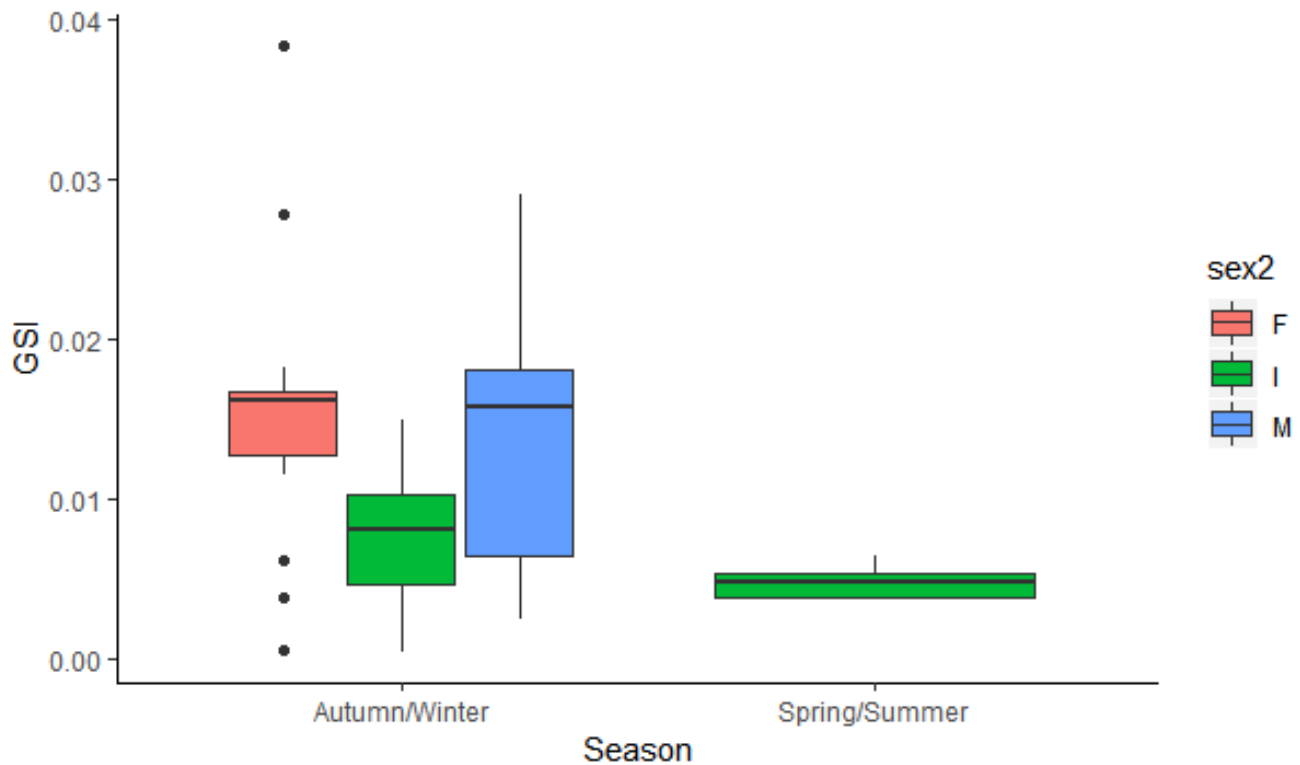
**Figure 7:** Boxplot showing seasonal variation in FL of male (M), female (F) and immature (I) *Scomber japonicus* processed for parasites off South Africa (2016 to 2017).

Condition factor did not vary significantly between male, female and immature fish ( $\chi^2 = 4.0034$ ,  $df = 2$ ,  $p - \text{value} = 0.1351$ ). Males presented a mean CF of 1.01 (SD = 0.08), females a mean CF of 1.02 (SD = 0.06) and immature fish a mean CF of 1.01 (SD = 0.23). No seasonal variation in the CF of immature fish was detected ( $U = 1904.5$ ,  $p - \text{value} = 0.3854$ ).



**Figure 8:** Boxplot showing the seasonal variation in CF of *Scomber japonicus* processed for parasites off South Africa (2016 and 2017) (one outlier with a CF of 3.2 from summer removed)

The GSI differed significantly between sexes ( $\chi^2 = 15.982$ ,  $df = 2$ ,  $p - \text{value} = 0.0003$ ), and while mean values of males ( $0.013 \pm 0.0075$ ) and females ( $0.016 \pm 0.0085$ ) were not significantly different, both differed significantly from the mean GSI of immature fish ( $0.007 \pm 0.0040$ ). There was no significant seasonal variation in GSI immature fish ( $U = 72$ ,  $p - \text{value} = 0.1483$ ).



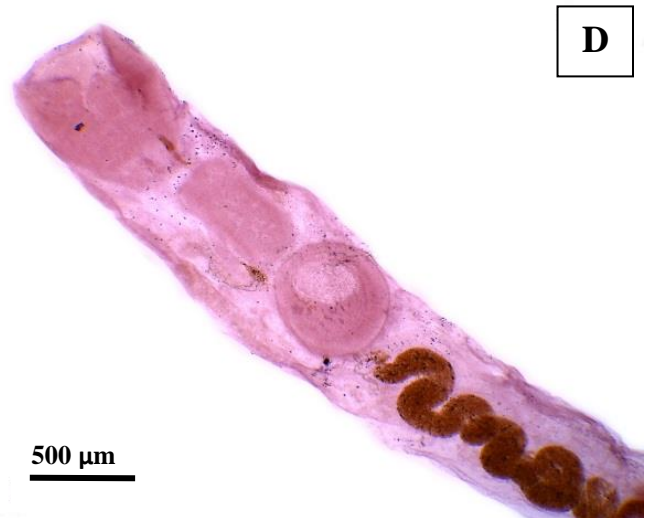
**Figure 9:** Boxplot showing seasonal variation in GSI of *Scomber japonicus* processed for parasites off South Africa (2016 and 2017)

## Parasite assemblage

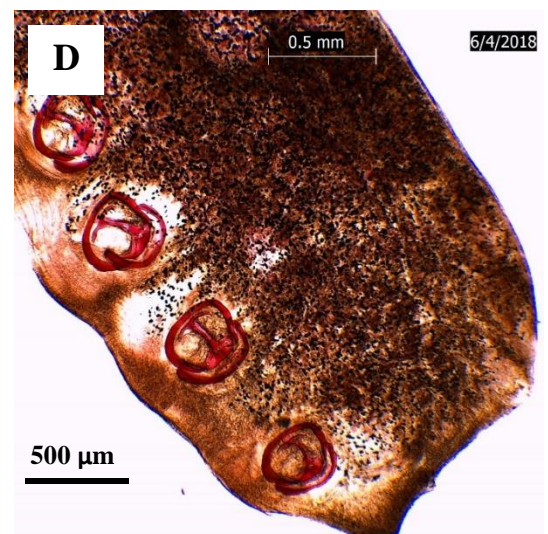
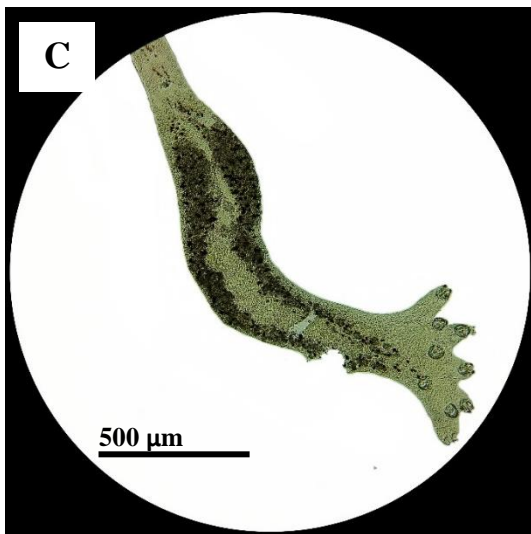
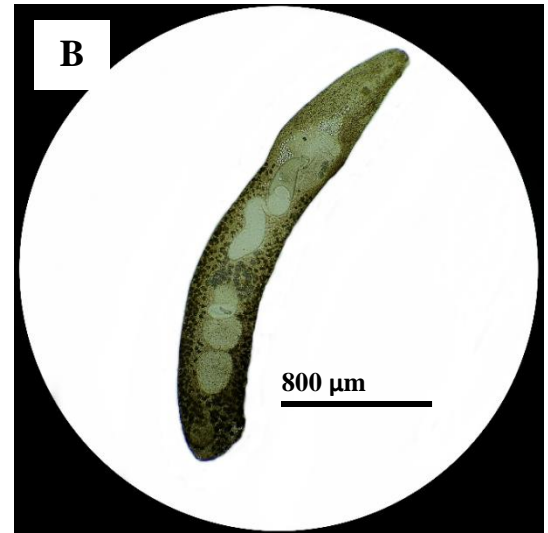
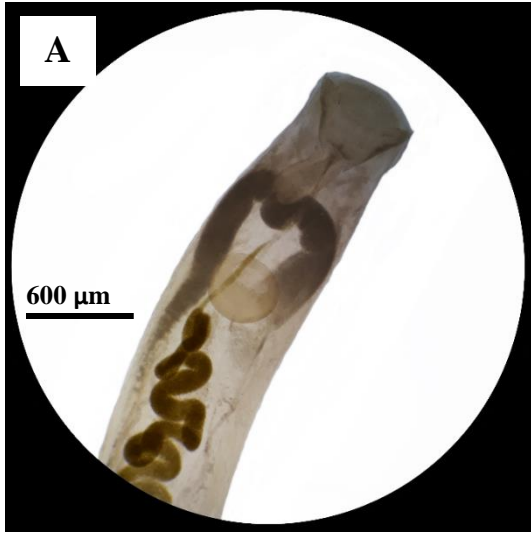
A total of 10 536 parasite specimens belonging to 17 taxa were found infecting *Scomber japonicus* from South Africa. Not all taxa were identified to the same level, nine taxa were identified to species level, six were identified to genus level, and a cyst and a metacercaria could not be identified. The parasite assemblage was made up of two nematode species [*Anisakis simplex* (Rudolphi, 1809) and *Contracaecum* sp. (Railliet & Henry, 1912)], six digenean species [*Lecithocladium* sp. (Lühe, 1901), *Opechona bacillaris* (Molin, 1859), *Nematobothrium faciale* (Baylis, 1938), *Halvorsenius* sp. (Gibson, MacKenzie & Cottle, 1981), *Didymocystis* sp. (Ariola, 1902) and an unidentified metacercaria], three monogenean species [*Pseudokuhnia minor* (Goto, 1984), *Kuhnia* sp. (Sproston, 1945) and *Grubea cochlear* (Diesing, 1858)], one acanthocephalan species [*Rhadinorhynchus pristis* (Rudolphi, 1802)], one cestode species [*Tentacularia coryphaenae* (Bosc, 1802)], two myxozporean species [*Kudoa thyrsites* (Gilchrist, 1924) and *Ceratomyxa* sp. (Thélohan, 1892)], one copepod species [*Clavellisa scombri* (Kurz, 1877)] and one unidentified encysted species (Cyst 1).

**Table 5:** Table showing the taxon, site of infection, prevalence, mean infection intensity and range of abundance of the parasite assemblage of *Scomber japonicus* off South Africa (November 2016 – August 2017). BC: body cavity, PC: pyloric caeca, S: stomach, FG: foregut, O: operculum, K: kidney, G: gills, MG: midgut, M: muscle, MO: Mouth. \* = New locality record

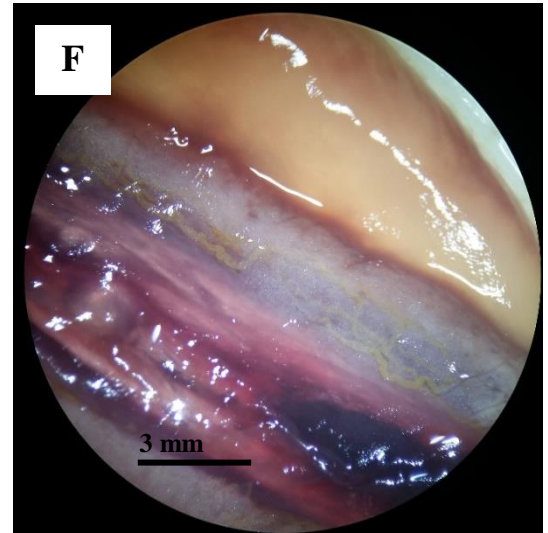
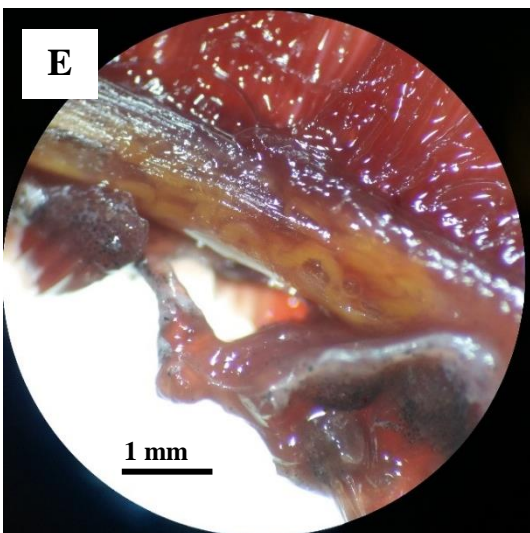
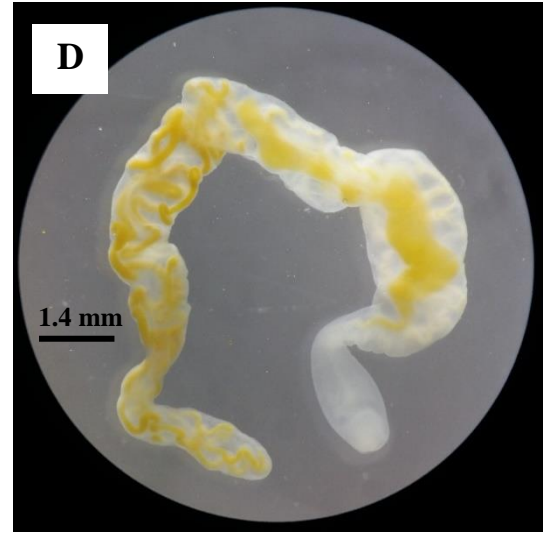
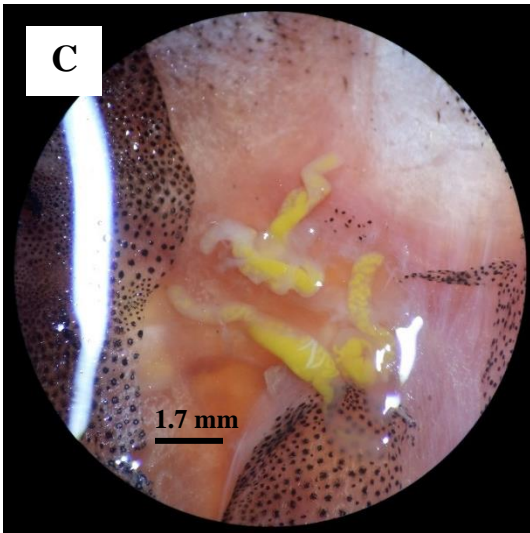
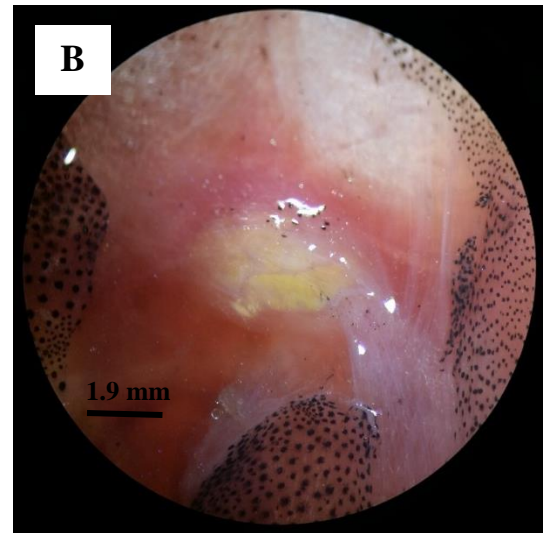
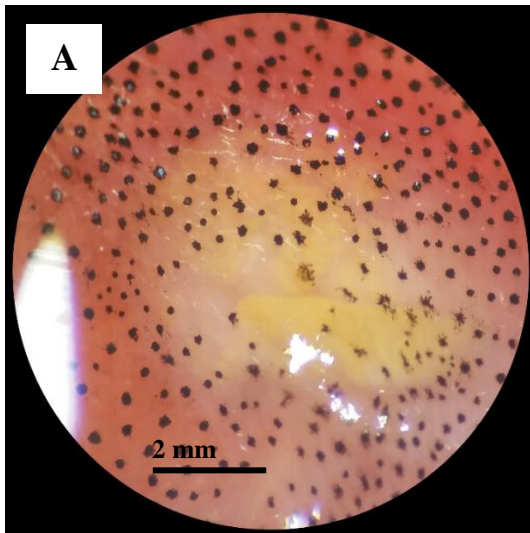
Parasite species	Site of infection	Prevalence	Mean infection intensity (SD)	Range
<b>Myxozoa</b>				
<i>Kudoa thyrsites</i> (Gilchrist, 1924)	M	9.2	13.6 (19.37)	0 – 74
<i>Ceratomyxa</i> sp. (Thélohan, 1892)	GB	19.1	41.6 (65.22)	0 – 250
<b>Monogenea</b>				
<i>Pseudokuhnia minor</i> (Goto, 1984)	G	86.2	47.5 (44.0)	0 – 208
<i>Kuhnia</i> sp. (Sproston, 1945)	G	16.5	1.8 (1.05)	0 – 5
<i>Grubea cochlear</i> (Diesing, 1858)	G	4.0	1.3 (0.52)	0 – 2
<b>Digenea</b>				
<i>Lecithocladium</i> sp. (Lühe, 1901)	S	21.1	2.6 (2.33)	0 – 11
<i>Opechona bacillaris</i> (Molin, 1859)	PC, FG	32.9	9.0 (8.30)	0 – 33
<i>Nematobothrium faciale</i> (Baylis, 1938) *	O	9.2	2.9 (1.49)	0 – 6
<i>Halvorsenius</i> sp. (Gibson, MacKenzie & Cottle, 1981) *	K, MO, G	11.8	1.5 (0.71)	0 – 3
Metacercariae	BC	3.3	2.8 (0.84)	0 – 4
<i>Didimocystis</i> sp.	G	2.0	1.3 (0.05)	0 – 2
<b>Cestoda</b>				
<i>Tentacularia coryphaenae</i> (Bosc, 1802)	BC	2.0	2.0 (1.73)	0 – 4
<b>Nematoda</b>				
<i>Anisakis simplex</i> (Rudolphi, 1809)	BC	62.5	18.5 (32.13)	0 – 150
<i>Contraecaecum</i> sp. (Railliet & Henry, 1912)	PC	1.3	1.0 (0.0)	0 – 1
<b>Acanthocephala</b>				
<i>Rhadinorhynchus pristis</i> (Rudolphi, 1802)	MG	30.3	3.2 (5.31)	0 – 35
<b>Copepoda</b>				
<i>Clavellisa scombri</i> (Kurz, 1877)	G	9.9	1.8 (1.08)	0 – 4
<b>Cysts</b>				
Cyst 1	BC, S	27.6	7.2 (8.34)	0 – 41



