The occurrence of a testicular coccidian *Eimeria sardinae* (Thélohan, 1820) (Reichenow, 1921) in southern African sardine *Sardinops sagax* (Jenyns, 1842)

Nurudean Norman Ssempa

This thesis is presented in partial fulfilment for the MSc degree in Applied Marine Science in the Department of Zoology, University of Cape Town
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Supervisor: Dr. Cecile C. Reed, Department of Zoology, University of Cape Town
Co-supervisor: Dr. Carl D. van der Lingen, Department of Agriculture, Forestry and Fisheries; and Marine Research Institute, University of Cape Town.

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ABSTRACT

A total of 553 male southern African sardine *Sardinops sagax* collected between 2010 and 2012 from around southern Africa, including from the northern Benguela off Namibia and from the South African west, south and east coasts, were examined for the occurrence of a testicular coccidian *Eimeria sardinae* to assess its potential use as a "biological tag" in order to differentiate between hypothesized discrete sardine stocks within the region. Sardine from Namibia and from the South African east coast showed no infection by *E. sardinae*, whereas fish from the South African west and south coasts were infected. Average prevalence infection values of 48.9 ± 6.3 and 48.1 ± 9.7% were recorded for sardine from the west and south coasts. Average infection intensity of 49.5 ± 15.8 oocysts per sample was recorded for the west coast and 69.8 ±33.5 oocysts per sample for the south coat sardine. There was no significance difference in *E. sardinae* infection (t = 0.08; p = 0.94) in *S. sagax* from the South African west coast and south coast. These results provide some support for the hypothesis of multiple sardine stocks off southern Africa and hence are important for fisheries management. The intensity of infection had a significant (Analysis of Variance-NOVA, $p<0.05$) negative effect on the gonadosomatic index values of infected fish from both the west and south coasts. There was a declining trend in condition factor values with increasing intensity of infection category, however no significant effect was observed. The marked negative effect of infection intensity on GSI, with heavy infection possibly leading to parasitic castration, has potentially important implications for sardine population dynamics and requires further research.

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Fisheries for small pelagic fishes

Trade in fish is a major source of foreign currency earnings for many countries in addition to provision of employment. China is the leading country in terms of fisheries capture volume followed by Indonesia, India, the United States and Peru respectively (FAO, 2010). Small pelagic fishes such as sardine, anchovy and herring contribute about a third of the total annual world marine fisheries catch, which in 2010 was 63.7 million tonnes (FAO, 2010) compared to 65.7 million tonnes captured in 2009. Of the total world marine fish annual catch in 2010, sardines, anchovy and herring contributed 17 096 817t or 26.8%. The single most important species caught was *Engraulis ringens* (anchoveta) with landings of 4 205 979t or 6.6%. This was followed by *Clupea harengus* (Atlantic herring) with 2 201 334t accounting for 3.5% and *Sardina pilchardus* (European sardine) with 1 219 663t or 1.9% of the total catch that year. Most of the global small pelagic fish landings are used for fishmeal production as feed for livestock industries and aquaculture (Alder *et al*., 2008). Small pelagic fish like sardine (*Sardinops*, *Sardina*, and *Sardinella*) and anchovy (*Engraulis*) occur in most of the upwelling regions of the world's oceans and exhibit an anti-tropical distribution. They are found in all the eastern boundary current systems and in the western boundary current systems of the Indo-Pacific region (Beckley and van der Lingen, 1999). The upwelling regions, which represent less than 1% of the total area of the oceans, are suitable habitants for sardine and anchovy. This is because of the high biological production due to wind-driven upwelling, which provides the necessary nutrients that stimulate phytoplankton growth (Longhurst *et al*., 1995; Chavez and Toggweiler, 1995). The major small pelagic fishes regions include: the California Current system off California and Mexico, the Humboldt Current system off Chile, Peru and Ecuador, the Kuroshio-Oyashio Current system off Japan, the Canary Current system off Morocco and Portugal, and the Benguela Current system off the west coast of southern Africa (Figure 1.1).

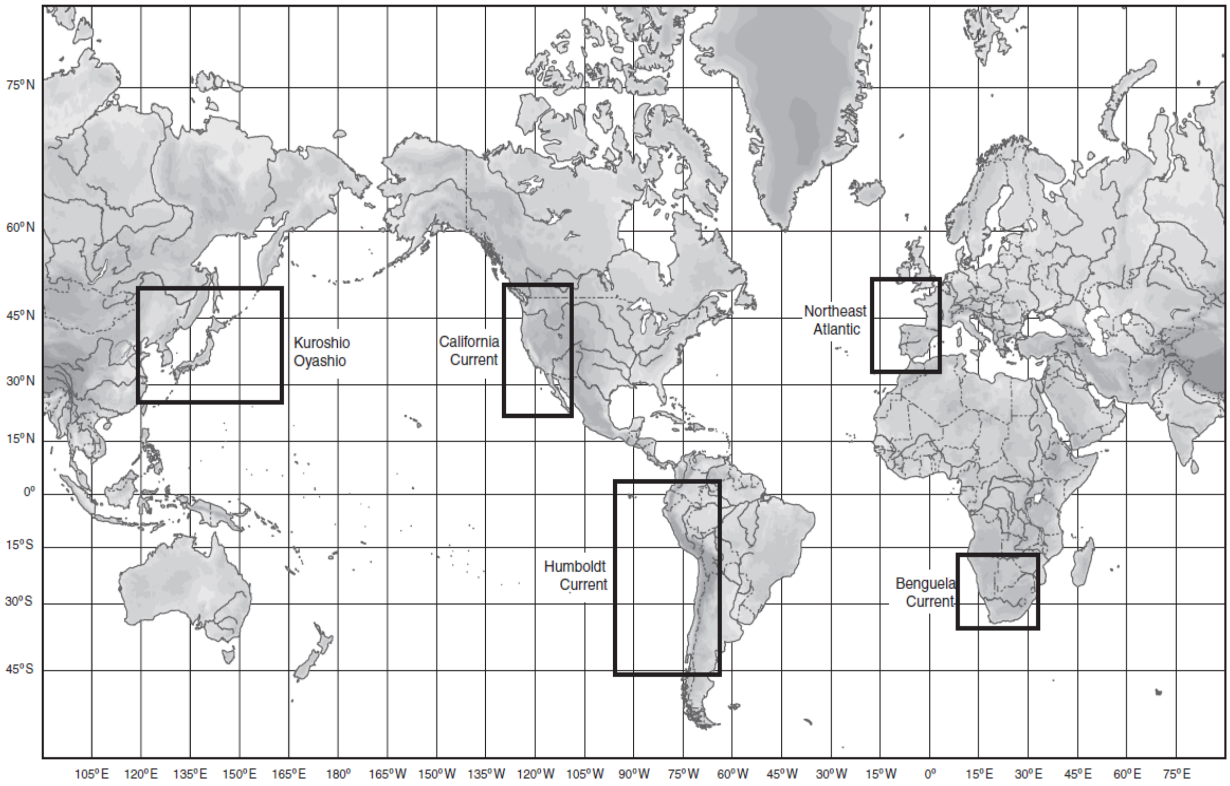


Fig. 1.1: World map showing locations of major small pelagic fishes regions (From Checkley *et al*., 2009).

The two dominant small pelagic fish species occurring in the California Current off the west coast of North America are *Engraulis mordax* (northern anchovy) and *Sardinops sagax* (Pacific sardine). *Engraulis ringens* (anchoveta) is widely distributed in the Humboldt Current off the coast of Chile, Peru, and Ecuador and *Sardinops sagax* (Pacific sardine) are found in the northern Humboldt. Sardine (*Sardina pilchardus*) are limited within the northeast Atlantic oceanographic area but widely distributed along the eastern Atlantic shelf (Checkley *et al*., 2009). The common small pelagic fish species found in the Kuroshio-Oyashio area are *Engraulis japonicus* (Japanese anchovy) and *Sardinops melanostictus* (Japanese sardine). In the Benguela Current system off the coast of southern Africa, the most common small pelagic fish species are *Engraulis encrasicolus* (anchovy) and *Sardinops sagax* (southern African sardine).

1.2 Taxonomy, distribution and life history of southern African sardine

Taxonomy

The southern African sardine *Sardinops sagax*, formerly known as *S. ocellatus* (Pappe, 1854), is one of the 13 species in the family Clupeidae that occur in southern African waters (Whitehead 1985; Beckley and van der Lingen, 1999).

Distribution of *Sardinops sagax* in the Benguela Current system

The Benguela Current system is one of the major ocean currents off the southern African coast, the other being the southward-flowing Agulhas Current on the east and south coasts of South Africa (van der Lingen *et al*., 2006c). The Benguela is located off the southwest coast of southern Africa and extends off the coasts of Angola, Namibia and South Africa, and is divided into the northern Benguela and the southern Benguela subsystems. The northern Benguela extends from the Angola-Benguela Front located between 14°S and 16°S to the Lüderitz upwelling cell, while the southern Benguela extends from Lüderitz to the Agulhas Bank off the South African south coast (Figure 1.2; Shannon, 2006; van der Lingen *et al*., 2006c; Checkley *et al*., 2009).

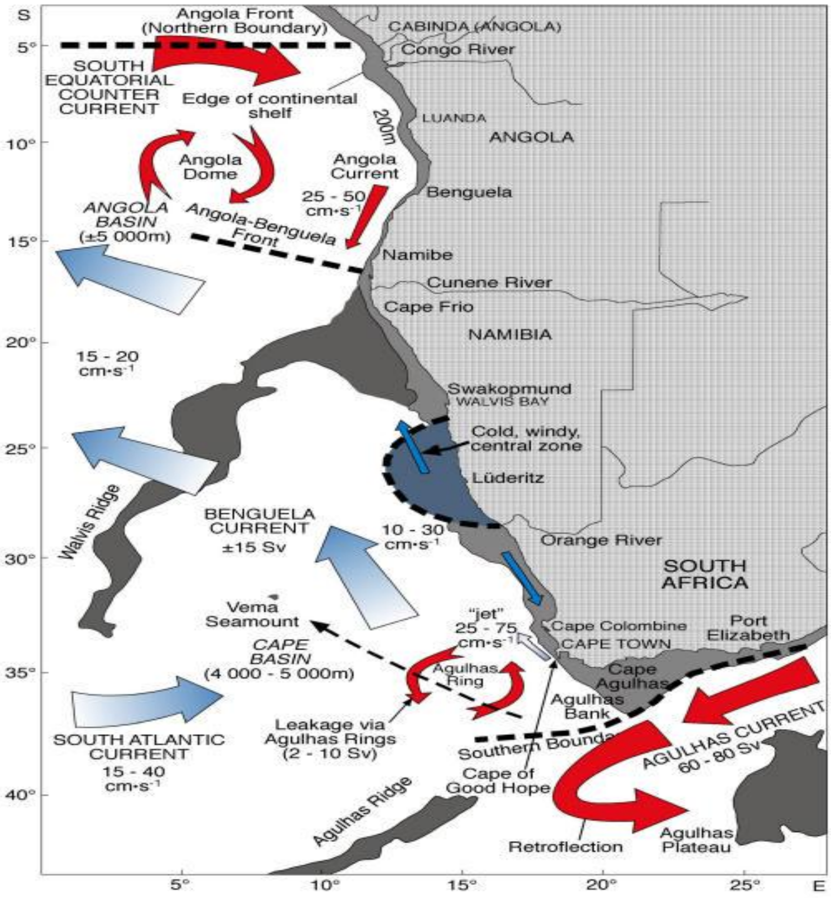


Fig. 1.2: Currents and boundaries of the Benguela Current ecosystem (From Shannon, 2006).

The habitat for the southern African sardine *S. sagax* in southern Africa extends from Baia dos Tigres in Southern Angola (14°S and 10 °E), down the west coast to Cape Town, across the Agulhas Bank and up to north of Durban on the east coast of South Africa. Other than *S. sagax,* anchovy (*Engraulis encrasicolus*) and the west coast redeye round herring (*Etrumeus whiteheadi*) are also found in the Benguela Current system.

