Temporal variation in infection of male sardine (*Sardinops sagax)* by a coccidian testicular parasite (*Eimeria sardinae*)

Nwabisa Malongweni

MSc degree in Applied Marine Science

Department of Biological Sciences

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Supervisor: Dr. Cecile C. Reed, Department of Biological Sciences, University of Cape Town

Co-supervisor: Dr. Carl D. van der Lingen, Department of Agriculture, Forestry and Fisheries; and Marine Research Institute, Department of Biological Sciences, University of Cape Town.

University of Cape Town

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Abstract

Temporal variability in infection of South African male sardines (*Sardinops sagax*) by a testicular coccidian parasite *Eimeria sardinae* was examined between putative western and southern stocks of this fish species. Samples were collected by commercial vessels from five localities; Gansbaai, St Helena Bay, Mosselbay, Port Alfred and Port Elizabeth (west and south coast) between 2012 and 2013. A total of 461 sardines were examined for the presence of *Eimeria sardinae*, including 185 males and 41 females from west coast and 180 males and 55 females from the south coast. Sardine females did not show any infection by the parasite. For males, prevalence of infection was 74.9% for the western stock and 76.5% for the southern stock. Mean infection intensity and standard error of the western stock was 6.7±0.7 and for the southern stock was 8.3±1.0. Parasite abundance and standard error of the western stock was 5.3±0.2 and 6.1±0.3 for the southern stock. A significant difference was observed within testes position (anterior, middle and posterior), with anterior being highly infected followed by middle and posterior (KW chi-square = 86.029, df = 2, $p<0.05$). Infection from the left and right testes did not show a significant difference (W= 623, $p = 0.13$). There was no significant difference in prevalence of infection, infection intensity index and abundance index per region across seasons. Seasonal pattern was the same in both stocks. There was a significant difference in monthly average GSI data of male sardines from west and south coast between 1996-2014 (KW = 5416.9, df = 11, $p < 0.01$). There was no significant difference between seasonal GSI and seasonal infection intensity index.

Keywords: Seasonal variability, Gonadosomatic index (GSI), Infection intensity index, *Eimeria sardinae*, *Sardinops sagax*, Parasite abundance index, Prevalence of infection.

CHAPTER 1: LITERATURE REVIEW

1. INTRODUCTION

1.1 The sardine *Sardinops sagax* (Jenyns, 1842)

Sardines (*Sardinops* spp. and *Sardina* spp.) are schooling epipelagic fish of the order Clupeiformes found in the upper layers of the ocean and are particularly abundant in upwelling regions because of high nutrient production that stimulates phytoplankton and zooplankton growth, which is the food source of these and other small pelagic fish. *Sardinops sagax* are short–lived, grow fast and have high levels of natural mortality (Barange *et al*., 2009). *Sardinops sagax* is found distributed in five upwelling regions globally (Figure 1.1) (Checkley *et al*., 2009).

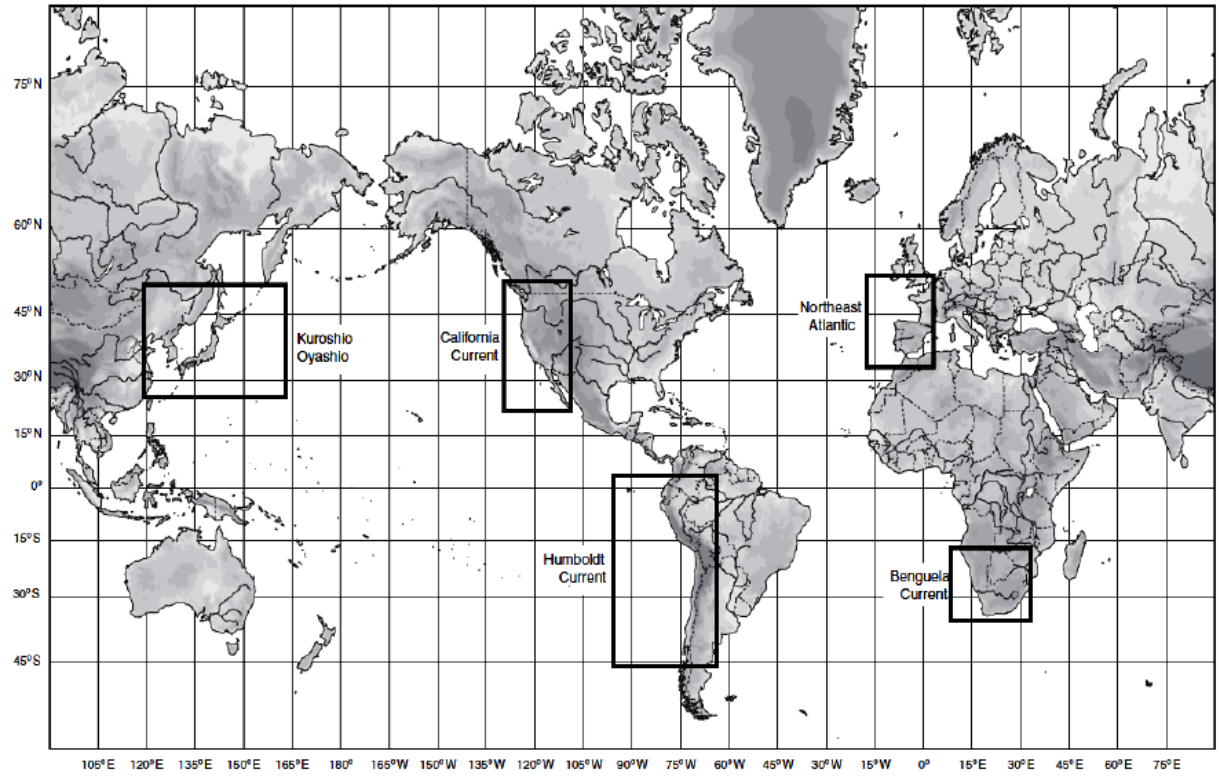

Figure 1.1: Map of the world showing upwelling regions where small pelagic fish such as sardines are found (Checkley *et al*., 2009).

1.1.1 Fisheries for small pelagic fish globally

Japanese sardine (*Sardinops melanostictus*) which is found along the Pacific coast of Japan is an important target species of the Japanese small pelagic fishery, although catches are dominated by the north-west Pacific anchovy (*Engraulis japonicus*) which accounts for more than 75% of landings (Barange *et al*., 2009). For the Californian fishery, sardine *S. sagax caerulea* is the dominant small pelagic fish species as well as anchovy *Engraulis mordax* which is found distributed from the central Baja California to central California. Fishery of the Humboldt Current system is sustained by sardine *S. sagax* which is found distributed from south Peru/north Chile and anchovy *Engraulis ringens* (Barange *et al*., 2009). European sardine *Sardina pilchardus* found along shelf waters along the north-east Atlantic and European anchovy (*Engraulis encrasicolus*) which is distributed from the Bay of Cadiz to the North Sea and western Baltic Sea dominates the European fishery. The Benguela small pelagic fishery is distributed from southern Angola to South Africa's east coast and consists of two dominant fish species; sardine *S. sagax* and anchovy *E. encrasicolus* (Barange *et al*., 2009; Checkley *et al*., 2009).

1.1.2 Phylogeny of Clupeiformes

Within the Clupeiformes, the suborder Clupeoidei consists of 397 species which are classified into five families (Lavoué *et al*., 2013), namely the Clupeidae with 55 genera and 205 species, the Engraulidae with 145 species from 17 genera, the Pristigateridae with nine genera and 38 species, the Sundasalangidae with one genus consisting of 7 species and the Chirocentridae with two species from one genus. The Clupeidae is divided further into four subfamilies, namely the Alosinae, Clupeinae, Ehiravine and Dorosomatinae. A temperate sub-family Alosinae is divided into four genera, with *Sardinops* and *Sardina* being more closely related than are *Alosa* and *Brevoortia*.

1.1.3 Sardinops regional forms

The genus *Sardinops* is found off Southern Africa, Australia and New Zealand, South America, North America and Japan (Figure 1.2). Whilst Parrish *et al.* (1989) considered *Sardinops* to be monotypic, five regional forms have been suggested (Figure 1.2) which may be separate species or sub-species (Bowen and Grant, 1997), and more recent work has revealed three monophyletic clades: (i) South Africa and Australia (ii) Chile and California, and (iii) Japan that shared a common ancestry some 200-500 thousands years ago (Kasapidis, 2014). Three subspecies of *Sardinops* were recommended by Grant *et al*. (1998) and those are: *S. sagax ocellatus* in Southern Africa, Australia and New Zealand; *S. sagax sagax* in Chile, Peru, Ecuador, Mexico, the United States and Canada; and *S. sagax melanostictus* in China, Korea, Japan and Russia.

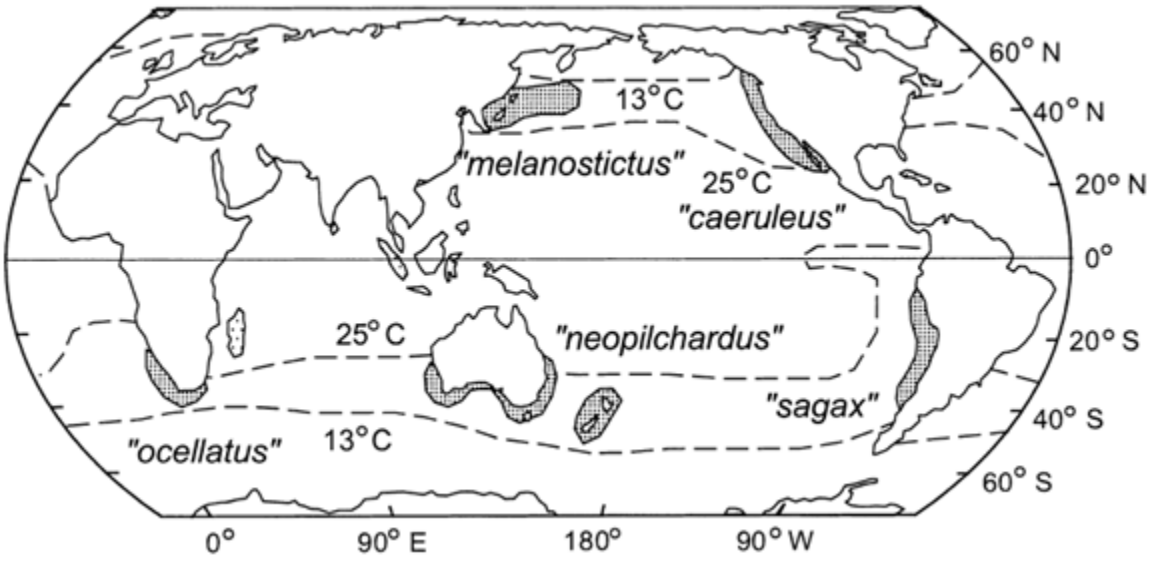

Figure 1.2: Distribution of *Sardinops* (from Bowen and Grant, 1997).

Sardinops populations in the northern and southern hemisphere are separated by a warm tropical water of >25°C which makes it difficult for sardines to move in between the spheres as they cannot survive in warm sea surface temperatures (Bowen and Grant, 1997; Grant and Bowen, 1998). A

series of studies incorporating genetics have been done to show the lineage and dispersal of *Sardinops* from the five upwelling regions and support the theory of sister taxa. Bowen and Grant (1997) reported diverse but shallow mtDNA lineages and low allozyme diversity from the five regions and thought this might be due to a series of population fluctuations caused by climatic regime shifts. However, allozyme clock which estimates the history of separation within regions between 15 and 23 million years ago BP; and some control regional clocks which is a lineage estimation from 15 to 20 million years ago BP showed that five *Sardinops* populations arise from a common ancestor within the Pleistocene.

1.2 *Sardinops sagax* off Southern Africa

Sardinops sagax is one of 13 species in the family Clupeidae occurring in Southern African waters (Beckley and van der Lingen, 1999), and was previously known as *Sardinops ocellatus*, *Clupea ocellatus* and *Sardinops occelata* (Bowen and Grant, 1997; Beckley and van der Lingen, 1999). It is distributed from southern Angola (14°S and 10°E) off the west coast of Southern Africa, to north of Durban off the east coast of Southern Africa (Beckley and van der Lingen, 1999). *Sardinops sagax* are only found off the east coast during the 'sardine run', which is a migration where sardines leave the Agulhas Bank and move to the east coast in late May/early June every year (van der Lingen *et al*., 2010a; 2010b).

1.2.1 Oceanography of the Benguela (North and South) and Northern and Southern Benguela sardine stocks

The Benguela Current system is bounded at each end by warm water systems; the tropical eastern Atlantic in the north and the equatorial Indian Ocean's Agulhas Current on the south end (Shannon, 2006). The Benguela Current upwelling system is divided into southern and northern sub-systems by a cell of concentrated and cold intense upwelling at Lüderitz off southern Namibia which forms

a barrier to the transportation of fish eggs and larvae between the two sub-systems (Figure 1.3) (Shannon, 2006; Checkley *et al*., 2009; Hutchings *et al*., 2009; van der Lingen *et al*., 2015). This division results in two discrete stocks of sardine *S. sagax*, one in the southern and one in the northern Benguela.

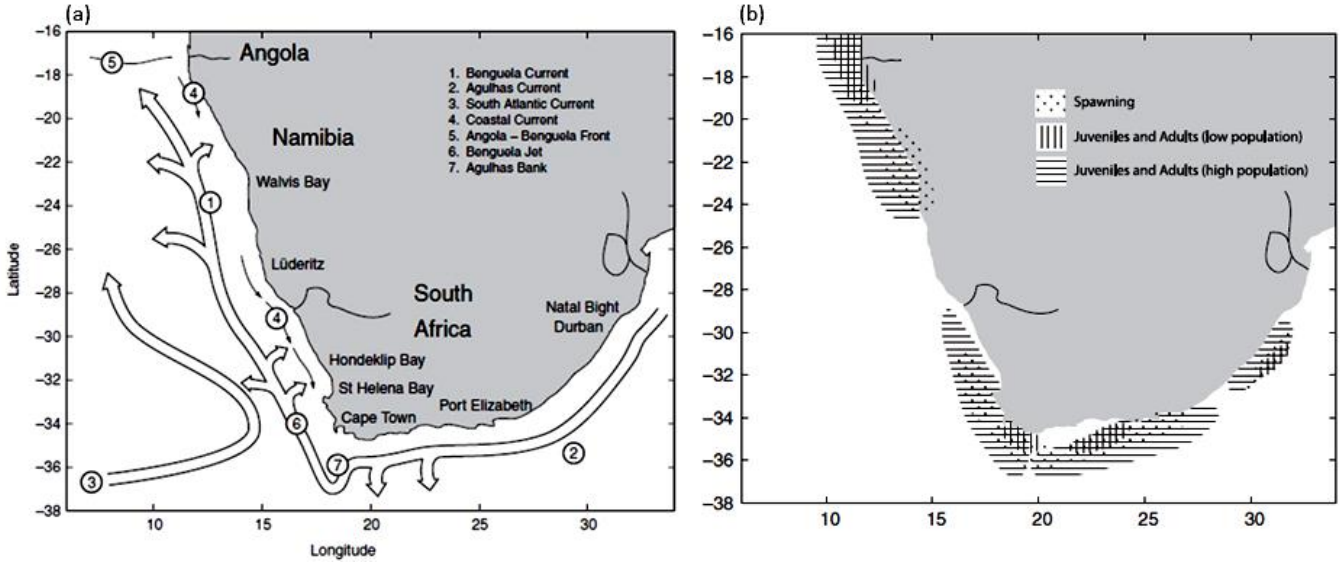

Figure 1.3: Southern African map showing: (a) the Benguela Current system and main oceanographic features (1-7), and (b) the distribution of *Sardinops sagax* around Southern Africa (from Checkley *et al*., 2009).

The southern Benguela is characterized by pulsed coastal upwelling which results in nutrient-rich waters and high productivity (van der Lingen and Huggett, 2003; Coetzee *et al*., 2008; Checkley *et al*., 2009). Intense upwelling in the southern Benguela occurs from late spring (September-November) to early autumn (March-May) (van der Lingen and Hugett, 2003; Mc Laverty, 2012; Ssempa, 2013), while upwelling in the northern Benguela occurs year-round. The southern Benguela *S. sagax* stock is found from the Orange River mouth (29°S) to KwaZulu-Natal (27°S) (Beckley and van der Lingen, 1999), and the northern Benguela *S. sagax* stock occurs from the northern edge of Lüderitz found at 25°S, and extends to the southern edge of the Angola/Benguela front which is usually located between 16 and 17°S (Boyer *et al*., 2001; Cury and Shannon, 2004).