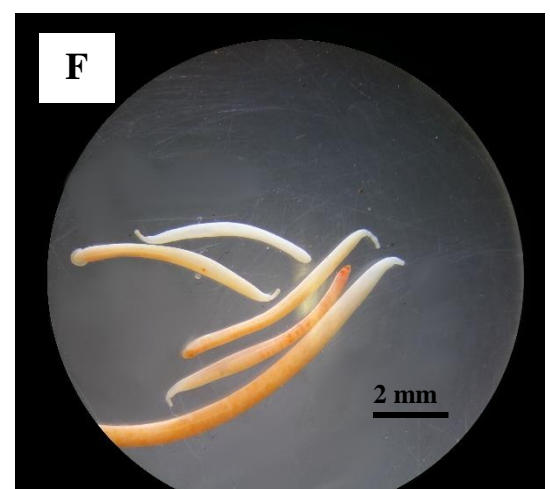
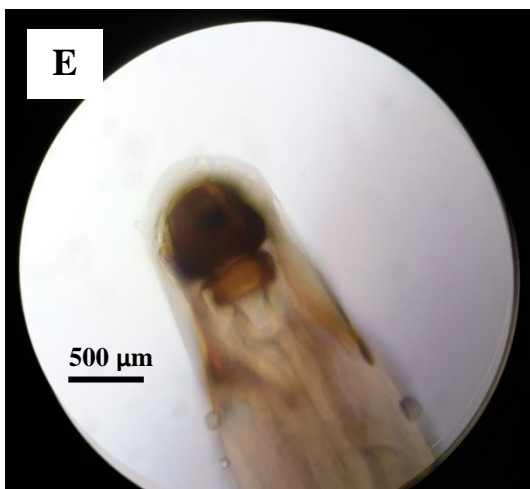
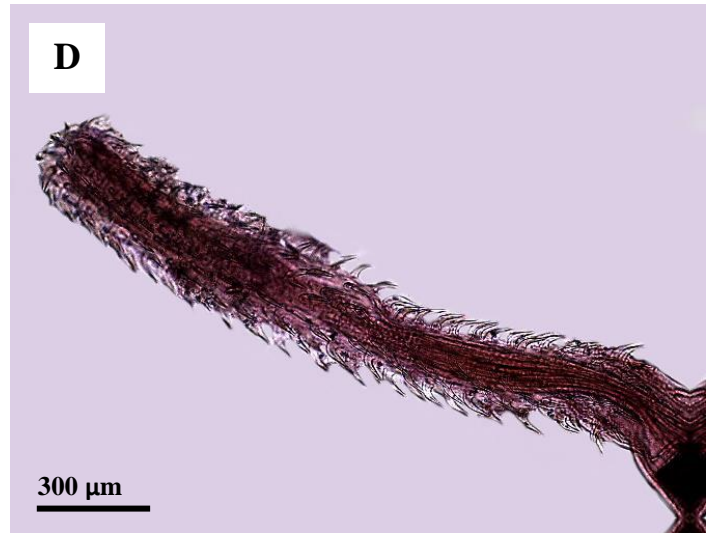
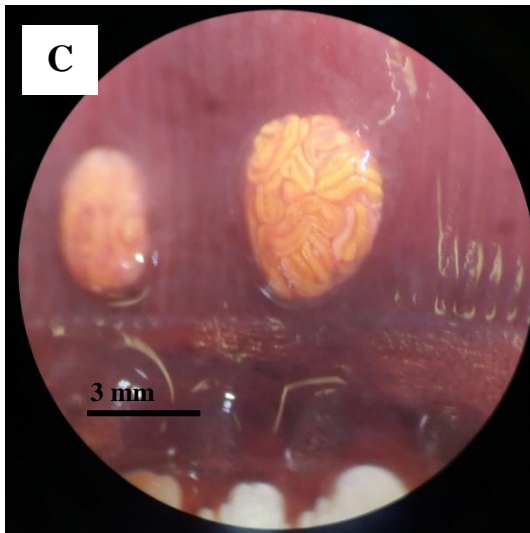
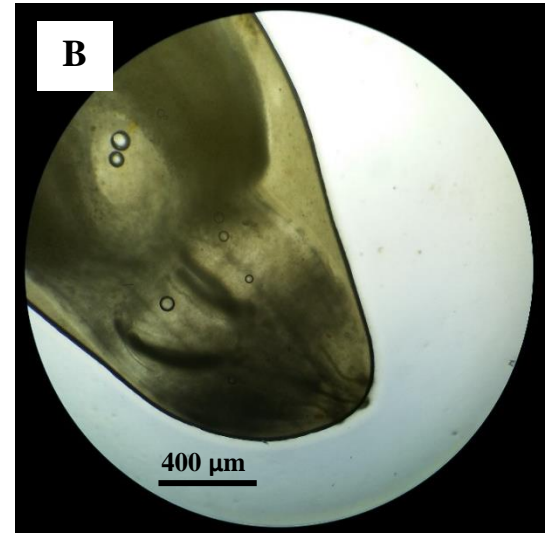
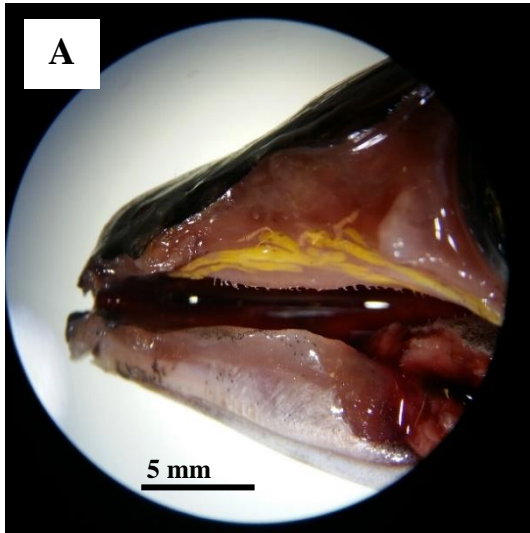
**Figure 10:** Various parasites found infecting *Scomber japonicus* off South Africa (2016 to 2017). A: *Kudoa thrysites* from muscle, B: *Ceratomyxa* sp. from gall bladder, C: *Lecithocladium* sp. from stomach (unstained), D: *Lecithocladium* sp. ventral and oral suckers (stained), E: *Lecithocladium* sp. (whole) (stained).



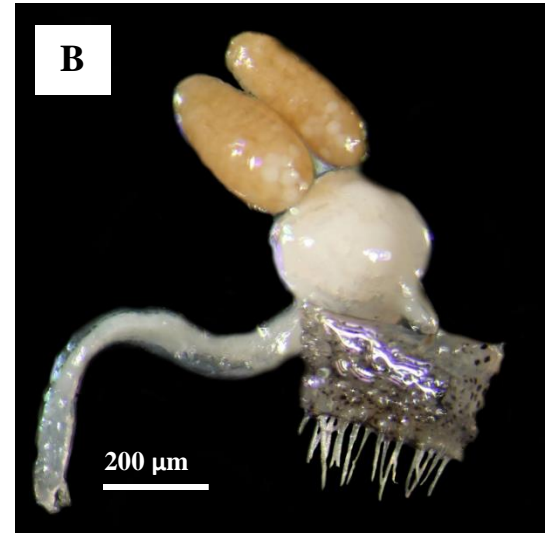
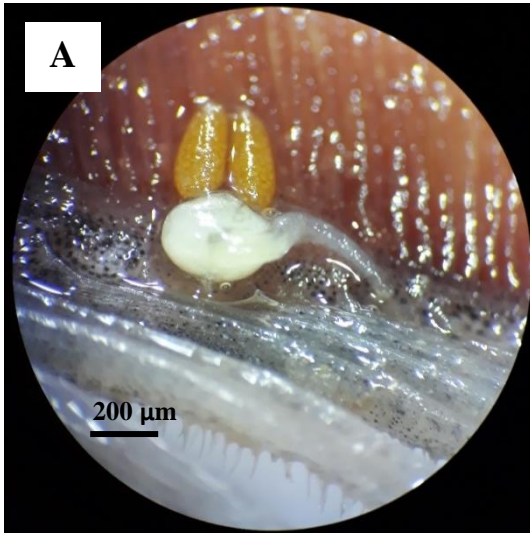
**Figure 11:** Various parasites found infecting *Scomber japonicus* off South Africa (2016 to 2017). A: *Lecithocladium* sp. (unstained), B: *Opechona bacillaris* from pyloric caeca (unstained), C: *Pseudokuhnia minor* from gills, D: *Grubea cochlear* (stained), E: *Grubea cochlear* (stained).



**Figure 12:** Various parasites found infecting *Scomber japonicus* off South Africa (2016 to 2017). A: encysted *Nematobothrium faciale* on the operculum (intact dermal layer), B: encysted *N. faciale* (dermal layer removed), C: *N. faciale* being removed from cyst, D: *N. faciale*, E: *Halvorsenius* sp. inside gill arch, F: *Halvorsenius* sp. in kidney tissue.



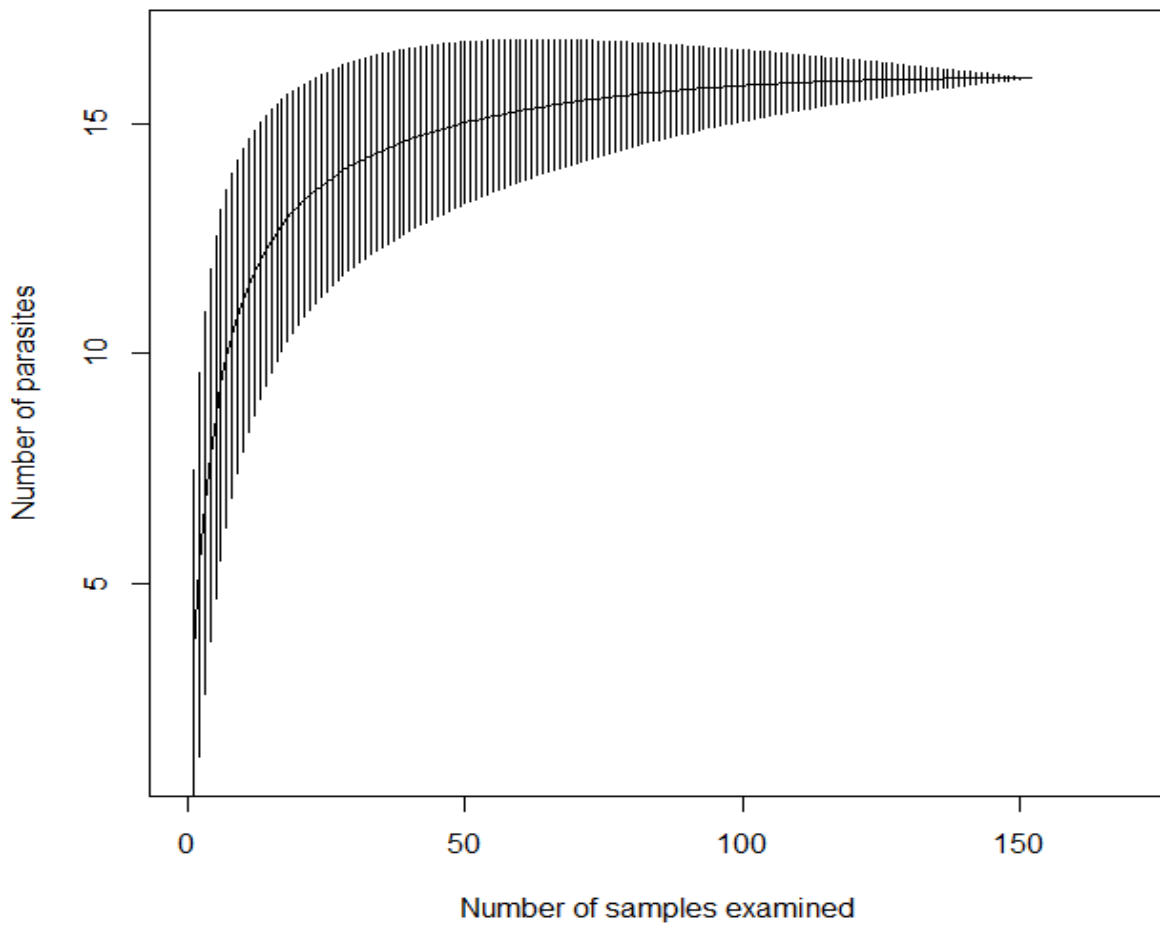
**Figure 13:** Various parasites found infecting *Scomber japonicus* off South Africa (2016 to 2017). A: *Halvorsenius* sp. in the mouth, B: *Tentacularia coryphaenae* from the body cavity, C: *Didymocystis* sp. on gill filaments, D: *Rhadinorhynchus pristis* (proboscis extruded), E: *Contracaecum* sp. head (stacked image), F: *R. pristis* (proboscis not extruded).



**Figure 14:** Various parasites found infecting *Scomber japonicus* off South Africa (2016 to 2017). A: *in situ* *Clavellisa scombri* on gill arch, B: *C. scombri* still attached to portion of gill arch (removed from gill), C: *in situ* image of several Cyst1 cysts.

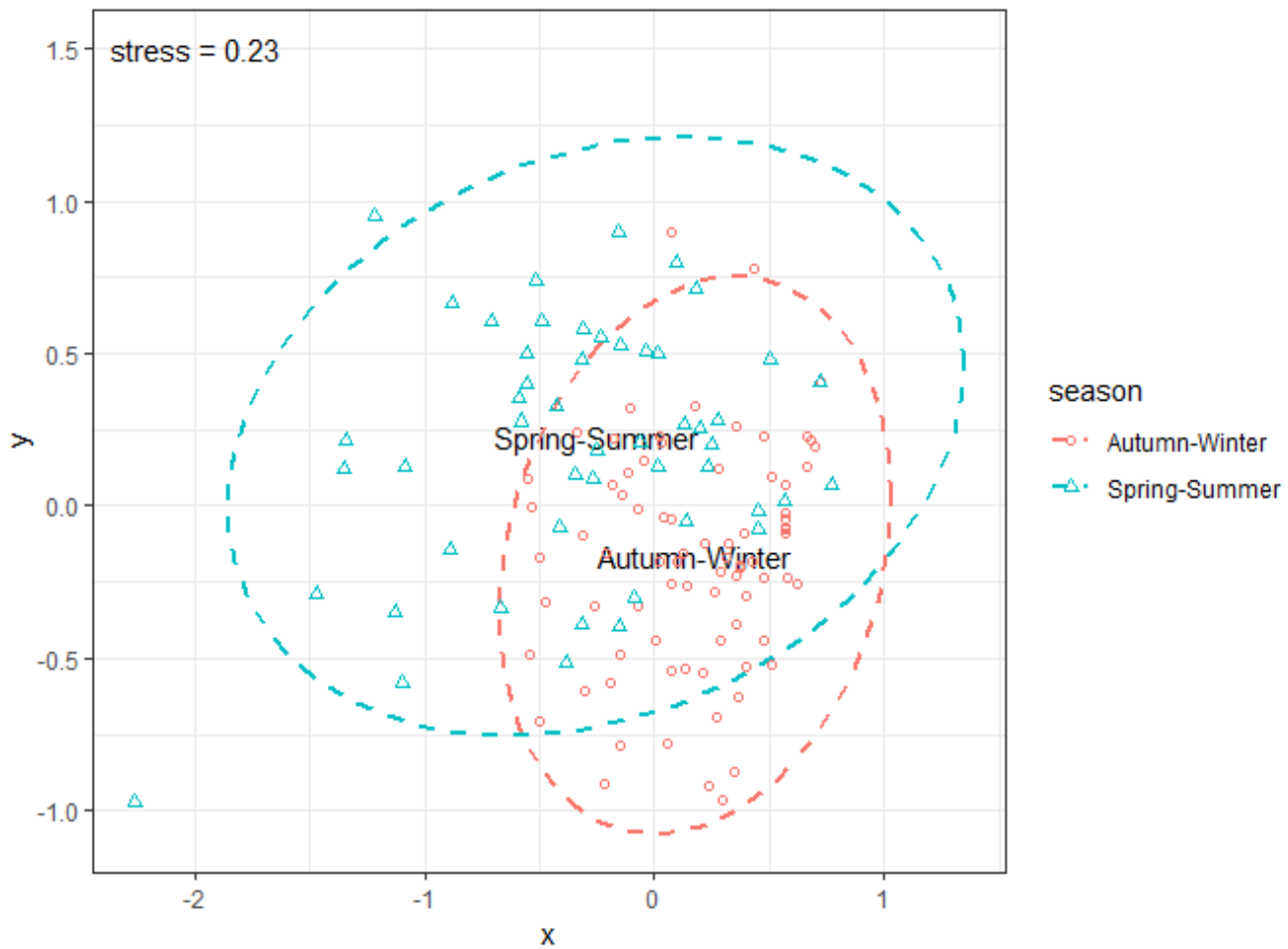
### Parasite community analysis

All the parasite taxa that were recorded infecting *Scomber japonicus* in this study were found infecting the first 70 fish sampled and processing a further 70 fish revealed no other parasite taxa, implying that a sufficient number of samples were processed to permit full documentation of parasites infecting this species off South Africa (Figure 15)



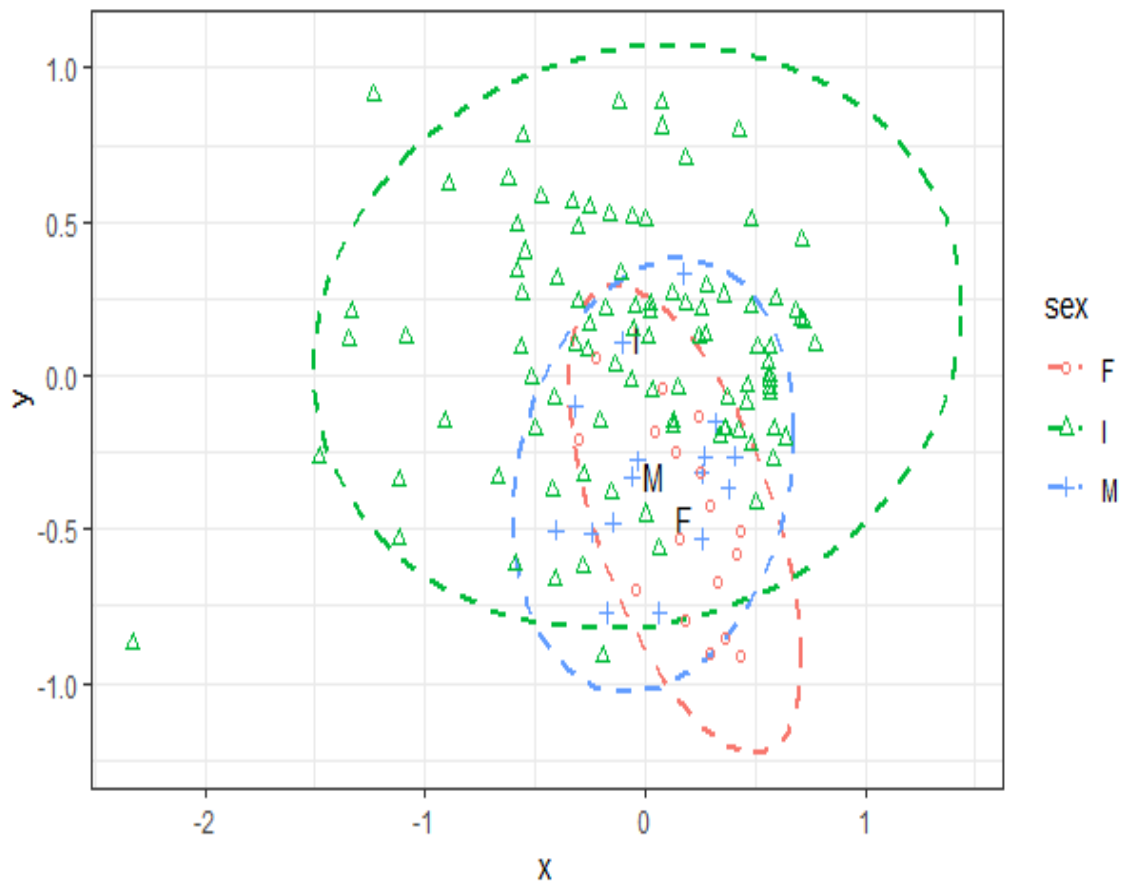
**Figure 15:** Species accumulation curve of the parasite assemblage of *Scomber japonicus* off South Africa (2016 to 2017)

The analysis of variance (ANOSIM) revealed that the parasite assemblage of *S. japonicus* a slight but significant seasonal variability ( $R = 0.124$ ,  $p - \text{value} = 0.002$ ) (Figure 16). The similarity percentage (SIMPER) test identified *Anisakis simplex* and *Pseudokuhnia minor* as the strongest drivers of the seasonal variation, contributing to over 71 % of the dissimilarity between seasons.