Biology of *Sardinops sagax*

Sardinops sagax is a low trophic level marine species that feeds on phytoplankton and zooplankton, but derives the majority of its dietary carbon from zooplankton (van der Lingen, 2002). The species reaches sexual maturity at about 2-3 years old, and approximately 15-20 cm caudal length (van der Lingen *et al*., 2006a). Its growth, weight and fat content may be affected by environmental fluctuations and food supply (Blaxter and Hunter, 1982). *Sardinops sagax* are very important in the marine food web acting as a link between plankton and larger predatory fish, seabirds and mammals (Cury *et al*., 2000).

Life history of *Sardinops sagax* in the Benguela Current system

Sardinops sagax eggs and the resultant larvae are transported from South Africa’s south coast to the west coast nursery grounds by a shelf-edge jet current (van der Lingen and Huggett, 2003). From the nursery grounds on the west coast juveniles migrate against food gradients to the south coast where they attain sexual maturity at the end of their second year and spawn. A stock, which various studies suggest is distinct, migrates to South Africa’s east coast during winter (May–July) from the Agulhas Bank to spawn each year, constituting the KwaZulu – Natal sardine run (Connell, 2010). Sardines that survive the run return to the Cape along a deeper, offshore route, supported by the Agulhas Current (Davies, 1957; Connell, 2010; Freon *et al*., 2010).

Less seems to be documented on the life history of pelagic fish off Namibia, nonetheless Beckley and van der Lingen (1999) point out that the spawning habitat for the Namibian sardine stock is found north of Walvis Bay. The adult fish tend to move towards Angola in a northern direction before returning to the spawning habitat. Tjizoo (2008) points out that northern Benguela sardine select multiple ranges of temperatures for spawning, and that spawning is separated in time and space.

Fishery for *Sardinops sagax* in South Africa

In South Africa, the fishing sector is dominated by pelagic and demersal fisheries which contribute about 68% of the total value of the sector. The fishing sector production represents about 0.5% of the national gross domestic product (GDP), and it employs approximately 27 000 people with an additional 81 000 people employed indirectly (DAFF, 2012). The pelagic sector has an average allowable annual catch of 400 000t and contributes approximately R1 billion to the South African economy. Commercial fishing of *S. sagax* started in the Western Cape in the 1930s using purse-seiners operating mainly from St Helena Bay and Lambert's Bay. The industry grew rapidly during the 1940s and expanded to Namibia, with vessels fishing from Walvis Bay and Luderitz. Fishing for sardines also takes place along South Africa's south and east coast, although beach-seine and not purse-seine nets are used off the east coast to catch sardine during the sardine run (Beckley and van der Lingen, 1999; van der Lingen *et al*., 2010a). Sardine, and initially adult horse mackerel *Trachurus capensis*, were the primary target of the purse-seiners in the early years of the fishery, and sardine catches off South Africa peaked at 410 000 metric tons in 1962 (Figure 1.3).

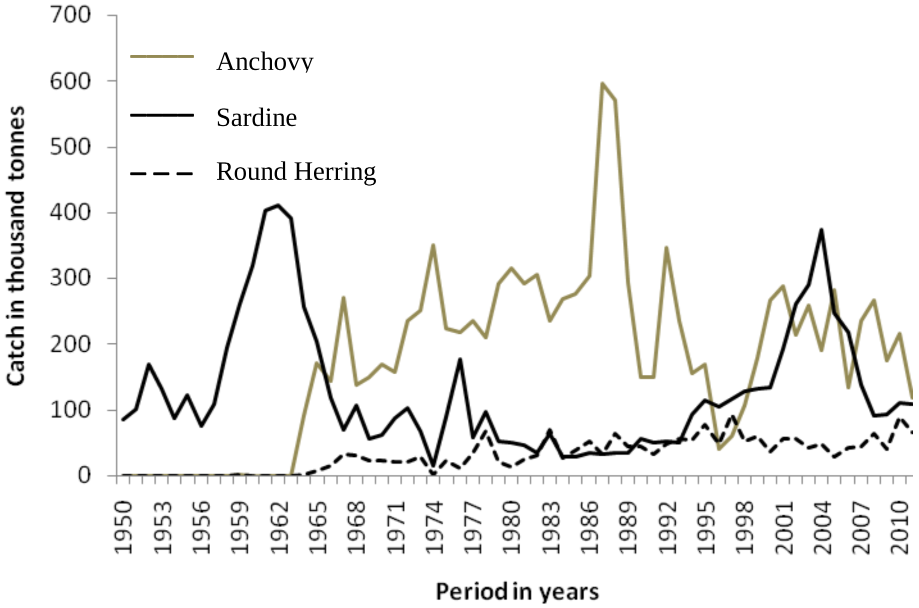


Fig. 1.3: Annual catches (000t) of anchovy, sardine and redeye round herring taken off South Africa over the period 1950-2010 (From van der Lingen *et al*. (2006c), updated using data supplied by Department of Agriculture, Forestry and Fisheries).

Sardine catches declined rapidly thereafter to below 100 000t although there was a slight recovery in the mid 1970s with catches reaching almost 200 000t (van der Lingen *et al*., 2006c). However, this recovery was short-lived and catches remained low, being around 50

000t from the end of the 1970s to the end of the 1980s. The effect on the fishing community was loss of employment, reduced earnings and bankruptcy of some companies involved. Following this decline in sardine catches during the 1960s, the fishery switched to smaller-meshed nets and began to target anchovy. Catches of this species soon became dominant and increased to a peak of about 600 000t in 1987 and 1988 (Figure 1.3). Sardine catches increased steadily from early the 1990s with ≥ 100 000t landed from 1995 onwards and peaked at almost 400 000t in 2004, before declining rapidly to current levels of around 100 000 metric tons (van der Lingen *et al*., 2006c; Figure 1.3).

1.3 Stock structure of *Sardinops sagax* in the southern Benguela Current system

Previously the South African sardine *S. sagax* was believed to comprise a single stock throughout its range, but recent studies have suggested the existence of multiple stocks. Coetzee *et al*. (2008) observed sardine distribution patterns at different biomass levels and noted a consistent separation in their distribution in the area of Cape Agulhas. That led those authors to suggest the occurrence of two discrete sardine stocks in the southern Benguela, one found to the west side of Cape Agulhas and the other located to the east. Miller *et al*. (2006) used an individual based model coupled to a 3D hydrodynamic model to examine spatial variability in sardine spawning and recruitment grounds, and concluded that sardine life history strategy could be divided into two main systems that were separated by Cape Agulhas. These observations have provided more evidence for the existence of two possible stocks of South African sardine off the west and south coasts. Additionally, a distinct sardine spawning area is found off the east coast during the annual winter, KwaZulu-Natal sardine run (Connell, 2010). Sardine found in this area show differences in some biological characteristics compared to fish from the putative western and southern stocks (van der Lingen *et al*., 2010b). Hence, fish that participate in the sardine run may represent a third stock (Connell, 2010; Freon *et al*., 2010). The South African sardine population may therefore consist of three stocks; a western stock found on west coast, a southern stock found on the south coast, and an eastern stock found off the eastern coast.

1.4 Parasites as biological tags for determining stock structure

In fisheries management, parasites can provide useful information about the stock structure and thus facilitate efficient fishery management. Fish stock discrimination is particularly important for economically valuable species as it may help to establish sustainable use of the fisheries resource, and 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 has several advantages 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). For example McGladdery and Burt (1985) used parasites to establish the migration, feeding and spawning behaviours of the Atlantic herring (Clupea harengus). Studies where parasites have been used successfully to distinguish fish stocks include those by Sindermann (1957a, 1957b and 1961; all reported in Sindermann, 1983) who used cestode larvae as a biological tag and showed that there is no intermingling between Atlantic herring (*Clupea harengus*) occurring in the Gulf of St. Lawrence and Gulf of Maine in the Northwest Atlantic. Moser and Hsier (1992) used parasite assemblages to suggest separation of Pacific herring (*Clupea harengus pallasi*) spawning stocks in California. Similarly, Braicovich and Timi (2008) used the same technique to identify three stocks of the Brazilian flathead *Percophis brasiliensis* off the coast of Argentina and Uruguay. The criteria for selection of a parasite or group of parasites involve examining infection level in different localities, and understanding the parasite biology (MacKenzie, 1983; MacKenzie and Abaunza, 1998 and 2005).

1.5 The Coccidia parasite *Eimeria sardinae*

The high diversity of organisms inhabiting the marine environment contributes to high diversity of marine parasites, and all marine fish can be potential hosts depending on the locality, season, local ecology and parasite species in question (Rohde, 2005). Marine parasites effects on hosts and infection intensity vary depending on the nature of the parasite, target organ and the habitat of the host (Sindermann, 1990). In the subkingdom protozoa, coccidia are among the most studied group because of the economic importance of the hosts they infect. The genus *Eimeria* (Schneider, 1875) which belongs to subclass coccidia is characterised by the presence of oocysts with 4 sporocysts, each containing 2 sporozoites (Figure 1.4).

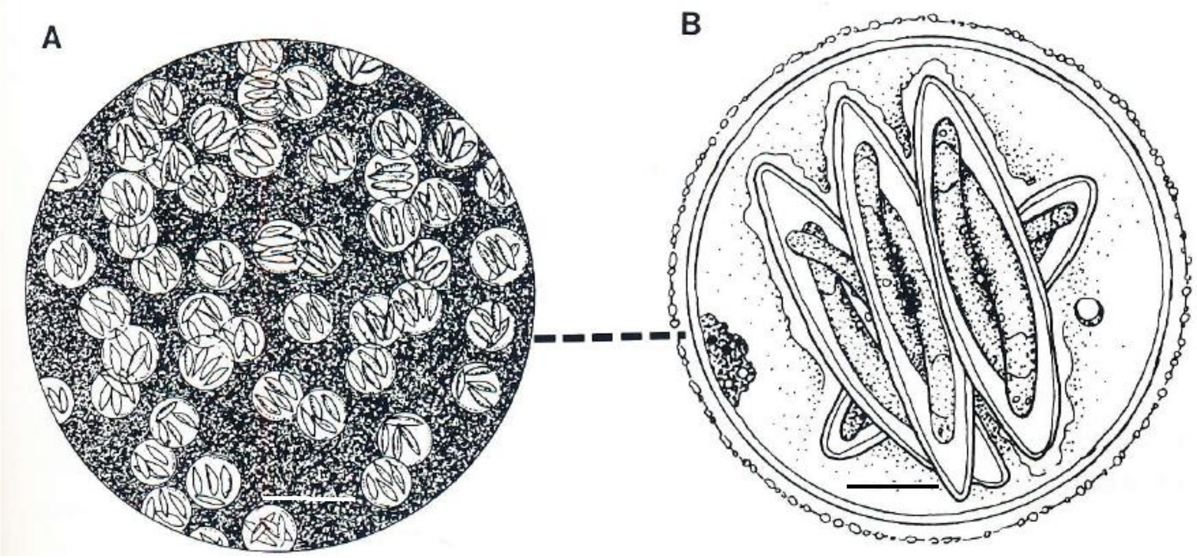


Fig. 1.4: A: Severe infection by *Eimeria sardinae* oocysts of the testes of herring *Clupea harengus* L. as observed at low power under a light microscope (scale bar = 250 *μ*m); B: Mature oocyst enclosing four sporocysts each containing two sporozoites (scale bar = 5 *μ*m). (From Sindermann, 1990).

Different species of *Eimeria* can be identified based on the host species they infect, the target organs and the morphology of oocysts (Pinto, 1956; Joyner, 1982). In fact many of the *Eimeria* species are known based on morphology of the parasite development stages observed in the host (Dykova and Lom, 1981; Joyner 1982**).**

Distribution of *Eimeria*

The genus *Eimeria* contains about 1,340 named species distributed largely in mammals, birds, and reptiles, while over 100 species from the genus *Eimeria* have been described from marine and freshwater fishes (Molnar, 1979; Levine, 1982; Sindermann, 1990). One of these is *Eimeria sardinae* (Thélohan, 1820) (Reichenow, 1921) which has been identified in several species of marine fish from different parts of the world as shown in Table 1.1.