In both the northern and southern Benguela, the habitat of *S. sagax* is confined to the continental shelf due to the strong offshore currents (Checkley *et al*., 2009). Whilst there is one stock of Namibian *S. sagax* in the northern Benguela, there is evidence of three distinct stocks within South African waters (see below).

1.2.2 Biology and ecology of Sardinops sagax in the southern Benguela

Sardinops sagax occurs in temperatures of 13-22°C around the SA coast, can live up to six to eight years (van der Lingen *et al*., 2006), and can reach close to 25 cm caudal length (van der Lingen *et al*., 2009; Hampton, 2014). They are ecologically important as they form a link in the food web between lower and upper trophic levels, being filter feeders that feed on phytoplankton and zooplankton (van der Lingen, 2002) and important prey of fish, seabirds and large marine mammals (Beckley and van der Lingen, 1999).

Sardinops sagax reaches sexual maturity at two or three years, and female *S. sagax* spawn repeatedly and can release up to 27 500 eggs per spawning event (Beckley and van der Lingen, 1999; Hampton, 2014). When *S. sagax* spawn, they tend to avoid the areas of intense offshore transport where reproductive products may be lost from the shelf area, and they also avoid areas of strong mixing of upper water column where food patches may be disrupted (Lluch-Belda *et al*., 1989). *Sardinops sagax* eggs are found around the SA coast, with spawning areas off the west and south coast separated by the Central Agulhas Bank (Figure 1.4) (van der Lingen *et al*., 2015), and there is also evidence of a third spawning area off the east coast of South Africa during the sardine run (Connell, 2010).

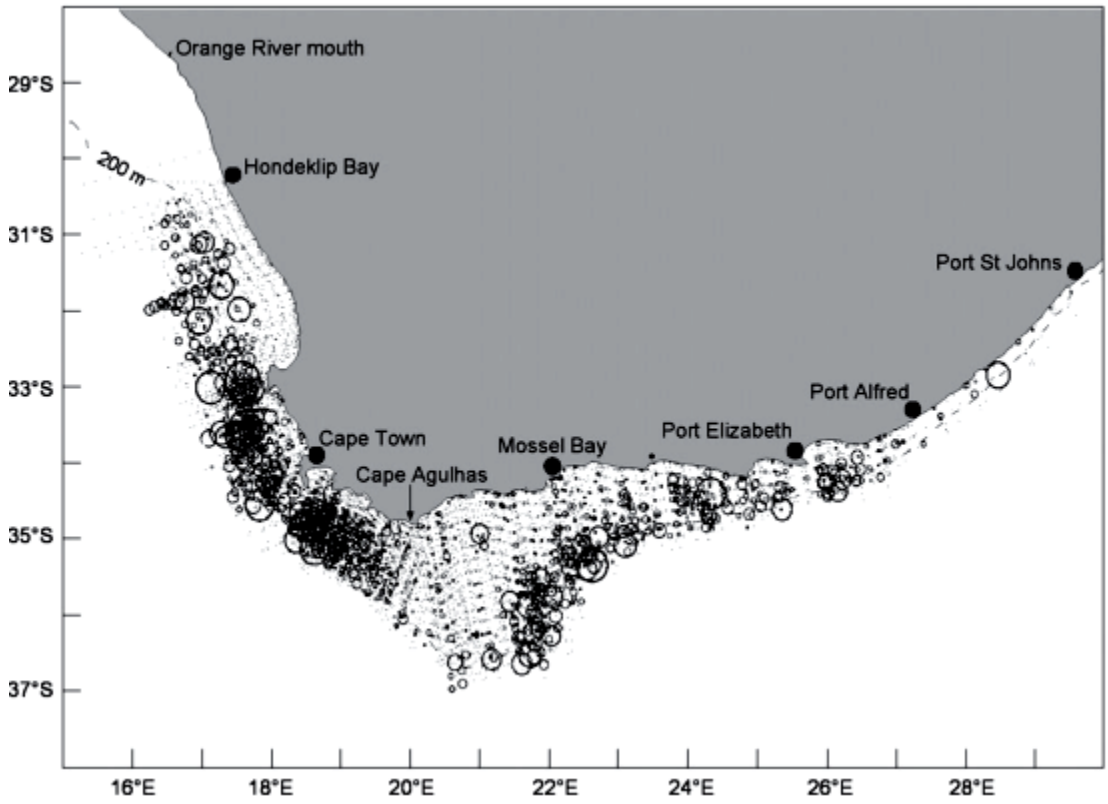

Figure 1.4: Composite *Sardinops sagax* egg distribution map showing two spawning areas separated by the central Agulhas Bank from 1986-2009 (van der Lingen *et al.*, 2015). Circles = egg abundance, maximum size = 4232 eggs m^{-2}.

Spawning of the west and south coast *S. sagax* occurs in spring and late summer (Beckley and van der Lingen, 1999; van der Lingen and Hugget, 2003; Miller *et al*., 2006). Miller *et al*. (2006) suggested that *S. sagax* in the west coast system spawn on the western Agulhas Bank and off the west coast, and recruit on west coast nursery grounds, while *S. sagax* in the Agulhas Bank system spawn on the central and eastern Agulhas Bank and recruit on south coast nursery grounds. Eggs and larvae are transported by currents from spawning areas to the nursery grounds (van der Lingen & Hugget, 2003; Coetzee *et al*., 2008). Juveniles then migrate from the nursery grounds to the south and/or west coast where they reach sexual maturity after two years.

There is a possibility of a third *S. sagax* stock occurring off the east coast of KZN during the annual winter migration of *S. sagax* (van der Lingen *et al*., 2010a). *Sardinops sagax* eggs occur in winter during the sardine run off Park Rynie (70 km south of Durban) (Connell, 2010; Fréon, *et al*., 2010), but are not found there during the remainder of the year (Connell, 2010).

1.2.3 Fishery for Sardinops sagax in South Africa

Sardinops sagax are commercially harvested in upwelling and other regions around the globe and together with other small pelagic fish such as anchovy (*Engraulis* spp.) and round herring (*Etrumeus* spp.) account for around ¼ of the world's marine fish catch (Barange *et al*., 2009; Checkley *et al*., 2009).

Sardinops sagax, locally known as pilchard, has been exploited by purse-seine fisheries in the Benguela system off Namibia and South Africa (Lluch-Belda *et al*., 1989) for several decades. The purse-seine fishery off SA became commercial in 1943 during World War II when the demand for canned fish increased, and is currently the largest fishery in South Africa in terms of landings. The fishery targets *S. sagax* off the west and southwest coasts (van der Lingen and Hugget, 2003), the combined marine fishery employs about 27 000 people and is worth around six to seven billion rand per annum (DAFF, 2014). The fishery serves as a source of food and income to many people therefore, maintaining sustainable utilization is of utmost importance. Catches of *S. sagax* by the purse-seine fishery have fluctuated between 15 000 t and 400 000 t per annum (Figure 1.5) with periods of high catches followed by rapid declines. *Sardinops sagax* catches increased again throughout the 1990s and reached 374 000 t during the early 2000s (DAFF, 2014). In 2012 the *S. sagax* catch was about 98 000 t which was to be the highest catch since 2007. There was a reduction of the total allowable catch (TAC) in 2013 to 90 000 t which is the minimum allowed catch under the current operational management procedure (OMP) used to manage this resource (DAFF, 2014).

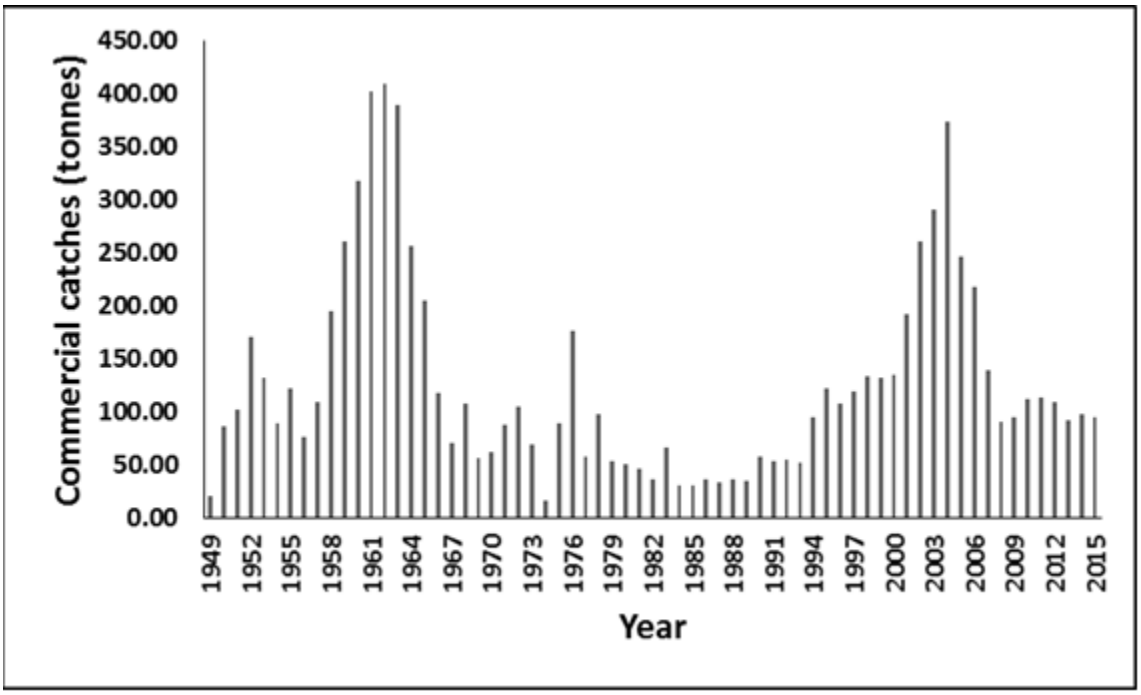

Figure 1.5 South African sardine *(Sardinops sagax*) catches from 1949 -2015 (DAFF).

There is also a small-scale, beach-seine fishery with about 25 right holders that catches *S. sagax* off the east coast during the sardine run, with fish primarily used for bait. KZN beach-seine catches have been <1% of purse-seine fishery catches off the west and south coast of South Africa since the 1950s and have not exceeded 1000 tons per year since 1951 (van der Lingen *et al*., 2010a; 2010b). Although beach-seine catches are unimportant compared to *S. sagax* catches made by the purse-seine fishery off South Africa, the sardine run has been beneficial to the ecotourism industry (Hutchings *et al*., 2010; van der Lingen *et al*., 2010a; 2010b).

1.3 Management of the fishery for South African sardine

The fisheries in the northern and southern Benguela are managed independently as the stocks are considered to be separate. In South Africa, however, whilst the fishery for *S. sagax* has been initially managed assuming a single stock using an operational management procedure (OMP) to set

total allowable catch levels (TACs), there is recent evidence for the occurrence of three *S. sagax* stocks (Coetzee *et al*., 2008; van der Lingen *et al*., 2015; Weston *et al*., 2015) which has management implications. Coetzee *et al*. (2008) described a separation between the west and southern *S. sagax* sub-populations or stocks at low and medium biomass levels where the western sardine occupied the Western Agulhas Bank (WAB) at all biomass levels and were distributed northwards over the shelf and shelf-edge up the west coast with increasing biomass and also eastwards onto the inner shelf of the Central Agulhas Bank (CAB). The southern *S. sagax* occupied the Eastern Agulhas Bank (EAB) at low biomass levels and extended westwards along the outer shelf of the CAB at moderate and high biomass (Coetzee *et al*., 2008), with an overlap on the Central Agulhas Bank between western and southern *S. sagax* being observed only at high biomass levels. There is a possibility of a third stock on the east coast of KZN.

Numerous studies including meristic, morphometric and other biological characteristics analyses have been considered to support the multiple sardine stock hypothesis, which are synthesized in van der Lingen *et al*. (2015). The use of parasites as biotags in *S. sagax* population structure studies has also been explored (Reed *et al*., 2012; van der Lingen *et al*., 2015; Weston *et al*., 2015), which have also added support to the multi-stock hypothesis.

1.4 Parasites as biological tags in studies of fish population structure

The use of parasites as biological tags to distinguish stocks has increased greatly in the recent past (MacKenzie and Abaunza, 1998). Using parasites as biological tags is advantageous in that it is less expensive than using artificial tags, and parasite assemblages within the host can also be used to study other aspects of the fish (MacKenzie and Abaunza, 1998) such as migration patterns, feeding and spawning behaviours. The use of parasites as biotags is based on the principle that a host can only be infected with a particular parasite when it is within the endemic area of that parasite

(MacKenzie and Abaunza, 1998; MacKenzie, 2002; Reed *et al*., 2012; Weston *et al*., 2015). If an infected fish is found outside of the parasite endemic area it can be assumed that it was once in the endemic area of the parasite (MacKenzie and Abaunza, 2005). Also, MacKenzie and Abaunza (2005) mentioned that in order for a parasite to be a good biotag for stock identification, it should meet the following criteria: the parasite should be detected and identified easily, should show different infection levels in the host population across its distribution, have a long life span within the host, must not affect the behaviour of the host and should preferably have a single-host life cycle.

Parasites have been successfully used as biotags in many parts of the world. For example, some of the studies done using parasites as biotags showed the occurrence of two distinct jack mackerel stocks (*Trachurus symmetricus murphyi*) along the Pacific coast of South America (see MacKenzie, 2002). In another study parasite assemblages were used to identify three stocks of the Brazilian flathead *Percophis brasiliensis* off the coast of Argentina and Uruguay (Braicovich and Timi 2008). Some of the studies include the use of parasites of the genus *Anisakis* as biomarkers to study stock structure of some demersal and pelagic fish species from the Mediterranean Sea (Mattiucci *et al*., 2015).

In South African waters, parasites have been used as biotags primarily for sardine stock assessment following an initial study by Reed *et al*. (2012) to document *S. sagax* parasites and identify suitable biotags. Out of seven parasite taxa recorded, a digenean ‘tetracotyle’ type metacercariae which infects the humors of the fish eyes and is considered to be of the genus *Cardiocephaloides*, best met the criteria for a biotag, in particular because sardines from the west coast showed higher infection prevalence than sardines from the south coast. A subsequent study of infection by this metacercaria (Weston *et al*., 2015) corroborated a significant difference in parasite loads between western and

southern *S. sagax*, further supporting the hypothesis of multiple stocks. Very few metacercaria have been found in the eastern *S. sagax* and none in fish from Namibia (van der Lingen *et al*., 2015). Weston *et al*. (2015) also showed a clear seasonal pattern in infection of S. *sagax* by this metacercaria, where higher infection levels were observed in spring than any other seasons in the south coast samples and higher infection levels were observed in winter for west coast samples.

Another parasite that may have utility as a biotag is the coccidian *Eimeria sardinae* (Thélohan, 1890) Reichenow, 1921. Reed *et al*. (2012) reported an overall infection prevalence of 40% by this parasite in male fish which is found in the testes of South African *S. sagax*. Subsequently, Ssempa (2013), examined a larger number (553) of male *S. sagax* from the South African west, south and east coast, and also from Namibia, and reported no infection by the coccidian parasite in *S. sagax* from either Namibia or the South African east coast, and prevalence of infection levels of 49% and 48% for *S.s sagax* from the west and south coast respectively. That author considered that spatial variability in infection by *E. sardinae* provided support for the *S. sagax* multistock hypothesis although it did not differentiate between west and south stocks. However, all but one of the *S. sagax* samples from the south coast examined by Ssempa (2013) were collected in spring/summer (Nov/Dec), and the remaining sample that was collected in late summer (February) had substantially and significantly higher mean infection intensity than all other south coast samples combined. Samples collected from the west coast during winter (June) had slightly lower mean infection intensity than those collected there in spring (Oct/Nov) although differences were not significant (Ssempa, 2013). Those data (particularly from the south coast samples) possibly suggest a seasonal pattern in infection of *S. sagax* by this parasite that is likely linked to the fishes reproductive cycles (see below). Should seasonal variability in *S. sagax* infection by *E. sardinae* occur, as has been observed for the digenean tetracotyle type metacercariae (Weston *et al*., 2015), then this could confuse interpretations of the data collected if seasonality is not accounted for. This

study investigates seasonal variability in *S. sagax* infection by *E. sardinae.*

1.5 The coccidian *Eimeria sardinae* (Thélohan, 1890) Reichenow, 1921

Eimeria sardinae is classified as follows*:*

Kingdom - Protozoa

Phylum - Apicomplexa

Class - Coccidea

Order - Eucoccida

Suborder - Eimeriorina

Family - Eimeriidae

Genus - *Eimeria*

Species - *sardinae*

The family Eimeriidae consists of nearly 1,400 named species of which about 75% belong to the genus *Eimeria* (Upton *et al.*, 1984), and over 100 species from the genus *Eimeria* have been described from marine and freshwater fish (Upton *et al*., 1984; Molnar, 1979). One of those *Eimeria* species is *E. sardinae,* which has been observed in the following marine fish from different parts of the world: *Sardina pilchardus* (see Pinto, 1956); *Clupea harengus L*. (see Sindermann, 1961); *Sprattus sprattus* (see Turovsky *et al*., 1993); *S. sagax* (see Reed *et al*., 2012); and *Sardinella aurita* (see Draoui *et al*., 1995).