**Figure 16:** Plot of the Non-metric Multidimensional Discriminate Scaling analysis comparing the seasonal variability in the parasite assemblage of *Scomber japonicus* off South Africa (autumn-winter = red circles, spring-summer = blue triangles) (2016 to 2017)

Another ANOSIM showed no significant difference ( $R = 0.075$ ;  $p = 0.098$ ) between the parasite assemblages of male, female and immature fish (Figure 17). The NDMS ordination did not expose any clear patterns, however the immature fish did appear to have a very variable parasite load compared to males and females (Figure 17). As no clear pattern was revealed in the NMDS and ANOSIM analysis, no SIMPER test was conducted.



**Figure 17:** Plot of the Non-metric Multidimensional Discriminate Scaling analysis comparing the parasite assemblages of male (M, blue cross), female (F, red circle) and immature (I, green triangle) *Scomber japonicus* off South Africa (2016 to 2017).

## Determinants of infection

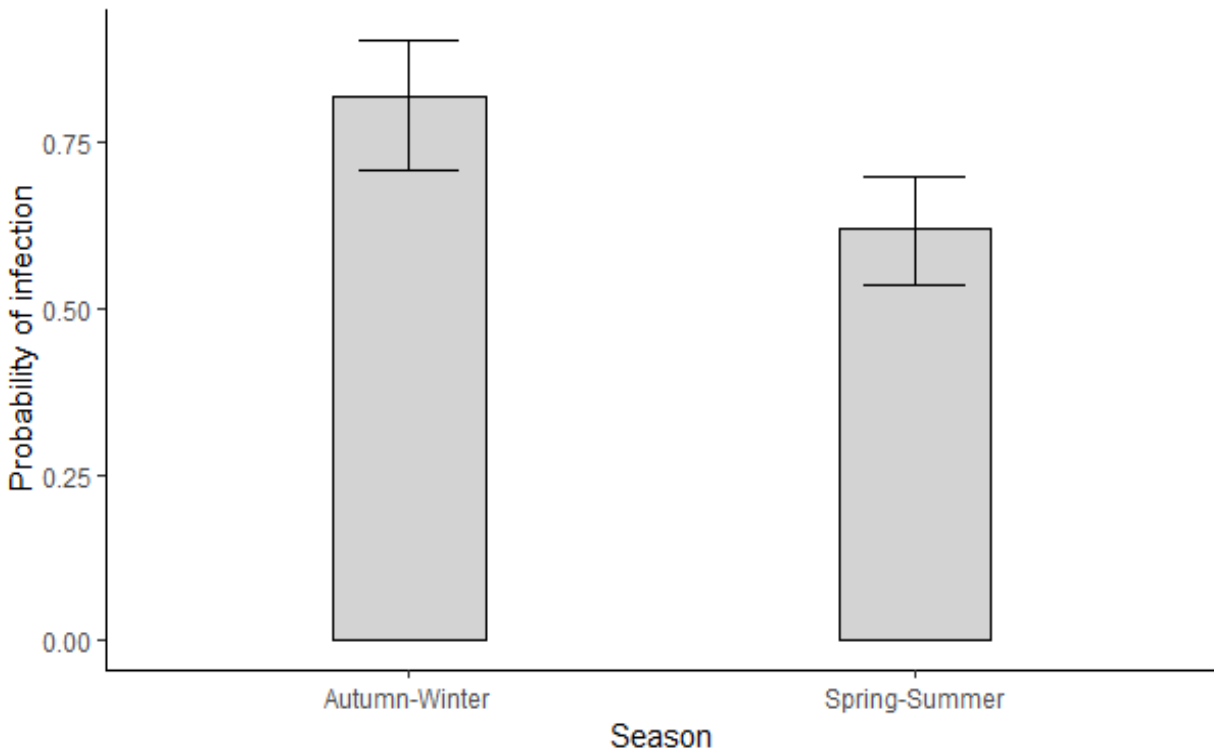
Generalized linear models were developed using prevalence data for *Anisakis simplex* (62.5%), *Pseudokuhnia minor* (86.2%), *Lecithocladium* sp. (21.1%), *Opechona bacillaris* (32.9%), *Rhadinorhynchus pristis* (30.3%) and Cyst 1 (27.6%) and intensity data for *A. simplex*, *P. minor*, *R. pristis* and Cyst 1 was modelled. Infection intensity of *Lecithocladium* sp. and *Opechona bacillaris* were not modelled as their data did not show any trends that this model design was able to identify. All other parasite species observed did not fulfil the model restrictions stated above.

### *Anisakis simplex*

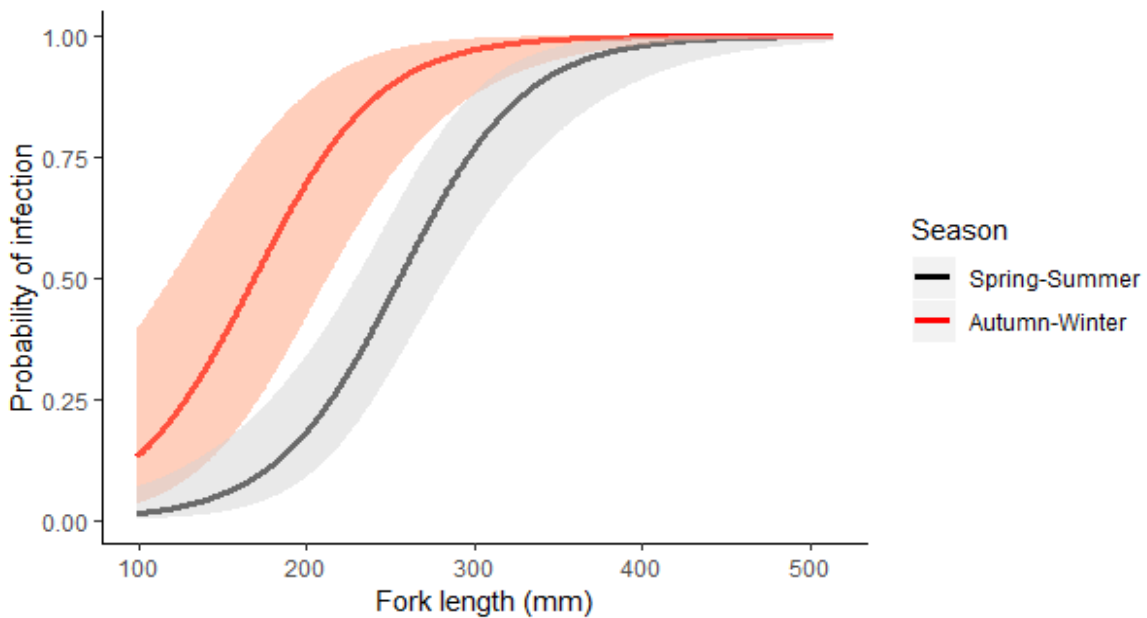
*Anisakis simplex* prevalence was most strongly influenced by two variables, namely fork length and season, while longitude had a marginal effect (Table 6). The model that included these three variables was found to be the most appropriate according to the model selection procedure and explained 58.2% of the variation observed in *A. simplex* prevalence. The residuals of the model revealed that all assumptions were met. The prevalence of infection by *A. simplex* showed a positive, non-linear relationship with host FL (Figure 19), and prevalence was higher in autumn-winter compared to spring-summer (Figure 18). Prevalence decreased with an eastward shift in sample location and this spatial variation was considerably larger during spring-summer (Figure 20)

**Table 6:** Summary of the analysis of deviance for the binomial GLM predicting the prevalence of *Anisakis simplex* infecting *Scomber japonicus* off South Africa between 2016 and 2017. The residual degrees of freedom (Residual df), degrees of freedom (df), residual deviance (Residual Dev.) and the associated significance (p – value) for a  $\chi^2$  test are shown.

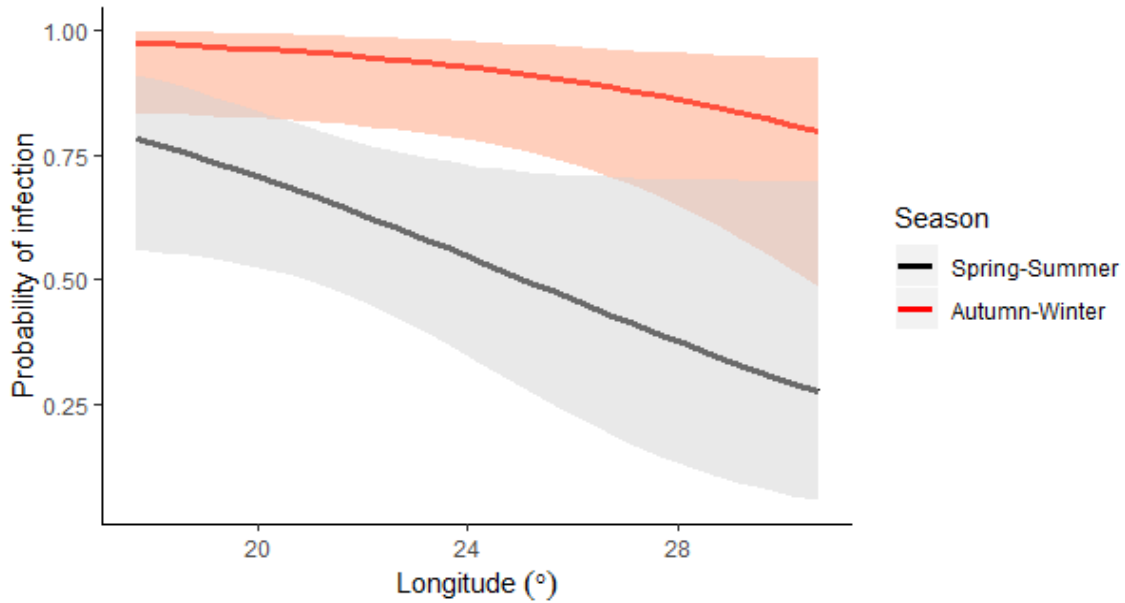
Predictor variable	Residual df	df	Residual deviance	p – value
NULL	151		201.115	
FL	150	1	95.699	$2.2 \times 10^{-16}$
Season	149	1	87.620	0.004
Longitude	148	1	84.073	0.059



**Figure 18:** Bar plot showing the seasonal variation in predicted mean probability of infection by *Anisakis simplex* of *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown).



**Figure 19:** Plot showing the seasonal relationship between host size (FL) and the predicted probability of infection by *Anisakis simplex* of *Scomber japonicus* off South Africa (2016 to 2017) (95% confidence intervals are shown).

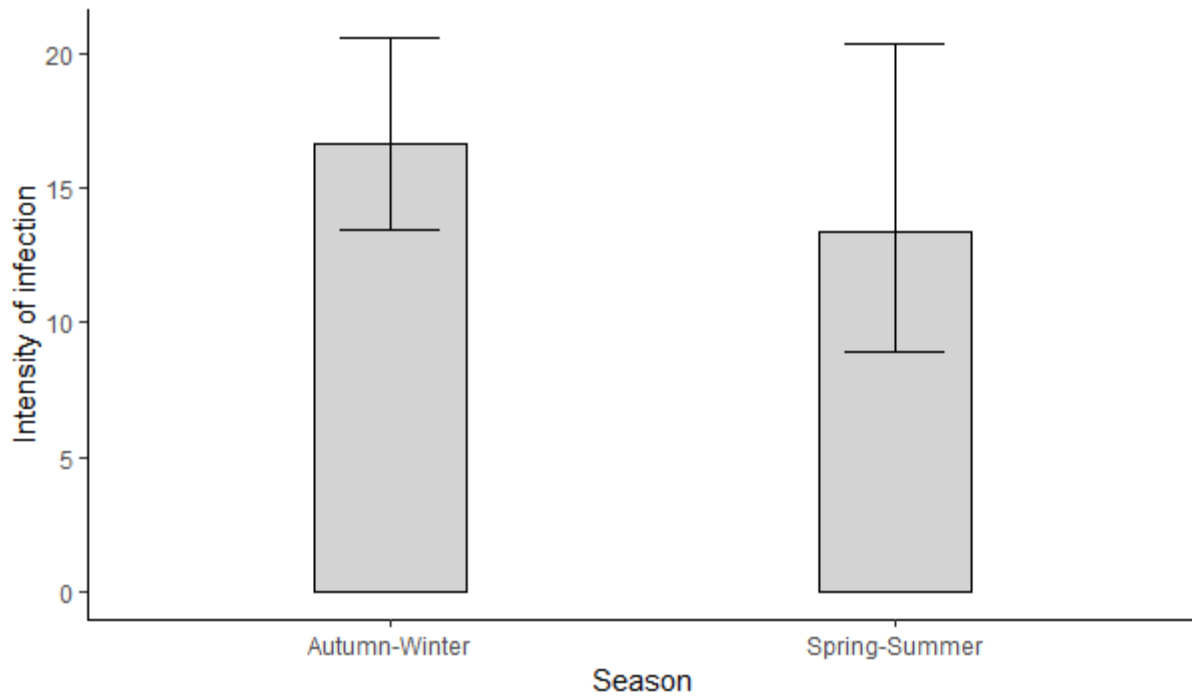


**Figure 20:** Plot showing the seasonal relationship between sample location and predicted probability of infection by *Anisaksis simplex* of *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown).

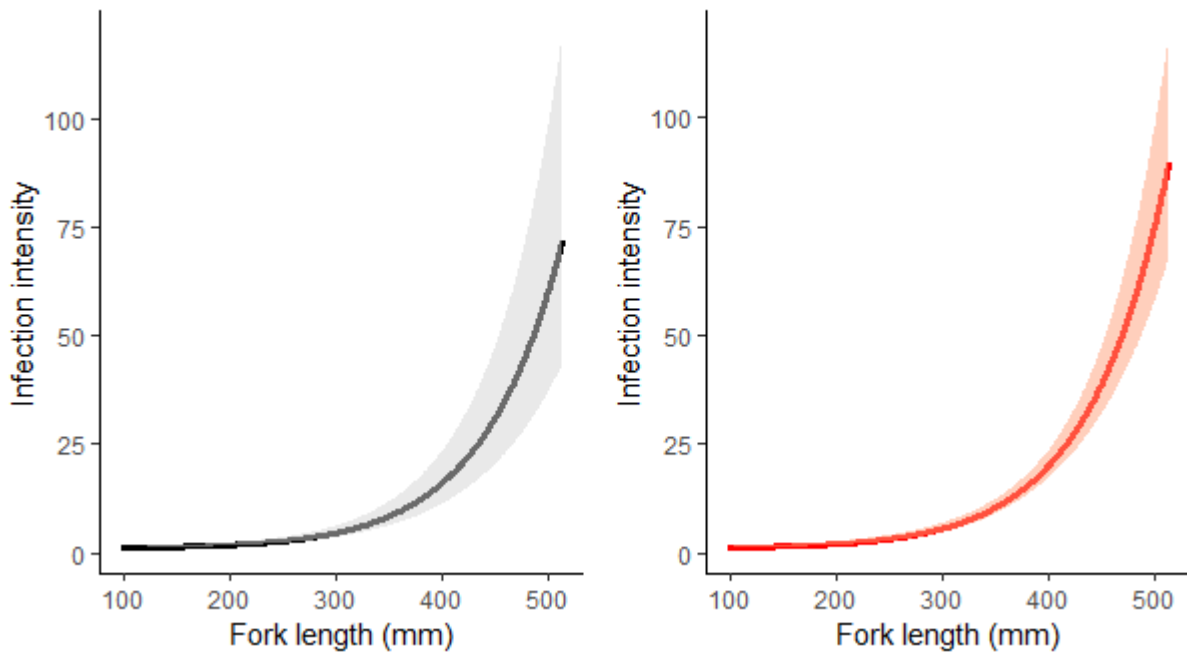
According to the AIC, the most appropriate model for predicting the infection intensity of *A. simplex* in *Scomber japonicus* contains fork length and season as predictor variables and explained 79.5% of the variation in infection intensity. Infection intensity showed an exponential relationship with FL and a slight seasonal difference (Figure 21 and 22), with fish over 400mm predicted to be infected with at least 18 *A. simplex* larvae in both seasons.

**Table 7:** Summary of the analysis of deviance for the negative binomial GLM predicting the infection intensity of *Anisakis simplex* in *Scomber japonicus* off South Africa between 2016 and 2017. The residual degrees of freedom (Residual df), degrees of freedom (df), residual deviance (Residual Dev.) and the associated significance (p – value) for a  $\chi^2$  test are shown.

Predictor variable	Residual df	df	Residual deviance	p – value
NULL	94		460.23	
FL	93	1	95.62	$2.0 \times 10^{-16}$
Season	92	1	94.41	0.2711



**Figure 21:** Bar plot showing the seasonal variation in predicted mean infection intensity of *A. simplex* off *Scomber japonicus* in South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown).



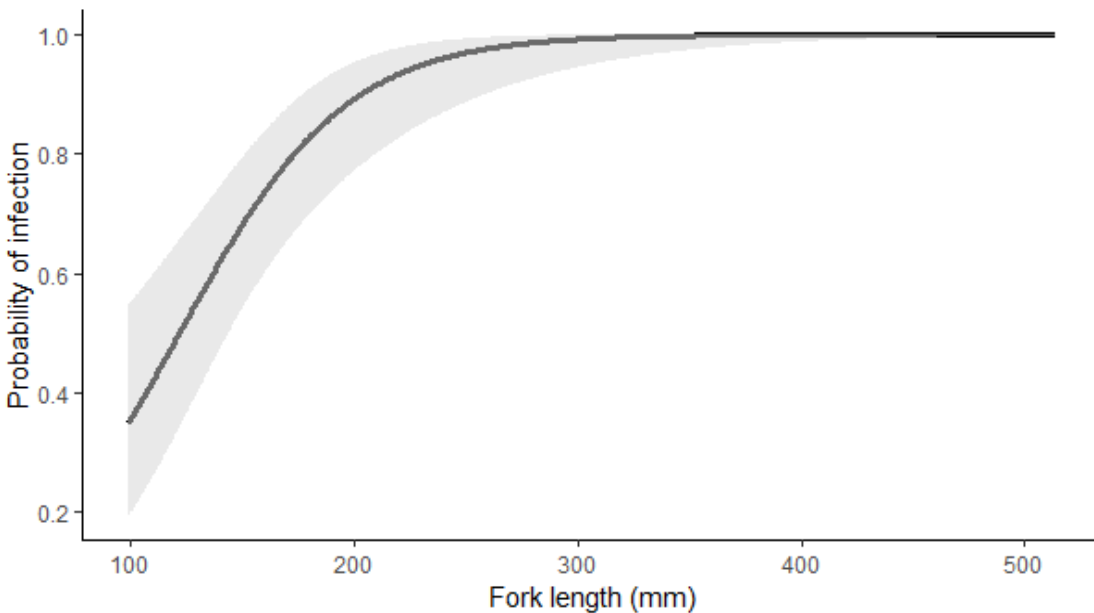
**Figure 22:** Plots showing the seasonal relationships between host size (FL) and the predicted mean infection intensity of *A. simplex* in *Scomber japonicus* off South Africa (2016 to 2017) (95% confidence intervals are shown). Black and Grey = Spring-Summer, Red and Pink = Autumn-Winter.

*Pseudokuhnia minor*

The AIC model selection protocol identified fork length as the only significant variable able to predict the prevalence of *Pseudokuhnia minor* in *Scomber japonicus* off South Africa (Table 8). The model was able to explain 43.4% of the observed variation in *P. minor* prevalence and the residuals of the model were found to not deviate significantly from the assumptions. Host size shared a strong, positive, non-linear relationship with *P. minor* prevalence and hosts that exceed 300mm were predicted to have a 100% prevalence of infection (Figure 23).