Table 1.1: *Eimeria sardinae* (Thélohan, 1820) (Reichenow, 1921) identified in different fish hosts from different parts of the world

Marine host fish where *Eimeria sardinae* was recorded	**Site of infection**	**Locality/Geographic area**	**Reference**
Sardina pilchardus (European pilchard)	Testes	Off Portugal	Pinto (1956)
Clupea harengus L. (Atlantic herring) *Clupea harengus* L. (Atlantic herring)	Testes Testes	Western North Atlantic Nova Scotia area	Sindermann (1961) in Sindermann (1990) Morrison and Hawkins (1984)
Clupea harengus (Scottish herring)	Testes	North sea and off Scottish west coast	Kabata (1963)
Clupea harengus membras (Baltic sea herring)	Testes	Estonian Archipelago and Gulf of Finland	Turovsky *et al*. (1993)
Sprattus sprattus (Sprat)	Testes	Estonian Archipelago and Gulf of Finland	Turovsky *et al*. (1993)
Sardinella maderensis (Madeiran sardinella)	Testes	Off Senegal Coast	Diouf and Toguebaye (1994)
Sardina pilchardus (Mediterranean sardine)	Testes and ovaries	Off Tunisia	Draoui *et al*. (1995)
Sardinella aurita (Round sardinella)	Testes and ovaries	Off Tunisia	Draoui *et al*. (1995)
Sardinops sagax (South African sardine)	Testes	Off South Africa	Reed *et al*. (2012)

Effects of *Eimeria sardinae* on infected marine fish

One of the earliest studies on the effects of *E. sardinae* was done by Pinto (1956) who concluded that heavy infection by the testicular coccidian parasite could lead to reduced fecundity of the host fish. Since then several studies have been done. Diouf and Toguebaye (1994) reached a similar conclusion based on the study of occurrence of *E. sardinae* in male *Sardinella maderensis*, while Mackenzie (1981) reported that an unnamed species of *Eimeria* caused Blue whiting (*Micromesistius poutassou*) to weigh less than uninfected fish of similar age. Other studies include those of Odense and Logan (1976) who pointed out that *Eimeria gadi* intensity in the swim bladder of haddock (*Melanogrammus aeglefinus*) inhibits their locomotion and spawning behaviour; and of Solangi and Overstreet (1980) who believed *Eimeria funduli* may render the organs of killifish (*Fundulus heteroclitus*) in which they occur non-functional. Some coccidians like *Eimeria sinensis*, *E. subepitheliali* and *Goussia carpelli* are economically important as they may cause death of commercially important species such as reared carp (Molnar 1979; Dykova and Lorn 1981), but mass mortalities of marine fish due to parasite infection are rare (Rohde, 1984).

In southern Africa, virtually nothing is known about the incidence of fish-infecting coccidia and their impacts. A recent study by Reed *et al*. (2012) identified *E. sardinae* from South African sardine but the authors did not examine its effect on the host fish. *Eimeria sardinae* infection occurs through ingestion by the potential host. The oocysts are released with sperm at spawning grounds by infected fish (Draoui *et al*., 1995). In the host fish gut, the oocystic and sporocystic wall ruptures releasing sporozoites which go through a series of development stages. The developing oocysts finally settle in the testes where they undergo further development before being released with sperm during spawning (Dykova and Lom, 1981; Draoui *et al*., 1995).

1.6 Using parasites as biological tags in southern Africa

There is little information on the use of parasites as biological tags in South African fish. One of the few studies done so far was that of Linde (2011), who examined the occurrence of *E. sardinae* in 141 male sardines from the South African west and south coasts and reported an overall prevalence of 7.1%, with sardine from the South African west coast showing higher prevalence than those collected from the south coast. Based on these results Linde (2011) considered that *E. sardinae* could be used as a biomarker to distinguish between stocks of South African sardine. Fish which had a caudal length of less than 13 cm showed no signs of infection (Linde, 2011).

Reed *et al*. (2012) identified seven parasite taxa in the South African sardine, including *E. sardinae*, with forty percent of male sardines examined being infected by *E. sardinae*, and fish from the west coast showing higher infection prevalence than those collected from the south coast. Those authors recommended a digenean parasite found in the eyes to have the greatest potential as a biological tag.

1.7 Aims of the study

The aims of the present study were;

1) To increase knowledge on the distribution, prevalence and infection intensity of the coccidian *E. sardinae* infecting sardine off the coast of southern Africa.
2) To assess the usefulness of *E. sardinae* as a potential biological tag for examining stock structure of the southern African sardine.
3) To determine the possible effects of infection *E. sardine* on the host. This objective was examined using the following hypotheses;
    i) Null Hypothesis (H_0): There are no significant physiological effects of *E. sardinae* infection on the host.
    ii) Alternative Hypothesis (H_A): There are significant physiological effects of *E. sardinae* infection on the host.

CHAPTER 2: MATERIALS AND METHODS

2.1 Sample collection

Sardines collected from around the southern African coast (Figure 2.1) between June 2010 and February 2012 were examined during this study.

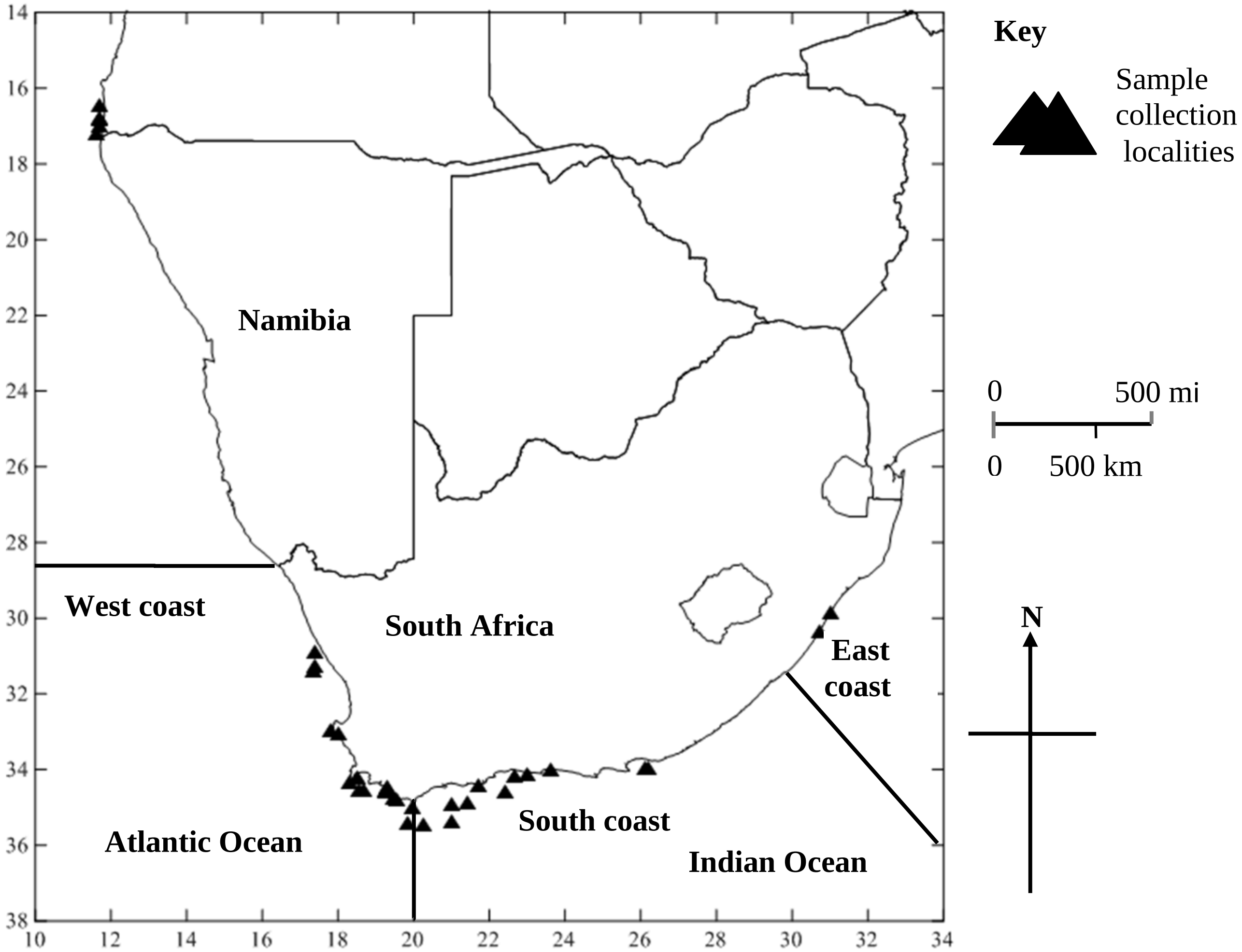


Fig. 2.1: Map of southern Africa showing locations where samples were collected from the northern Benguela, and off the west, south and east coasts of South Africa.

Most of the samples from the South African west and south coast were collected from mid-water trawls made during surveys to estimate pelagic fish biomass off South Africa conducted by the Department of Agriculture, Forestry and Fisheries (DAFF), with one south coast sample taken from a commercial purse-seine catch. East coast samples were taken from commercial beach-seine net catches made during the sardine run. Samples from the northern Benguela were collected from mid-water trawls made during a survey by the Ministry of Fisheries and Marine Resources (MFMR) to estimate pelagic fish biomass off northern

Namibia and southern Angola. For all samples, fish were placed in plastic bags and labelled with date and collection locality, and were frozen soon after collection.

A sub-total of 57 fish was collected from 5 stations in the northern Benguela, 248 fish from 18 stations off the South African west coast 151 fish from 11 stations off the South African south coast and 97 fish from 2 stations off the South African east coast. Sample collection details are given in Table 2.1.

Table 2.1: Sample collection details of Southern African sardine examined in this study including region, number of stations, station code, latitude and longitude, and date of collection per station.

Region	Code	Latitude ºS	Longitude ºE	Date
Northern Benguela (5)	54	16.4460	11.6968	11-Aug-11
	63	16.7863	11.6975	13-Aug-11
	64	16.8363	11.6840	13-Aug-11
	70	16.9913	11.6802	14-Aug-11
	80	17.1862	11.6298	15-Aug-11
S. A. west coast (18)	A30609	33.0622	18.0270	4-Jun-10
	A30944	34.3178	18.2853	23-Oct-10
	A30877	31.2672	17.4063	15-Oct-10
	A31837	34.4608	19.2987	12-Jun-11
	A31820	34.2158	18.4913	10-Jun-11
	A31823	34.5450	18.6713	11-Jun-11
	A31822	34.5443	18.5575	10-Jun-11
	A31839	34.7447	19.4637	12-Jun-11
	A31955	35.4033	19.8433	17-Nov-11
	A31943	34.7883	19.5550	16-Nov-11
	A31944	35.2252	19.5255	16-Nov-11
	A31934	34.5500	19.2683	15-Nov-11
	A31932	34.6000	18.8510	15-Nov-11
	A31891	32.9467	17.7933	9-Nov-11
	A31881	31.3983	17.3627	8-Nov-11
	A31924	34.9327	18.7498	15-Nov-11
	A31954	35.0150	19.9717	17-Nov-11
	A31878	30.9050	17.3883	7-Nov-11
S. A. south coast (11)	A32128	33.9730	26.1152	07-Dec-11
	A32086	34.0178	23.6168	03-Dec-11
	A32063	34.1562	22.6695	01-Dec-11
	A31995	35.3643	20.9977	25-Nov-11
	A31965	35.4435	20.2438	21-Nov-11
	A32031	34.4125	21.7223	27-Nov-11
	A32050	34.5962	22.4060	29-Nov-11
	A32006	34.8592	21.4043	26-Nov-11
	A31996	34.9327	20.9977	25-Nov-11
	A32075	34.1258	22.9845	02-Dec-11
	PECOM	33.9625	26.2167	27-Feb-12
S. A. east coast (2)	Addington Beach	29.8392	31.03660	01-Jul-11
	Rocky Bay	30.3340	30.7343	26-Jun-11

2.2 Sample processing and examination

Sardine specimens were thawed and processed in the Department of Zoology laboratory, at the University of Cape Town. Since other studies have shown that *E. sardinae* target the testes of adult fish, only male sardines with caudal length (CL) of ≥ 13.0 cm were considered for this study. Fish were weighed using an electronic scale and their wet body mass (WBM) recorded to the nearest 0.1g and their CL was then measured to the nearest centimetre. The sardines were then cut open and their testes removed and weighed to the nearest 0.1 g. Pea-sized portions from the anterior, middle and posterior regions of the left and right testes were removed and placed on glass slides. A drop of distilled water was added to each section which was then squashed under a cover slide and the prepared sections were then examined for *E. sardinae* infection using a light microscope (LEICA DM 500; magnification range 4X-1000X). Infected gonads were recognised by the presence of oocysts scattered in the gonad tissue. The number of oocysts was recorded from each section of each testis and these were then summed to give the relative number of *E. sardinae* oocysts in the fish examined. Photomicrographs of infected and non-infected testes tissue were taken under a camera fitted to the light microscope. To illustrate the physiological effects of *E. sardinae*, photographs of severely infected fish gonads were taken using a digital camera Canon EOS 350D 18-55 mm lens. Infected testes were finally preserved in 70% ethanol contained in labelled vials. The sardine specimens were repackaged in plastic bags and frozen at -20 °C for later examinations for additional parasites.