Two genera which belong to the family Eimeriidae, *Eimeria* and *Isospora*, are classified according to how many sporocysts they have within the oocyst and the number of sporozoites within sporocysts (Upton *et al*., 1984). Members of the genus *Eimeria* are characterized by having four sporocysts within an oocyst and with each sporocyst containing two sporozoites (Baker, 1969;

Upton *et al*., 1984) (Figure 1.6).

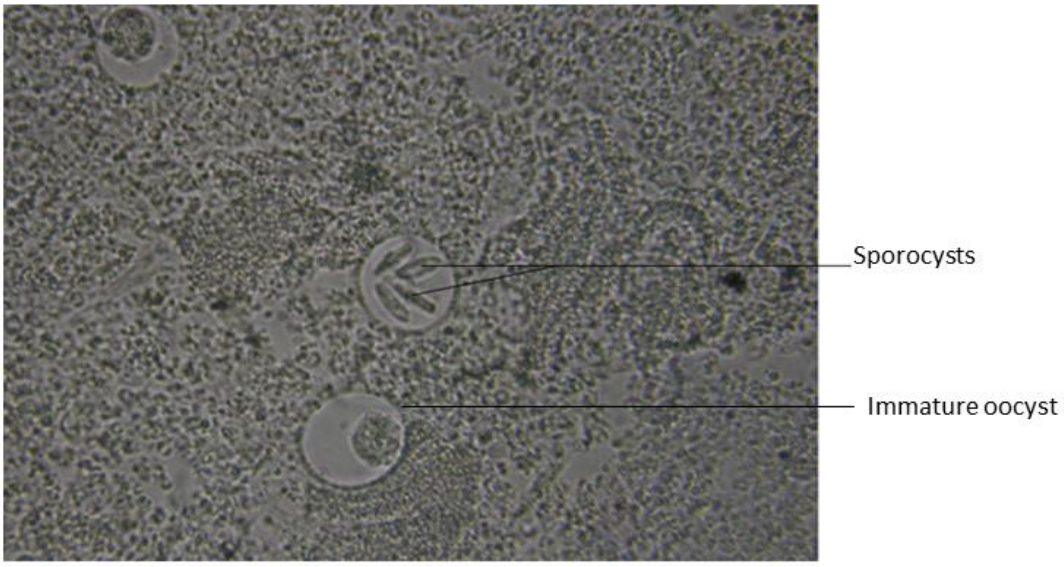

Figure 1.6: Photograph of *Eimeria sardinae* from a *Sardinops sagax* testis showing sporocysts within an oocyst and immature oocysts (courtesy of C. Reed).

Most coccidian parasites infect the intestinal epithelium (Baker, 1969; Molnar, 1979), but some coccidians, especially those with large oocysts, develop at non-intestinal sites such as the kidney, spleen, liver and swim bladder (Molnar, 1979). Infection by *E. sardinae* in *S. sagax* ovaries has been reported by Draoui *et al*. (1995).

Sporulated oocysts of *Eimeria* species released by an infected fish get swallowed by a new host. Once ingested, sporozoites are released from the sporocysts by the action of digestive enzymes and bile salts, and penetrate cells of the intestinal mucosa following which they grow inside the intestinal cells or get transported by macrophages to the site of infection in the host (Baker, 1969; Upton *et al*., 1984). Sporozoites reproduce asexually inside the intestinal epithelial cells by schizogony, forming small nuclear organisms called merozoites by multiple fission (Figure 1.7).

The number of produced merozoites varies between 16 and 1000 during each schizogony (Baker, 1969).

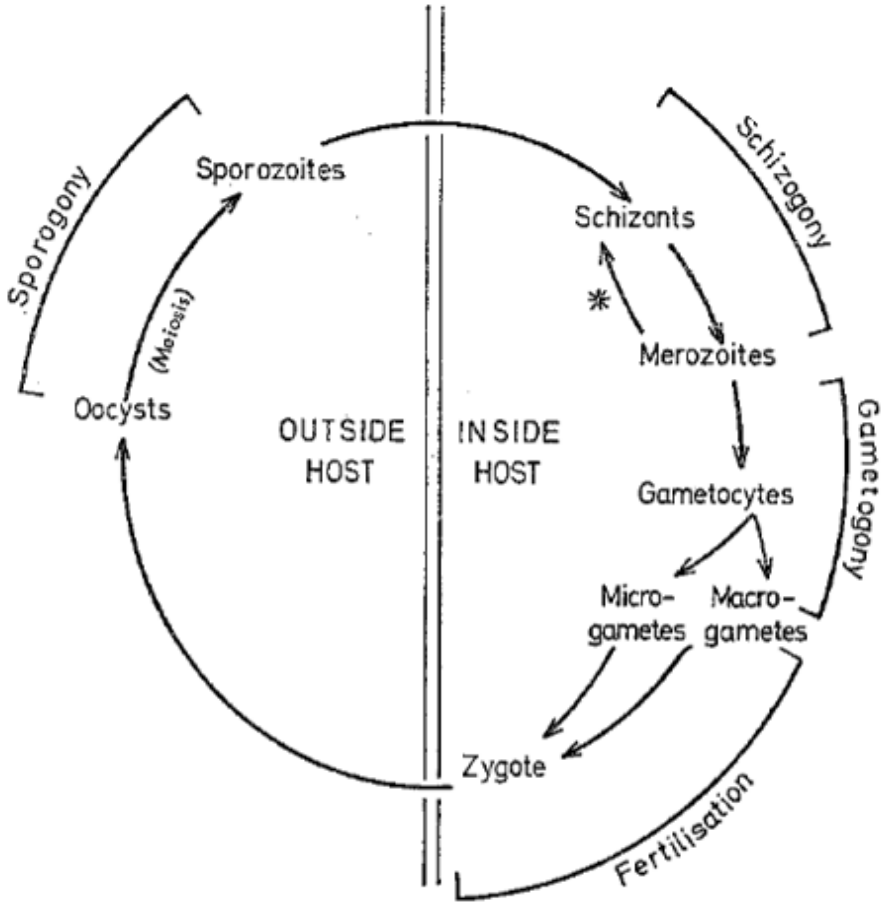

Figure 1.7 Diagram of the life cycle in the suborder Eimeriorina, (*Not in all species) from Baker (1969).

When the host cell and schizonts rupture, merozoites are released to near-by or distant cells depending on the host species, and the schizogony process starts again (Baker, 1969; Upton *et al*., 1984). Merozoites then enter a new host cell and grow by a process called gametogony (formation of gametes in the sexual cycle of sporozoans by schizogony) into sexual individuals called gametocytes. Female macro-gametocytes remain uninuclear while male micro-gametocytes go through several nuclear divisions to produce numerous flagellated microgametes (Baker, 1969; Upton *et al*., 1984). After being released by the gametocyte, microgametes fertilize macrogametes

to form a zygote which then encysts within another host cell. Within the thick cyst wall, nucleus division occurs (diploid as a result of fertilization), then cytoplasmic division occurs resulting to four sporoblasts (Baker, 1969). Each sporoblast encysts within the oocyst to form a sporocyst; and the nucleus and cytoplasm of sporocysts divide again to form two sporozoites. During differentiation of the oocyst contents (sporogony), the oocyst leaves the host cell, passes down the intestine and is released to the outside world where it can survive for some time before being ingested by the host again and the life cycle starts (Baker, 1969; Upton *et al*., 1984). Draoui *et al*. (1995) mention that *E. sardinae* oocysts leave the host (*Sardina* and *Sardinella*) during spawning when they get released with the sperm and/or oocytes.

1.5.1 Pathology of Eimeria sardinae

Heavy infection by *E. sardinae* has been known to cause reduced fecundity of the host fish (Pinto 1956). Draoui *et al.* (1995) reported parasitic castration following infection by *E. sardinae* in males of both *Sardina pilchardus* and *Sardinella aurita*, and Ssempa (2013) reported that heavily infected male *S. sagax* had significantly lower smaller testes (reduced gonadosomatic index) compared to lightly or un-infected fish. Other studies showed varied effects of *Eimeria* infection on marine fish species: a study by Odense and Logan (1976) revealed that infection by *E. gadi* in the swim bladder of haddock (*Melanogrammus aeglefinus*) inhibited their locomotion and spawning behaviour, and Mackenzie (1981) reported that unnamed species of *Eimeria* caused blue whiting (*Micromesistius poutassou*) to weigh less than uninfected fish of the same age.

1.6 Thesis aims and objectives

The aims and objectives of this study were:

- To examine seasonal variability in the infection of South African male *S. sagax* from the west and south coast by the testicular parasite *E. sardinae*.
- To compare prevalence of infection, infection intensity index and parasite abundance index by *E. sardinae* between coasts.
- To investigate whether infection by *E. sardinae* is related to fish size.
- To examine if there is any difference in infection intensity index between the left and right testes and between the anterior, middle and posterior portions of the testes in order to test whether sub-sampling of the testes may introduce bias and possibly help in terms of identifying the infection pathway as this is unclear for *E. sardinae*.
- To determine whether seasonality in infection (if it occurs), is related to seasonal cycles in gonadosomatic index (GSI).
- To assess whether female *Sardinops sagax* are infected by this parasite, as has been reported for female fish in some species (e.g. *Sardina* and *Sardinella*; see Draoui *et al.*, 1995).

The null hypotheses are that:

- There is no seasonal variation in infection levels in *S. sagax* off the west coast and south coast.
- Prevalence of infection, infection intensity index and abundance index is the same for *S. sagax* off both coasts.
- There is no relationship between fish size and infection.
- Infection intensity index between the right and left testis do not differ, and there is no difference in infection intensity index between anterior, middle and posterior part of the

testes.

- There is no relationship with seasonal GSI and seasonal infection intensity index.
- There is no infection in female sardine.

CHAPTER 2: MATERIALS AND METHODS

2.1 Sample and data collection

Sardinops sagax were collected from commercial purse-seine vessel catches from around the South African coast between January 2012 and November 2013 for this study (Figure 2.1, Table 2.1). The date and location of sample collection was recorded and samples were preserved in 70% ethanol for later processing.

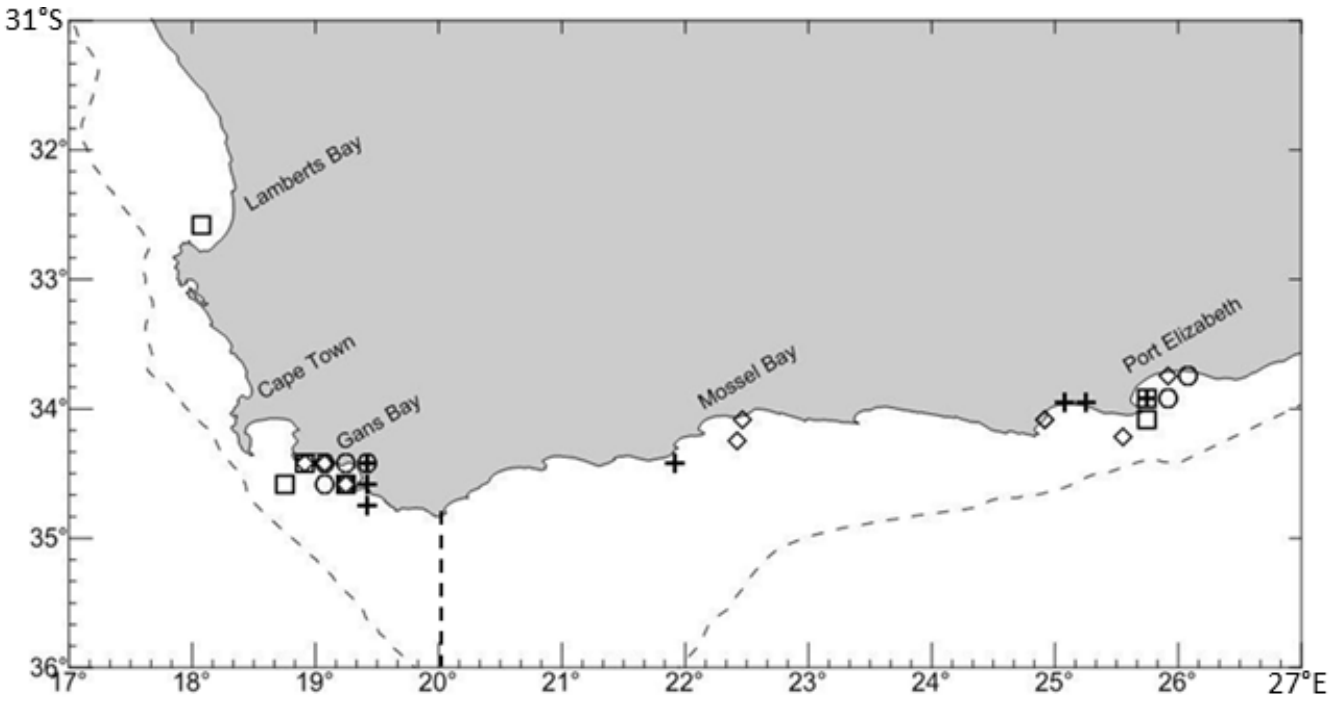

Figure 2.1: A map showing the locations of *Sardinops sagax* samples collected during 2012 and 2013 from the west and south coast of South Africa (squares = spring, circles = summer, diamonds = autumn, crosses = winter). A dashed line from 20°E longitude separates the west coast from south coast.

Table 2.1: Summary of *Sardinops sagax* samples processed for this study, showing region (west or south), sample ID, boat name, date of collection and number of male and female *Sardinops sagax* examined for *Eimeria sardinae*.

Region	Sample ID	Boat name	Date of collection	Number of males	Number of females
West	1	Silver Snapper	25/01/2012	10	3
West	2	Bella Prima	01/02/2012	14	3
West	3	Silver Snapper	01/02/2012	10	3
West	4	Starcrest	06/02/2012	10	3
West	5	Kolgans	15/02/2012	10	3
West	6	Oom Arthur	21/02/2012	10	4
West	7	Ocean Blue	29/03/2012	10	3
West	8	Emerald Isle	23/04/2012	10	3
West	9	Toliko	10/05/2012	10	3
West	10	Bordered	11/06/2012	10	3
West	11	Emerald Isle	18/07/2012	10	3
West	12	Starcrest	23/08/2012	10	4
West	13	Starcrest	05/09/2012	10	3
West	14	Emerald Isle	17/09/2012	10	0
West	15	Alert	08/10/2012	10	0
West	16	Southern Belle	20/11/2012	10	0
West	17	Ruiwekus	12/02/2013	11	0
West	18	Wafra	06/05/2013	10	0
TOTAL				**185**	**41**

Region	Sample ID	Boat name	Date of collection	Number of males	Number of females
South	1	Antonie w2	27/03/2012	10	3
South	2	Umfondini	08/05/2012	10	3
South	3	Antonie w2	26/06/2012	10	6
South	4	Fred Marie	25/07/2012	10	3
South	5	Scomber	18/08/2012	10	3
South	6	Zolani	13/11/2012	20	3
South	7	Antonie w2	25/01/2013	10	3
South	8	Scomber	29/01/2013	10	3
South	9	Antonie w2	08/02/2013	10	3
South	10	Zolani	27/02/2013	10	3
South	11	Zolani	20/03/2013	10	3
South	12	Umadala	25/03/2013	10	3
South	13	Sagax	04/04/2013	10	3
South	14	Castella	29/05/2013	10	4
South	15	Umfondini	08/07/2013	10	3
South	16	Antonie w2	29/08/2013	10	3
South	17	Fred Marie	04/09/2013	10	3
TOTAL				**180**	**55**

Seasonal cycles in *S. sagax* GSI were documented using data on gonad and fish mass collected from commercial catches sampled by DAFF over the period 1996-2013. The catch location and date were recorded for each sample. This was done to establish the normal seasonal GSI for this species of sardine, but GSI data and infection data were not collected from the same fish.

2.2 Dissections

Fish were removed from ethanol, rinsed in tap water, and measured to the nearest 1mm (caudal length). They were then dissected and testes and ovaries removed. In general ten male and three female fish were processed from each sample, but in some samples additional male fish were added to increase the sample size. Pea-sized portions of approximately 1.5 mm * 1.5 mm * 1.5 mm were cut from the anterior, middle and posterior portions of the left and right testes of each male *S. sagax* for wet slide preparation (Figure 2.2).