**Table 8:** Summary of the analysis of deviance for the binomial GLM predicting the prevalence of *Pseudokuhnia minor* infecting *Scomber japonicus* off South Africa between 2016 and 2017. The residual degrees of freedom (Residual df), degrees of freedom (df), residual deviance (Residual Dev.) and the associated significance (p – value) for a  $\chi^2$  test are shown.

Predictor variable	Residual df	df	Residual deviance	p – value
NULL	151		122.088	
FL	150	1	69.045	$3.26 \times 10^{-13}$

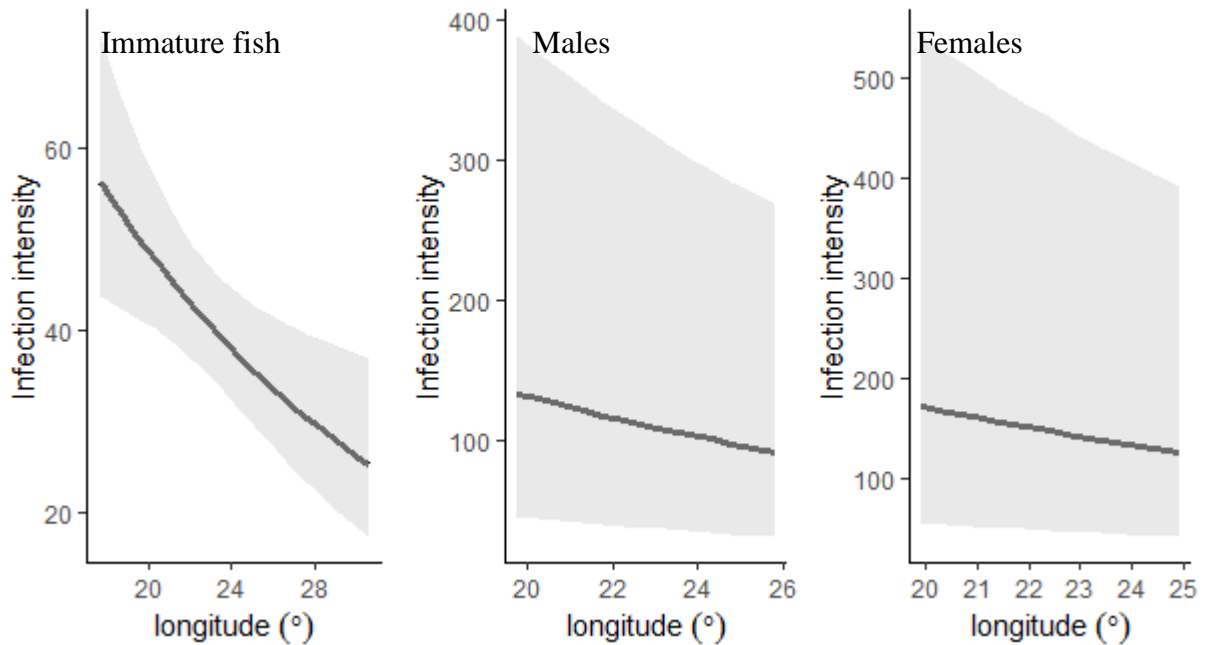


**Figure 23:** Plot showing the relationship between host size (FL) and predicted probability of infection by *Pseudokuhnia minor* off *Scomber japonicus* in South (2016 to 2017) (95% confidence intervals are shown).

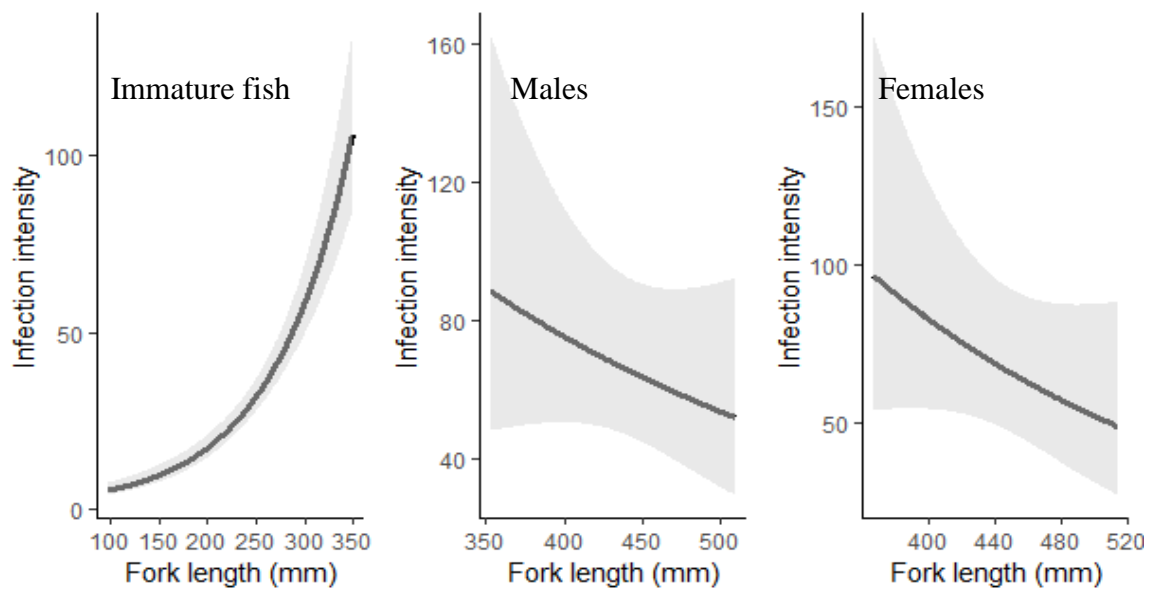
According to the AIC model selection, the infection intensity of *P. minor* was most adequately predicted by a model that included fork length, sex, longitude and an interaction between fork length and sex (Table 9). The residuals did not differ significantly from the model's assumptions and the model explained 50.7 % of the variability observed in *P. minor* infection intensity. According to the model's predictions, *P. minor* infection intensity is significantly greater in males and females than in immature fish and males and females exhibited greater variation in *P. minor* infection intensity than immature fish (Figure 26). However, whereas infection intensity was predicted to have a strong positive, exponential relationship with host size for immature fish, the opposite was observed for males and females, which were predicted to experience decreased *P. minor* infection intensity with increased host size (Figure 25). For all sexes, infection intensity was predicted to decrease with an eastward shift in sample location, with the decrease more rapid in immature fish (Figure 24)

**Table 9:** Summary of the analysis of deviance for the negative binomial GLM predicting the infection intensity of *Pseudokuhnia minor* infecting *Scomber japonicus* off South Africa between 2016 and 2017. The residual degrees of freedom (Residual df), degrees of freedom (df), residual deviance (Residual Dev.) and the associated significance (p – value) for a  $\chi^2$  test are shown.

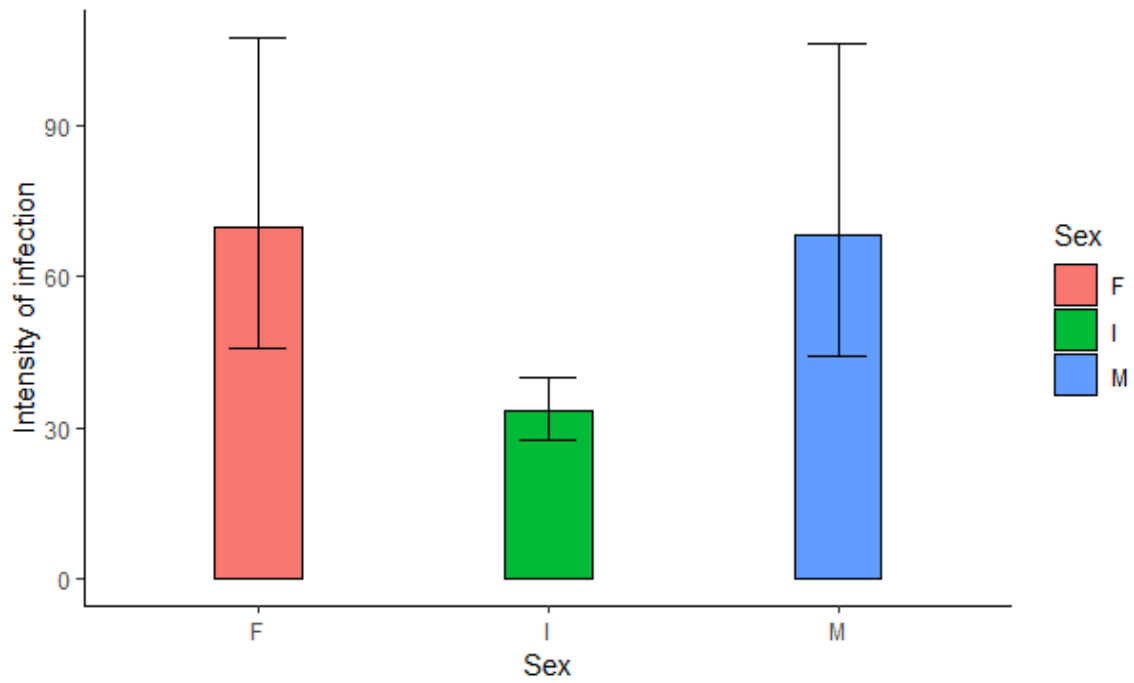
Predictor variable	Residual df	df	Residual deviance	p – value
NULL	130		283.44	
FL	129	1	199.68	$2.2 \times 10^{-16}$
Sex	127	2	184.39	0.0004
Longitude	126	1	179.00	0.0203
FL * Sex	124	2	139.70	$2.92 \times 10^{-9}$



**Figure 24:** Plots showing the relationships between the longitude of sample collection and the predicted mean infection intensity of *Pseudokuhnia minor* in immature, male and female *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown). Note: the x and y axes are different.



**Figure 25:** Plots showing the relationships between host size (FL) and the predicted mean infection intensity of *Pseudokuhnia minor* in immature, male and female *Scomber japonicus* off South Africa (2016 to 2017) (95% confidence intervals are shown). Note: the x and y axes are different.



**Figure 26:** Bar plot showing the predicted mean infection intensity of *Pseudokuhnia minor* in male (M), female (F) and immature (I) *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown).

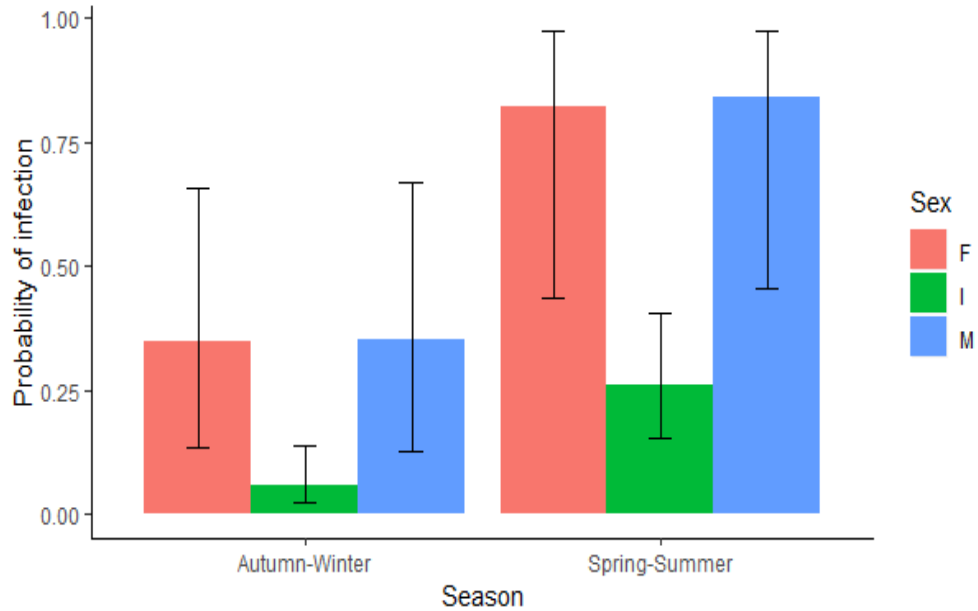
***Lecithocladium* sp.**

The prevalence of *Lecithocladium* sp. was best predicted by a model containing fork length, season and the interaction between fork length and sex, and was able to explain 27 % of the variation observed in the prevalence of *Lecithocladium* sp. (Table 10). All residuals were checked and were found to not differentiate significantly from assumptions. The probability of infection varied between the sexes, with males and females having a much higher chance of infection than immature fish (Figure 27) and fish that were caught during autumn-winter were predicted to exhibit lower prevalence levels compared to those caught during spring-summer (Figure 27). The relationship between fork length and prevalence differed between sexes, with both males and females showing decreased prevalence with increased host size whereas, immature fish had a greater prevalence of infection with increased host size (Figure 28).

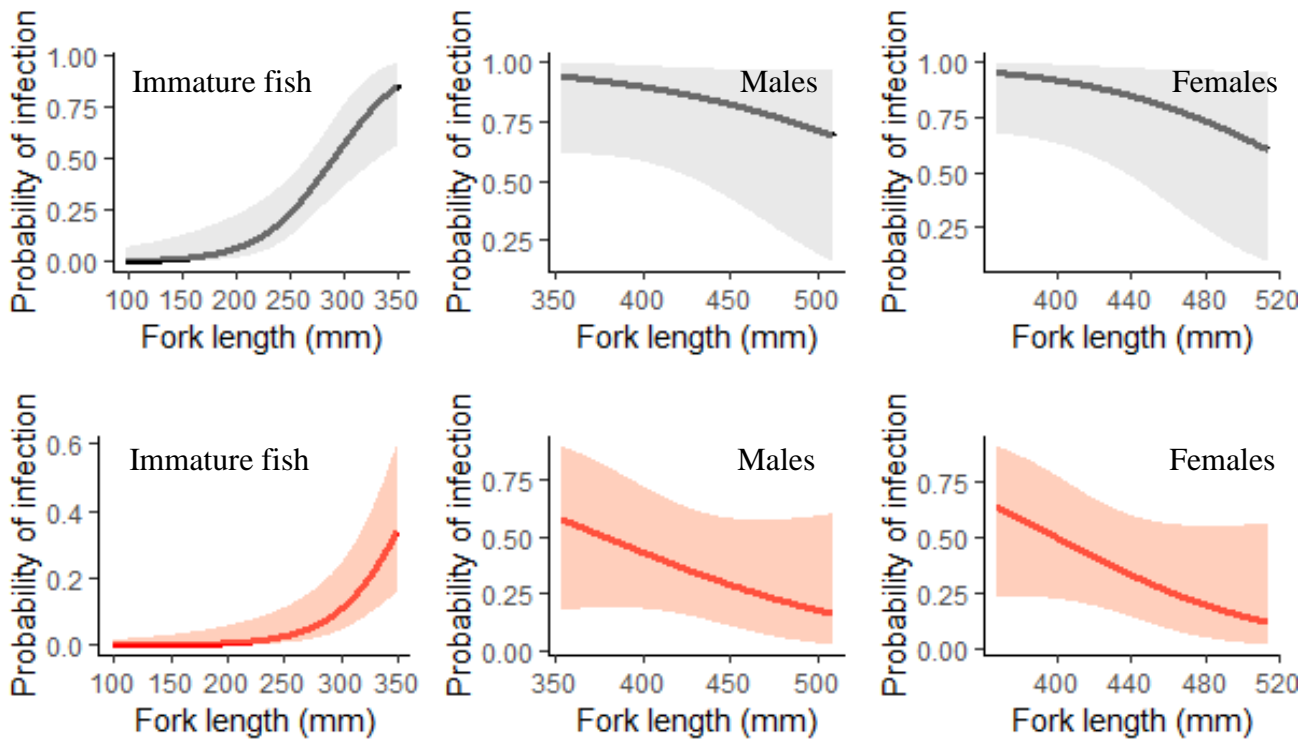
No suitable model was found to model the infection intensity of *Lecithocladium* sp.

**Table 10:** Summary of the analysis of deviance for the binomial GLM predicting the prevalence of *Lecithocladium* sp. infecting *Scomber japonicus* off South Africa between 2016 and 2017. The residual degrees of freedom (Residual df), degrees of freedom (df), residual deviance (Residual Dev.) and the associated significance (p – value) for a  $\chi^2$  test are shown.

Predictor variable	Residual df	df	Residual deviance	p – value
NULL	151		156.46	
FL	150	1	143.51	0.0003
Season	149	1	134.85	0.0032
Sex	147	2	134.53	0.8503
FL * Sex	145	2	113.53	2.76 x 10 <sup>-5</sup>



**Figure 27:** Bar plot showing the seasonal variability in the predicted mean probability of infection by *Lecithocladium* sp. of immature, male and female *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown).



**Figure 28:** Plots showing the seasonal relationships between host size (FL) and the predicted probability of infection by *Lecithocladium* sp. of immature, male and female *Scomber japonicus* in spring/summer– black and grey and autumn/winter– red and pink (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown). Note: the x and y axes are different.

### *Opechona bacillaris*

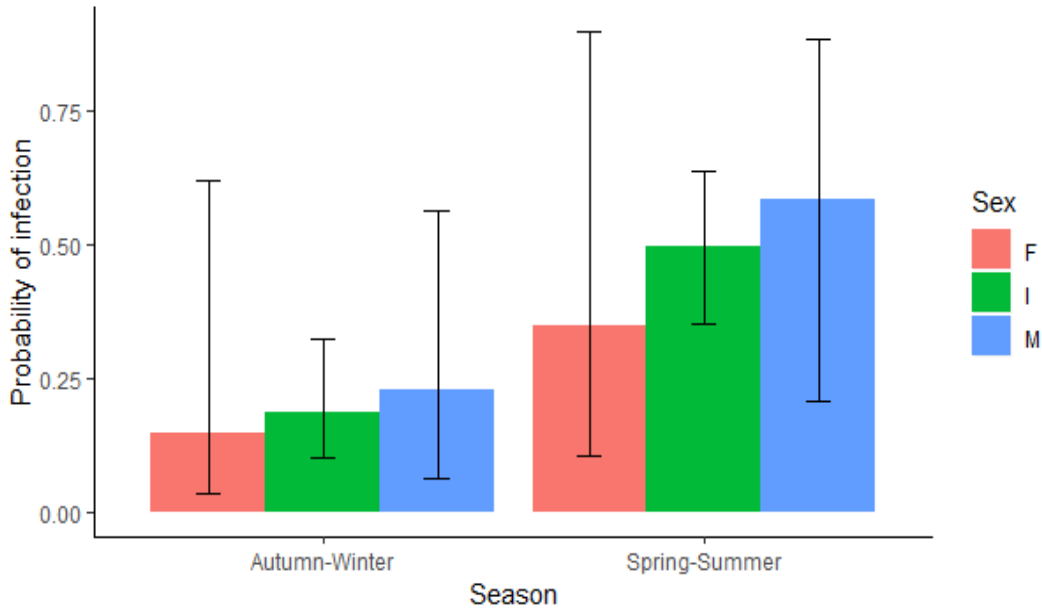
After AIC model selection was complete the most optimal model for predicting the prevalence of *Opechona bacillaris* included season, sex and an interaction between sex and fork length. The model was able to explain 12.4% of the observed variation in *O. bacillaris* prevalence, and the plot of the residuals revealed that the model did not differ significantly from the assumptions.

**Table 11:** Summary of the analysis of deviance for the binomial GLM predicting the prevalence of *Opechona bacillaris* infecting *Scomber japonicus* off South Africa between 2016 and 2017. The residual degrees of freedom (Residual df), degrees of freedom (df), residual deviance (Residual Dev.) and the associated significance (p – value) for a  $\chi^2$  test are shown.