2.3 Data analysis

Sardines were recorded as being infected if one or more *E. sardinae* oocysts were observed in their testes, and infection prevalence and mean infection intensity determined for each station following Bush *et al*. (1997). Infection prevalence was calculated as the number of infected fish divided by sample size, and then multiplied by one hundred. Mean infection intensity was calculated by dividing the total number of oocysts counted by the number of infected fish from each station. Histograms, bar graphs and scatter plots were made to examine the trends of *E. sardinae* infection.

Spatial variability in infection prevalence and mean infection intensity of male sardine by the coccidian parasite was assessed by plotting histograms of *E. sardinae* infection throughout the study area. And then t-tests done on data from the west coast and south coast to assess whether there was a significant difference in mean infection prevalence and mean infection intensity between the South African west and south coasts.

The physiological effects of *E. sardinae* infection were assessed by first examining the gonadosomatic index (GSI) and condition factor (CF) of both infected and un-infected fish. Fish were assigned to one of the five categories based on their infection intensity, the categories being un-infected (no parasites), low infection (1-10 oocysts per fish), moderate infection (11-100 oocysts per fish), high infection (101-1000 oocysts per fish) and severe infection intensity (>1000 oocysts per fish). Scatter plots and bar graphs of GSI and infection intensity categories were obtained to establish possible relationship. A single factor analysis of variance (ANOVA) was used to assess the mean relationship between mean GSI values and infection category on the combined data set of west coast and south coasts, followed by a *post-hoc* multiple comparison Tukey test conducted to indicate which categories differ from each other (Zar, 1999).

GSI was obtained from the expression:

$$GSI = \frac{Gonad\ mass\ (g)}{Observed\ wet\ body\ mass - gonad\ mass\ (g)} x\ 100 \quad \text{..................................} (1)$$

Condition factor (CF) of each sardine was calculated using the expression:

$$CF = \frac{Observed\ wet\ body\ mass\ (g)}{Expected\ wet\ body\ mass\ (g)} \quad \text{..} (2)$$

Expected wet body mass was estimated from a length mass relationship derived by fitting a non-linear regression to the untransformed wet body mass in grams (as the y-axis) and caudal length in cm (x-axis) data of sardine from all four regions (n=553).

The relationship obtained was; $y = 0.0144\ x^{2.9091}$ ………………………………………. (3)

$r^2 = 0.8402$

The parameters from equation (3) were used to estimate expected body weight of the fish, and fish condition factors for all four regions were obtained using equation (2).

Scatter plots and bar graphs of CF and infection intensity categories were obtained to establish possible relationships. A single factor analysis of variance (ANOVA) was used to compare the mean relationship between the CF values among infection categories on the combined data set of west coast and south coasts. Before ANOVA tests were done, assumptions for normality and homoscedasticity were tested and found to be met for both GSI and CF data (results are shown in Appendices I and II). One way ANOVA was preferred since we had more than one groups drawn from the same population and comparing their means.

CHAPTER 3: RESULTS - SPATIAL PATTERNS IN *EIMERIA* INFECTION OF SARDINE AND RELATIONSHIP WITH FISH SIZE

3.1 Results

A total of 553 sardines from the four regions (the northern Benguela (NB), and the South African west coast (SAWC), south coast (SASC) and east coast (SAEC) were examined. Individual oocysts were clearly visible through the light microscope (Figure 3.1).

Fig. 3.1: Photomicrograph of an *Eimeria sardinae* from oocyst enclosing four sporocysts observed in the testis of *Sardinops sagax.*

Only sardines collected from the South African west and south coasts were infected by *E. sardinae,* with 101 infected sardines out of 248 from the SAWC and 89 infected sardines out of 151 from the SASC (Table 3.1). The intensity of infection ranged from 1 to 2054 oocysts on the west and south coasts of South Africa. The average prevalence (±SE) for sardine off the SAWC was 49.3 ± 6.3% and the mean infection intensity (± SE) was 49.5 ± 15.8 oocysts per fish. Sardine from the SASC had an average prevalence (± SE) of 48.1 ± 8.3%, and a mean infection intensity (± SE) of 69.8 ± 33.5 oocysts per fish. One station on the South African south coast stood out starkly because fish were heavily infected with a mean infection intensity (± SE) of 397.3 ±11.8 oocysts per fish. No infected sardines were observed from the northern Benguela or from the South African east coast.

Table 3.1: Number of sardine examined (n), mean caudal length (CL), ± standard error (SE), prevalence (%), infection range(Minimum to maximum), and mean intensity and ± standard error (SE) of *Eimeria sardinae* in *Sardinops sagax* per locality in each of the four study regions off southern Africa.

Region	**Sample size (n)**	**Mean CL (cm) ± SD**	**Prevalence (%)**	**Infection Range Min - Max**	**Mean Infection intensity± SE**
Northern Benguela	14	15.3 ±0.6	0.0	0	0.0 ± 0.0
Northern Benguela	15	16.6 ±0.6	0.0	0	0.0 ± 0.0
Northern Benguela	10	17.1 ±0.5	0.0	0	0.0 ± 0.0
Northern Benguela	7	16.2 ±0.8	0.0	0	0.0 ± 0.0
Northern Benguela	11	13.9 ±1.1	0.0	0	0.0 ± 0.0
Total	**57**	**15.8 ±0.7**	**0.0**	**0**	**0.0 ± 0.0**
S. A. west coast	29	19.1 ±1.3	24.1	1 - 22	6.0 ±0.3
S. A. west coast	45	16.4 ±1.8	24.4	1 - 20	6.6 ±0.1
S. A. west coast	13	19.1 ±0.5	0.0	0	0.0 ± 0.0
S. A. west coast	12	19.4 ±0.6	41.7	1 - 22	8.0 ±0.6
S. A. west coast	11	16.5 ±0.6	63.6	1 - 491	122.3 ±15.7
S. A. west coast	9	18.4 ±0.7	66.7	7 - 47	20.3 ±1.7
S. A. west coast	24	18.5 ±1.2	50.0	1 - 127	20.4 ±1.5
S. A. west coast	7	16.7 ±0.6	50.0	3 - 22	9.8 ±1.1
S. A. west coast	9	15.7 ±1.8	55.6	6 - 121	50.2 ±10.6
S. A. west coast	7	18.1 ±0.7	28.6	15 - 458	236.5 ±44.7
S. A. west coast	16	18.3 ±1.1	12.5	3 - 4	3.5 ±0.04
S. A. west coast	11	18.1 ±0.7	63.3	15 - 458	92.3 ±8.3
S. A. west coast	7	18.3 ±1.9	51.1	3 - 4	58.8 ±3.5
S. A. west coast	7	18.1 ±1.1	71.4	8 - 256	21.1 ±2.3
S. A. west coast	6	18.2 ±0.3	100.0	4 - 35	21.5 ±2.7
S. A. west coast	7	16.4 ±1.6	28.6	16 - 291	153.5 ±27.7
S. A. west coast	6	17.6 ±0.8	100.0	5 - 210	46.8 ±13.3
S. A. west coast	22	18.9 ±0.5	50.0	3 - 68	12.8 ±1.7
Total	**248**	**17.8 ±0.9**	**49.3 ±6.3**		**49.5 ±15.8**
S. A. south coast	8	8.4 ±1.4	75.0	4 - 157	42.6 ±7.5
S. A. south coast	11	17.6 ±1.2	90.9	4 - 137	63.4 ±5.0
S. A. south coast	6	18.3 ±0.7	50.0	4 - 84	36.0 ±7.1
S. A. south coast	7	14.6 ±0.6	0.0	0	0.0 ± 0.0
S. A. south coast	16	13.8 ±0.8	12.5	6 - 35	24.3 ±0.1
S. A. south coast	15	17.7 ±0.9	40.0	4 - 194	80.0 ±5.3
S. A. south. coast	7	18.8 ±0.5	42.9	2 - 9	5.3 ±0.5
S. A. south coast	14	18.3 ±0.7	21.4	13 - 45	23.0 ±1.4
S. A. south coast	10	15.3 ±2.4	30.0	4 - 121	47.7 ±6.3
S. A. south coast	12	18.1 ±0.6	66.7	8 - 121	48.4 ±3.6
S. A. south coast	45	17.9 ±0.7	100.0	11 - 2054	397.3 ±11.8
Total	**151**	**17.1 ±0.9**	**48.1 ±9.7**		**69.8 ±33.5**
S. A. east coast	30	16.2 ±1.1	0.0	0	0.0 ± 0.0
S. A. east coast	67	17.5 ±0.5	0.0	0	0.0 ± 0.0
Total	**97**	**16.9 ±0.8**	**0.0**		**0.0 ± 0.0**

3.2 Variation of *Eimeria* infection with sardine size

Prevalence of infection and *S. sagax* size

Sardinops sagax caught on both the west and south coasts ranged from 13.5 to 20.5 cm caudal length, dominated by *S. sagax* of caudal length between 18.0 and 19.5 cm. For west coast fish, apart from a 100% infection prevalence value at 13.5 cm caudal length (which is from a single fish), there was a gradual increase in *E. sardinae* infection prevalence in *S. sagax* with increasing size up to 17.5 cm then infection levels declined with increasing size to caudal length 20.5 cm (figure 3.2).

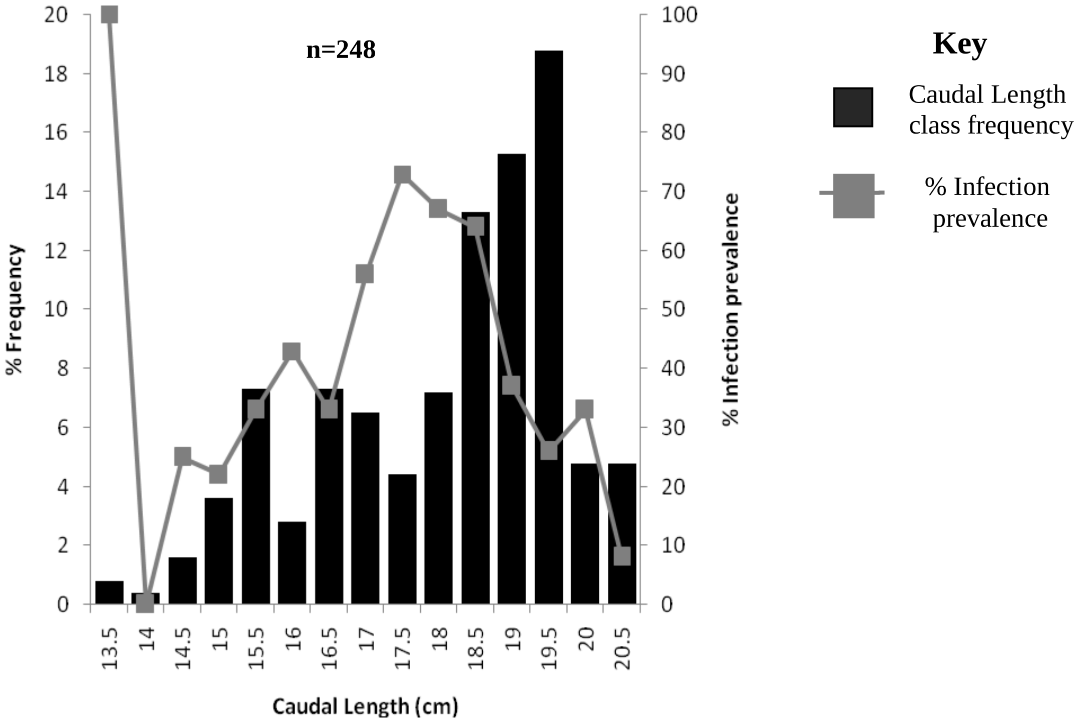


Fig. 3.2: Length class frequency distribution of *Sardinops sagax* and infection prevalence of *Eimeria sardinae* on the South African west coast.