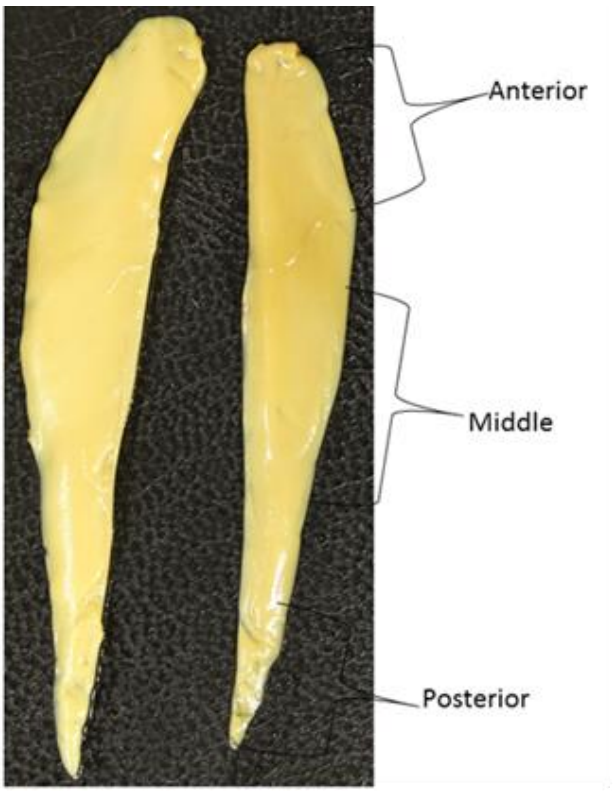

Figure 2.2: *Sardinops sagax* testes (left and right) showing anterior, middle and posterior portions (picture captured using Canon EOS 360D digital camera).

Although not precisely similarly-sized, portions were always removed from the same part of a testis from each fish. For wet slide preparation, a drop of distilled water was added to each portion of testis placed on a slide and the portion was carefully squashed under a cover slip. Six slides were used for each fish, clearly indicating left and right testes positions where extraction was done. Slides were then examined under a light compound microscope (LEICA DM 500) at a magnification range of 4X-1000X) to determine infection levels. Infected gonads were recognized by the presence of *E. sardinae* oocysts in the gonad tissue, and parasite identification was done with the help of Dr. C. Reed from the Department of Biological Sciences in the University of Cape Town. Each of the six portions of testis was assigned to one of five infection intensity index categories based on the numbers of oocysts estimated in each portion (Table 2.2). The average of infection indices was used to analyze data.

Table 2.2: Five categories of infection intensity index.

Index	**Infection Intensity Category**
0	Uninfected (0 parasites)
1	Low infection (1-10 oocysts per fish)
2	Moderate (11-100 oocysts)
3	High (101-1000 oocysts)
4	Severe infection (>1000 oocysts)

For collecting GSI data, fish wet body mass was recorded to the nearest 0.1 g. Fish were dissected and biologicals including sex, gonad maturity stage and gonad mass to the nearest 0.1 g were recorded.

2.3 Data analysis

Samples were assigned a season based on their month of collection, namely summer (December, January, and February), autumn (March, April, and May), winter (June, July, and August) and spring (September, October, and November).

Infection prevalence, mean infection intensity index and mean parasite abundance index were determined for each sample following Bush *et al*. (1997) as below. Mean infection intensity index was adapted from mean infection intensity in Bush *et al*. (1997); the sum of indices from all six portions examined was used instead of total number of oocysts because the data were categorical. Also, to calculate mean parasite abundance index, the sum of indices from all six portions examined was used instead of total number of oocysts (see below).

$$\textbf{Mean Infection Intensity Index} = \frac{\textit{sum of indices}}{\textit{number of infected fish from each sample}}$$

$$\textbf{Infection prevalence (\%)} = \frac{\textit{number of infected fish}}{\textit{number of fish examined from each sample}} * 100$$

$$\textbf{Mean Parasite Abundance Index} = \frac{\textit{sum of indices}}{\textit{number of fish examined from each sample}}$$

A table of males and females from both coasts with calculated prevalence of infection, mean intensity index, and mean parasite abundance index per sample, was designed to compare infection indices between *Sardinops sagax* from the west and south coast. Datasets were tested for normality in R studio statistical software version 3.2.3 using the Shapiro-Wilk test. As the data were not

normal a Kruskal-Wallis test was done to test for differences between the anterior, middle, and posterior part of testes, and a Mann Whitney test was used to test for significant differences in infection between the left and right testes from samples collected from the west and south coast. Scatterplots to assess the relationship between sum of infection intensity index (summation of infection indices per each fish) and caudal length, and between parasite abundance index and caudal length, were done in Excel, and regressions of infection intensity categories in each of the three portions from the left and right testes were done in Excel. Sum of infection intensity index was used because it showed good sample coverage than mean intensity index. The data on fish caudal length were tested for normality using a Kolmogorov-Sminorov test.

Histograms of mean infection intensity index per season for western and southern *S. sagax* stocks (2012-2013 data combined) were plotted to visualize the temporal distribution of infection in *S. sagax* from the two coasts. A Generalized Linear Model (GLM) was applied to model the influence of period and location of samples (season and region) on parasite prevalence, infection intensity index and total parasite abundance index. GLMs possess two important attributes: (1) they allow for the specification of error distribution in addition to the Gaussian (normal) distribution that is assumed in simple linear regression, including other families of distribution such as binomial, poison, lognormal, gamma, etc.; and (2) they permit the use of link function, the simplest being *identity*, that allows different types of response variables (proportions, binary data, continuous variables, survival data) to be modelled. The link function allows for the transformation of the response variable so that the relationship between it and the set of predictors is linear. All statistical analyses were done in R statistical analysis programming environment (R Core Team, 2015). Boxplots for mean prevalence of infection, mean infection intensity index and parasite abundance index from west and south coast across seasons were plotted to visualize the mean of the variables.

Sardine gonadosomatic index was calculated using the following equation from Kreiner *et al*. (2001):

$$\mathbf{GSI} = \frac{\text{Gonad mass}}{\text{Observed wet body mass} - \text{gonad mass}} * 100$$

Monthly and seasonal averages of GSI were calculated separately for western and southern sardine, and time-series of monthly average GSI for the two stocks (1996-2013 data) were plotted to visualize the temporal distribution of spawning (indicated by higher GSI values) in sardine from the two coasts. A Kruskal Wallis test was used to test for significant differences in monthly/seasonal average GSI values between western and southern fish. Furthermore, Kruskal Wallis multiple independent samples by groups test was done to see which seasons differed. Seasonal average infection intensity was plotted against seasonal average GSI in a scatterplot and a fitted regression was plotted to verify if there was any relationship and between the two.

CHAPTER 3: RESULTS

3.1 Infection by *Eimeria sardinae* on sardines from the west and south coast of South Africa

A total of 461 *Sardinops sagax* were examined for the occurrence of *Eimeria sardinae*, including 185 males from 18 samples and 41 females from 14 samples collected off the West coast, and 180 males from 17 samples and 55 females from 17 samples collected off the South coast of South Africa (Tables 3.1 & 3.2). The fish data on caudal length showed normality (see Appendix for normality results). Mean CL (± SD) for the western male *S. sagax* was 18.0±0.8 cm and 18.4±0.6 cm for the western female *Sardinops sagax*. For southern male *S. sagax*, mean CL was 17.6±0.7 cm and mean CL of the southern female *S. sagax* was 18.1±0.6 cm (Tables 3.1 & 3.2). Size range for the male *S. sagax* western stock was between 12 cm and 20 cm, for the southern stock it was between 15 cm and 20 cm (Figure 3.1a). There was a peak at 18 cm for male from the western stock and at 17 cm for males from the southern stock.

Table 3.1: Date of sample collection, season, number of fish examined (n), mean caudal length (cm) ± standard deviation (SD), infection prevalence (%) and mean infection intensity index ± standard error (SE), mean parasite abundance index and SE of *Eimeria sardinae* in male *Sardinops sagax* samples from two localities; west and south coast of South Africa in 2012 and 2013.

WEST

Date of collection	Season	Sample size (n)	MeanCL±SE	Prevalence (%)±SE	Mean Infection intensity index± SE	Mean Abundance index± SE
25/01/2012	Summer	10	18.7±0.3	30	4.7±0.5	1.4±0.1
01/02/2012	Summer	10	18.2±0.2	90	9.7±0.9	8.7±0.2
06/02/2012	Summer	10	18.1±0.2	70	4.3±0.7	3.0±0.2
10/02/2012	Summer	10	18.4±0.3	60	6.8±0.9	4.1±0.2
15/02/2012	Summer	11	18.3±0.2	36	5.5±0.7	2.0±0.2
21/02/2012	Summer	10	18.2±0.2	80	3.9±0.6	3.1±0.2
29/03/2012	Autumn	10	18.6±0.3	100	9.1±0.6	9.1±0.2
23/04/2012	Autumn	10	17.9±0.2	70	4.9±0.6	3.4±0.2
10/05/2012	Autumn	10	18.3±0.6	30	5.0±0.7	1.5±0.2
11/06/2012	Winter	10	17.5±0.2	80	5.9±0.8	4.7±0.2
18/07/2012	Winter	10	17.8±0.3	90	8.7±0.9	7.8±0.3
23/08/2012	Winter	10	18.3±0.1	100	9.2±0.7	9.2±0.2
05/09/2012	Spring	10	18.0±0.3	100	10.9±0.6	10.9±0.3
17/09/2012	Spring	10	17.4±2.1	100	5.9±0.7	5.9±0.2
08/10/2012	Spring	14	18.9±0.3	71	5.0±0.5	3.6±0.2
20/11/2012	Spring	10	18.3±0.2	100	8.1±0.1	8.1±0.2
12/02/2013	Summer	10	18.2±0.2	90	4.1±0.5	4.0±0.2
06/05/2013	Autumn	10	15.7±0.2	50	8.0±1.1	4.0±0.3
TOTAL		**185**	**18.0±0.3**	**75.0±1.8**	**6.7±0.7**	**5.3±0.2**

SOUTH

Date of collection	Season	Sample size (n)	MeanCL±SE	Prevalence (%)±SE	Mean Infection intensity index± SE	Mean Abundance index± SE
27/03/2012	Autumn	10	17.7±0.2	100	6.0±0.5	6.0±0.2
08/05/2012	Autumn	10	18.7±0.2	100	9.0±0.8	9.0±0.2
26/06/2012	Winter	10	18.3±0.1	100	9.1±0.8	9.1±0.2
25/07/2012	Winter	10	18.4±0.2	100	10.9±0.7	10.9±0.3
18/08/2012	Winter	10	17.7±0.2	80	7.3±0.9	5.8±0.3
13/11/2012	Spring	20	16.5±0.3	80	9.5±0.9	3.6±0.2
25/01/2013	Summer	10	17.6±0.1	60	10.5±1.3	6.3±0.3
29/01/2013	Summer	10	17.2±0.2	80	6.3±1.0	5.0±0.3
08/02/2013	Summer	10	16.8±0.3	90	5.3±0.6	4.8±0.2
27/02/2013	Summer	10	16.8±0.2	60	8.8±1.0	5.3±0.3
20/03/2013	Autumn	10	17.6±0.1	100	11.2±1.6	11.2±0.4
25/03/2013	Autumn	10	17.0±0.2	40	11.3±1.5	4.5±0.4
04/04/2013	Autumn	10	17.2±0.3	50	9.4±1.4	4.7±0.3
29/05/2013	Autumn	10	19.0±0.2	40	6.3±0.8	2.5±0.2
08/07/2013	Winter	10	18.5±0.4	80	10.4±01.1	8.3±0.3
29/08/2013	Winter	10	17.4±0.3	80	6.3±1.0	5.0±0.3
04/09/2013	Spring	10	17.4±0.4	60	4.0±0.6	2.4±0.2
TOTAL		**180**	**17.6±0.2**	**76.0±1.6**	**8.3±1.0**	**6.1±0.3**

Table 3.2: Date of sample collection, season, number of fish examined (n), mean caudal length (cm) ± standard deviation (SD), prevalence (%) and mean infection intensity index ± standard error (SE), mean parasite abundance index and SE of *Eimeria sardinae* in female *Sardinops sagax* samples from two localities; west and south coast of South Africa in 2012 and 2013.

WEST

Date of collection	Season	Sample size (n)	MeanCL±SE	Prevalence (%)±SE	Mean Infection intensity index± SE	Mean Abundance index± SE
06/02/2012	Summer	3	17.6±0.2	0.0	0.0	0.0
21/02/2012	Summer	3	18.4±0.6	0.0	0.0	0.0
29/03/2012	Autumn	3	18.8±0.2	0.0	0.0	0.0
23/04/2012	Autumn	3	19.1±0.3	0.0	0.0	0.0
10/05/2012	Autumn	3	18.3±0.2	0.0	0.0	0.0
11/06/2012	Winter	4	18.5±0.2	0.0	0.0	0.0
23/08/2012	Winter	3	18.6±0.6	0.0	0.0	0.0
05/09/2012	Spring	3	17.7±0.7	0.0	0.0	0.0
17/09/2012	Spring	3	18.7±0.4	0.0	0.0	0.0
08/10/2012	Spring	3	19.3±0.6	0.0	0.0	0.0
20/11/2012	Spring	3	18.7±0.4	0.0	0.0	0.0
12/02/2013	Summer	4	19.2±0.2	0.0	0.0	0.0
06/05/2013	Autumn	3	16.2±0.2	0.0	0.0	0.0
TOTAL		**41**	**18.4±0.4**	**0.0±0.0**	**0.0**	**0.0**

SOUTH

Date of collection	Season	Sample size (n)	MeanCL±SE	Prevalence (%)±SE	Mean Infection intensity index± SE	Mean Abundance index± SE
27/03/2012	Autumn	3	19.2±0.1	0.0	0.0	0.0
08/05/2012	Autumn	6	18.8±0.1	0.0	0.0	0.0
26/06/2012	Winter	4	18.2±0.2	0.0	0.0	0.0
25/07/2012	Winter	3	18.5±0.3	0.0	0.0	0.0
18/08/2012	Winter	3	19.5±0.4	0.0	0.0	0.0
13/11/2012	Winter	3	16.3±0.3	0.0	0.0	0.0
27/11/2012	Spring	3	16.5±0.2	0.0	0.0	0.0
25/01/2013	Summer	3	18.1±0.2	0.0	0.0	0.0
29/01/2013	Autumn	3	18.1±0.3	0.0	0.0	0.0
08/02/2013	Summer	3	17.2±0.1	0.0	0.0	0.0
20/03/2013	Winter	3	16.3±0.3	0.0	0.0	0.0
25/03/2013	Autumn	3	18.9±0.1	0.0	0.0	0.0
04/04/2013	Autumn	3	20.4±0.7	0.0	0.0	0.0
29/05/2013	Summer	3	19.6±0.7	0.0	0.0	0.0
08/07/2013	Spring	3	17.4±0.3	0.0	0.0	0.0
29/08/2013	Summer	3	17.0±0.4	0.0	0.0	0.0
04/09/2013	Autumn	3	17.9±0.9	0.0	0.0	0.0
TOTAL		**55**	**18.1±0.3**	**0.0±0.0**	**0.0**	**0.0**

For female *S. sagax*, the size range for the western stock was between 16 cm and 19 cm, and the southern stock was between 16 cm and 20 cm (Figure 3.1b). For female *S. sagax*, a peak was observed at 18 cm from both coasts.

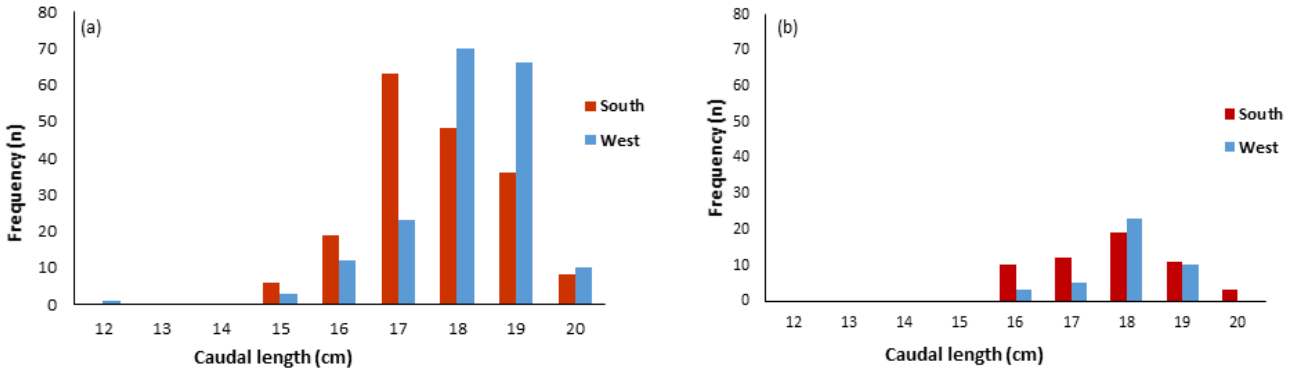

Figure 3.1: Length frequency distribution of (a) male and (b) female S*ardinops sagax* processed for this study.