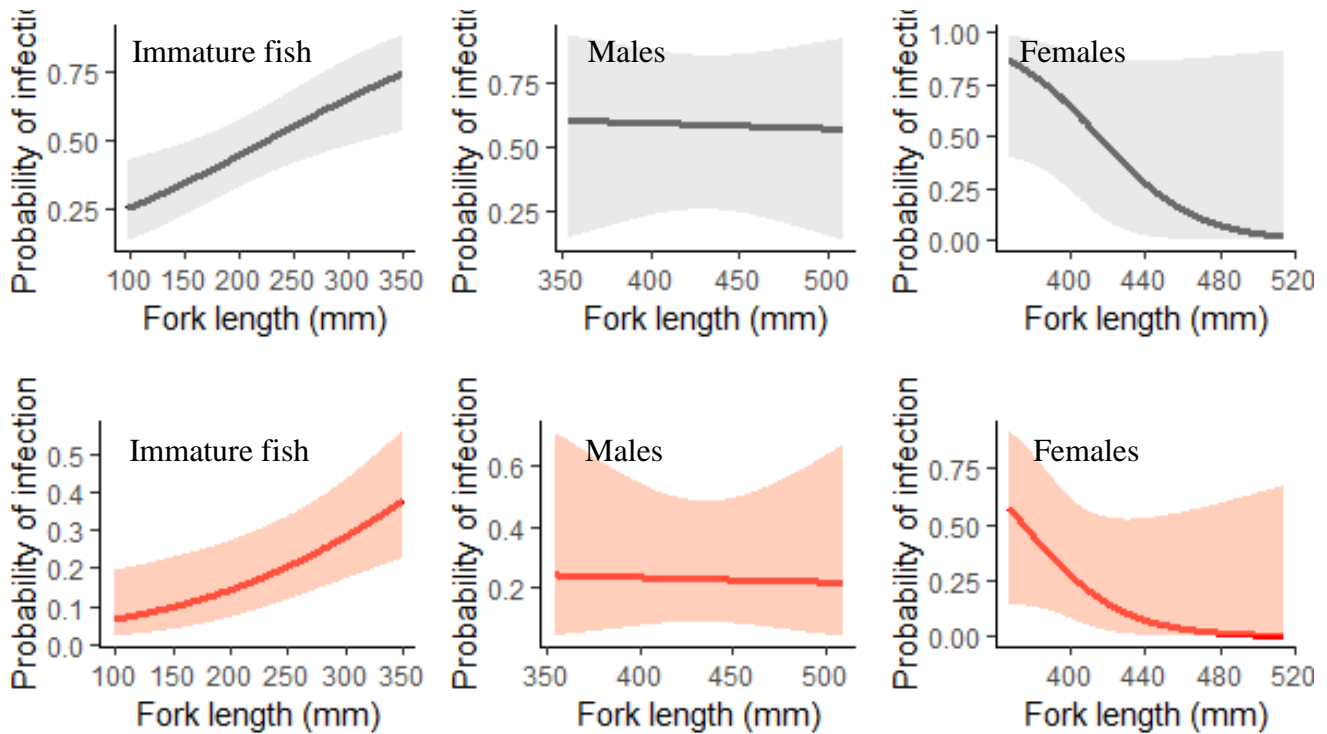
Predictor variable	Residual df	df	Residual deviance	p – value
NULL	151		192.56	
Season	150	1	183.56	0.0026
Sex	148	2	183.24	0.8542
FL	147	1	176.47	0.0092
FL * Sex	145	2	168.65	0.0200

Season had the strongest effect, with *O. bacillaris* displaying higher prevalence levels during spring-summer than autumn-winter (Figure 29), and males were predicted to exhibit higher prevalence levels than females and immature fish (Figure 29). *O. bacillaris* prevalence was strongly related to host size, however this relationship was not consistent between the three sexes. Immature fish displayed a strong positive, non-linear relationship between host size and prevalence, but males and females displayed very different trends; size did not appear to affect *O. bacillaris* prevalence in males and was negatively correlated with host size in females (Figure 30).

No suitable model was found to predict the infection intensity of *O. bacillaris* in *Scomber japonicus*.



**Figure 29:** Bar plot showing the seasonal variation in predicted mean probability of infection by *Opechona bacillaris* in immature, male and female *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals shown).



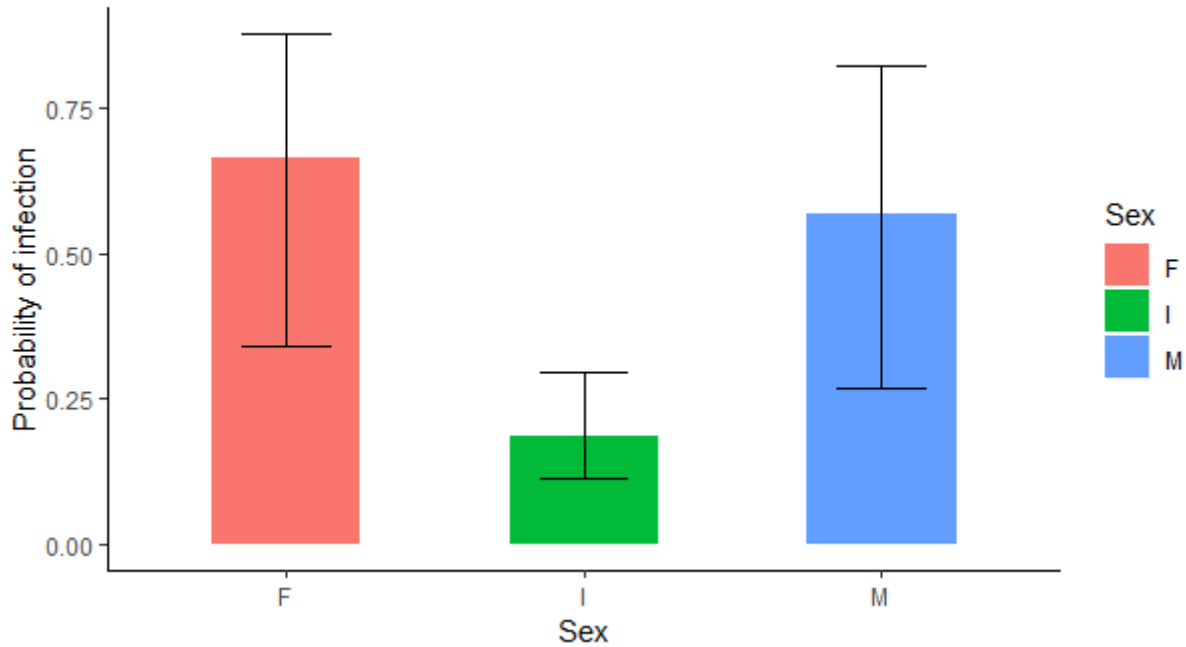
**Figure 30:** Plots showing the seasonal relationships between host size (FL) and the predicted mean probability of infection by *Opechona bacillaris* of immature, male and female *Scomber japonicus* off South Africa in spring/summer – black and grey and autumn/winter – red and pink (2016 to 2017) (95% confidence intervals are shown). Note: the x and y axes are different.

### *Rhadinorhynchus pristis*

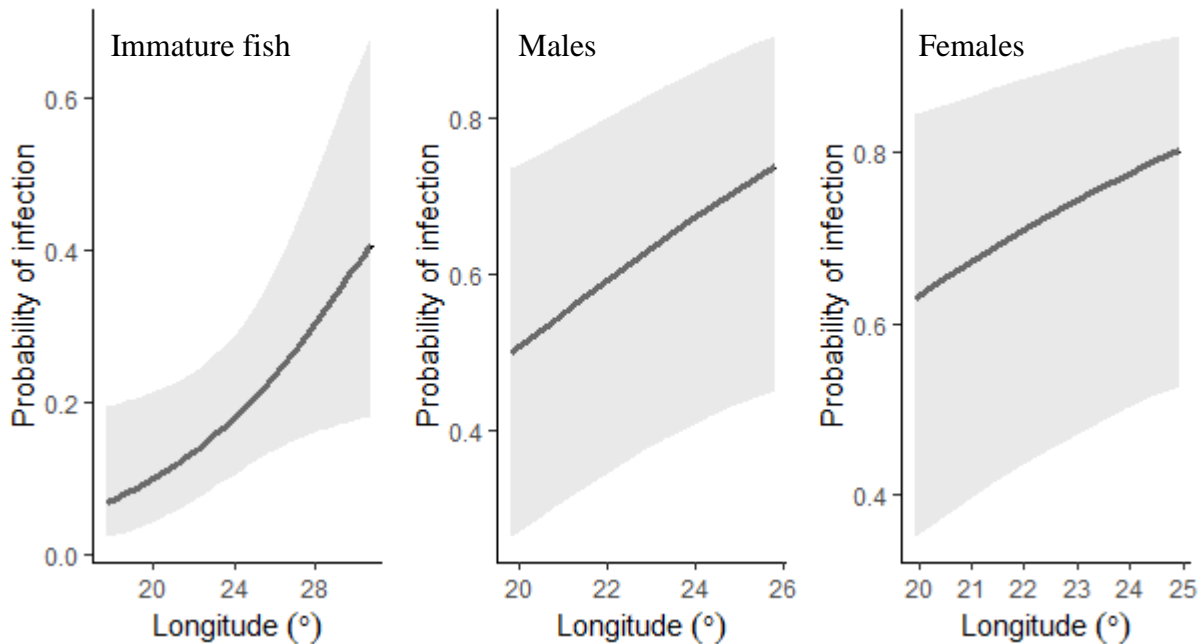
The optimal model for the prevalence of *Rhadinorhynchus pristis* identified fork length, longitude and an interaction between fork length and sex to be responsible for explaining 23.2% of the variation present in the prevalence of *R. pristis* (Table 12). Prevalence was strongly impacted by host size, but whilst prevalence was positively correlated with fork length in immature fish it was negatively correlated with FL for males and females (Figure 33), with the negative relationship being considerably stronger in females than in males. Both males and females were predicted to have a greater mean probability of infection than immature fish (Figure 31), and predicted prevalence of *R. pristis* infection increased eastwards in all sexes (Figure 32)

**Table 12:** Summary of the analysis of deviance for the binomial GLM predicting the prevalence of *Rhadinorhynchus pristis* infecting *Scomber japonicus* off South Africa between 2016 and 2017. The residual degrees of freedom (Residual df), degrees of freedom (df), residual deviance (Residual Dev.) and the associated significance (p – value) for a  $\chi^2$  test are shown.

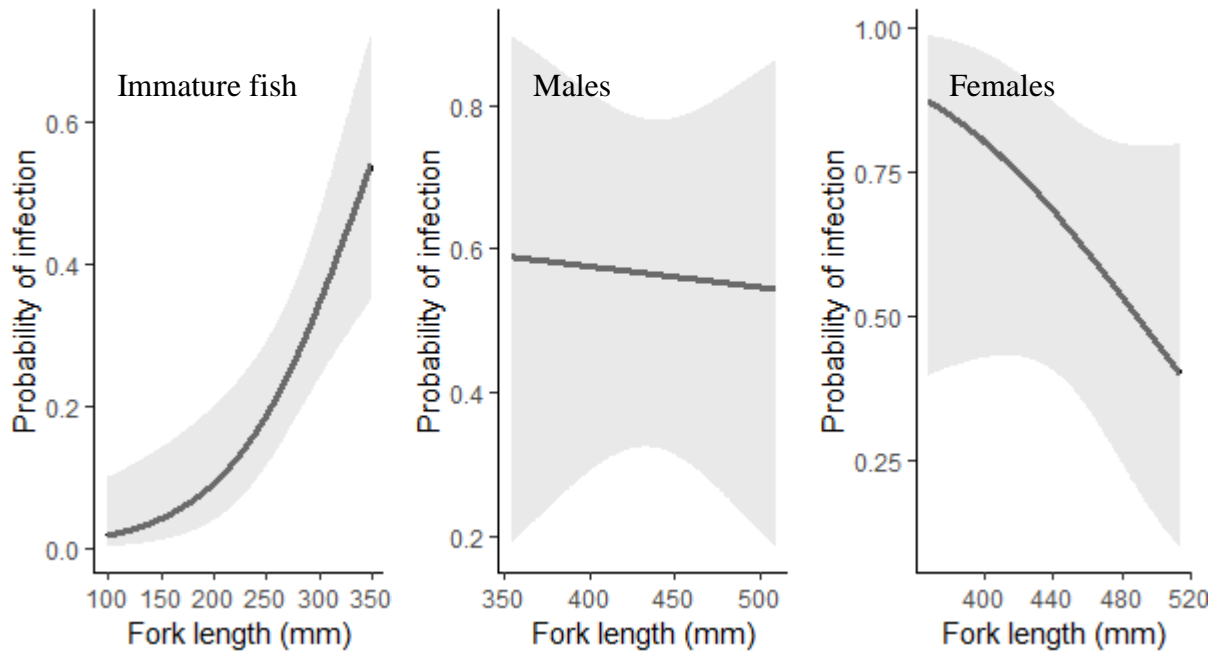
Predictor variable	Residual df	df	Residual deviance	p – value
NULL	151		186.38	
FL	150	1	158.26	1.14 x 10 <sup>-7</sup>
Longitude	149	1	152.98	0.0214
Sex	147	2	152.77	0.9018
FL * Sex	145	2	143.18	0.0082



**Figure 31:** Bar plot showing the predicted mean probability of infection by *Rhadinorhynchus pristis* in immature, male and female *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown).



**Figure 32:** Plots showing the relationships between the longitude of sample collection and the predicted mean probability of infection by *Rhadinorhynchus pristis* of immature, male and female *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown). Note: the x and y axes are different.

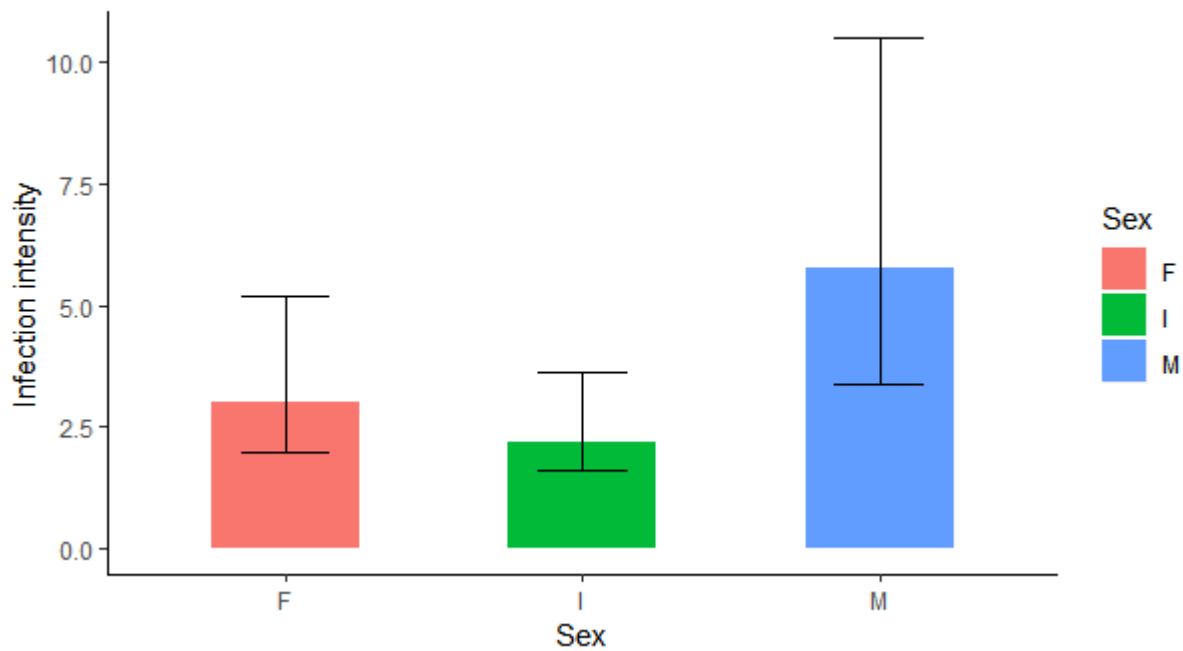


**Figure 33:** Plots showing the relationship between host size (FL) and the predicted mean probability of infection by *Rhadinorhynchus pristis* of immature, male and female *Scomber japonicus* off South Africa (2016 to 2017) (95% confidence interval are shown). Note: the x and y axes are different.

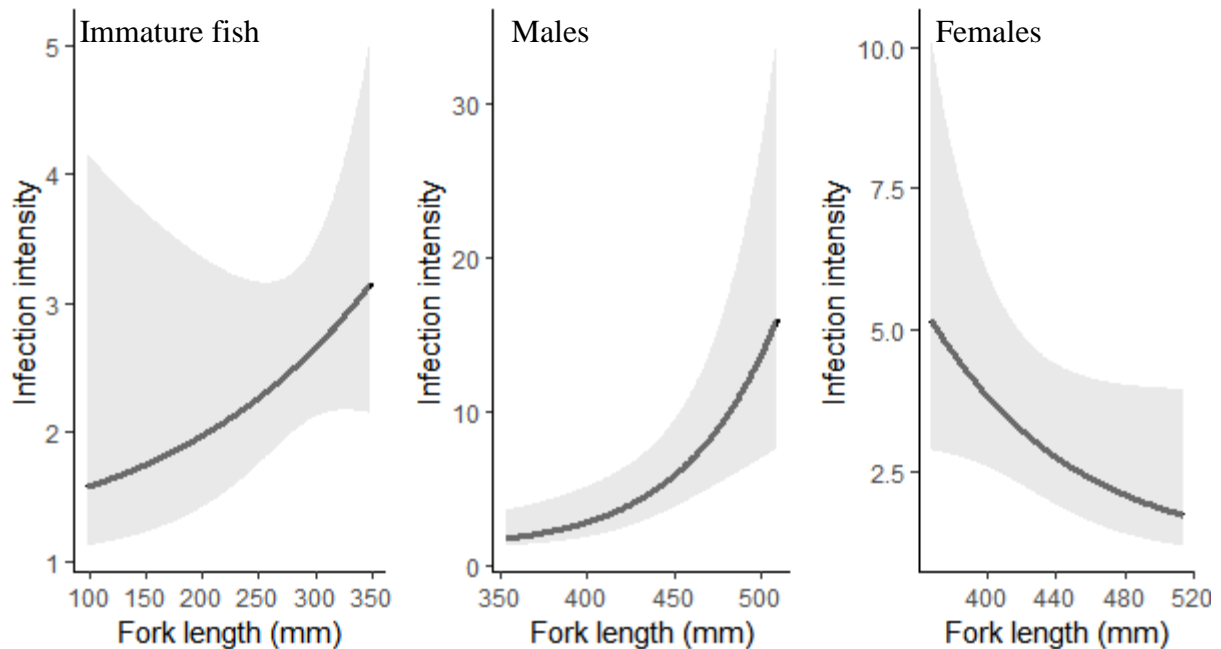
The AIC protocol identified fork length and an interaction between fork length and sex as the best model for predicting infection intensity of *R. pristis* in *Scomber japonicus*, and explained 49.1% of the variation present in the infection intensity (Table 13). Both male and immature fish were predicted to display a positive relationship with host size, while intensity of infection was predicted to decrease with increased size in females (Figure 35). Males were predicted to be infected with a greater number of *R. pristis* than both females and immature fish (Figure 34).

**Table 13:** Summary of the analysis of deviance for the negative binomial GLM predicting the infection intensity of *Rhadinorhynchus pristis* infecting *Scomber japonicus* off South Africa between 2016 and 2017. The residual degrees of freedom (Residual df), degrees of freedom (df), residual deviance (Residual Dev.) and the associated significance (p – value) for a  $\chi^2$  test are shown.

Predictor variable	Residual df	df	Residual deviance	p – value
NULL	45		72.409	
FL	44	1	54.071	1.85 x 10 <sup>-5</sup>
Sex	42	2	51.798	0.3209
FL * Sex	40	2	36.874	0.0006



**Figure 34:** Bar plot showing the predicted mean infection intensity (number of parasites per infected fish) by *Rhadinorhynchus pristis* of male (M), female (F) and immature (I) *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown).