On the south coast, a similar trend of infection prevalence with increasing size class was observed. *Sardinops sagax* of the smaller size classes (<16 cm CL) tended to have low prevalence, with the exception of fish of (13.5 cm CL where two thirds of fish were infected. High infection prevalence was recorded with increasing size. Infection prevalence declined in sardine with caudal length of 19.5 to 20 cm (Figure 3.3).

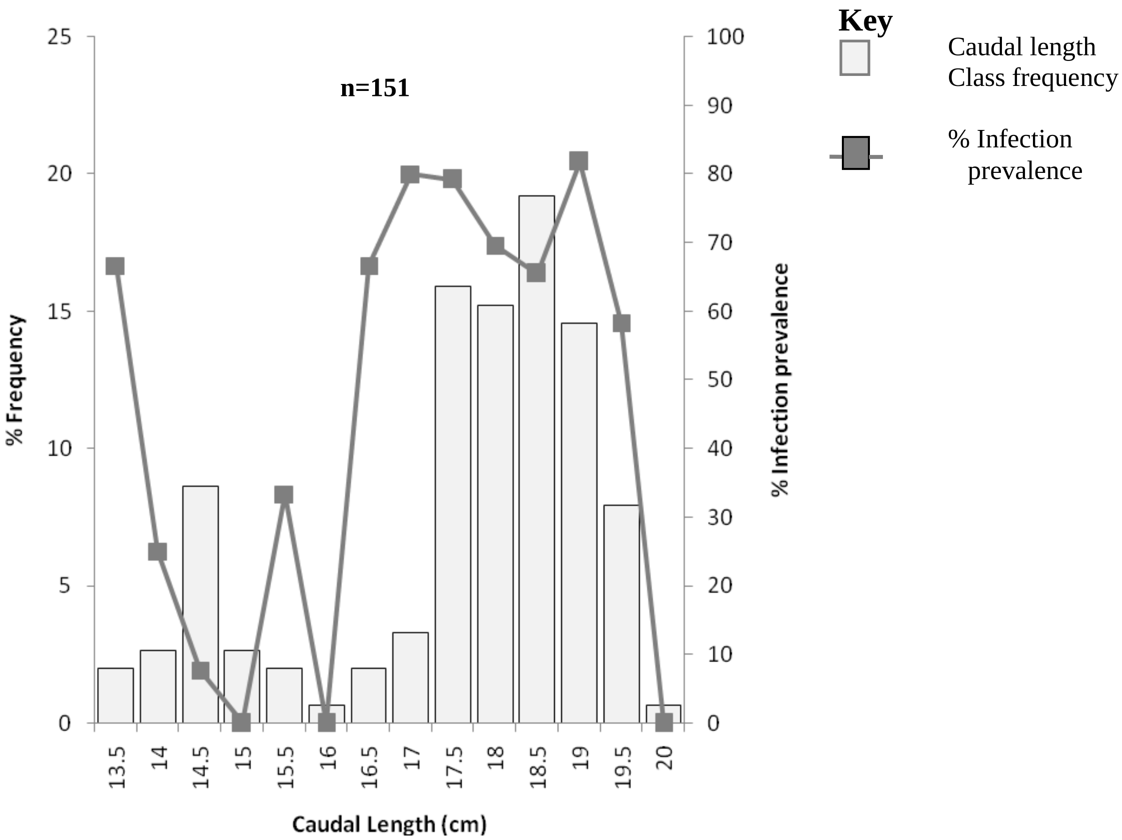


Fig. 3.3: Length class frequency distribution of *Sardinops sagax* and infection prevalence of *Eimeria sardinae* on the South African south coast.

Infection intensity and *S. sagax* size

On the west coast, infection intensity of *E. sardinae* increased with size class (Fig. 3.4) from sardine of 14 cm caudal length and reached the peak in sardine of 16 cm caudal length. After this size, infection intensity decreased gradually with increasing caudal length. On the south coast however, large fish show a higher infection intensity of *E. Sardinae* (Figure 3.5).

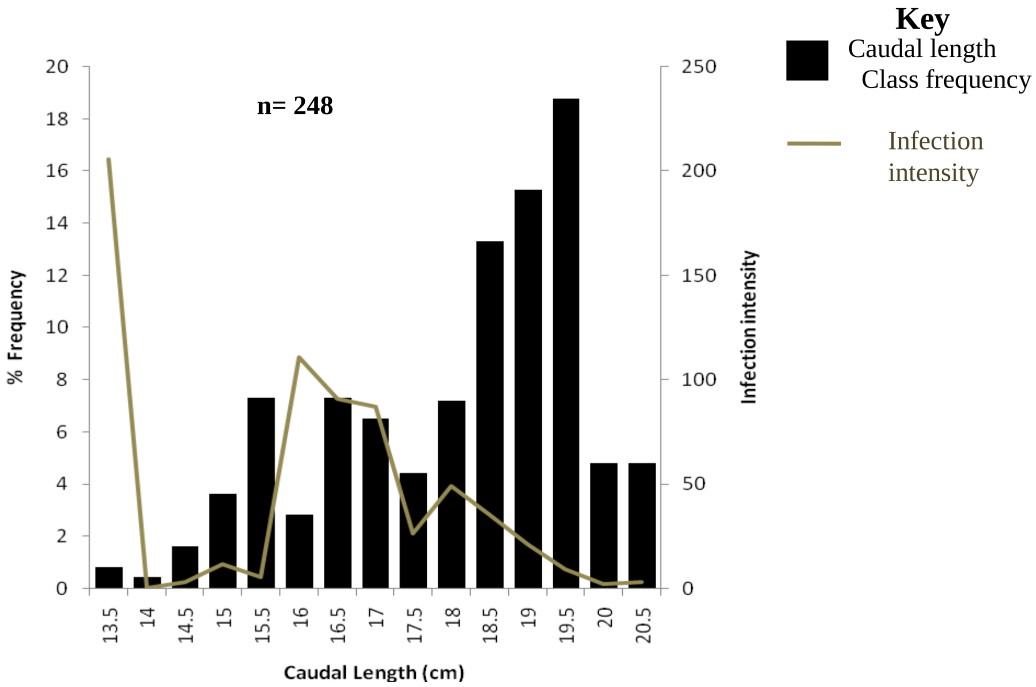


Fig. 3.4: Length class frequency distribution of *Sardinops sagax* and infection intensity of *Eimeria sardinae* on the South African west coast.

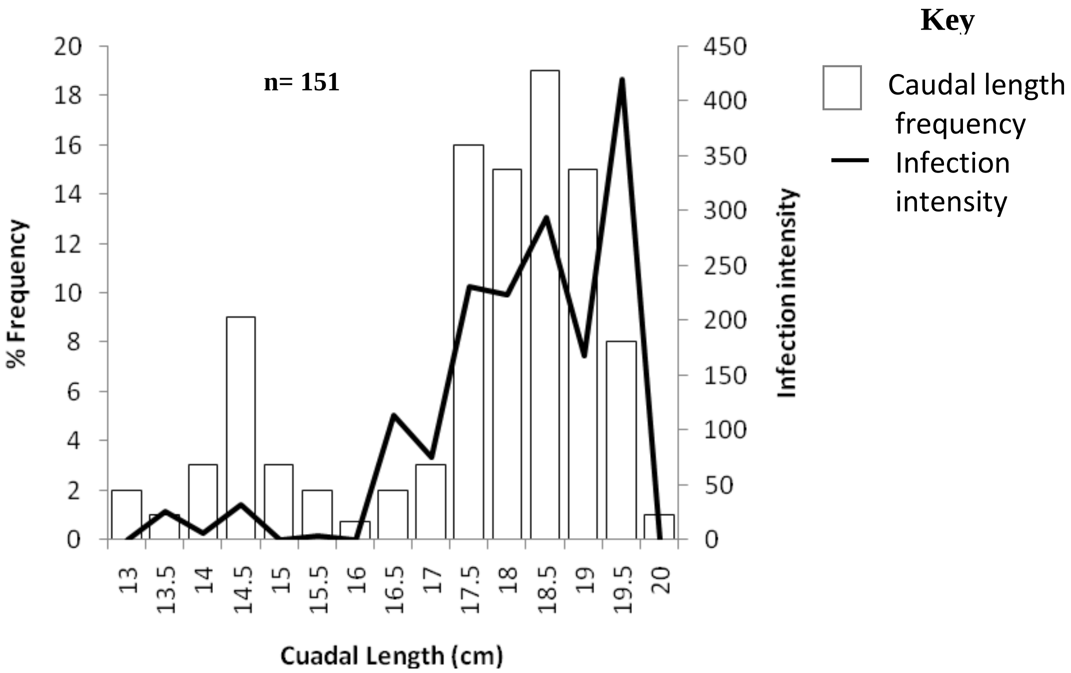


Fig. 3.5: Length class frequency distribution of *Sardinops sagax* and infection intensity of *Eimeria sardinae* on the South African south coast.

Spatial variability of *Eimeria sardinae* infection prevalence in *S. sagax*

Eimeria sardinae infection prevalence in southern African sardine varied spatially, from zero prevalence in *S. sagax* from the northern Benguela and South African east coast, to high and severe prevalence in sardine on the west and south coasts. Mean infection prevalence for South African west coast and south coast is shown in Figure 3.6.

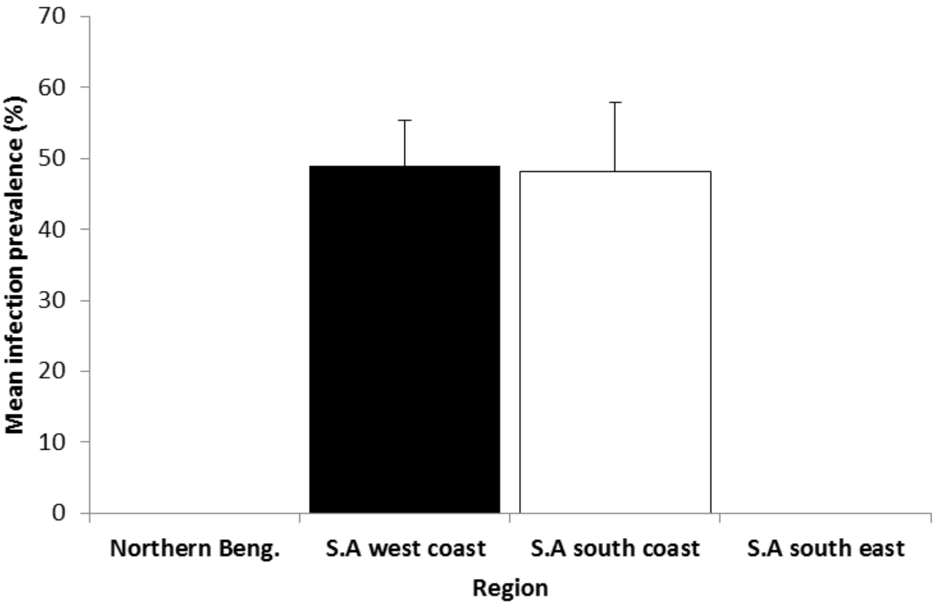


Fig. 3.6: Mean infection prevalence of *Eimeria sardinae* in *Sardinops sagax* from the northern Benguela, the South African west, south and east coasts, including standard error bars.