Length frequency distribution of west and south male *S. sagax* showed a significant difference; t = -4.29, df = 363, p < 0.01 (Table 3.3), with western fish having a bigger mean CL than the southern stock.

Table 3.3: Summary of t-test results between length frequency of male *Sardinops sagax* collected from west and south coast from 2012-2013.

T-test Grouping: Location (west and south males)							
Variable	Mean South	Mean West	n South	n West	t-value	df	p
Caudal length	17.55	18.05	180	185	-4.29	363	**<0.01**

Western male *S. sagax* had an average prevalence and standard error of 75.0±1.8 % while the southern male *S. sagax* had an average prevalence of 76.0±1.6 % (Table 3.1). Overall mean infection intensity index of the western male sample was 6.7±0.7 while the southern sample had

mean infection intensity index of 8.3±1.0 (Table 3.1). Mean parasite abundance index of the western male sample was 5.3±0.2, and for the southern male sample it was 6.1±0.3. *Eimeria sardinae* were not observed in the ovaries of female *S. sagax* from either the west or south coast (Table 3.2).

Infection prevalence data per sample data, sum of infection intensity index per fish data and sum of parasite abundance index per fish data (calculated using categories shown in Table 2.2) for the south and west coast were tested for normality using a Shapiro Wilk test respectively. All datasets were not normally distributed; infection prevalence (West: $W = 0.869$, $n = 18$, $p<0.05$; South: $W = 0.879$, $n = 17$, $p < 0.05$), infection intensity index (West: $W =0.963$, $n = 138$, $p<0.05$; South: $W = 0.975$, $n = 138$, $p<0.05$), and parasite abundance index (West: $W =0.967$, $n = 185$, $p<0.05$; South: $W = 0.978$, $n = 180$, $p<0.05$) (see Figures 1, 2 and 3 in Appendix I). A Mann-Whitney Wilcox test was therefore done to test for differences in the three factors between south and west coast samples respectively and found no significant difference between infection prevalence and coasts, however there were significant differences between infection intensity index and coasts, and parasite abundance index and coasts (Table 3.4).

Table 3.4: Summary of Mann-Whitney Wilcox test of infection prevalence, mean infection intensity index and parasite abundance index in S*ardinops sagax* testes from west and south coast.

	Wilcoxon rank sum test with continuity correction **Data: Infection prevalence/Mean Infection intensity index/Mean Parasite abundance index by Region**					
Variable	**Mean West**	**Mean South**	**n West**	**n South**	**W**	**p**
Infection Prevalence	**74.83**	76.47	18	17	157	**0.906**
Mean Infection intensity index	**6.94**	8.41	138	138	11232.5	**<0.05**
Mean Parasite abundance index	**6.91**	8.35	185	180	11454	**<0.05**

3.2 Infection in sardine by *Eimeria sardinae* in relation to fish size

There was no linear or any other trend in these data at all for either stock. Fish from both coasts did not any relationship between infection index and caudal length as fish of various sizes showed both high and low infection levels by *E. sardinae* (Figure 3.2). This lack of a clear trend was seen in infection intensity index (Figures 3.2b & d) and parasite abundance index (Figures 3.2a &c).

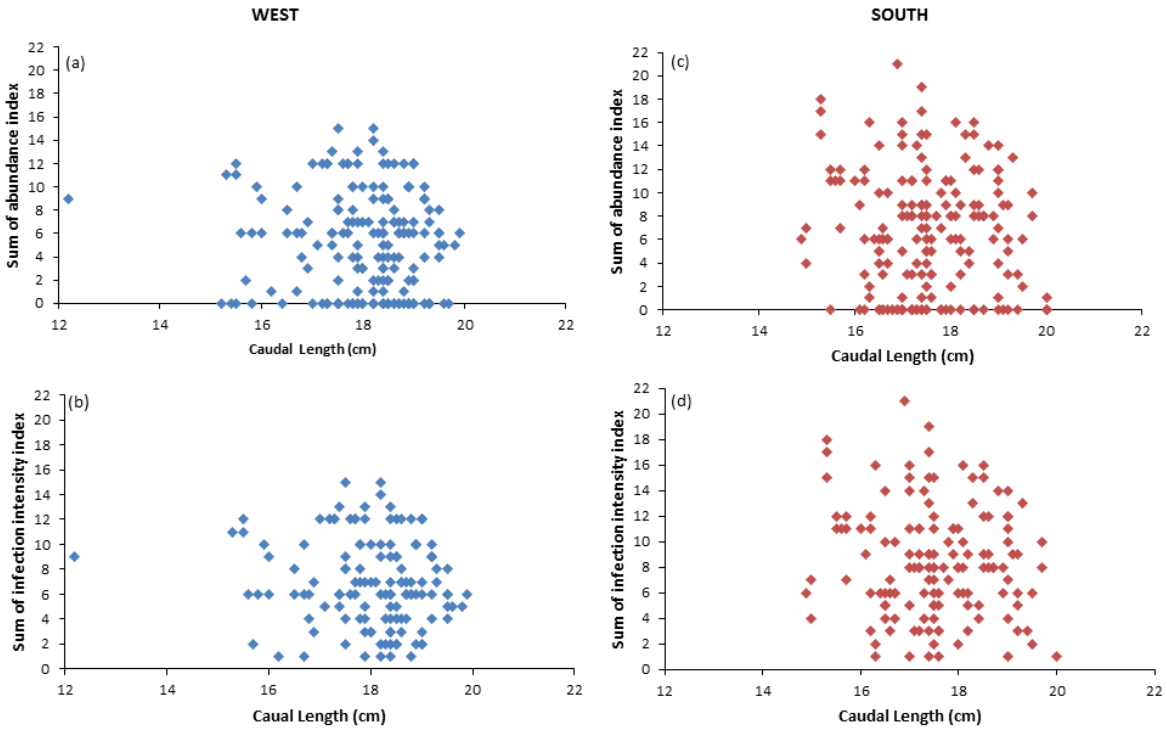

Figure 3.2: Scatterplots of caudal length and sum of abundance index (a&c) and with sum of infection intensity index (b&d)), for male *Sardinops sagax* collected from the west and south coast from 2012-2013 commercial samples.

3.3 Comparing infection within and between testes

Infection data within each of the testes positions (anterior, middle and posterior) was not normally distributed, so a Kruskal-Wallis analysis was performed, which showed a significant difference in infection within testes positions (KW chi-square = 86.029, df = 2, $p < 0.05$). The anterior portion had the highest average abundance index (1.2±0.04), whilst the middle portion had an average of 1.0±0.03 and posterior portion was the least infected with 0.7±0.03 average abundance index (Figure 3.3). The KW comparison was done for the combined left and right testes from both west and south coast male sardines. A chi-square test of independence was done to verify whether the location of highest infection differed in fish from the west and south coast but this was found not to be different ($X^2 = 0.588$, df = 2, $p = 0.74$) (see Table 4 in Appendix I).

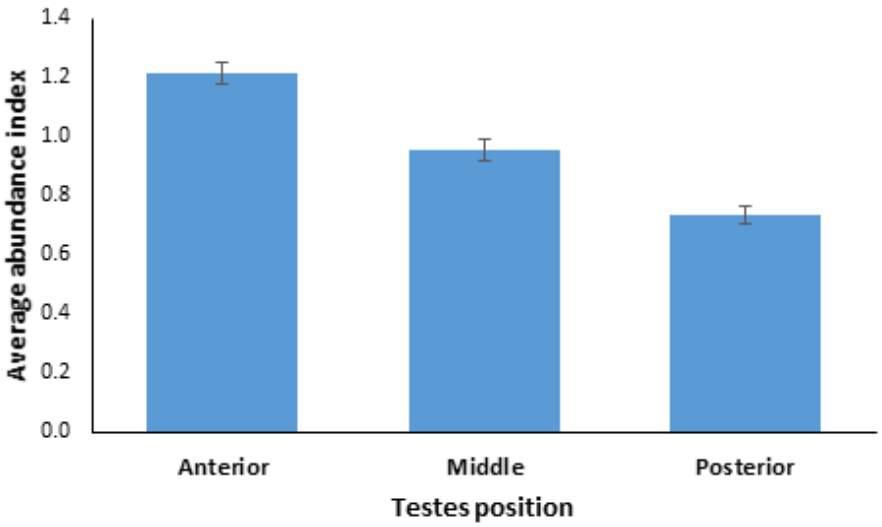

Figure 3.3: Average abundance index (± standard errors) by *Eimeria sardinae* for each of three testes positions (anterior, middle and posterior) from *Sardinops sagax* samples collected from the SA west and south coast in 2012-2013.

Scatterplots of infection intensity index between different portions of the left and right testes for all coastal data combined are shown in Figure 3.4. Significant positive linear relationships between left and right testes for all three portions were observed (Table 3.5), with the portion from the right

testes having higher infection than that from the left testes. Also, scatterplots of infection intensity index between different portions of the left and right testes for separate western and southern stocks showed significant positive linear relationships (see Figure 5 and Table 1 of Appendix I). However, a Mann-Whitney test did not show any significant difference in infection between the right and left testes separately (W = 623.5, nLeft =n 365, nRight = 365, p = 0.13).

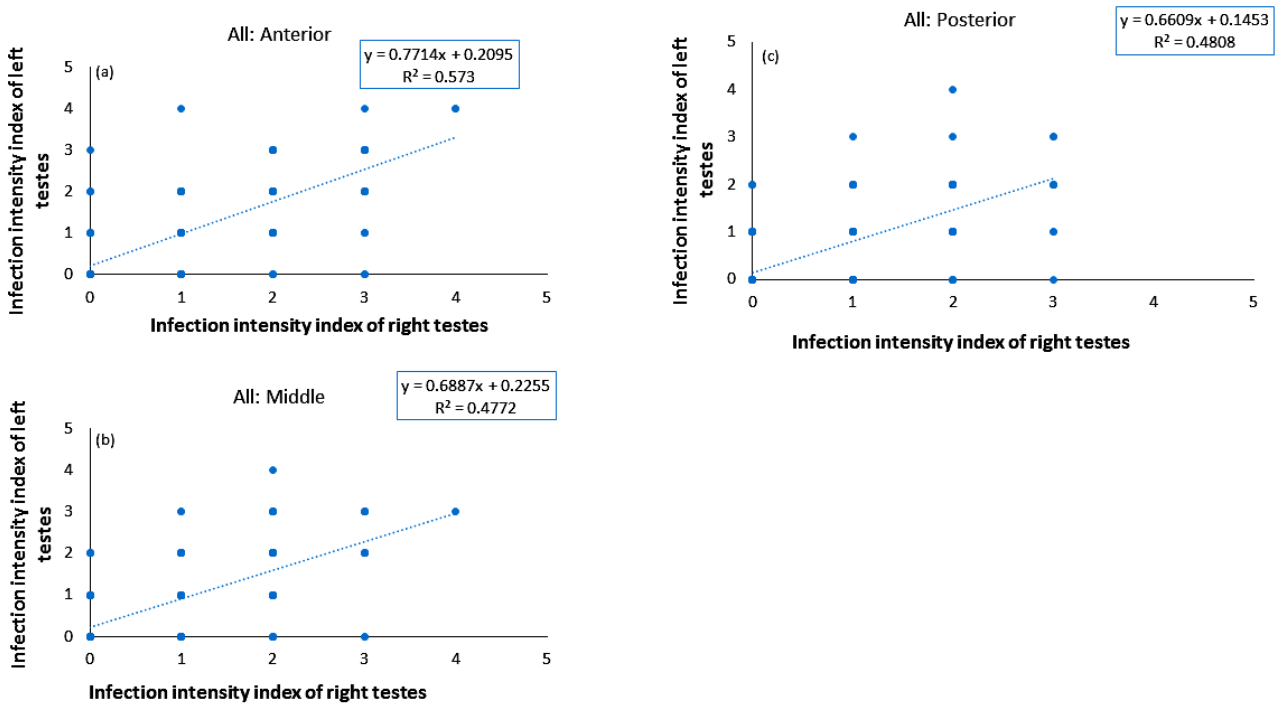

Figure 3.4: Scatterplot and fitted linear regression between infection intensity index in (a) anterior, (b) middle, and (c) posterior of the right and left testes for *Sardinops sagax* collected from west and south coast during 2012-2013.

Table 3.5: Summary of regression statistics for fitted linear regressions between infection intensity index in the anterior, middle and posterior portions of the left and right testes from combined samples.

All: Anterior						
	Coefficients	**Standard Error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**
Intercept	0.210	0.056	3.745	<0.05	0.100	0.320
X Variable 1	0.771	0.035	22.070	<0.05	0.703	0.840
All: Middle						
	Coefficients	**Standard Error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**
Intercept	0.226	0.051	4.426	<0.05	0.125	0.326
X Variable 1	0.689	0.038	18.201	<0.05	0.614	0.763
All: Posterior						
	Coefficients	**Standard Error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**
Intercept	0.145	0.041	3.529	<0.05	0.064	0.226
X Variable 1	0.661	0.036	18.335	<0.05	0.590	0.732

3.4 Seasonal variation in infection intensity index

Mean infection intensity index of the western stock was highest in winter, followed by spring, autumn and then summer with the lowest infection (Figure 3.5a). The southern stock also showed the highest mean infection intensity index in winter and the lowest in summer (Figure 3.5a). Mean infection intensity index of the southern stock was higher than that of the western stock in all seasons except spring.

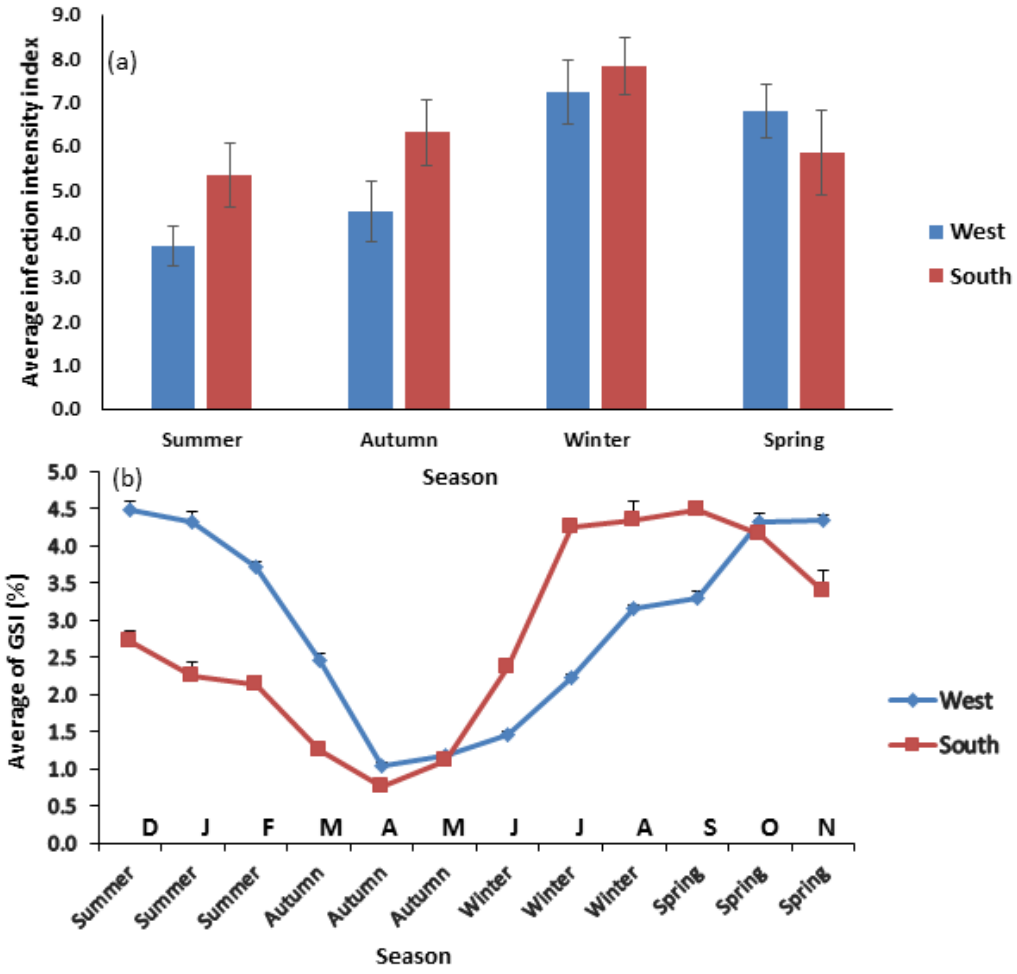

Figure 3.5: Seasonal average infection intensity index of male *Sardinops sagax* with standard error between 2012 and 2013 (a); and (b) monthly average gonadosomatic index (GSI) with standard error of male *Sardinops sagax* between 1996 and 2013 off the west and south coast of South Africa.