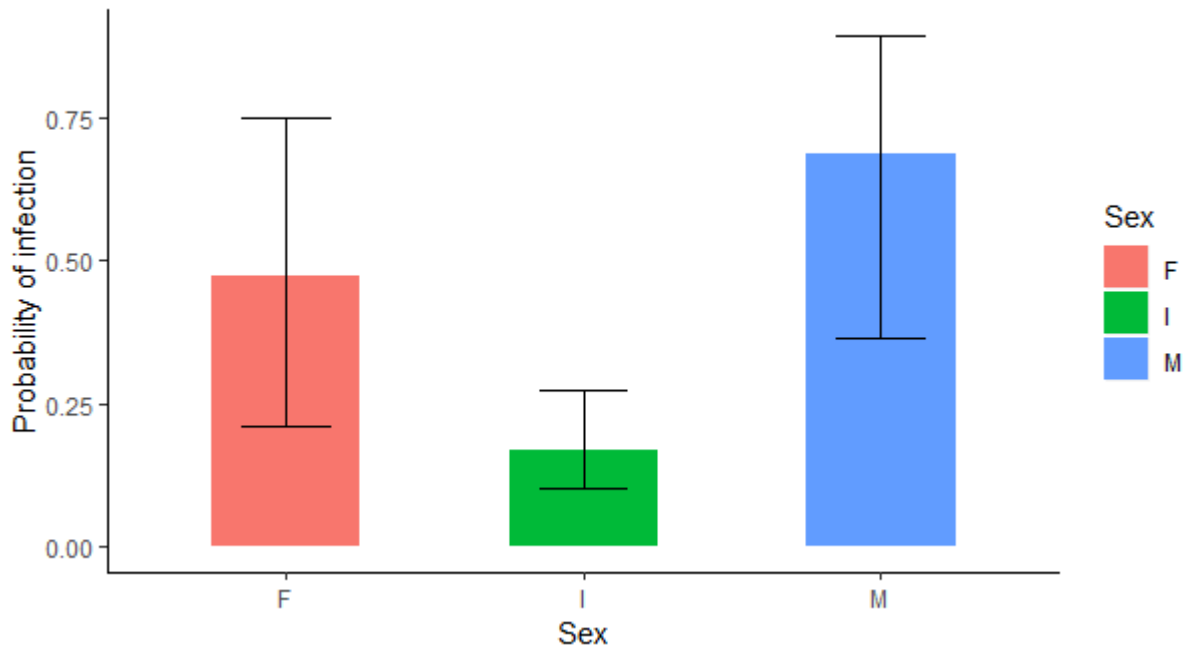
**Figure 35:** Plots showing the relationships between the host size (FL) and the predicted mean infection intensity by *Rhadinorhynchus pristis* of immature, male and female *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown). Note: the x and y axes are different.

### Cyst 1

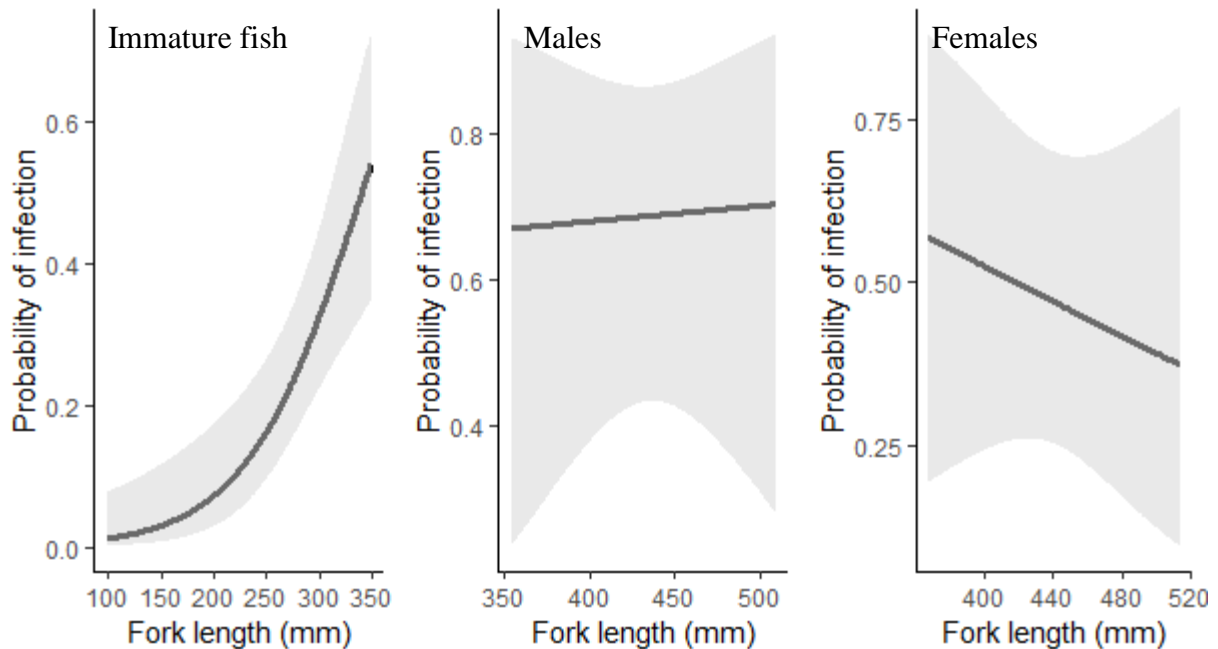
The AIC model selection procedure revealed that the most appropriate model for the prevalence of Cyst 1 contained fork length and an interaction between sex and fork length (Table 14). The selected model explained 23.4% of the variance observed in the prevalence of the cyst and the residuals were found to not deviate significantly from the assumptions of the model. Males and females were predicted to have higher prevalence levels than the immature fish (Figure 36). Prevalence of infection by this cyst increased with host size in both males and immature fish but this increase was power and rapid in immature fish and linear and slow in males (Figure 37). Females exhibited a negative, linear relationship in cyst prevalence with fork length.

**Table 14:** Summary of the analysis of deviance for the binomial GLM predicting the prevalence of Cyst 1 infecting *Scomber japonicus* off South Africa between 2016 and 2017. The residual degrees of freedom (Residual df), degrees of freedom (df), residual deviance (Residual Dev.) and the associated significance (p – value) for a  $\chi^2$  test are shown.

Predictor variable	Residual df	df	Residual deviance	p – value
NULL	151		179.19	
FL	150	1	146.06	8.6 x 10 <sup>-9</sup>
Sex	148	2	143.52	0.2815
FL * Sex	146	2	137.34	0.0454



**Figure 36:** Bar plot showing the predicted mean probability of infection by Cyst 1 of male (M), female (F) and immature (I) *Scomber japonicus* off South Africa (2016 to 2017) (mean FL of 274.4 mm) (95% confidence intervals are shown)

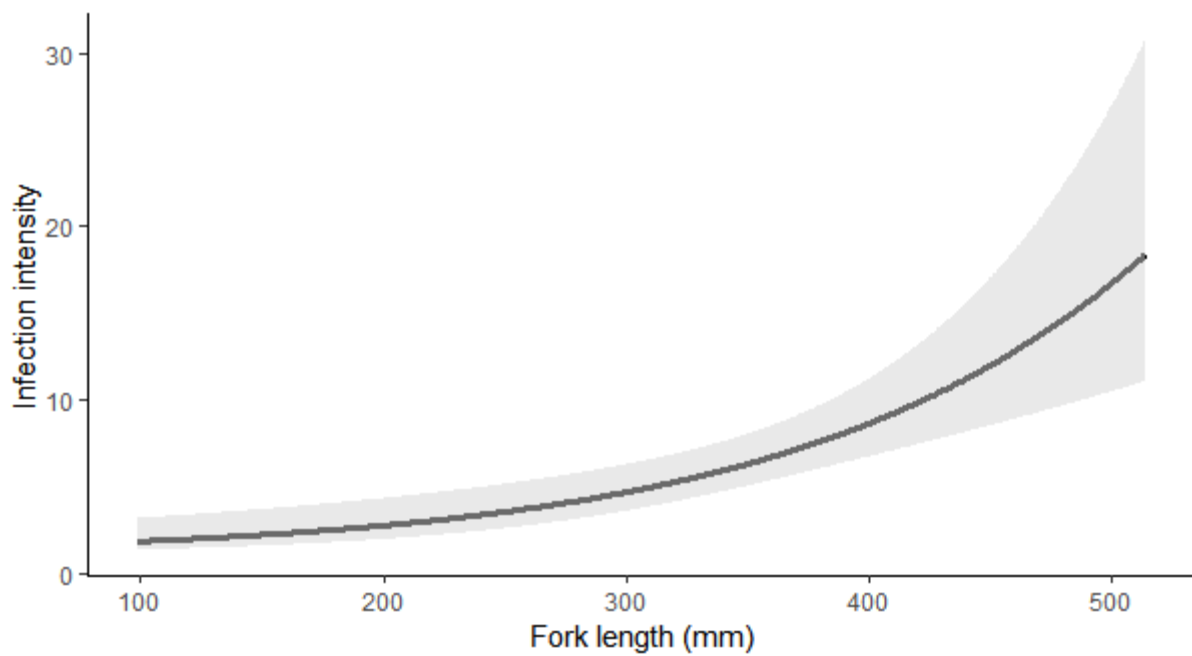


**Figure 37:** Plots showing the relationships between the host size (FL) and the predicted probability of infection by Cyst 1 of immature, male and female *Scomber japonicus* off South Africa (2016 to 2017) (95% confidence intervals are shown). Note: the x and y axes are different.

Only fork length was identified by the AIC model selection as being a significant predictor of the infection intensity of Cyst 1 (Table 15). The model explained 28.89% of the variation observed in the infection intensity of this parasite in *S. japonicus* and plotting the residuals indicated that the model assumptions were met. Cyst 1 infection intensity showed an exponential increase with increased host size, with the rate of increase low for fish of 100 to 300 mm FL and around 10 cysts being predicted to infect fish of 400 mm FL (Figure 38)

**Table 15:** Summary of the analysis of deviance for the negative binomial GLM predicting the infection intensity of Cyst 1 infecting *Scomber japonicus* off South Africa between 2016 and 2017. The residual degrees of freedom (Residual df), degrees of freedom (df), residual deviance (Residual Dev.) and the associated significance (p – value) for a  $\chi^2$  test are shown.

Predictor variable	Residual df	df	Residual deviance	p – value
NULL	41		58.701	
FL	40	1	41.150	$2.8 \times 10^{-5}$



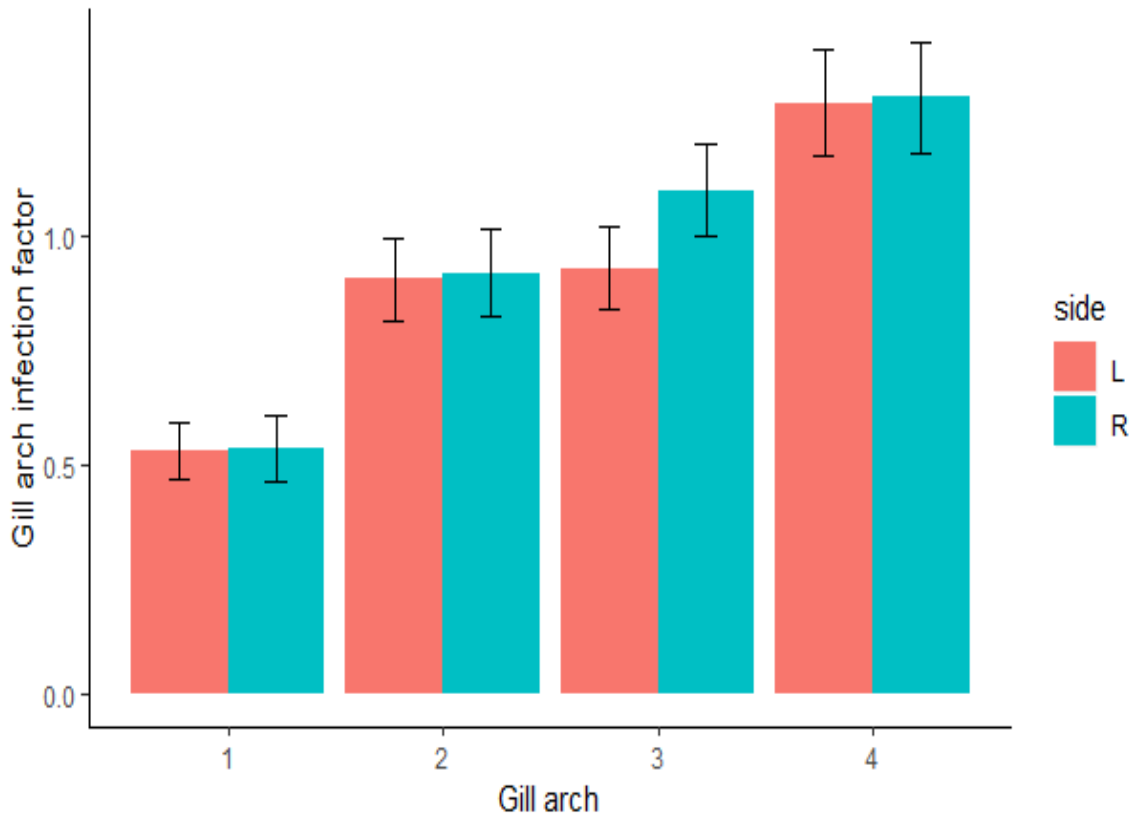
**Figure 38:** Plot showing the relationship between host size (FL) and predicted mean infection intensity by Cyst 1 of *Scomber japonicus* off South Africa (2016 to 2017) (95% confidence intervals are shown).

### Gill parasite analysis

Mean gill arch infection factor (GAIF) of *Pseudokuhnia minor* was not significantly different between the left and right gill arches for all gill arches (p – value >0.05). The analysis of variance (ANOVA) comparing the mean gill arch infection factor (GAIF) of the four gill arches found that all were significantly different from one another (p – value <0.05) except for the second and third gill arches, which were found to be significantly similar (Table 16).

**Table 16:** Table showing the difference in mean gill arch infection factor (GAIF) (number *Pseudokuhnia minor* individuals per mm) results of the *post hoc* Tukey Honest Significant Difference test comparing mean GAIF between gill arches in *Scomber japonicus* off South Africa (2016 to 2017).

Gill arch	Diff	Lower	Upper	p - value
gill2-gill1	0.378	0.133	0.624	0.0005
gill3-gill1	0.479	0.234	0.725	0.0000
gill4-gill1	0.761	0.516	1.006	0.0000
gill3-gill2	0.101	-0.144	0.346	0.7144
gill4-gill2	0.383	0.137	0.628	0.0003
gill4-gill3	0.282	0.036	0.527	0.0169



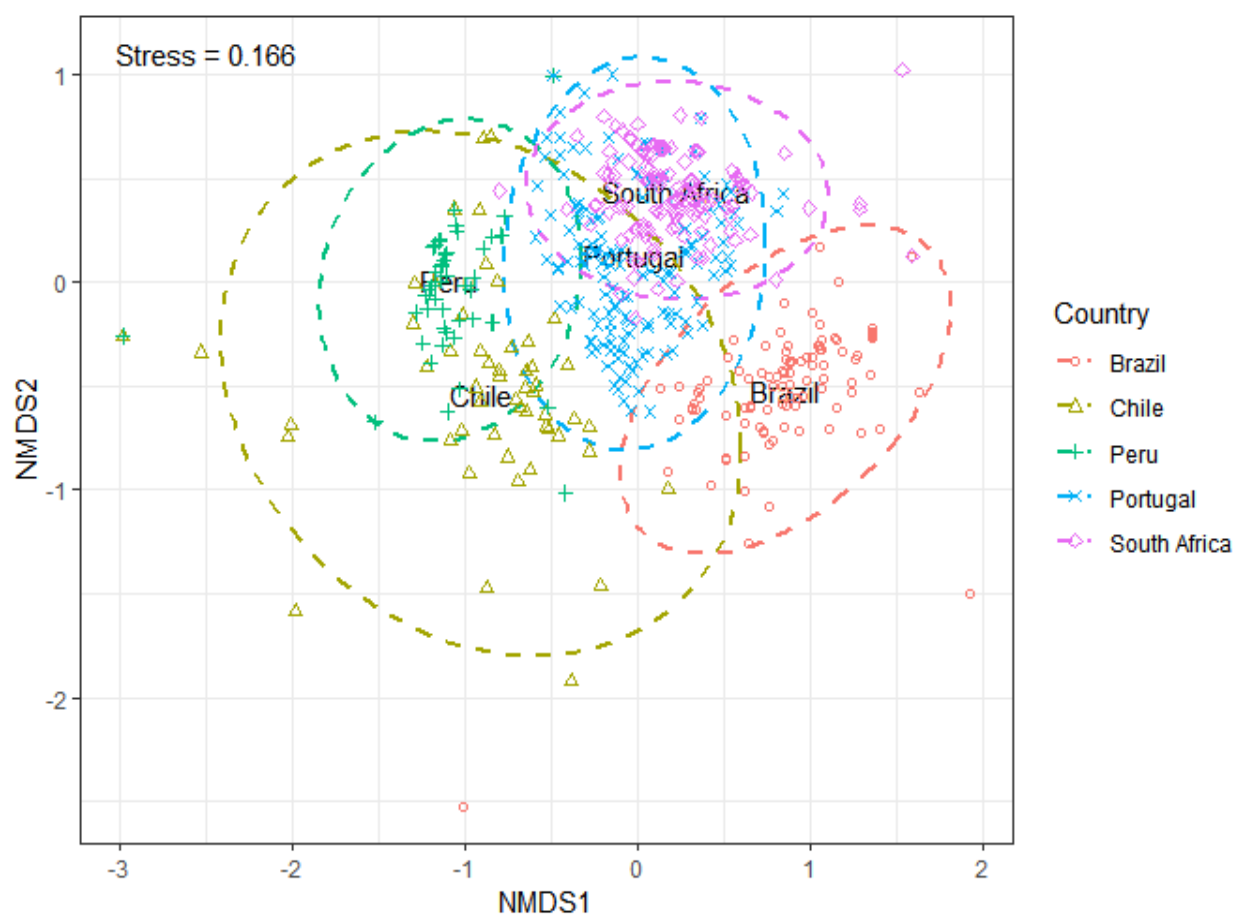
**Figure 39:** Bar plot showing the mean gill arch infection factor (number *Pseudokuhnia minor* individuals per mm) of left (red) and right (blue) gill arches of *Scomber japonicus* off South Africa (2016 to 2017)

### Global comparison of the parasite assemblages of *Scomber japonicus* populations

The NMDS plot (Figure 40) and the ANOSIM of the parasite assemblages of *Scomber japonicus* off Brazil, Chile, Peru, Portugal and South Africa revealed that the different fish populations off these countries have very different parasite community structures ( $R = 0.705$ ,  $p$  – value = 0.001). The pairwise ANOSIM revealed that the parasite assemblages of each *S. japonicus* population was significantly different from all countries but that *S. japonicus* from Portugal and South Africa, and from Chile and Peru were the least dissimilar whilst *S. japonicus* from Brazil and Portugal, and from Brazil and Peru, were the most dissimilar (Table 17).

**Table 17:** Table showing the pairwise ANOSIM results comparing the parasite assemblage of *Scomber japonicus* off Brazil, Chile, Peru, Portugal and South Africa.

Comparison	R statistic	p – value
Chile - Brazil	0.81	0.001
Chile - Peru	0.32	0.001
Chile - Portugal	0.89	0.001
Chile - South Africa	0.79	0.001
Brazil - Peru	0.91	0.001
Brazil - Portugal	0.94	0.001
Brazil - South Africa	0.76	0.001
Peru - Portugal	0.92	0.001
Peru - South Africa	0.88	0.001
Portugal - South Africa	0.28	0.001



**Figure 40:** Plot of the Non-metric Multidimensional Scaling analysis of the parasite assemblages of *Scomber japonicus* off Brazil (red circles), Chile (yellow triangles), Peru (green crosses), Portugal (blue crosses) and South Africa (pink diamonds).

The SIMPER analysis identified 16 parasite taxa as being responsible for 80% of the dissimilarity between *S. japonicus* from the five countries, namely *Ovarionematobothrium saba*, *Anisakis* sp., *Nematobotrium scombri*, *Corynosoma australe*, *Kuhnia sprostonae*, *Kuhnia scombri*, *Didymocystis* sp., *Corysoma* sp., *Scolex pleuronectis*, *Raphidascaris* sp, *Prodistomum orientalis*, *Digenea* sp., *Pseudokuhnia minor*, *Kuhnia scombercolias*, *Opechona bacillaris* and *Lecithocladium harpodontis* (Table 18).