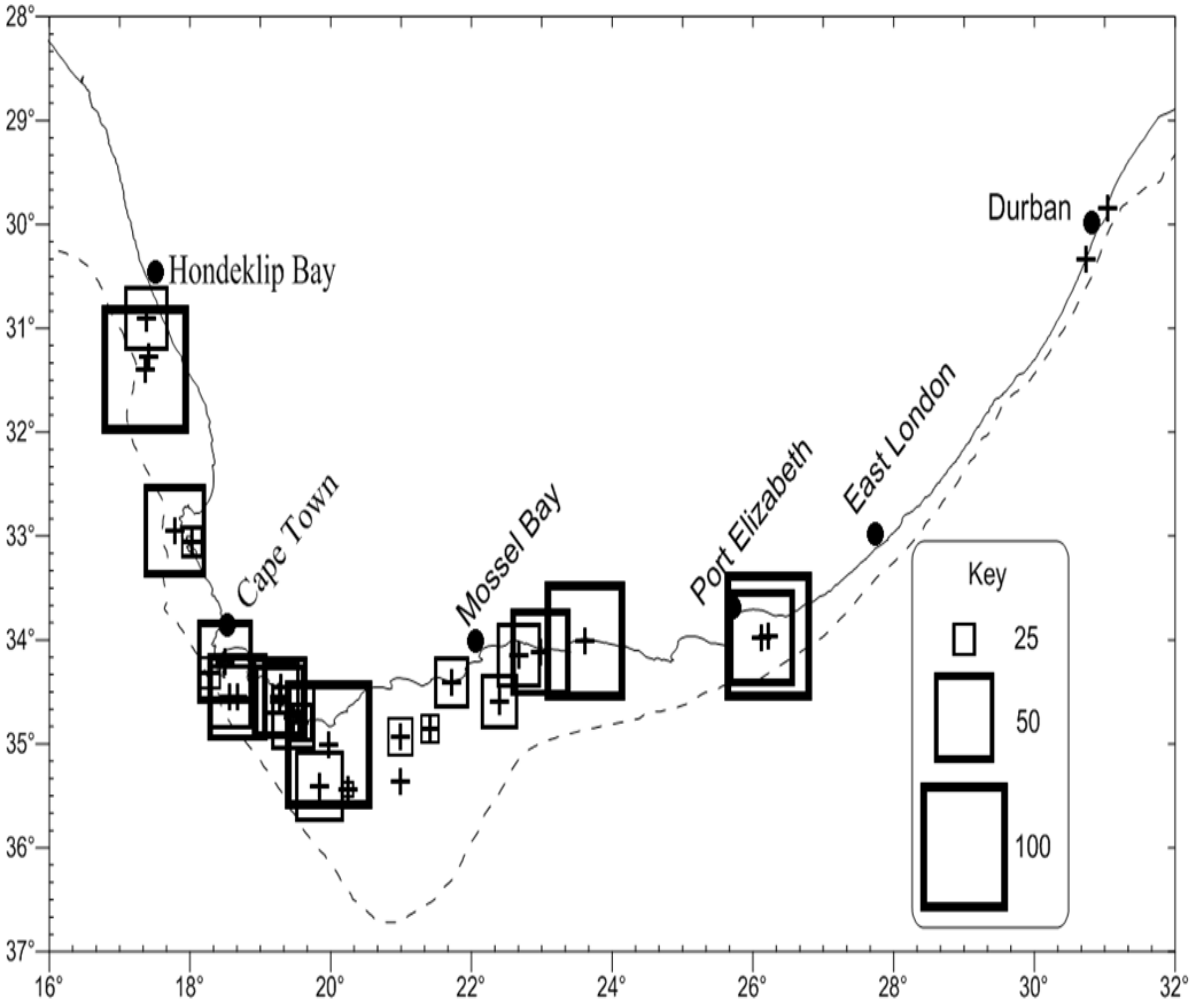


Fig. 3.7: Map showing infection prevalence (black boxes) of *Eimeria sardinae* in *Sardinops sagax* collected from the South African west, south and east coasts. Also shown (---) is the 200-m isobath. Symbol size is proportional to infection prevalence, and black crosses show sample locations. Note that data from the northern Benguela are not shown.

Spatial variability of *Eimeria sardinae* infection intensity in *S. sagax*

No *E. sardinae* infection levels were recorded in fish from the east coast. The mean infection intensity of *E. sardinae* in *S. sagax* from the South African west coast was 49.5, which was lower than that of the South African South coast (69.8) as shown in figure 3.8.

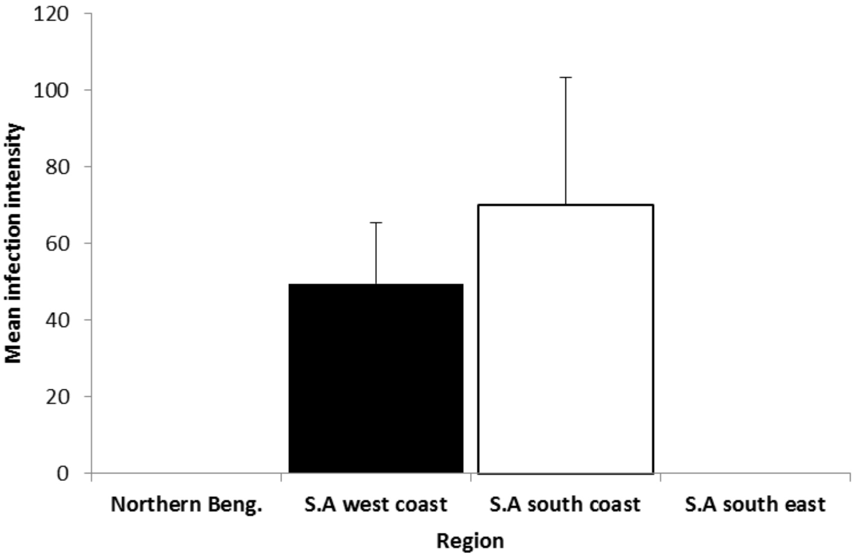


Fig. 3.8: Mean infection intensity of *Eimeria sardinae* in *Sardinops sagax* from the northern Benguela, South African west, south and east coasts, including standard error bars.

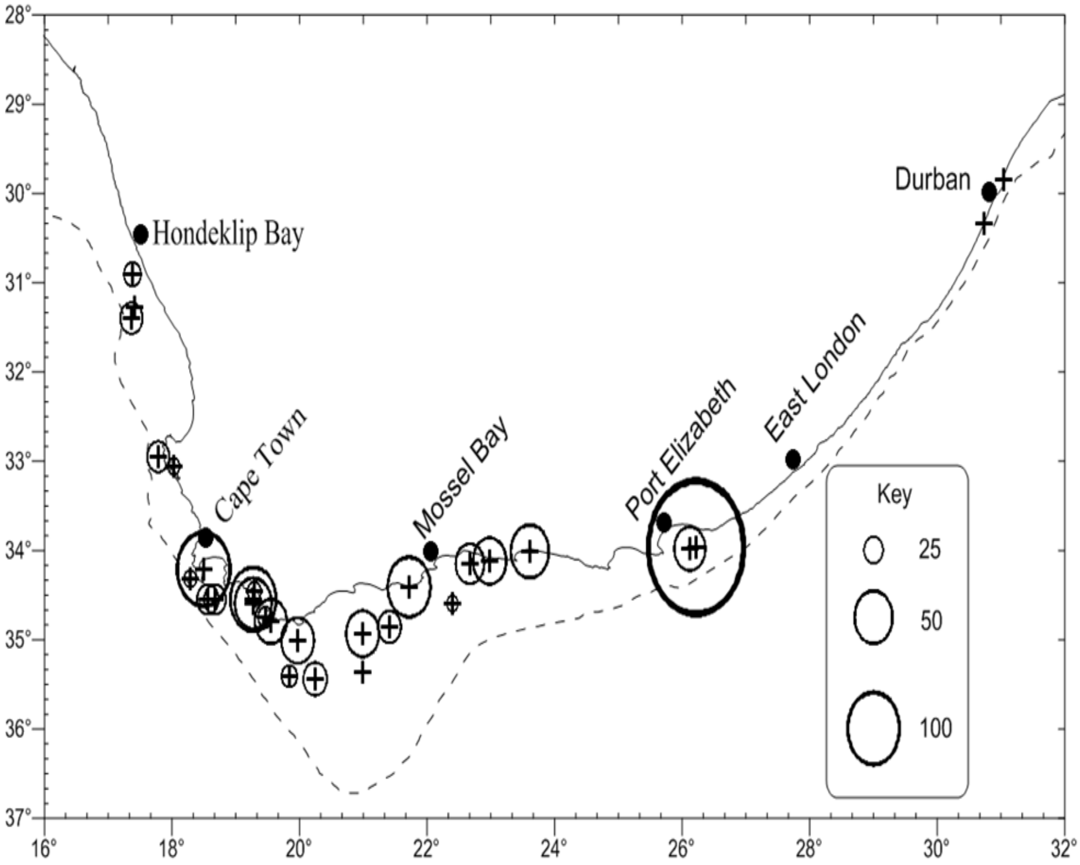


Fig. 3.9: Map showing infection intensity (black circles) of *Eimeria sardinae* in *Sardinops sagax* collected from the South African west, south and east coasts. Symbol size is proportional to infection intensity, and black crosses show sample locations. Note that data from the northern Benguela are not shown.

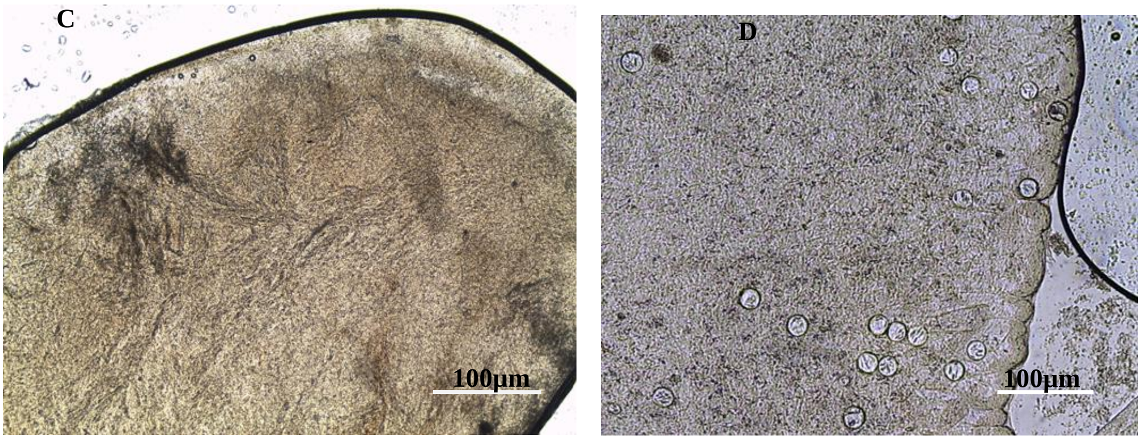
Results of t-tests showed no significance difference between mean infection prevalence of *Eimeria sardinae* in *Sardinops sagax* collected from the South African west coast and south coast (t = 0.08; p = 0.94). Similarly, t-tests results showed no significance difference (t = -0.63; p = 0.53) between mean infection intensity of *Eimeria sardinae* in *Sardinops sagax* collected from the South African west coast and south coast.

CHAPTER 4: RESULTS - PHYSIOLOGICAL EFFECTS OF *EIMERIA* INFECTION

4.1 Effects on gonadosomatic index (GSI)

Testes of severely infected *S. sagax* appeared atrophied compared to those from uninfected sardine or those with low or no *E. sardinae* infection (Figure 4.1).

Fig. 4.1: Macroscopic view of the testes of an uninfected *Sardinops sagax* (A) and a severely infected with *Eimeria sardinae* (B).



C) Photomicrograph of uninfected *Sardinops sagax* testis, D) infected testis showing oocysts scattered in the host tissue.

Uninfected fish from the combined data set from the west coast and south coast had various percentage GSI values. Infected fish had low GSI values with the majority having GSI of less than 4% although there is a considerable number of infected fish with GSI between 4% and 8% (Figure 4.2).