3.5 Seasonal variation in gonadosomatic index

Data from between 1996 and 2013 used for calculating GSI were available for a total of 15 384 male fish from the west coast and 6 583 male fish from the south coast (Table 3.6). Overall average GSI was higher in western male *S. sagax* (3.0±0.1%) than south coast (2.8±0.1%) (Table 3.6). The gonadosomatic index of the western stock was highest in late spring (October-November) and summer (December) and lowest in autumn and winter (April-July; Table 3.6 and Figure 3.5b). The gonadosomatic index of the southern fish was highest in late winter and spring (July-October) and

lowest and lowest in autumn (March-May; Table 3.6 and Figure 3.5b).

Table 3.6: Month, season, average GSI, standard error (SE) and number of fish examined (n), of male sardine *Sardinops sagax* samples from two localities; west and south coast of South Africa in 1996 and 2013. Red and bold numbers show the highest monthly average in each season.

		West			South		
Month	**Season**	**Average GSI**	**Standard error (SE)**	**Sample size (n)**	**Average GSI**	**Standard error (SE)**	**Sample size (n)**
January	Summer	4.3	0.1	417	2.3	0.1	291
February	Summer	3.7	0.1	523	2.1	0.2	201
March	Autumn	**2.5**	0.1	1808	**1.3**	0.1	379
April	Autumn	1.0	0.1	2232	0.8	0.1	512
May	Autumn	1.2	0.0	1672	1.1	0.1	278
June	Winter	1.5	0.1	1555	2.4	0.1	583
July	Winter	2.2	0.0	1584	**4.3**	0.1	617
August	Winter	**3.2**	0.1	1477	**4.3**	0.1	686
September	Spring	3.3	0.1	1310	**4.5**	0.3	628
October	Spring	4.3	0.1	487	4.2	0.1	1507
November	Spring	**4.4**	0.1	1301	3.4	0.2	540
December	Summer	**4.5**	0.1	1018	**2.7**	0.3	361
TOTAL		**3.0**	**0.1**	**15384**	**2.8**	**0.1**	**6583**

Levene's test of normality was done on the GSI data separately by season for western and southern *S. sagax*. Data deviated from normality ($F = 45.218$, $df = 7$, $p < 0.05$), and did not show normality after being transformed with n-root, log and square-root transformation (See Figure 5 in Appendix 1).

Because of the non-normal data a Kruskal-Wallis test was conducted, which showed an overall significant difference in GSI by season (KW-chi square = 5288.5, $df = 7$, $p < 0.05$), and multiple comparison of the Kruskal-Wallis showed a significant difference in seasonal GSI values between all months and coasts except for (i) summer-west and spring-south, (ii) summer-south and winter-west, and (iii) spring-west and spring-south (Table 3.7).

Table 3.7: Multiple comparisons of Kruskal-Wallis test for seasonal GSI of west and south coast collected from 1996-2013. Red highlighted values show significant differences.

GSI	SummerWest	SummerSouth	AutumnWest	AutumnSouth	WinterWest	WinterSouth	SpringWest	SpringSouth
SummerWest		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	**1**
SummerSouth	<0.05		<0.05	<0.05	**1**	<0.05	<0.05	<0.05
AutumnWest	<0.05	<0.05		<0.05	<0.05	<0.05	<0.05	<0.05
AutumnSouth	<0.05	<0.05	<0.05		<0.05	<0.05	<0.05	<0.05
WinterWest	<0.05	**1**	<0.05	<0.05		<0.05	<0.05	<0.05
WinterSouth	<0.05	<0.05	<0.05	<0.05	<0.05		<0.05	<0.05
SpringWest	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		**0.06**
SpringSouth	**1**	<0.05	<0.05	<0.05	<0.05	<0.05	**0.06**	

3.6 Seasonal Infection intensity index and seasonal gonadosomatic index

There was no correlation between GSI and seasonal mean infection intensity index ($p = 0.863$). For the western stock, there was a positive relationship between GSI and mean infection intensity index whilst the southern stock showed a weak negative linear relationship between mean infection intensity index and GSI in both regions (Figure 3.6). Mean infection intensity index was high in winter and spring, and low in summer and autumn, while GSI was high in spring and low in autumn in both regions.

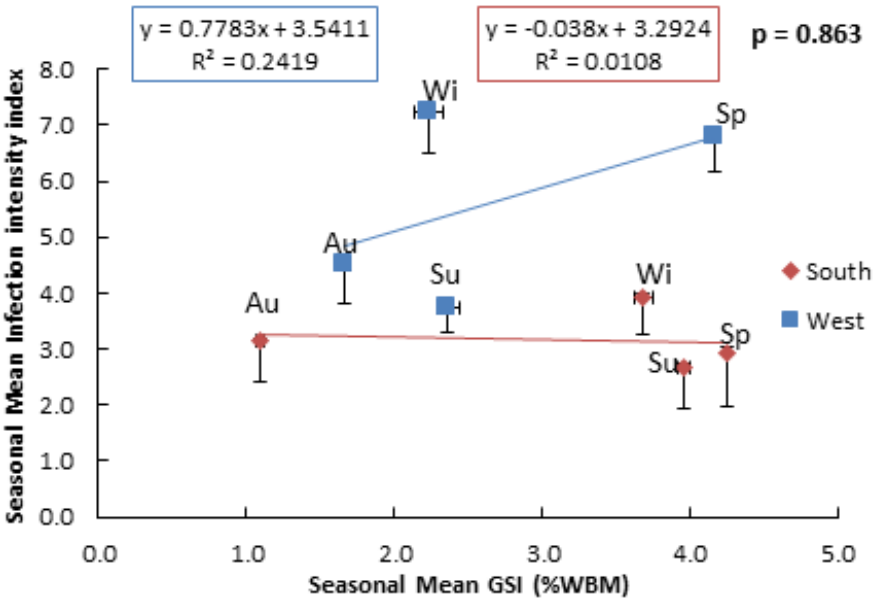

Figure 3.6: Scatterplot of seasonal mean infection intensity and seasonal mean GSI (std errors shown for both) with fitted regression lines and equations for *Sardinops sagax* from the west coast and south coast. Su = summer, Au = autumn, Wi = winter and Sp = spring. Statistical significance between GSI and seasonal mean infection intensity index is represented by p=0.863.

3.7 Prevalence of infection, Infection intensity index and Parasite abundance index across seasons by regions

The estimated parameter for each factor was compared to the reference level using General Linear Model (GLM). The factor that is not shown in the table is called the reference level. Tables 3.8, 3.9 and 3.10 show parameter estimates and the test of significance for each factor (season and region). For example in table 3.8, region west is compared to region south which is not shown in the table the significance level shows no difference between regions (p = 0.67). Likewise, season spring did not show a difference when compared to all the other three seasons (p = 0.95). There was no significant difference when summer was compared to the other three seasons (p = 0.97) and no significant difference when winter was compared to the other three seasons (p = 0.48). Therefore, prevalence of infection did not vary with seasons and regions (Figure 3.7) and seasonal cycles were

not different in prevalence from the west and south coast (Table 3.8).

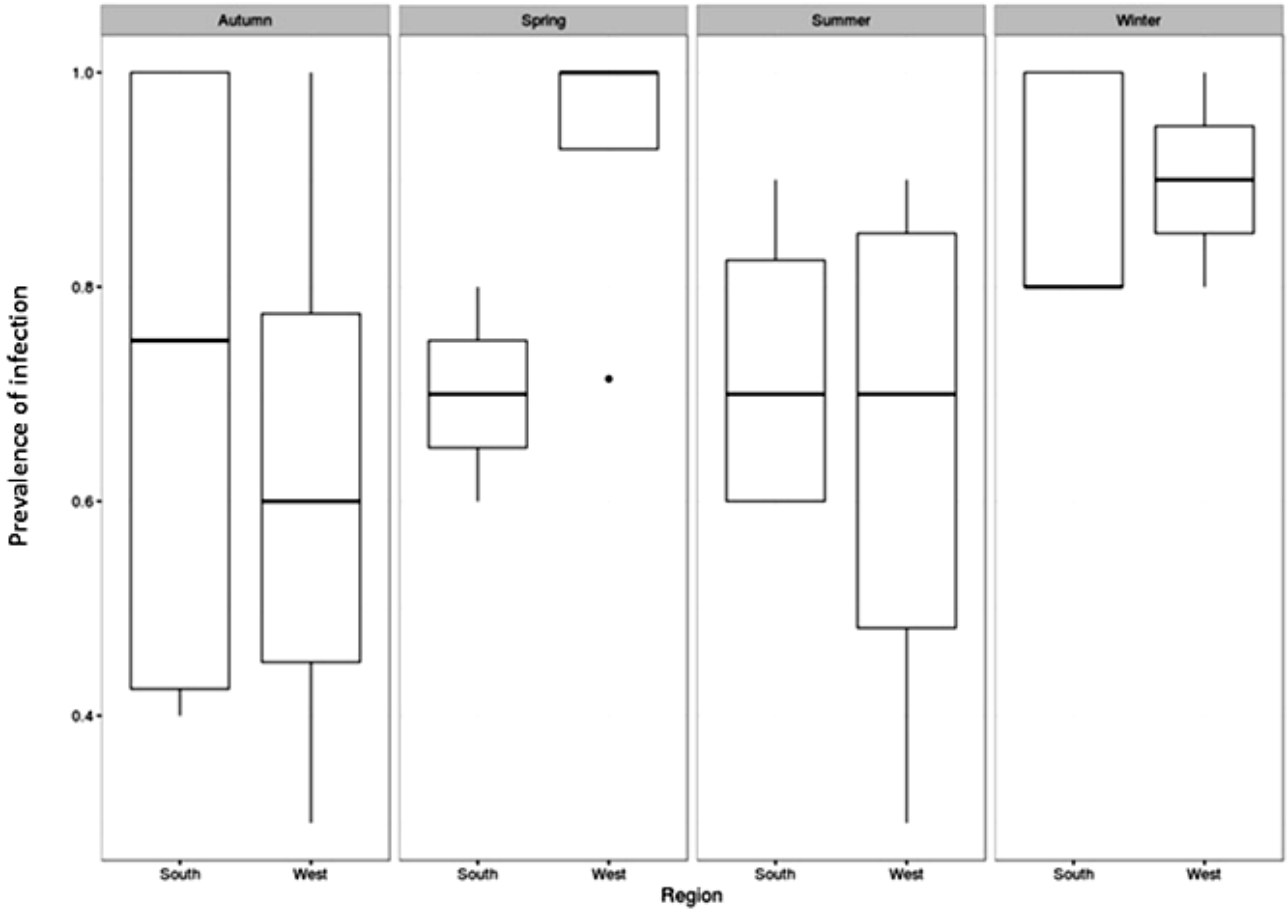

Figure 3.7: Boxplot of seasonal mean prevalence of infection (expressed as proportion) by *Eimeria sardinae* of *Sardinops sagax* off the west and south coast during 2012-2013.

Table 3.8: Summary of GLM with parameter estimates and significance on the effect of region and season on the prevalence rate of *Eimeria sardinae*.

	Estimate	SE	z value	p
(Intercept)	-0.33	0.20	-1.67	0.10
RegionWest	-0.14	0.32	-0.42	0.67
SeasonSpring	0.02	0.34	0.07	0.95
SeasonSummer	0.01	0.32	0.04	0.97
SeasonWinter	0.21	0.29	0.71	0.48
RegionWest:SeasonSpring	0.35	0.48	0.73	0.46
RegionWest:SeasonSummer	0.02	0.45	0.05	0.96
RegionWest:SeasonWinter	0.16	0.47	0.34	0.73

Both coasts showed the same seasonal pattern in infection intensity index (Figure 3.8 & Table 3.9).

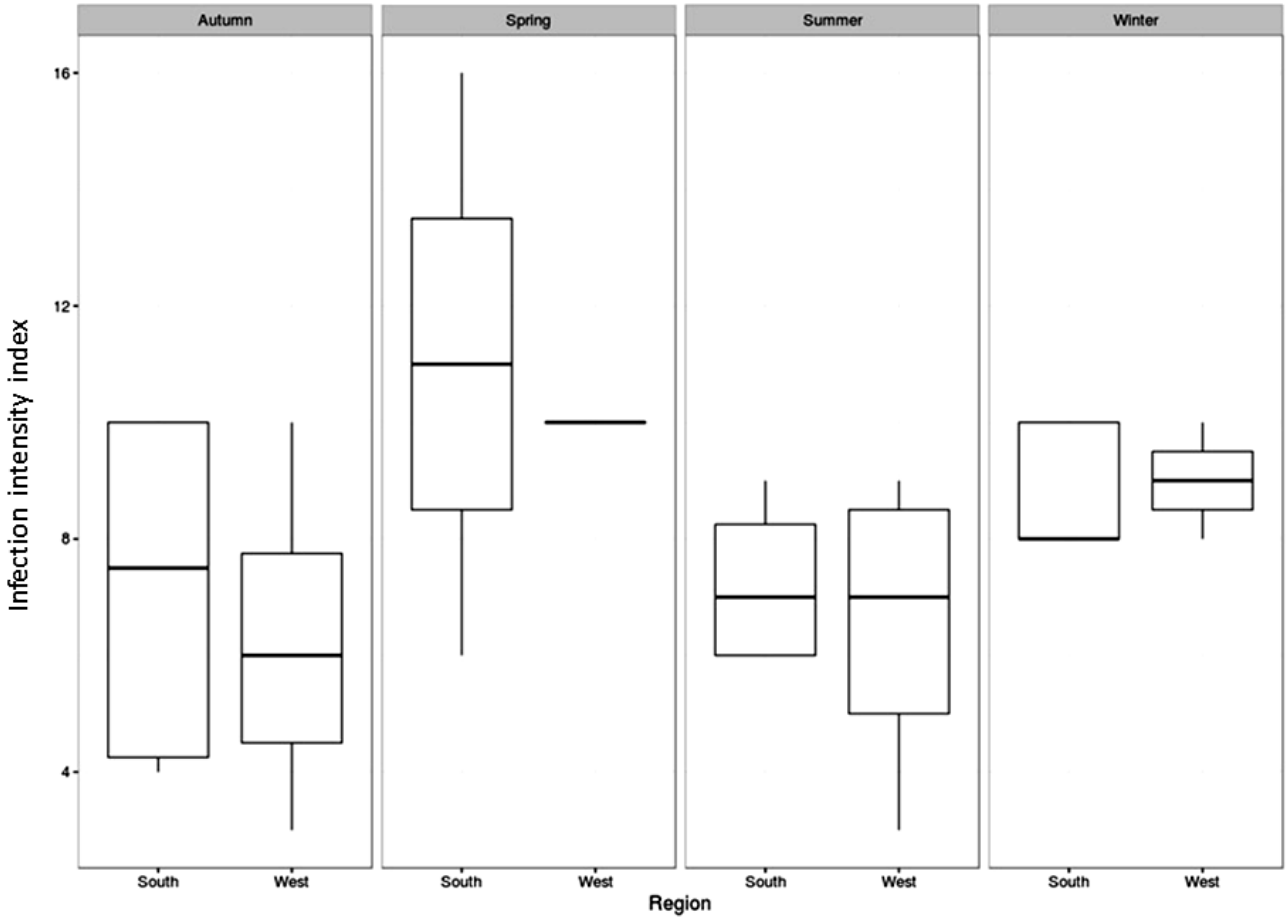

Figure 3.8: Boxplot of mean infection intensity index of *Eimeria sardinae* across seasons between 2012-2013 from west and south coast.

From the table below, there were no significant differences in infection intensity index between seasons. Also, regions did not show a difference in infection intensity index; $p = 0.60$.

Table 3.9: Summary of GLM with parameter estimates and significance on the effect of region and season on infection intensity index of *Eimeria sardinae*.

	Estimate	SE	z value	p
(Intercept)	4.43	1.25	3.54	0.00
RegionWest	-1.06	1.98	-0.53	0.60
SeasonSpring	-1.73	2.30	-0.75	0.46
SeasonSummer	-0.01	2.06	0.00	1.00
SeasonWinter	-0.04	1.85	-0.02	0.98
RegionWest:SeasonSpring	2.09	3.16	0.66	0.51
RegionWest:SeasonSummer	0.53	2.91	0.18	0.86
RegionWest:SeasonWinter	0.62	2.98	0.21	0.83

Mean parasite abundance index of *E. sardinae* also did not show any variation by season between the west and south coast (Figure 3.9). All the factors showed no significant difference with $p > 0.05$ (Table 3.10).