**Table 18:** Table showing the prevalence of the 16 parasite taxa responsible for 80% of the dissimilarity between *Scomber japonicus* from five countries.

Species	Brazil	Chile	Peru	Portugal	South Africa
<i>Ovarionematobothrium saba</i>	0.0	28.6	30	0.0	0.0
<i>Anisakis</i> sp.	4.0	48.2	10	52.3	62.5
<i>Nematobotrium scombri</i>	55.0	5.3	0.0	27.8	0.0
<i>Corynosoma australe</i>	10.0	42.9	0.0	0.0	0.0
<i>Kuhnia sprostonae</i>	0.0	28.6	43.3	0.0	0.0
<i>Kuhnia scombri</i>	29.0	0.0	0.0	15.2	16.5
<i>Didymocystis</i> sp.	0.0	8.9	40.0	0.0	2.0
<i>Corysoma</i> sp.	10.0	42.9	0.0	0.0	0.0
<i>Scolex pleuronectis</i>	29.0	0.0	0.0	15.2	0.0
<i>Raphidascaris</i> sp.	52.0	0.0	0.0	0.0	0.0
<i>Prodistomum orientalis</i>	0.0	0.0	0.0	19.9	0.0
<i>Digenea</i> sp.	0.0	5.4	0.0	31.1	0.0
<i>Pseudokuhnia minor</i>	0.0	0.0	0.0	98.7	86.2
<i>Kuhnia scombercolias</i>	46.0	0.0	0.0	39.1	0.0
<i>Opechona bacillaris</i>	85.0	0.0	0.0	0.0	32.9
<i>Lecithocladium harpodontis</i>	80.0	0.0	0.0	0.0	21.2

## DISCUSSION

The ecology, biology and parasitology of *Scomber japonicus* has been extensively studied in several parts of the world, partly due to their cosmopolitan distribution but also due to their wide use as a food source in many countries, especially in eastern Asia. South African *S. japonicus* are relatively understudied in comparison, and thus far their parasite assemblage has not been documented. This study aimed to describe the parasite assemblage of *S. japonicus* off South Africa, to identify the drivers of parasite infection in this species, and to compare the parasite assemblage of *S. japonicus* off South Africa to those of *S. japonicus* populations elsewhere.

### *Scomber japonicus* parasites

#### **Myxozoa**

Myxozoans are parasitic Cnidarians commonly found infecting both freshwater and marine fish species (Rohde, 2005). They primarily parasitize teleosts, but have been recorded in several amphibians, reptiles, invertebrates and even mammalian hosts (Rohde, 2005). Initially considered protists, they are currently classified and widely accepted as metazoan organisms related to Cnidaria (Rohde, 2005). Myxozoan life cycles are largely unknown and only six from marine ecosystems have been described in detail. All involve a polychaete alternate host, however there is evidence of direct fish-fish infection (Rohde, 2005). Two myxozoan parasites were found infecting *S. japonicus* off South Africa, *Kudoa thrysites* and *Ceratomyxa* sp.

#### *Kudoa thrysites*

There are 44 known species in the genus *Kudoa*, which infect a wide range of teleosts and have a cosmopolitan distribution (Moran *et al.*, 1999). *Kudoa* myxosporeans are of a particular concern to fisheries and aquaculture as they are responsible for the accelerated muscle degradation, or post-mortem myoliquifaction of many commercially exploited fish species around the world (Moran *et al.*, 1999;

Levsen, 2008; Henning *et al.*, 2013; Nunkoo *et al.*, 2016). This can potentially lead to substantial economic losses as the muscle of the fish become soft and unpalatable and is often rejected or can be sold at lower prices (Moran *et al.*, 1999; Henning *et al.*, 2013; Nunkoo *et al.*, 2016; Levsen, 2008). *Kudoa thrysites* has been recorded in Scombroid teleosts in the North Atlantic and is the cause of much concern due to the value of the mackerel fishery there and the potential damage the parasite can cause (Levsen, 2008). Several species of *Kudoa* have previously been recorded in South Africa, including *K. paniformes* in Cape hake *Merluccius paradoxus* and *M. capensis* and snoek *Thrysites atun*, and *K. thrysites* in sardine *Sardinops sagax* and *T. atun* (Gilchrist, 1923; Reed *et al.*, 2012; Henning *et al.*, 2013; Nunkoo *et al.*, 2016). In this study the presence of *K. thrysites* was greatest in older, larger fish and no fish smaller than 445 mm FL were infected, suggesting that *K. thrysites* infects *S. japonicus* only when the latter reach a certain age or length. This contrasts with the *K. thrysites* infections of *T. atun* observed by Nunkoo *et al.* (2016), who reported that *K. thrysites* infects *T. atun* from a young age, possibly in their nursery grounds. It is not possible to predict the origin of *K. thrysites* infections in *S. japonicus* due to the limited knowledge of the life cycle of *K. thrysites*. Henning *et al.* (2013) listed various methods of detection of *Kudoa* parasite infections and stated that microscopy is a useful method when three tissue samples per fillet are examined. It is possible that insufficient sampling is a cause of the relatively low prevalence of *K. thrysites* found in *S. japonicus* in this study.

#### *Ceratomyxa* sp.

With almost 200 species, the genus *Ceratomyxa* is extremely diverse and has a worldwide distribution (Gunter *et al.*, 2009). These parasites are known to infect many marine fish species and are mostly found in the gall bladder (Rohde, 2005; Gunter *et al.*, 2009). Several species of *Ceratomyxa* have previously been recorded in South Africa, including *Ceratomyxa shulmanii* (Dubina and Isakov, 1976), and *C. cottoidii*, *C. dehoopi* and *C. honckenii* (Reed *et al.*, 2007). These species were all found infecting intertidal fish along the coast of South Africa which would have a minimal chance of interacting with *S.*

*japonicus*. Preliminary examinations suggest that the species recorded in this study is possibly *Ceratomyxa inconstans*, which infects *S. japonicus* in the North Atlantic (Jameson, 1929). The genus *Ceratomyxa* has been described as pathogenic and can cause irritation and damage to the gall bladder of their host (Rohde, 2005), however no obvious reaction was observed on the gall bladders recovered in this study. The low prevalence of *Ceratomyxa* sp. meant that modelling was not possible, however it is possible that these parasites were more prevalent than reported in this study as not all 152 gall bladders were examined due to damage or not being found (small frozen specimens meant gall bladders were extremely fragile).

## **Monogenea**

Monogeneans, an entirely parasitic class within the phylum Platyhelminthes, almost exclusively infect the gills and skin of both marine and freshwater fish and feed on blood, mucus and epithelial cells (Schmidt *et al.*, 1989; Rohde, 2005; Reed *et al.*, 2009). A few monogeneans have adapted to be able to enter the rectal cavity, ureter, body cavity or circulatory system (Reed *et al.*, 2009). Their haptors are specialized attachment structures, which are unique to monogeneans, and help them retain a firm hold on their hosts. Monogeneans are separated into two groups based on their haptor structure; monopisthocotyleans have hooked haptor attachment structures, and polyopisthocotyleans have clamp-like attachment structures (Schmidt, 1989; Rohde, 2005; Reed *et al.*, 2009). Monogenean infections are rarely detrimental to their hosts however, farmed populations of fish, which are more at risk of extreme monogenean infestations due to dense populations, have been known to suffer from skin or gill irritations and can even face mortalities (Reed *et al.*, 2009). This is due to their direct life cycle (moving from one host directly to the next) unlike trematodes and cestodes which have complex life cycles which involve intermediate and final hosts (Rohde, 2005; Reed *et al.*, 2009). Three monogeneans, namely *Pseudokuhnia minor*, *Kuhnia* sp. and *Grubea cochlear* (all polyopisthocotyleans) were found in this study.

### *Pseudokuhnia minor*

Monogeneans from the genera *Kuhnia* and *Pseudokuhnia* have been recorded and studied in several *S. japonicus* populations around the world and are closely associated with the *Scomber* genus (Rohde & Watson, 1985; Rohde, 1989). These two polyopisthocotylean monogeneans share many morphological traits, are both part of the family Mazocraeidea and are often found together. *Pseudokuhnia minor* is very similar to the parasites in the genus *Kuhnia* and was initially part of the *Kuhnia* genus until Rohde and Watson (1985) removed it, based on *P. minor* having two vaginae, instead of the monogeneans in the genus *Kuhnia* which characteristically have none. With a prevalence of 86.2%, *Pseudokuhnia minor* were by far the most common parasite species found in this study. This is common among *S. japonicus* populations, where *P. minor* is consistently among the most prevalent parasite species found (Rohde, 1985; Oliva *et al.* 2008; Costa *et al.*, 2011). Host size and probability of infection shared a strong positive relationship and *S. japonicus* over 300 mm FL were predicted to have a 100% chance of infection. Adults showed a higher intensity of infection than immature fish, which is to be expected since they would have more opportunities to encounter and become infected by a *P. minor* individual.. Infection intensity also showed a spatial pattern, with fish caught further east having lower infection intensities than those fish caught further west. The decrease in infection intensity from the west to the east coast is possibly related to shift from a temperate to more tropical temperature range. The decreasing trend was not attributed to a size difference or an imbalance in the sampling effort since the east and west coast samples were not significantly different in size, and the decreasing trend was observed throughout the sampling area. The gill analysis of *P. minor* infections revealed that the intensity of *P. minor* infections was greater on gill arches closer to the anterior of the fish, and furthest from the spinal cord (gill arch number four). Not all the gill arches of *S. japonicus* are the same size, with the innermost gill arches being the smallest and the gill arch size increasing outward. The innermost gill arches receive the fastest water currents directly from the mouth and by the time the water reaches the fourth set of gill arches the water current is much

slower. Gutiérrez & Martorelli (1999) highlight are two leading hypothesis explaining gill arch preferences of parasites, the first considering that water currents are the leading driver behind gill arch preference with, parasites being able to remain on their hosts more securely on gill arches which experience less water flow. The second predicts that space availability drives gill arch preference, and as there is more space on larger gill arches this means that more parasites will be present. In this study the intensity of *P. minor* infections was standardized for space availability by using the gill arch infection factor (GAIF), therefore the pattern of higher intensity on the fourth set of gill arches observed in this study supports the hypothesis that *P. minor* prefer gill arches with reduced waterflow.

There are no recorded cases of *Pseudokuhnia* species affecting the health of *S. japonicus* negatively, however *S. japonicus* off South Africa do appear to harbour extremely high levels of *P. minor*, with a mean infection intensity of 47.5 and a maximum of over 200 *P. minor* individuals per infected fish. Monogeneans are known to cause respiratory problems as well as gill epithelial cell irritations when infections reach extreme levels (Reed *et al.*, 2009), but gills did not appear to be conspicuously affected by any monogenean infections in this study.

#### *Kuhnia scombri*

Initial examinations of the *Kuhnia* sp. found in this study indicate that this is likely *Kuhnia scombri*, however with the limited knowledge and samples of *Kuhnia* sp. collected it was not possible to make a definitive identification. *Kuhnia* sp. had a much lower prevalence (16.5%) than *P. minor* and also a lower prevalence than many other parasitic taxa including digeneans and acanthocephalans. This is atypical, since *Kuhnia* species are usually the most prevalent and numerous parasites infecting *S. japonicus* populations around the world (Rohde, 1989; Oliva *et al.*, 2008).

## *Grubea cochlear*

*Grubea cochlear* is a polyopisthocycean monogenean which almost exclusively infects members of the genus *Scomber* (Rohde, 1986; Lyndon and Martinez-Vidal, 1994) and has been recovered from populations of *S. japonicus* throughout the Mediterranean and the Atlantic Ocean (Brazil, Portugal and North America) (Rohde, 1986; Lyndon and Martinez-Vidal, 1994). Erimina (1970) and Solonchenko (1968) recovered *G. cochlear* from horse mackerel *Trachurus trachurus capensis* and Atlantic chub mackerel *Scomber colias* respectively, off South Africa, however no other subsequent studies have recorded *G. cochlear* in South African fish species or in *T. trachurus*. *Grubea cochlear* has the lowest prevalence (4%) out of the three monogeneans found infecting *S. japonicus* off South Africa, which appears to be a consistent trend in most *S. japonicus* populations where *G. cochlear* was found along with other species of monogeneans, but no studies have attempted to explain this phenomenon. Lyndon and Martinez-Vidal (1994) reported on *G. cochlear* microhabitat preferences in the gills of *Scomber scombrus* in the North Atlantic and determined that *Kuhnia scombri* and *G. cochlear* share a close relationship and that despite significant differences in total size, the reproductive organs of these monogeneans are in fact very similar in size and form and possibly has led to a close association or alignment in their microhabitat preferences. They also found that *G. cochlear* has a clear preference for the outermost gill arches and that prevalence decreases on gill arches closer to the spine. This was also observed in this study, as *G. cochlear* specimens were only recovered from the 4<sup>th</sup> gill arch, which was located furthest from the spinal column. This is either a result of increased space availability or reduced water flow as these monogeneans have large bodies that would potentially prefer gill arches with reduced water currents.

## Digenea

The class Trematoda, which includes both Aspidogastrea and Digenea, is highly diverse (Schmidt *et al.*, 1989; Cribb *et al.* 2002; Rohde, 2005). Their diversity is partly due to the wide range of organisms and sites of infections that these parasites have been able to exploit (Cribb *et al.* 2002; Rohde, 2005). Digeneans are generally endoparasitic gut parasites but are also commonly found in the swim bladder, gall bladder, body cavity, muscle, gonads or blood (Schmidt *et al.*, 1989; Rohde, 2005). Some digenea have even adopted a somewhat ectoparasitic lifestyle and have been observed infecting the scales and gills of fish, digeneans also infect tetrapod livers, blood, lungs, bladders, eyes and gonads (Rohde, 2005). Digenean life cycles are typically complex and involve both a free-living (egg, miracidium and cercaria) and parasitic component (sporocyst and adult). Most digenean sporocysts utilise a mollusc as their first intermediate host, these in turn produce cercaria and which continue on to infect a vertebrate as their final host. This study found five digenean parasites that were found to infect the *Scomber japonicus* off South Africa

### *Lecithocladium* sp.

*Lecithocladium* spp., part of the Hemiuridae family, are common stomach parasites found in many *S. japonicus* populations, mostly in the Atlantic Ocean (Køie, 1991; Oliva *et al.*, 2008; Ndiaye, 2012). Few studies have attempted to describe the life cycle of *Lecithocladium* species, but several invertebrates such as molluscs, polychaetes and ctenophores are suspected to be intermediate hosts of *Lecithocladium* Digeneans, while *Scomber* and possibly other teleosts are the most probable terminal hosts (Matthews & Matthews, 1988; Køie, 1990). Initial identifications indicate that the species recorded in this study is the cercaria of *Lecithocladium harpodontis*. Probability of infection by *Lecithocladium* sp. was predicted to increase with size in juvenile *S. japonicus* whereas in adult fish, prevalence decreased with increased host size. This trend is a possible result of the separation in diet between adult and juvenile *S. japonicus* in South Africa. Køie (1991) identified various copepods and the sea slug *Philine aperta* as

*Lecithocladium* intermediate hosts; *P. aperta* does occur in South Africa and would be able to transfer *Lecithocladium* cercaria to *S. japonicus*, however further research is needed to confirm the source of *Lecithocladium* sp. infections in the *S. japonicus* off South Africa. *Lecithocladium* sp. infections in *S. japonicus* are of little concern with regards to human health, as they were only been recorded infecting the stomach of *S. japonicus* in South Africa and would be removed through processing.

#### *Opechona bacillaris*

*Opechona bacillaris* is a typical parasite species found in scombrids and several other fish in the Atlantic Ocean (Williams, 1959; Bray & Gibson, 1990; Keser *et al.* 2007; Ndiaye, 2015). K ie (1975) described the life history of this Lepocreadiid digenean whose eggs initially infect a gastropod, *Nassarius pygmaeus*. The cercaria continue on to infect cnidarians medusae, ctenophores or chaetognaths, these intermediate hosts are consumed by mackerels or other planktivorous fish, the *O. bacillaris* metacercariae then mature in the foregut and release eggs. K ie (1974) described this digenean from Denmark, however South Africa has several species of gastropod which belong to the genus *Nassarius* and could possibly complete the life cycle of *O. bacillaris*. The prevalence of *O. bacillaris* was predicted to increase during spring-summer, which was expected due to their reliance on planktonic invertebrates for the second stage of their life cycle. The increased upwelling experienced in South Africa during the end of the year, increases productivity and results in a greater density of several species of zooplankton. This provides *O. bacillaris* with a greater opportunity to proliferate and infect *S. japonicus*, who are also more abundant in South Africa during the same time (Crawford & De Villiers, 1984). In juvenile *S. japonicus*, host size and probability of infection shared a positive relationship. Adult male prevalence was predicted to be independent of host size, while the probability of infection in females appeared to decrease with increased host size. These Digenea infect the foregut of *S. japonicus* and pose very little health risk to humans as they would be removed from the fish during processing. The effect on the fish is also expected to be minimal as no pathology has been recorded in fish infected by *O. bacillaris*.

## Didymozoids

Members of the family Didymozoidae are Digenea with a wide geographical distribution that are particularly common in the Scombridae family of tunas, mackerels and bonitos (Mota *et al.*, 2019 and Pozdnyakov *et al.*, 1990) in which they parasitized a variety of tissues including the gills, mouths, muscles and several internal organs such as the kidneys and pyloric caeca. While other teleosts in the Serranidae, Sphyraenidae and Carangidae families are infected by didymozoid digeneans, almost 65% of all didymozoid species are found in scombrids (Nikolaeva, 1985). *Scomber japonicus* alone are host to 11 species of Didymozoidae (Nikolaeva, 1980). Knowledge of the life history of didymozoid parasites is lacking, but Nikolaeva (1980) explained that didymozoid life cycles are complex and variable and that the first life stage is very poorly understood. Metacercaria stages have been recorded in several pelagic teleosts, squid, crustaceans and sharks, and free-living metacercaria have been found in plankton samples.

### *Nematobothrium faciale*

Initially called *Didymozoon faciale* by Baylis (1938), *Nematobothrium faciale* is a member of the Didymozoidae family of digeneans. *Nematobothrium faciale* belongs to the sub-family Nematobothriine and occurs in cysts located on the inner side of the operculum. These cysts, which contain between one and three individuals, often deform the cartilage below and are surrounded by a thin dermal layer of tissue (Baylis, 1938). Lester (1979) states that these didymozoids are of commercial importance as they can affect the marketability of fish due to the cysts being visible on the operculum. The health risks of this species are of minimal concern as consumption of the operculum is rare, but Nikolaeva (1980) predicted that some didymozoid digeneans might have developed the ability to develop inside humans if consumed. However, negative effects of consuming *Nematobothrium faciale* are yet to be recorded.

*Halvorsenius* sp.

*Halvorsenius* sp. also belongs to the Nematobothriine subfamily in Didymozoidae, which shares a very close association with scombrids (Nikolaeva, 1985; Pascual *et al.* 2006). There is a strong possibility that the species recorded in this study is *Halvorsenius exilis*, which was described by Gibson *et al.* (1981) as a filamentous digenean found mainly in the pericardium, but also in the kidney, eyes, mouth, operculum and musculature of Atlantic mackerel *Scombrus scombrus*. This is uncommon of digenean parasites as it is both ecto and endoparasitic and hasn't assumed a single lifestyle. The *Halvorsenius* sp. in this study were only found infecting the kidney, mouth and gill arches. Gibson *et al.* (1981) hypothesized that *H. exilis* infect *Scomber scombrus* when they are between one and two years old, and then develop and produce eggs which remain in the host and are only released when the host dies and decomposes or is digested. This was based on observations of only worms being found in young fish under two years and only eggs being found (where worms used to be were present) in fish older than three years. There were also no obvious mechanisms for egg release as well as a high degree of organ atrophy in all *H. exilis* worms. Gibson *et al.* (1981) noted that these parasites live in very close contact with their host's tissue, which does not appear to have any reaction to its presence, suggesting a long and close association between these species and possible co-evolution. The prevalence of *Halvorsenius* sp. was only 11.8%, which was below the required prevalence set in this study to model predictor variables, however no *Halvorsenius* sp. was observed in fish longer than 265 mm FL and worms were only recovered from three fish, all of which were under 231 mm FL. While no pathology of *H. exilis* has been conducted, Gibson *et al.* (1981) indicated minimal health risks of human infections by *H. exilis*, because whilst eggs in the musculature of the host could possibly be consumed by humans, it is unlikely that they will survive processing, freezing or sewerage systems.