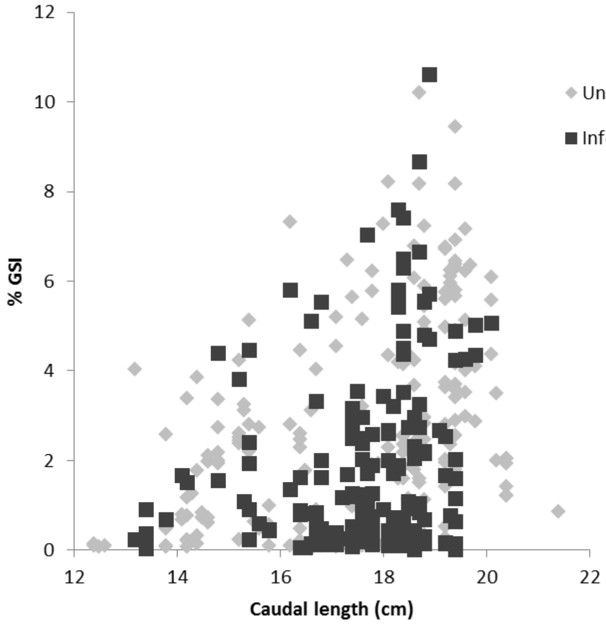


Fig. 4.2: Scatter plot of gonadosomatic index (GSI) and caudal length of infected and uninfected *Sardinops sagax* from both the South African west and south coasts.

Further assessment revealed a negative relationship between GSI and *E. sardinae* infection intensity (r^2 = 0.14), with a higher infection intensity associated with lower GSI values (Figure 4.3).

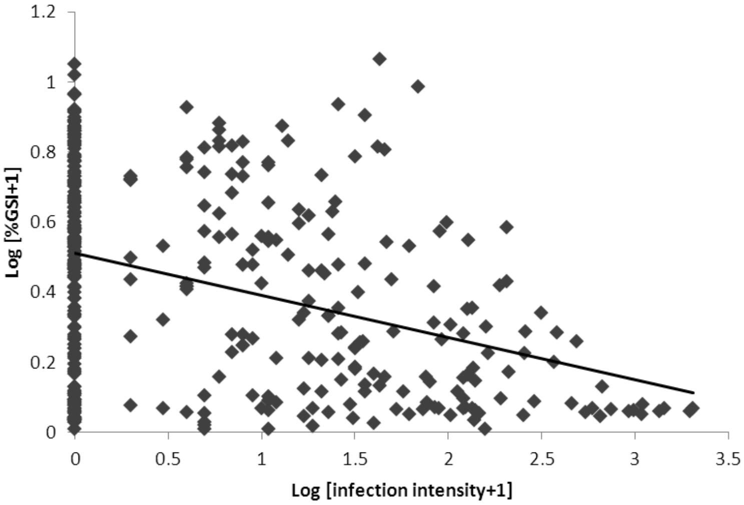


Fig. 4.3: Relationship between gonadosomatic index (GSI) and *Eimeria sardinae* infection intensity. Trendline equation; Log [%GSI+1] = -0.1196*Log [infection intensity +1] + 0.51. R^2 = 0.1364.

When mean sardine GSI values were plotted per categorised infection levels of *E. sardinae*, separately for fish from the west coast and south coast, west coast fish that had high infection intensity showed substantially lower mean GSI values than fish with no, low and moderate infection categories (Figure 4.4).

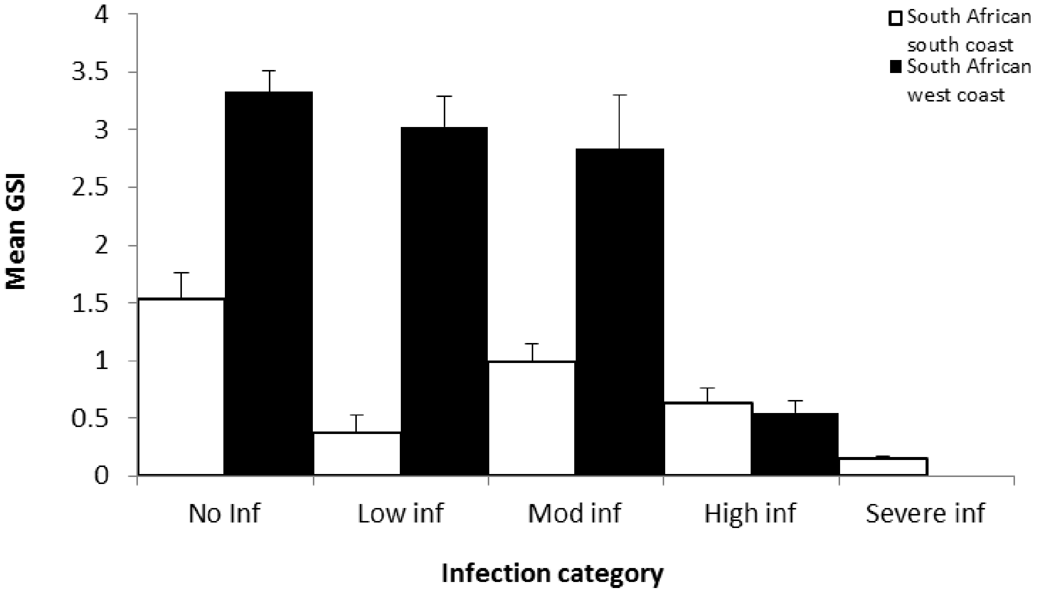


Fig. 4.4: Effect of infection intensity category on gonadosomatic index (GSI, ± standard error)) of *Sardinops sagax* from both the South African west and south coasts.

When data from both the South African west coast and south coast were combined and plotted, fish with no infection had an average GSI value of 2.8% and those with low infection had a GSI value of 2.5%. Severely infected *S. sagax* had the lowest GSI value of about 0.25% and highly infected fish had a mean GSI value of just above 0.5% (Figure 4.5).

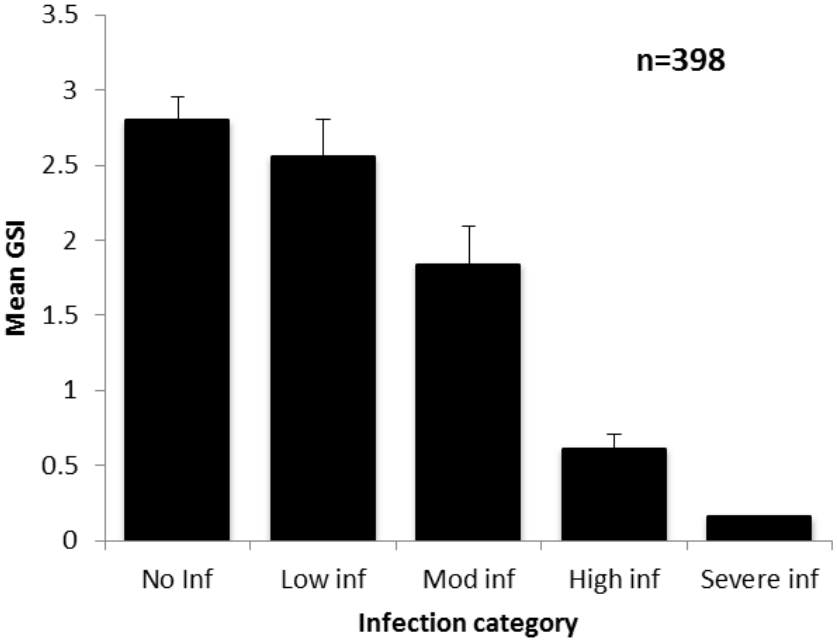


Fig. 4.5: Effect of infection intensity category on gonadosomatic index (GSI, ± standard error)) for combined data of the South African west and south coasts.

Results of a single factor ANOVA indicate that average GSI differs significantly among infection categories (F=12.5; p<0.005; see Table 4.1 for details; Figure 4.5). A *post hoc* Tukey test showed that means of the high infection category and severe infection category significantly differ from each other. GSI means of no infection category and low infection category did not differ (Table 4.2).

Table 4.1: Results of single factor ANOVA test for mean infection GSI values among groups.

Source of Variation	SS	df	MS	F	p-value
Between Groups	219.240	4	54.8100	12.5	0.0000001
Within Groups	1724.17	394	4.3761		
Total	219.24	398			

Table 4.2: Results of Tukey HSD Test for mean infection values among groups.

Infection Category	**No** 2.7989	**Low** 2.5568	**Mod inf.** 1.8394	**High** 0.61260	**Severe** 0.15774
No		**0.92**	**0.006**	**0.000017**	**0.02**
Low	**0.92**		**0.24**	**0.000037**	**0.06**
Moderate	**0.006**	**0.24**		**0.02**	**0.32**
High	**0.000017**	**0.00004**	**0.02**		**0.99**
Severe	**0.02**	**0.055**	**0.32**	**0.99**	

4.2 Effects on condition factor (CF)

There was a wide variation in the individual condition factor values of *S. sagax*, which ranged largely between 0.6 and 1.3 for uninfected fish and fish with infection intensity of <500 oocysts per sample. However, when *E. sardinae* infection intensity increased beyond 500 oocysts per fish condition factor values reduced to close to 0.9 (Figure 4.6).

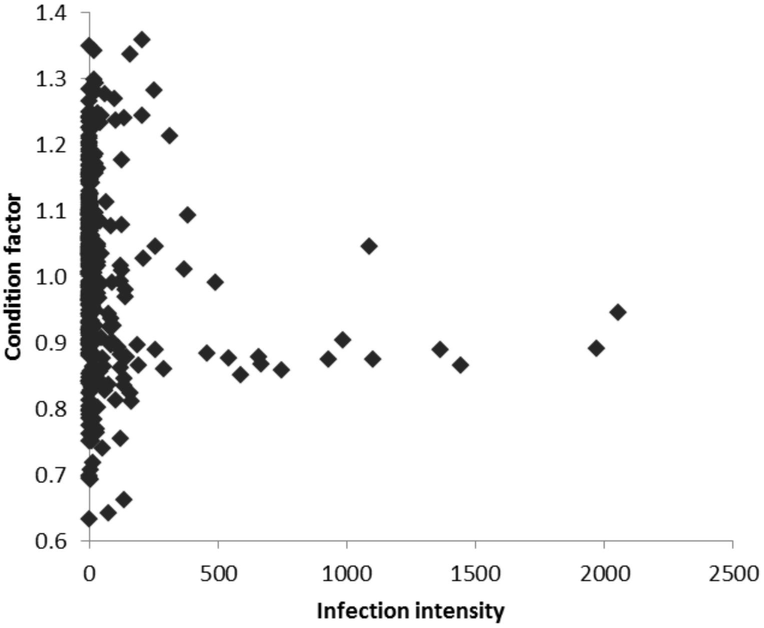


Fig. 4.6: Scatter plot of condition factor of *Sardinops sagax* and *Eimeria sardinae* infection intensity for the combined data of the South African west and south coasts.

Sardinops sagax from the South African west coast had higher average condition factor (CF) values compared to those from the south coast, and the effect of *E. sardinae* infection appeared to be less pronounced than for the fish from the South African south coast (Figure 4.7).

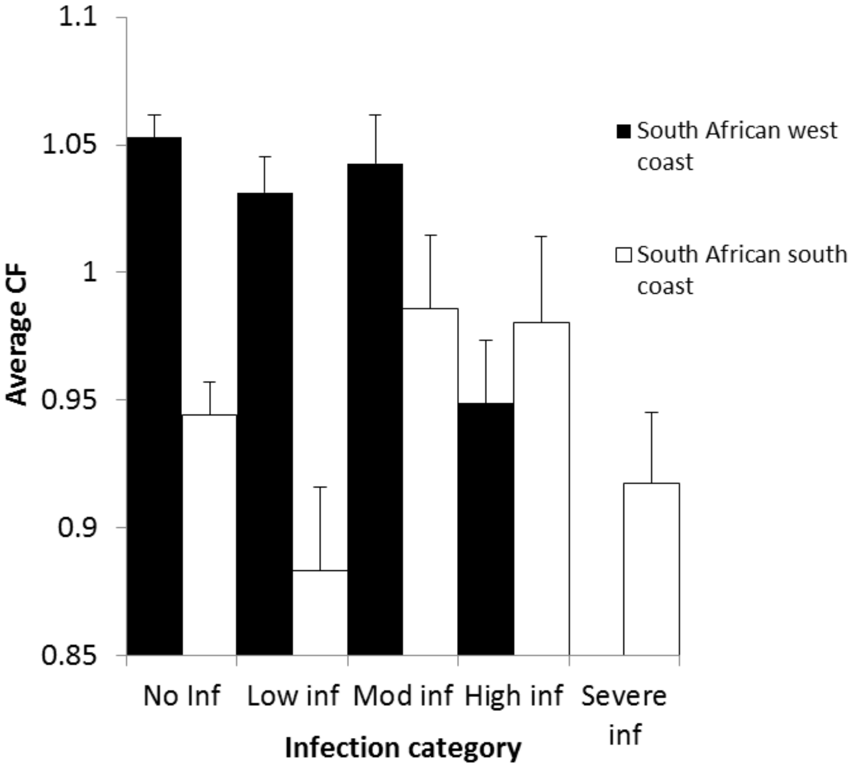


Fig. 4.7: Effect of *Eimeria sardinae* infection intensity category on the fish condition factor (± standard error) from the South African west and south coasts.