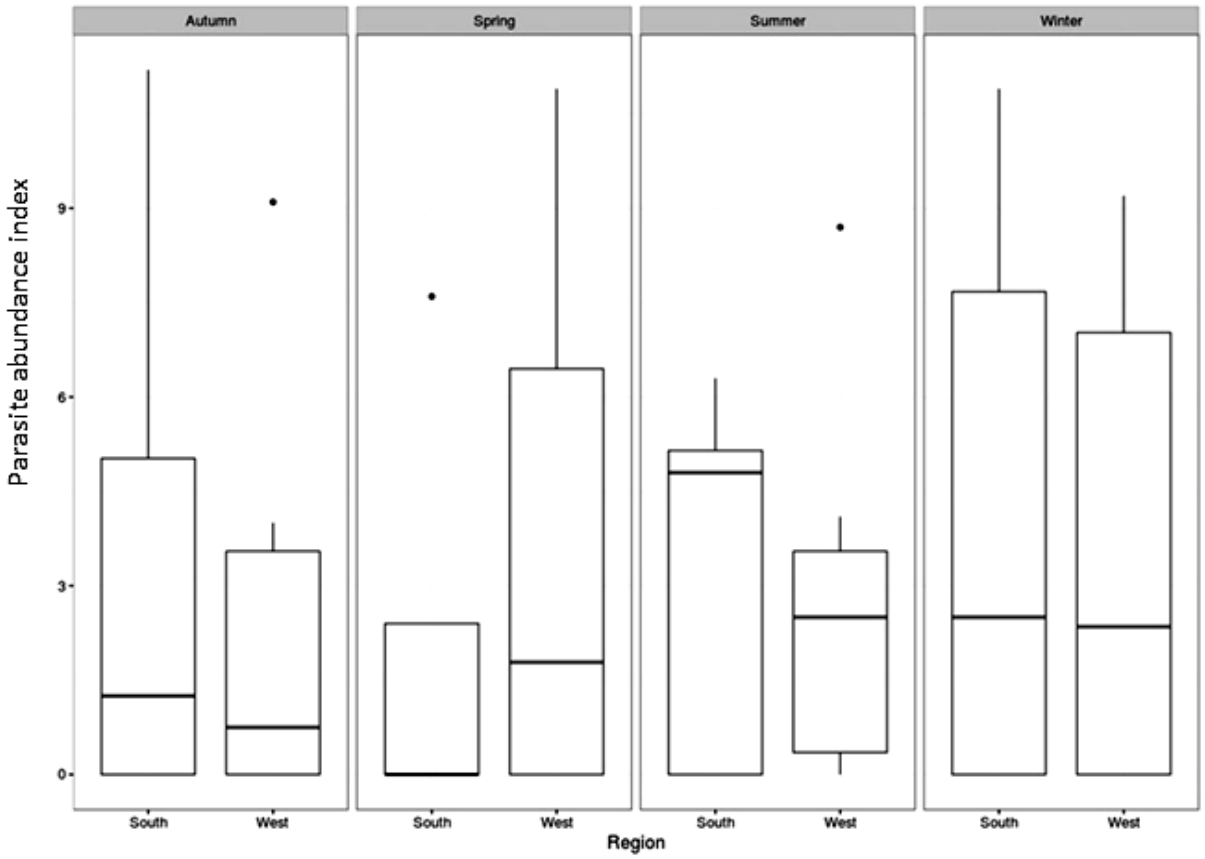

Figure 3.9: Boxplot of mean parasite abundance index of *Eimeria sardinae* across seasons between 2012-2013 from west and south coast.

Table 3.10: Summary of GLM with parameter estimates and significance on the effect of region and season on mean parasite abundance index of *E. sardinae*.

	Estimate	SE	z value	p
(Intercept)	3.16	1.07	2.95	0.00
RegionWest	-0.91	1.69	-0.54	0.59
SeasonSpring	-1.16	1.97	-0.59	0.56
SeasonSummer	-0.10	1.76	-0.06	0.95
SeasonWinter	0.75	1.59	0.47	0.64
RegionWest:SeasonSpring	2.47	2.71	0.91	0.37
RegionWest:SeasonSummer	0.45	2.49	0.18	0.86
RegionWest:SeasonWinter	0.62	2.55	0.24	0.81

CHAPTER 4: Discussion, Conclusion and Future Work

Discussion

Temporal variability in infection of male sardines (*Sardinops sagax*) by a testicular coccidian parasite (*Eimeria sardinae*) from the South African west and south coast was assessed during 2012 and 2013 to further examine suitability of this parasite as a potential biotag. Variation in infection intensity within testes and between left and right testes of individual fish were also examined. Furthermore, infection intensity was examined in relation to fish size and sex.

Results showed no significant seasonal differences in prevalence of infection, infection intensity index or parasite abundance index for sardine off either the west or south coast. These findings are in contrast with other *S. sagax* parasites (tetracotyle) studied by Weston *et al*. (2015). Significant differences in infection intensity index and parasite abundance index were however found between west and south coast *S. sagax* but not for infection prevalence. Ssempa (2013) did not find a significant difference in infection intensity index between coasts but did report higher infection abundance in southern fish.

Mean infection intensity was higher in southern sardine than western fish in all seasons except spring, and overall, *S. sagax* from the south coast showed a significantly higher mean infection intensity index than *S. sagax* from the west coast. This is in agreement with Ssempa (2013) who reported that the southern stock showed higher infection levels of *E. sardinae* than the western stock, although there was no significant difference. However, Linde (2011) reported higher infection by *E. sardinae* on *S. sagax* from the west coast than from the south. The absence of significant differences in infection indices between sardine from the western and southern stocks found in this study does not support the evidence for multiple stocks.

Despite the lack of a significant difference in overall seasonal averages of infection, the same pattern of highest infection in winter and the lowest infection in summer was observed for *S. sagax* off both west and south coasts, suggesting infection was higher before and during spawning and lower after spawning because oocysts were released during spawning. This is in agreement with McGladdery and Burt (1985) who postulated that infection by *Anisakis* spp. in Atlantic herring (*Clupea harengus harengus*) increased from before spawning to during spawning and declined in post-spawning males. According to Beckley and van der Lingen (1999), Kreiner *et al*. (2001) and Miller *et al*. (2006), spawning by *S. sagax* off the west coast occurs from spring (September) to late summer (February), while the gonadosomatic index data presented in this study confirmed that the spawning peak of the fish from the south coast extended from winter to spring. Higher infection during the spawning season may arise because infected fish release sperm and *E. sardinae* oocysts, which become abundant in the water column and get ingested by the other fish (McGladdery, 1987). Despite a strong seasonal signal in GSI there was no significant relationship between seasonally-averaged GSI and infection intensity index. This is in contrast to Seempa (2013) who found that highly-infected testes had very low GSI values. For the western stock, GSI was high when infection intensity was high except in winter and summer. However, southern stock showed a negative relationship between GSI and infection intensity index. Fish from both coasts showed higher GSI in spring (September) and lower GSI in autumn (March), which appears to follow spawning patterns. According to Pinto (1956), GSI is high during low infection levels because testes are concentrated with gonad cells. When testes are heavily infected with *E. sardinae* gonad cells become compressed as infection spreads throughout a large proportion of the gonad.

According to Kim *et al*. (2001) and Lamková *et al*. (2007), seasonal fluctuations in parasite abundance and prevalence of infection can be due to feeding patterns of the host, availability of the intermediate host, hormonal changes and temperature. Because there is a single host for *E. sardinae*

in *S. sagax* the availability of an intermediate host cannot explain non-significant seasonal pattern observed in this study. For example, Kim *et al*. (2001) concluded that the abundance of a monogenean parasite *Prosomicrocotyla gotoi* in a greenling *Hexagrammos otakii* was higher when the temperatures were cool in winter than in hot summer. This is in agreement with what was observed in this study; higher infection in winter than summer from both coasts. Also from Kim *et al*. (2001), there were high peak abundances of *P. gotoi* during spawning season than after spawning, which corresponds with high infection intensity of *E. sardinae* during spawning season of *S. sagax* in the west and south coast of South Africa.

Kruskal-Wallis tests showed a significant difference in infection by *E. sardinae* between the portions of the examined testis (anterior, middle and posterior), with the anterior portion being more highly infected than the other portions. This is in agreement with Linde (2011) who found that the anterior lobe of *S. sagax* testes was more infected with *E. sardinae* than the posterior lobe. Sub-sampling resulted to a significant difference within testes, meaning that when sampling, the position of sample collection within the testes matters and might provide clues about infection pathway. An assumption would be that oocysts complete their development stage in the anterior before they get released from the posterior part during spawning. It is not known which part of the testes do oocysts infect first after leaving intestinal walls but it is likely that they infect the posterior since its closer to the intestines. Baker (1969) and Upton *et al*. (1984) postulated that mature oocysts enter the host by being swallowed and after some time hatch in the small intestine. After developing sporozoites, oocysts will then be transferred to the site of infection (which is testes for *E. sardinae*) by penetrating inside the intestinal cells. Knowing the infection pathway of *E. sardinae* can be helpful in explaining why the anterior part is highly infected when intestines are in close proximity to the posterior part.

Mann-Whitney tests showed no significant difference in infection between the right and left testes of individual fish. In addition there was a significant positive relationship in infection intensity index of the anterior, middle and posterior portions between right and left testes, supporting the Kruskal-Wallis results. This is in contrast with Linde (2011) who suggested that infection by *E. sardinae* was higher on the right testes of *S. sagax* than on the left. On the other hand, McGladdery (1987) did not find any difference between right and left testes of herring *Clupea harengus harengus*. It needs to be taken into consideration that only zygote and oocyst stages can be identified by a light microscope, hence infection by other developmental stages of *E. sardinae* can go unnoticed (McGladdery, 1987). Therefore it is possible that infection can be higher in one testes more than the other or vice-versa due to identification techniques used. This however does not explain how oocysts infect testes and it is not known whether the oocysts enter the right or left testes first. According to these results it appears both testes get infected at the same time since when infection is high on the left it is also high on the right.

A larger sample size for male fish was collected for the west coast, hence that ample included more fish that were larger. Yet there was no correlation between infection intensity and mean abundance index relative to fish size observed in this study for fish from either west or south coast samples. This is in contrast with Ssempa (2013) who suggested that infection intensity increased with fish size. McGladdery (1987) also confirmed that mature Atlantic herring (*Clupea harengus harengus*) males were more highly infected by *E. sardinae* than small herrings and Draoui *et al*. (1995) reported that bigger fish show higher prevalence of infection than smaller fish of *Sardinella aurita* and *Sardina pilchardus*. However, McGladdery (1987) mentioned that high infection in mature males must be due to the efficient transport of parasites between hosts during spawning. Fishes sampled for this study were mostly sexually mature but showed low and high levels of infection in all size classes. Size range covered in this study was similar to Ssempa's (2013). Although infection

intensity and prevalence of infection did not show a trend with fish size in the current study the smallest fish (one 12 cm fish) from the western stock was found to be infected.

No female *S. sagax* from either coast showed any signs of infection. *Eimeria sardinae* were believed to be present in males only, but a study conducted by Draoui *et al*., (1995), found relatively high prevalence of *E. sardinae* in female gonads of *Sardinella aurita* and *Sardina pilchardus* near Tunisian shores. Infection of females by *E. sardinae* was last reported by Draoui *et al*. (1995), but no other reports have mentioned the presence of infected ovaries by the same species.

Parasites can alter the host's behaviour, physiology and morphology (Poulin and Thomas, 1999; Dejen *et al*., 2006). Some of the changes can be beneficial to both the host and the parasite. For example, the body size of a host could increase to accommodate a parasite such as in trematode parasite (*Probolocoryphe uca*) of the snail (*Cerithidea californica*) (Poulin and Thomas, 1999); or it could affect the behaviour of the host for example trematode (*Euhaplorchis californiensis*) found in killifish (*Fundulus parvipinnis*) resulted into a decreased schooling activity of the fish (Lafferty and Morris, 1996). *Eimeria sardinae* is known to cause reduced fecundity (Pinto, 1956) and deformities and lesions in testes of blue whiting *(Micromesistius poutassou)* (MacKenzie, 1981). Currently there are no known pathological effects of *E. sardinae* infecting *S. sagax* as they have not yet been studied in South Africa.

Conclusion

Potential exists for the use of *E. sardinae* as a biological tag to partly distinguish sub-populations of *S. sagax* off South Africa*; e.g*. differentiating eastern stock sardine from western and/or southern stocks, but not western from southern sardine. The results of the study showed no seasonal difference in prevalence, infection intensity index and abundance index of *E. sardinae* in *S. sagax* off the west and south coasts of South Africa. However, the unequal coverage of seasonal samples over the two coasts might bias the conclusions of this study and overall sample size was also possibly insufficient. Also, inter-annual effects may play a role, where both studies covered different timeframes which could affect the results. In Ssempa's (2013) study samples were collected from January 2010 to February 2012, whereas the current study samples were collected from January 2012 to December 2013.

Sardinops sagax GSI showed a strong seasonal cycle in both western and southern stocks, and although significant seasonal differences in infection were not found, higher infection levels appeared to be associated with higher GSI values. However, seasonal infection intensity index and seasonal GSI from the west and south coast did not show any correlation. Infection levels in the right and left testes of the same fish were not significantly different, but there was a significant difference in infection within each testes, the anterior portion having more oocysts, followed by middle and then posterior portions. These findings need to be investigated further as they may provide insight into the infection pathway of *E. sardinae.* There was no infection observed in *S. sagax* females.

Future work

Although this study revealed some interesting findings, it could be improved by including several aspects. Increasing samples sizes by increasing the timeframe and getting an even number of

seasonal samples of *S. sagax* from both coasts would provide more confidence to the results obtained. In addition, recording the actual number of parasites in testes sub-samples would likely provide better data than the infection categories used in this study, particularly if infection is unevenly distributed throughout the testes as was observed here. For example one could estimate a high infection in only the anterior part of the testes (with 4's) and zeros and low infection in other parts (with 3's and 2's). Summing these results will result in an unlikely high infection estimate. However counting individual parasites is more time consuming. Histological studies of *E. sardine* infection in *S. sagax* should be done in order to look for pathological effects caused by this parasite. Finally long term monitoring of this infection will be useful for examining the large-scale effects on inter-annual spawning.

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Appendix I

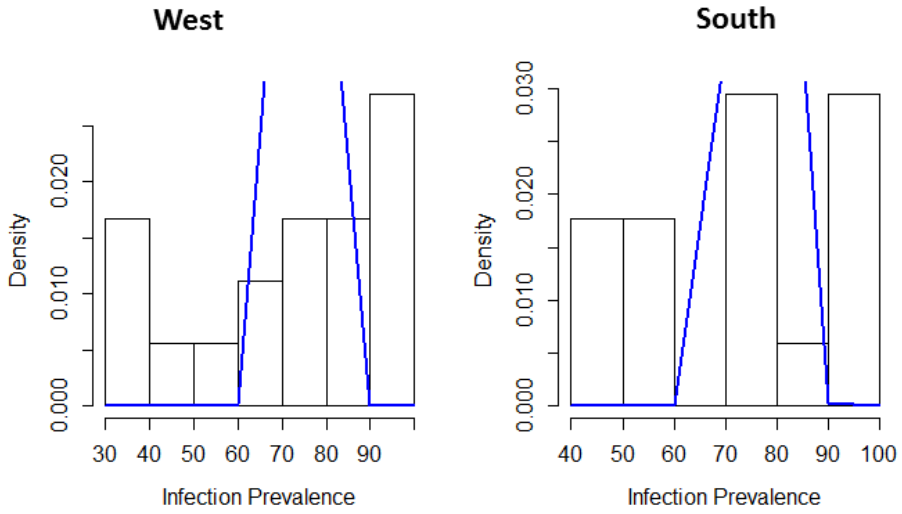

Figure 1: Normality probability plots of infection prevalence per sample and coasts (west and south) from data collected between 2012-2013.

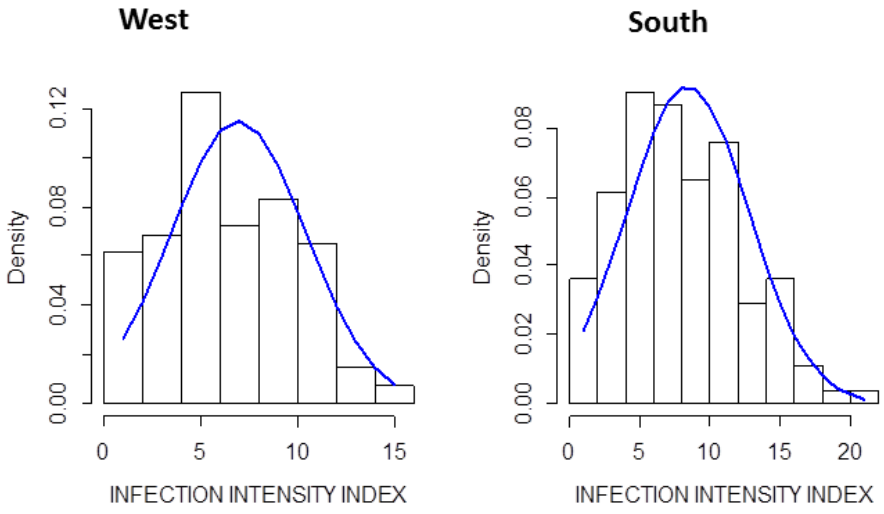

Figure 2: Normality plots of infection intensity index per fish and coasts collected from 2012-2013.