*Didymocystis* sp.

*Didymocystis* sp. has mostly been recorded infecting the gill filaments of their hosts but have also been found infecting the skin and opercula (Kohn *et al.*, 2001). *Didymocystis* parasites are rarely endoparasitic but have been found infecting the muscle of three different species (Nigrelli, 1939). In this study *Didymocystis* sp. was only found infecting the gill filaments of *S. japonicus*. Despite the low prevalence and intensity of infection, this is not considered an accidental infection due to the high degree of host specificity of the *Didymocystis* genus.

### **Cestoda**

The Cestoda is a large class consisting of over 5000 distinct species, 1 400 of which are associated with marine environments and infect various marine organisms at different life stages, including birds, pinnipeds, cetaceans, crustaceans and fish (Schmidt *et al.*, 1989; Rohde, 2005). A defining characteristic of cestodes is the presence of a scolex, which is a prominent holdfast organ located on the exterior of their body. Cestodes characteristically use vertebrates as their final hosts, are usually transmitted through predator-prey interactions and typically utilise between two and four intermediate hosts, meaning that their lifestyle is complex and affects numerous taxa (Schmidt *et al.*, 1989; Rohde, 2005).

*Tentacularia coryphaenae*

*Tentacularia coryphaenae*, belonging to the infraorder Trypanorhyncha, was the only cestode parasite recovered *Scomber japonicus* during this study. Initially infecting crustaceans and subsequently fish, trypanorhynch cestodes finally infect a vertebrate which is usually an elasmobranch (Knoff *et al.*, 2004). *T. coryphaenae* infected the body cavity of chub mackerel and were found at a very low prevalence, but trypanorhynch cestodes have also been found infecting the mesentery and musculature of other organisms. The low prevalence of *T. coryphaenae* meant that the probability and intensity of infection were not possible to model. *T. coryphaenae* has been found in several other *S. japonicus* populations

around the world, therefore it is unlikely this is an accidental infection, and has also been recorded in several species of commercially important fish in South Africa including *Thryxites atun* and *Sardinops sagax* (Reed *et al.*, 2012; Nunkoo *et al.*, 2016). *S. japonicus* feed on both crustaceans and smaller pelagic fish (Clupeoids) and are preyed upon by larger teleost species, meaning they are a potential intermediate host for *T. coryphaenae* between these two trophic levels. However, without more research into the parasite host interactions it is not possible to clearly describe this relationship.

## **Nematoda**

Two nematode species were found infecting the viscera of *Scomber japonicus* off the coast of South Africa, namely *Anisakis simplex* and *Contracaecum* sp. Both species are members of the family Anisakidae and are known to have cosmopolitan distributions (Audicana & Kennedy, 2008; Rohde, 2005). *Anisakis simplex*, and to a lesser degree, *Contracaecum* sp. have been extensively studied due to the high risk they pose of human infection and associated health impacts (Audicana *et al.*, 2002; Audicana & Kennedy, 2008; Santos *et al.*, 2017; Sakanari & McKerrow, 1989; Costa *et al.*, 2003). Parasites from the genera *Anisakis*, *Thynnascaris*, *Histerothylacium*, *Contracaecum* and *Pseudoterranova* have been recorded in *S. japonicus* populations across the world (Wysokinski *et al.*, 1987; Cremonte & Sardella, 1997; Suzuki *et al.*, 2009; Oliva *et al.* 2008; Eiras & Rego, 1987).

Both *A. simplex* and *Contracaecum* sp. have complex life histories, including several stages of infection in a wide range of marine organisms (Anderson, 2000; Klimpel & Palm, 2011; Rohde, 2005). Adult anisakids infect a variety of marine mammals, fish and birds and have been known to infect humans as well. Larvae are initially free living but infect several intermediate hosts, including crustaceans, fish and cephalopods, which are ingested by mammals and develop into adult stages (Anderson, 2000; Rohde, 2005; Klimpel & Palm, 2011; Audicana & Kennedy, 2008;). During processing of samples in this study, high numbers of euphausiids and cephalopods in the stomach contents of the processed *S. japonicus* were observed which are the likely origin of the anisakid infections. Baird (1978b) reported that euphausiids

make up as much as 42.6% of the diet of fish smaller than 250 mm FL, and that adult fish (> 250 mm in length) consume both euphausiids and small pelagic fish such as anchovy *Engraulis encrasicolus* in large quantities, both of which are known to harbour *A. simplex* larvae (Smith, 1983; Van Stavel, 2015). *Scomber japonicus* have a higher probability of *A. simplex* infection during autumn-winter, which may be due to increased feeding behaviour before spawning, which would make them more susceptible to infection, or another possibility is that the temperature range experienced during autumn-winter is more conducive to the growth of anisakid larvae than the temperatures during spring-summer. Fish larger than 200 mm FL also have a greater probability and intensity of infection which may be due to their older age and having a wider variety diet compared to their earlier life history meaning they encounter and consume more larvae. *Scomber japonicus* are known to school with snoek *Thyrsites atun* and anchovy *Engraulis encrasicolus* individuals of a similar size, and both of these species harbour anisakid parasites (Hennig, 1974; Van Stavel, 2015; Nunkoo *et al.*, 2016) and could transfer their parasites to *S. japonicus* when consumed (Nunkoo *et al.*, 2016; Crawford & De Villiers, 1984). The reason for the probability of anisakid infection decreasing with an eastward shift in sample location is likely a result of a shift in diet, environmental factors tend to not affect endoparasites like *A. simplex* since variability is limited due to their host.

Anisakid infections in *S. japonicus* are of particular concern, as parasites from this genus are closely associated with a number of human health risks (Audicana *et al.*, 2002; Audicana & Kennedy, 2008; Suzuki *et al.*, 2009). *Anisakis simplex* infections in humans have been investigated thoroughly and can cause a condition called anisakiasis which can have several severe symptoms including lethal anaphylactic reactions, gastrointestinal discomfort and sinus irritation. In this study *Anisakis* larvae were only observed to infect the body cavity of *S. japonicus* and not the muscle, meaning that processed fish should be safe to consume by humans. However, a study conducted by Niewehuizen *et al.* (2005)

determined that extended contact with anisakid proteins is sufficient to induce a reaction. Further research is needed to determine how *S. japonicus* could be processed safely for human consumption or use.

### **Acanthocephala**

The Acanthocephala is a small phylum, containing at least 1 000 species (Schmidt *et al.*, 1989; Rohde, 2005). These parasitic worms, commonly known as spiny-headed worms, have complex life cycles and have developed a variety of life strategies depending on systematic structure (Schmidt *et al.*, 1989; Rohde, 2005). All acanthocephalans use crustaceans such as crabs, amphipods or ostracods as an initial host. They then infect fish, which are either their final host or an intermediate host in cases where the final host is a marine mammal such as a pinniped or cetacean. Some sea birds have also been found to harbour acanthocephalan parasite species (Schmidt *et al.*, 1989; Rohde, 2005). Numerous studies have investigated the effects that acanthocephalans have on host populations, and many have found noteworthy results revealing that acanthocephalans are able to alter host behaviour and affect mortality (Bethel & Holmes, 1973; Schmidt *et al.*, 1989; Rohde, 2005; Perrot-Minnot *et al.*, 2007). Latham and Poulin (2002) report that an acanthocephalan, *Profilicollis* sp., affects the burrowing behaviour of their crab hosts which make them more susceptible to predation. Marine acanthocephalans occur exclusively in the gastrointestinal tract of their hosts and therefore infections in humans are rare (Adams *et al.*, 1997). A single species of acanthocephalan, *Rhadinorhynchus pristis*, was found infecting *S. japonicus* off South Africa.

#### *Rhadinorhynchus pristis*

There are 38 species in the genus *Rhadinorhynchus* which are characteristically intestinal parasites of several teleosts and marine mammals (Cable & Linderoth, 1963; Amin *et al.*, 2011). *Rhadinorhynchus pristis* has a complex life cycle and uses pelagic crustaceans such as amphipods and crab larvae as intermediate hosts, which are eaten by small pelagic fish, mostly from the family Myctophidae or by

their final hosts which are typically scombrids but may also be other pelagic fish such as the dolphinfish species *Coryphaena hippurus* and *C. equiselis* (Carbonell *et al.*, 1999; Gregori *et al.*, 2013). *Rhadinorhynchus pristis* has primarily been recorded in the North Atlantic Ocean but records from Brazil in the South Atlantic are also common (Carbonell *et al.*, 1999; Amin *et al.*, 2011), and this species was recorded as infecting *S. colias* off South Africa by Solonchenko (1968), although at very low prevalence. Several factors observed in this study suggest that *S. japonicus* is a definitive or final host of *R. pristis* in South African waters. *Rhadinorhynchus pristis* had a significantly greater probability of infecting larger, mature fish compared to smaller, immature *S. japonicus*. This difference is likely a result of the separation in diets between mature and immature fish, as seems to be the case with many of the parasites infecting *S. japonicus* off South Africa. The intensity of infection in South African *S. japonicus* is also generally higher than the intensity of *R. pristis* infections observed elsewhere in the world. This may possibly be due to lantern fish (myctophid *Lampanyctodes hectoris*) making up almost 45% of the diet of adult *S. japonicus* in South Africa (Baird, 1978). Myctophid species in the north Atlantic are suspected by Klimpel (2006) to be paratenic hosts of *R. pristis*, if adult *S. japonicus* off South Africa consume more lantern fish than *S. japonicus* populations elsewhere, this could explain their higher probability and intensity of infection by *R. pristis*. In addition to differences in infection of mature and immature *S. japonicus*, *R. pristis* displayed spatial differences in both prevalence and infection intensity, with fish caught further west having lower prevalence and fewer *R. pristis* than *S. japonicus* caught further east. The cause of this difference is probably not environmental, since *R. pristis* is endoparasitic, and a likely driver is difference in diet or food availability. The spatial difference in prevalence of *R. pristis* in *S. japonicus* off South Africa suggests that this parasite could be used in population studies of chub mackerel around South Africa, although a larger number of and more frequently collected samples would be required.

## Copepoda

Copepods are one of the most specious groups in the Crustacea, with approximately 11 500 species, of which over 3 600 are parasitic (Cressey & Cressey, 1980; Rohde, 2005). Copepods infect nearly all phyla including members of the Porifera, Cnidaria, Platyhelminthes, Nemertea, Sipunculida, Annelida, Mollusca, Arthropoda, Phoronida, Brachiopoda, Echinodermata, Hemichordata, and Chordata (Huys & Boxshall, 1991; Ho, 2001). They are ecologically diverse, displaying both endo and ectoparasitic lifestyles, whilst they have been studied extensively in commercially important teleost and invertebrate species, knowledge of parasitic copepods on less valuable vertebrates and invertebrates is lacking (Rohde, 2005). Most parasitic copepods are grouped into two orders, namely the Poecilostomatoida and Siphonostomatoida (Rohde, 2005). Only one copepod species, *Clavellisa scombri* from the order Siphonistomatoida, was found infecting *S. japonicus* in this study.

Copepods from the genus *Clavellisa* are typically closely associated with Clupeids, but *C. scombri* is an exception to this trend and exclusively infects the genus *Scomber*, having been observed infecting all four species in the genus (Cressey & Cressey, 1980). Thanks to the cosmopolitan distribution of its hosts, *C. scombri* is found attached to gill arches or filaments of *S. japonicus* around the world and has adapted to a range of habitats (Oliva *et al.*, 2008; Mele *et al.*, 2014). Almost 10% of the *S. japonicus* examined were found to be infected with *C. scombri*, with a very typical level of intensity compared to other populations of *S. japonicus* (Oliva *et al.*, 2008). The infection of copepods on teleosts can potentially have several implications on the host's condition. Copepod attachment mechanisms and feeding strategies are known to cause lesions on the attachment sites which are susceptible to secondary infections and reduced respiratory efficiency if gills are the site of infection, and reduced fecundity of their hosts (Rohde, 2005). No obvious detrimental effects of *C. scombri* infections were observed, but to do so adequately would require further research.

## Community analysis

The parasite assemblage of *S. japonicus* did not show seasonal variability in terms of parasite species composition. *Scomber japonicus* in the Benguela show large-scale migrations that are thought to be driven by prey availability and are more abundant off South Africa between austral spring and summer (Crawford & De Villiers, 1989). This migration may explain the observed lack of seasonal variability in parasitic composition, since the fish would likely encounter a wide range of environmental conditions during their migration which would require very specific group of parasites able to tolerate substantial environmental fluctuations and remain in or on their host throughout the year. Some parasite species, namely the nematode *Anisakis simplex* and digeneans *Lecithocladium* sp. and *Opechona bacillaris*, did display seasonal variability in their prevalence and infection intensities, possibly a result of *S. japonicus* switching between feeding and reproduction focused lifestyles. The probability of infection of both *Lecithocladium* sp. and *O. bacillaris* increased in *S. japonicus* during spring and summer, when *S. japonicus* are spawning in South Africa. The increased pressure of reproduction could hinder immune responses to parasite infections resulting in increased prevalence and intensity. The GSI of the examined fish did not display any seasonal variation, however hardly any mature fish were processed from spring-summer which could mask a reproductive effect on the prevalence. However, in contrast, the probability and infection intensity of *Anisakis simplex* was lower during spring-summer and increased during autumn-winter. This could possibly be due to other hosts of *Anisakis* changing in abundance or intensity, for example euphausiids or snoek. The increased gonad size during the reproductive season may also limit the space available to Anisakid larvae.

The main driver of patterns in parasite prevalence and infection intensity in almost all of the taxa studied was host size. This variable was the most significant predictor in all of the six taxa modelled for prevalence and the four taxa modelled for infection intensity, either by itself or as an interaction term with sex (Table 19). Increases in these parasite indices with increasing host size are common and have

been reported in many studies (Nunkoo *et al.*, 2016; Mackintosh *et al.*, 2018). Whilst both prevalence and infection intensity were positively correlated with *Scomber japonicus* size in most instances in this study, contrasting relationships between immature and mature fish were observed for several parasites, with prevalence and/or infection intensity in adults being negatively correlated with host size hence the importance of the significant interaction between host size and sex in these cases (Table 19). The location was a significant predictor in three of the models, indicating spatial patterns in infection. The reason behind these are not known, but spatial patterns in infection indices have been observed for South African sardine *Sardinops sagax* (Reed *et al.*, 2013; van der Lingen *et al.*, 2015; Weston *et al.*, 2015), which have been taken as evidence of population structure in that species.

**Table 19:** Significant predictors (listed in order of decreasing p-value) of prevalence and infection intensity from GLM analyses on six parasite taxa infecting *Scomber japonicus* off South Africa.

Parasite taxon	Significant drivers of prevalence	Significant drivers of Infection intensity
<i>Anisakis simplex</i>	FL, Season, longitude	FL, Season
<i>Pseudokuhnia minor</i>	FL	FL, FL * Sex, Sex, Longitude
<i>Lecithocladium</i> sp.	FL * Sex, FL, Season, Sex	
<i>Opechona bacillaris</i>	FL, FL * Sex, Sex	
<i>Rhadinorhynchus pristicus</i>	FL, FL * Sex, Longitude, Sex	FL, FL * Sex
Cyst 1	FL, FL * Sex, Sex	FL

The ANOSIM (Table 17) and NMDS plot (Fig. 40) of the parasite communities of *S. japonicus* from Brazil, Portugal, Chile, Peru and South Africa revealed that each population was unique. This result differ from that of Oliva *et al.* (2008), who used parametric correspondence and multivariate discriminant analyses and reported that the parasite communities of *S. japonicus* from Chile and Peru were not significantly different but that the assemblage of those fish was significantly different to the assemblages of fish from Brazil and Portugal. This difference may arise from the different analyses used by Oliva *et*

*al.*, (2008) and in this study (non-parametric). Results from this study (ANOSIM) indicated that the degree of inter-population difference differed, with fish populations off South Africa and Portugal and those from populations off Chile and Peru, being the least dissimilar, whilst *S. japonicus* off Brazil was not aligned with any other population analysed. The addition of data from South African *S. japonicus* to the study conducted by Oliva *et al.* (2008) has corroborated their results and increased the observed parasite diversity in this species from 34 to 36 parasite taxa. Oliva *et al.* (2008) considered that the biggest driver of a population's unique parasite assemblage is extended separation, and the relative similarity of assemblages of *S. japonicus* populations off South Africa and Portugal indicates more recent separation of these populations compared to the others due to the shared coastline along west Africa between the two regions. Present mixing between these populations is limited due to the anti-tropical distribution of *S. japonicus* in the Atlantic Ocean (Stepian & Rosenblatt, 1996). The Brazilian and South African populations of *S. japonicus* are separated by the Atlantic Ocean basin, and therefore the parasite assemblage of Brazilian *S. japonicus* is dissimilar to both South African and Portuguese *S. japonicus* populations despite being in the same ocean. Chile and Peru share a coastline and their relative similarity in parasite assemblage indicates a recent separation, but the NMDS plot also indicates strong differences in the parasite assemblages of *S. japonicus* from the Atlantic and Pacific Oceans. Oliva *et al.* (2008) hypothesized that *S. japonicus* from the Atlantic entered the Pacific over the Isthmus of Panama while it was submerged, but once the Isthmus was above sea level, Atlantic and Pacific *S. japonicus* populations were separated and developed unique parasite assemblages. Oliva *et al.* (2008) also hypothesized that the presence of highly host specific parasite genera, such as *Kuhnia*, *Didymocystis* and *Grubea* suggest that these populations share a common origin, and the inclusion of these parasites in the SIMPER analysis supports this.

## LIMITATIONS AND FURTHER RESEARCH

Since little is known about the diversity of marine parasites in South Africa, one limitation in this study was the inability to identify all parasite taxa found to species level. However, this study has increased our knowledge of parasites infecting *Scomber japonicus* off South Africa and has created an opportunity for taxonomists to completely describe species that we know are present. The identification of parasites to species level through genetic analysis would aid comparisons of South African *Scomber japonicus* parasite communities to populations elsewhere. The analysis of drivers behind parasite infections could also improve with a higher level of identification.

Due to the reliance of samples collected by the Department of Agriculture, Forestry and Fisheries (DAFF) research cruises, all samples were frozen when caught. Future studies, particularly taxonomic focused projects, should aim to use fresh samples that have never been frozen before as parasites from frozen fish samples could be damaged or harder to identify. Future projects should include a broader temporal and spatial coverage of *Scomber japonicus* habitat in South Africa, as very few adult fish from spring-summer, and fish of all sizes from the west coast between 27° and 30°E were insufficiently sampled. Fish should preferably be caught with a jig, since trawled fish often rub against one another when concentrated in a trawl net and ectoparasites can potentially be lost or damaged

The 152 *Scomber japonicus* processed during this study were predominantly (just under 80%) immature fish, as defined using the length-based criterion (immature if <351 mm FL) described previously and reflected the size distribution of available samples. Whilst fish of up to 514 mm FL were processed, the bias toward immature fish could suggest that the chub mackerel's parasite assemblage was not fully documented, although this is not supported by the fact that species accumulation curve which reached an asymptote.

Future studies could include an analysis of the stomach contents of the *S. japonicus* processed for parasites, as many parasites found in this study appear to be trophically transmitted. Including data on the parasite assemblages of *S. japonicus* from the north-eastern (USA) and western Pacific (Japan) Ocean as well as the Indian Ocean would likely be useful in further examining the taxonomy of this species.

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