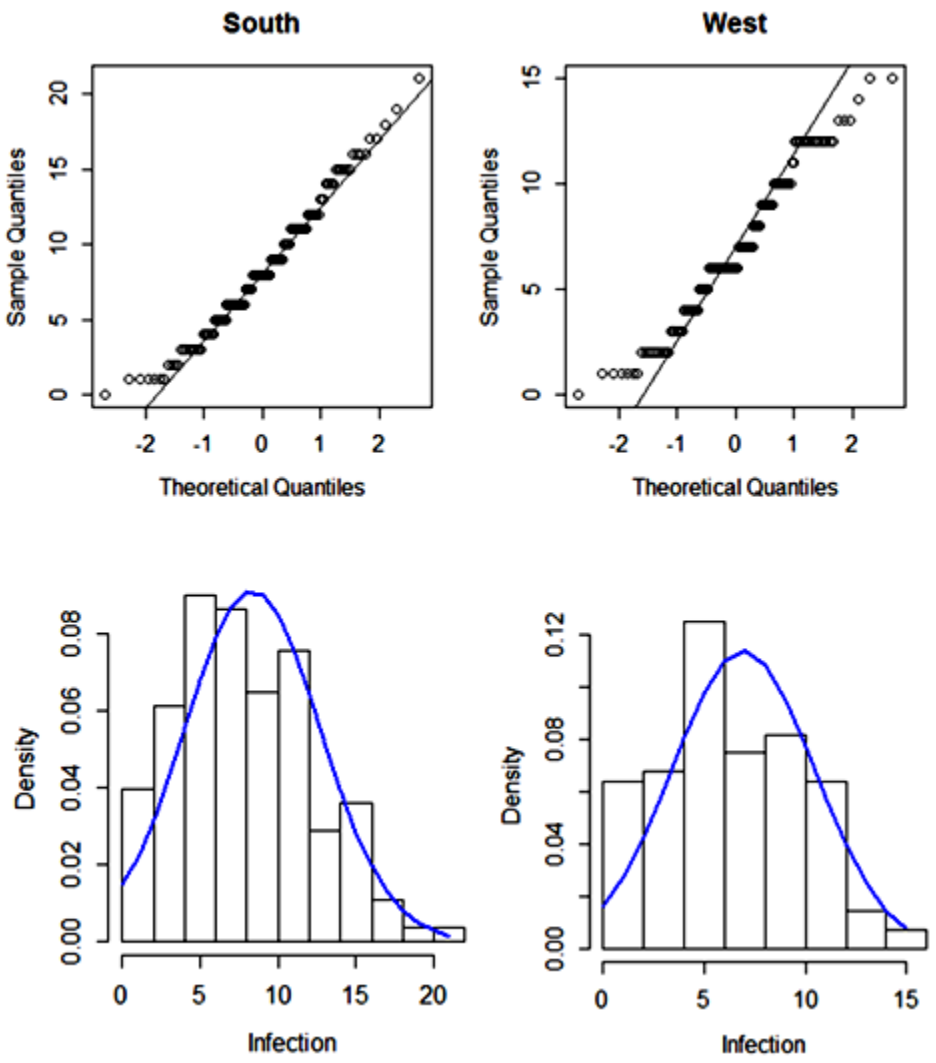

Figure 3: Q-Q plots and histograms of south and west coasts showing the distribution of the sum of mean abundance index data on the coasts.

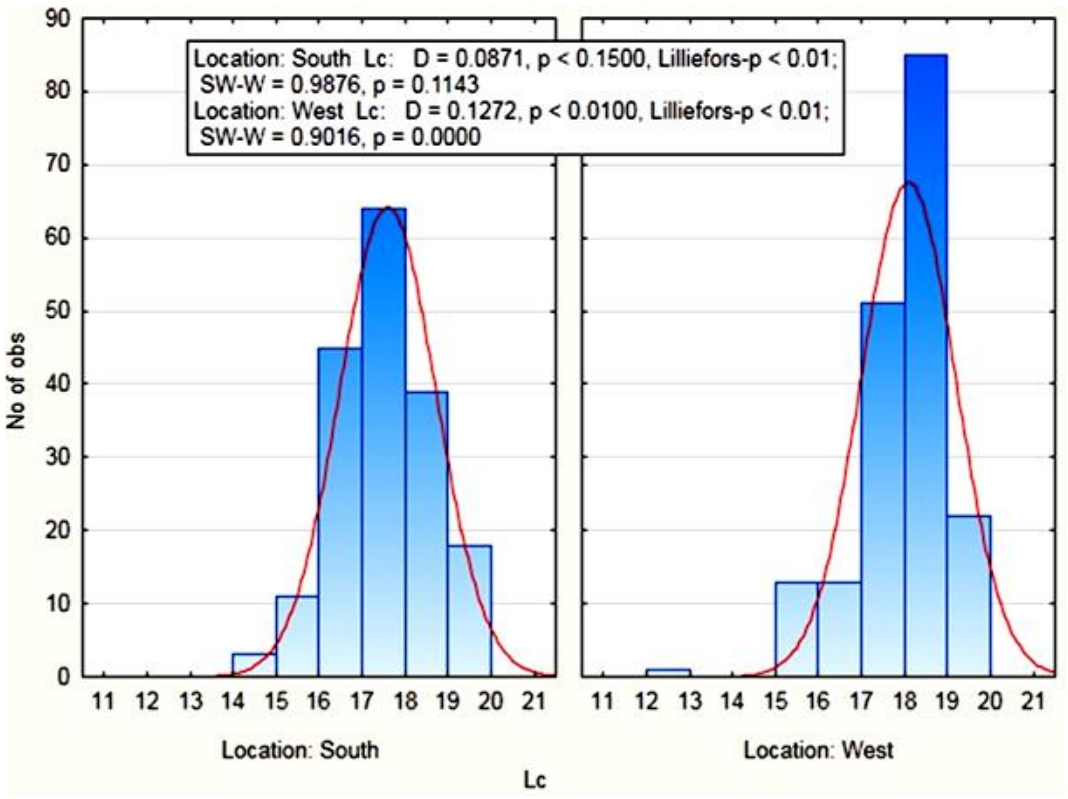

Figure 4: Histograms of length frequency distribution from west and south coast sardine with statistics results from Kolmogorov-sminorov and Shapiro Wilk normality test.

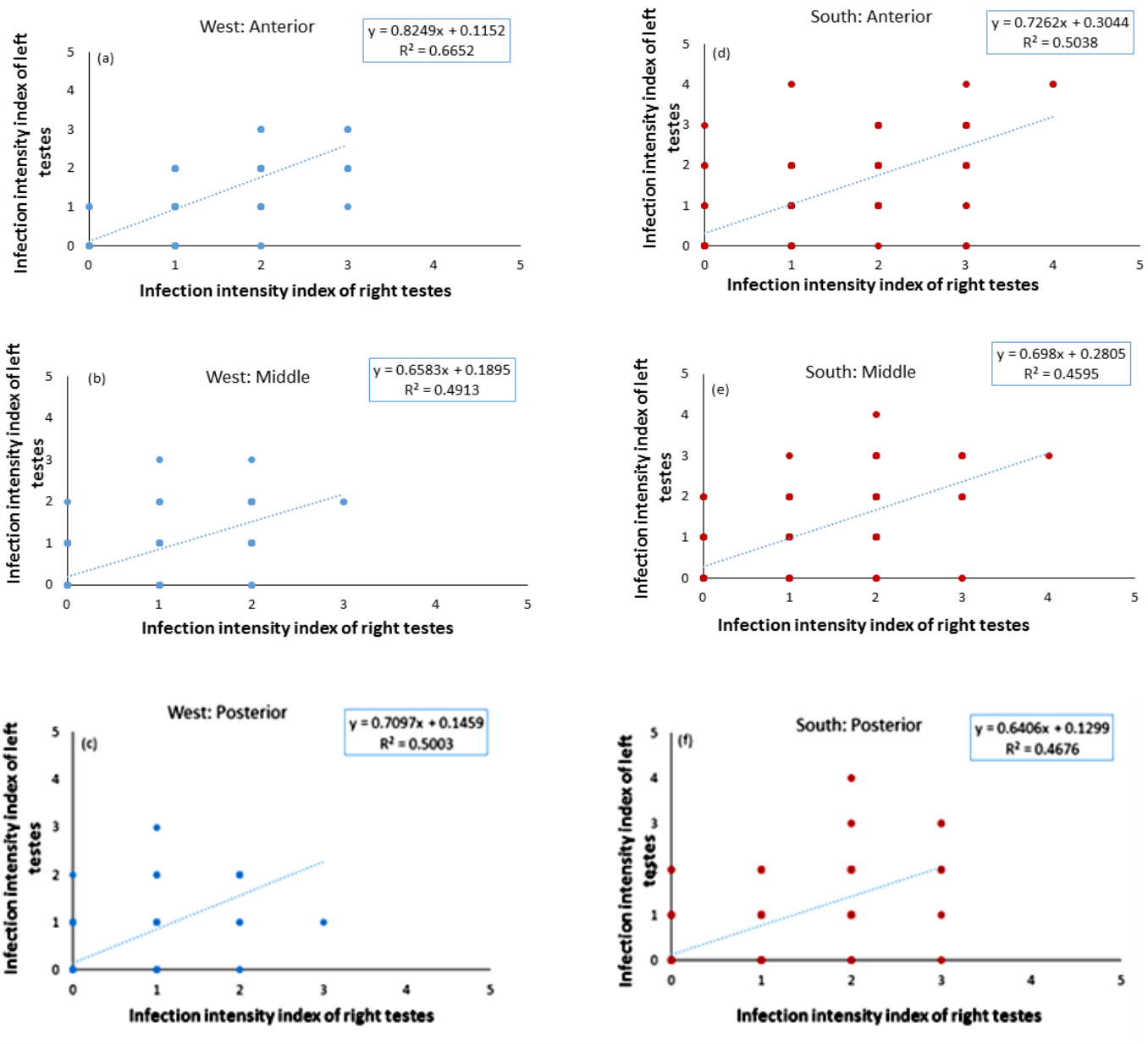

Figure 5: Scatterplots and fitted linear regression showing a relationship in infection intensity index between left and right testes in (a) and (d) anterior, (b) and (e) middle, and (c) and (f) posterior from *Eimeria sardinae* data.

Table 1: Summary of Regression in infection intensity index of anterior, middle and posterior of left and right testes from *Eimeria sardinae* data.

	Anterior: West					
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.115	0.063	1.817	< 0.05	-0.010	0.240
X Variable 1	0.825	0.043	19.070	< 0.05	0.740	0.910
	Middle: West					
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.190	0.061	3.128	< 0.05	0.070	0.309
X Variable 1	0.658	0.050	13.295	< 0.05	0.561	0.756
	Posterior: West					
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.146	0.051	2.854	< 0.05	0.045	0.247
X Variable 1	0.710	0.052	13.535	< 0.05	0.606	0.813
	Anterior: South					
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.304	0.093	3.259	< 0.05	0.120	0.489
X Variable 1	0.726	0.054	13.443	< 0.05	0.620	0.833
	Middle: South					
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.280	0.083	3.379	< 0.05	0.117	0.444
X Variable 1	0.698	0.057	12.300	< 0.05	0.586	0.810
	Posterior: South					
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.130	0.066	1.964	< 0.05	-0.001	0.260
X Variable 1	0.641	0.051	12.504	< 0.05	0.540	0.742

Table 2: Summary of Shapiro-Wilk normality test between infection and testes positions.

Shapiro-Wilk normality test Grouping: Infection by testes position			
	Mean	W	p
Anterior	1.21	0.87	**<0.01**
Middle	0.95	0.84	**<0.01**
Posterior	0.74	0.79	**<0.01**

Table 3: Summary of Pearson's chi-square results of testes position (anterior, middle and posterior) and coasts (west and south) with observed and expected frequencies of infection by *Eimeria sardinae*.

		Anterior	**Middle**	**Posteriror**	**Total**
West	Expected:	410	312	239	961
	Observed:	**401**	**316**	**244**	
South	Expected:	476	385	299	1160
	Observed:	**485**	**381**	**294**	
Total		886	697	538	2121

Table 4: Summary of Pearson's chi-square statistics between testes position (anterior, middle and posterior) and coasts (west and south).

Pearson chi-square			
Statistic	**df**	**value**	**Probability**
Chi-square	2	0.588	0.75

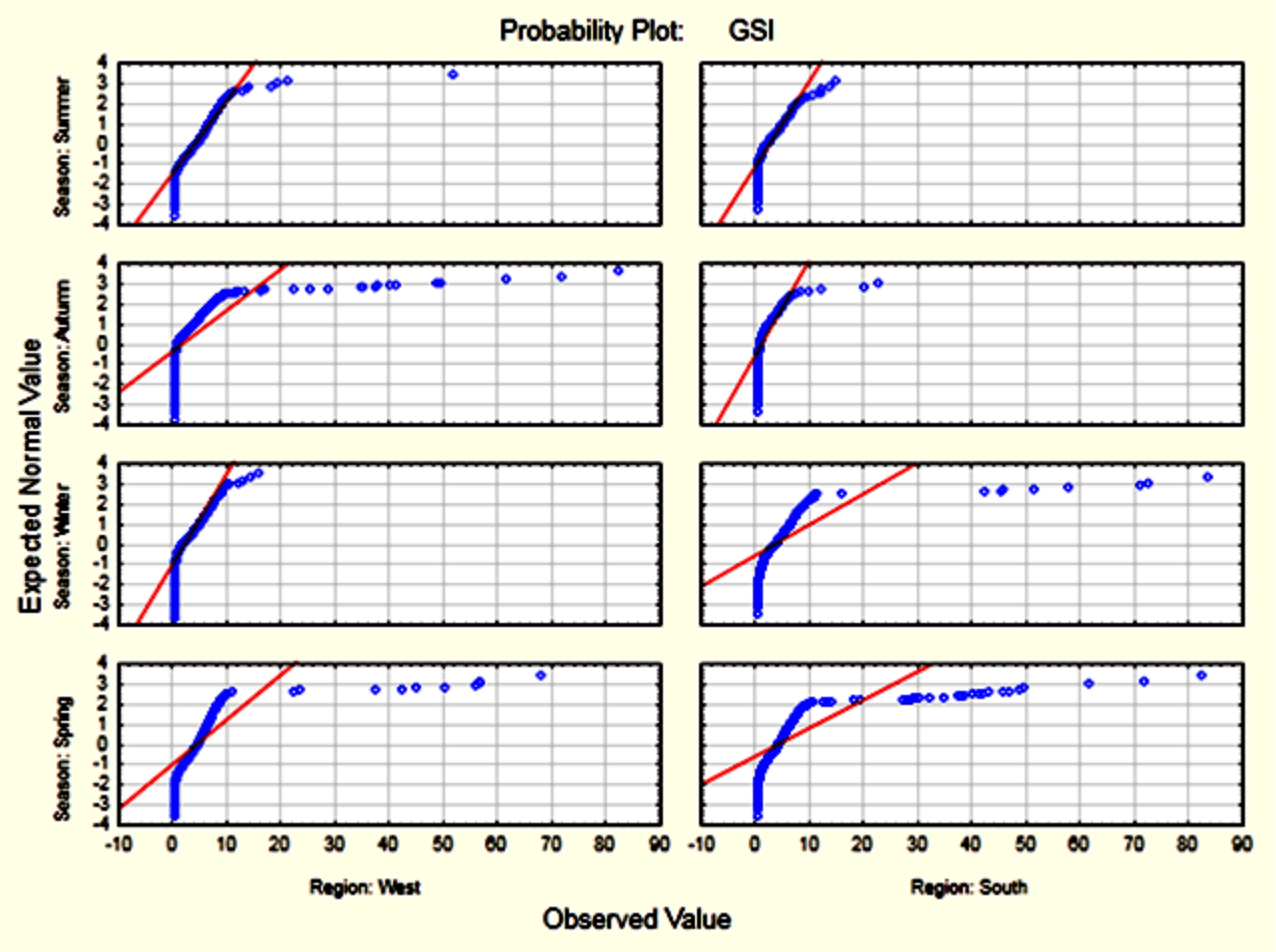

Figure 6: Probability plots of GSI across seasons from the west and south coast from data collected from 1996-2013.