However the trend between infection intensity and average condition factor was not consistent for the combined fish data (Figure 4.8). Fish with a high infection intensity category had an average low CF value of about 0.97, fish with moderate infection had an average CF value of 1.01 which was higher than fish with low infection levels which had an average CF of about 1. A one way ANOVA test showed no significant differences in mean CF among categories (F=2.0; p=0.09) of *E. sardinae* infection intensity, as shown in Table 4.3.

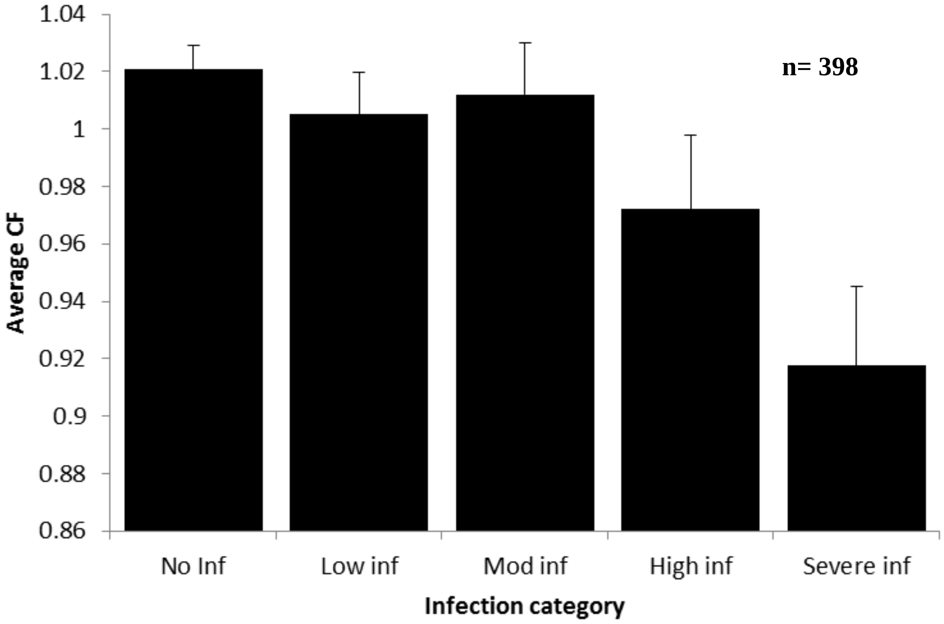


Fig. 4.8: Effect of infection intensity category on the condition factor (CF, ± standard error) of *Sardinops sagax* for the combined data of the South African west and south coasts.

Table 4.3: Results of single factor ANOVA test for mean infection CF values among categories.

Source of Variation	SS	df	MS	F	p-value
Between Groups	0.1376	4	0.0344	1.98	0.09713
Within Groups	6.8515	394	0.0174		
Total	6.9891	398			

CHAPTER 5: DISCUSSION AND CONCLUSION

5.1 Discussion

Spatial variability in infection intensity and prevalence

The results of this study show spatial variability both in infection prevalence and infection intensity of *E. sardinae* in *S. sagax* around the southern African coast (Figures 3.6 and 3.8). That no sardine from the northern Benguela and South African east coast were found to be infected with *E. sardinae*, suggests that these are not endemic areas of infection for *E. sardinae*. Parasites can provide information about migrations and intermingling of fish. The underlying theory is that fish can only be infected by a parasite when the fish is in the endemic area of that parasite, the endemic area being the geographic region in which conditions are suitable for the transmission of the parasite (MacKenzie and Abaunza, 1998). Infected fish found outside of the endemic area must previously have been within the endemic area in order for infection to have occurred.

The northern Benguela and southern Benguela are separated by a strong perennial upwelling cell at Lüderitz (27-28°S), which creates a semi-permanent environmental barrier thought to prevent mixing between the *S. sagax* populations from Namibia and those occurring in the southern Benguela, which are considered to be distinct stocks (Newman, 1970; van der Lingen *et al.*, 2006c; Coetzee *et al.*, 2008). The observed pattern of *E. sardinae* infection therefore suggests that sardine in these two regions are distinct sub-populations. This is in line with previous studies that documented differences in some biological characteristics of southern African sardine from the northern Benguela and the southern Benguela. Idris (2010) reported a significant difference in gill raker numbers and spacing between sardines from the northern Benguela and those obtained from the southern Benguela, and suggested that the South African and the Namibian sardine are independent stocks. Wessels (2009) examined the differences in vertebral count and body shape characters of sardines from Namibia and the southern Benguela and came to a similar conclusion. Differences also exist between the spawning areas of sardine found in the northern Benguela and the southern Benguela which further explains the separation of the fish stocks between the two regions (van der Lingen *et al.*, 2006c; Checkley *et al.*, 2009).

In the southern Benguela, well as small scale variability in both *E. sardinae* infection prevalence and infection intensity was observed (Figures 3.7 and 3.9), these results cannot be clearly demonstrated that sardine in this area are separate stocks. Since the majority of samples came from spring (October-November) 2011, seasonal and interannual variability could confound the results. In fact further t-tests showed no significant difference in *E. sardinae* infection prevalence and infection intensity in fish obtained from the west and south coasts. The marked decline in infection prevalence in sardine with caudal length of 19.5 cm on the south coast as shown in figure 3.2 could be due to post spawning effect. The oocysts are shed with the sperm during spawning and therefore infection prevalence in spent sardine drops significantly (MacGladdery and Burt, 1985). On the south coast the positive correlation between large fish and infection intensity of *E. Sardinae* (Figure 3.5) could be due to cumulative infection. The higher infection prevalence observed in the present study is much different from 7.1% reported by Linde (2011). The difference could be due to the fact that in the present study a bigger sample size (n=553) over a wide area was used, unlike the smaller sample size (n=141) used by Linde (2011) from a smaller study area. However, the *E. sardinae* infection prevalence recorded in the present study is not much different from the 40% reported by Reed *et al*. (2012).

The absence of *E. sardinae* in sardine samples from the South African east coast, which make up the sardine run, suggests this is a distinct subpopulation. The separation of *S. sagax* on the east coast as a separate stock from the west coast and south coasts is supported by Connell (2010), who sampled ichthyoplankton off the east coast and concluded that the sardines migrate from the south coast to the east coast in order to spawn in KwaZulu Natal waters. Connell (2010) further suggested that the sardines that survive the run return to the Agulhas Bank with the onset of summer. According to Freon *et al*. (2010), the sardine run is more likely to be a seasonal spawning migration of a genetically distinct subpopulation or functionally discrete adult assemblage. The separation of *S. sagax* populations of the east coast from those in the west and south coasts is further supported by van der Lingen *et al*. (2010b), who observed significant differences in some of the biological characteristics of *S. sagax* from the KwaZulu-Natal sardine run compared to those elsewhere in the southern Benguela.

Physiological effects

Overall there does not seem to be much difference in the pattern between CL and GSI for infected and un-infected fish (Figure 4.2), probably because the fish with high GSI have low infection levels. *Eimeria sardinae* infection had a significant negative effect on the gonadosomatic index (GSI) of *S. sagax* on both the South African west and south coasts. Severe infection could result in the possibility of reduced sperm production. This is supported by Pinto (1956), who observed serious destruction of testes tissue of the European sardine *Sardina pilchardus* infected with *E. sardinae*, leading to parasitic castration. Turovsky *et al*. (1993) also suggested that heavy infection by *E. sardinae* may result in testes deformation, leading to complete sterility. Similarly, Diouf and Toguebaye (1994) reported atrophy in testes of *Sardinella maderensis* (Madeiran sardinella) infected with *E. sardinae* and suggested a possible reduction in their fecundity potential. Morrison and Hawkins (1984) reported spermatic cords to be distorted by severe infection of *E. sardinae* in herrings (*Clupea harengus*). According to Pinto (1956), when oocysts invade the gonad tissue, they take up a large volume of the gonads by violently compressing gonad cells and thereby reducing space left for sexual cells. Internal hemorrhages are more frequent and heavily infected testes turn into a hard mass of tissues. This change in the testes physiology by *E. sardinae* infection could lead to total castration (Pinto, 1956). Based on results of the present study, it is only testes in high and severe infection categories that showed marked reduction in GSI values as further shown in a post hoc test (Table 4.2), probably indicating that their reproductive potential had been reduced partially or totally. Assuming the stocks are distinct in the southern Benguela and the relative infection is exact, those with severe or high infection will most likely reduce spawning success. This could affect recruitment strength, compared to a stock with low or no infection levels. High and severe *E. sardinae* infection levels could therefore affect the population dynamics of sardine in the southern Benguela.

Eimeria sardinae intensity of infection did not have a significant effect on the condition factor (CF) of South African sardine. This could be expected as the condition of sardine is least affected by presence of parasites as compared to the primary target organ, where tissues are violently compressed by parasitic forms (Pinto, 1956). It could be assumed that sardine were still in reasonable condition despite the infection. The observed high CF on west coast as compared to south coast was unexpected since van der Lingen *et al.* (2006a) reported that sardine on the south coast have high CF as compared to those on the west coast. Differences in sardine condition factor on the west coast and south coast could be due to, among others, fluctuations in primary productivity, sardine lipid content and density dependent effects (Shannon and Nelson, 1990; de Goede and van der Lingen, 2005; van der Lingen, *et al.*, 2006a). Condition factor influences the reproductive cycle in fish (Welcome, 1979) with high CF allowing efficient energy transfer to gonads resulting in more eggs and sperm production which ensures more survival (van der Lingen, 2006a).

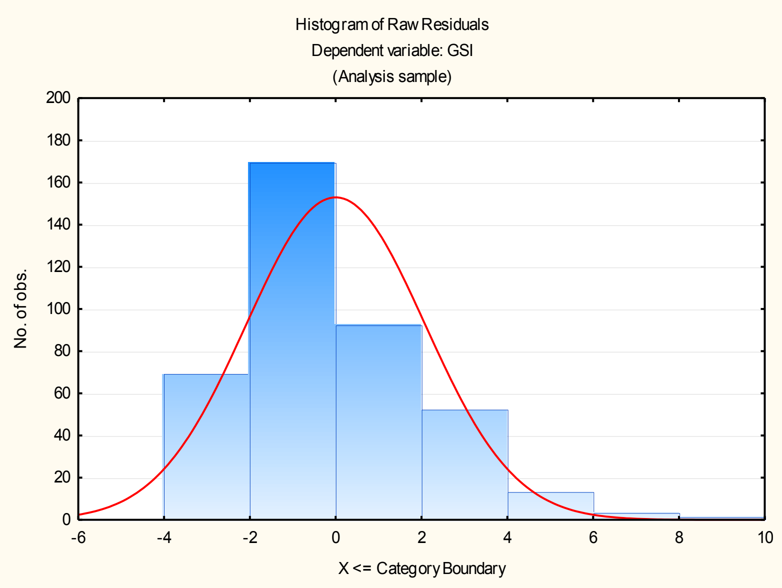
5.2 Conclusion

The results of this study show that there is spatial difference in the infection by *E. sardinae* of sardine off the southern African coast. These findings suggest that the testicular coccidian parasite could potentially be used in a biological tagging study and corroborates the hypothesis that sardine in the northern Benguela and the southern Benguela are separate subpopulations. *Eimeria sardinae* appears to be useful in discriminating between *S. sagax* populations from the putative east coast stock and those from the South African western and southern populations. No marked difference in *E. sardinae* infection was noted between sardine from the western and southern stocks, most likely due to overlap as both of these two regions are endemic coccidian parasite areas or possibly due to seasonal variation.

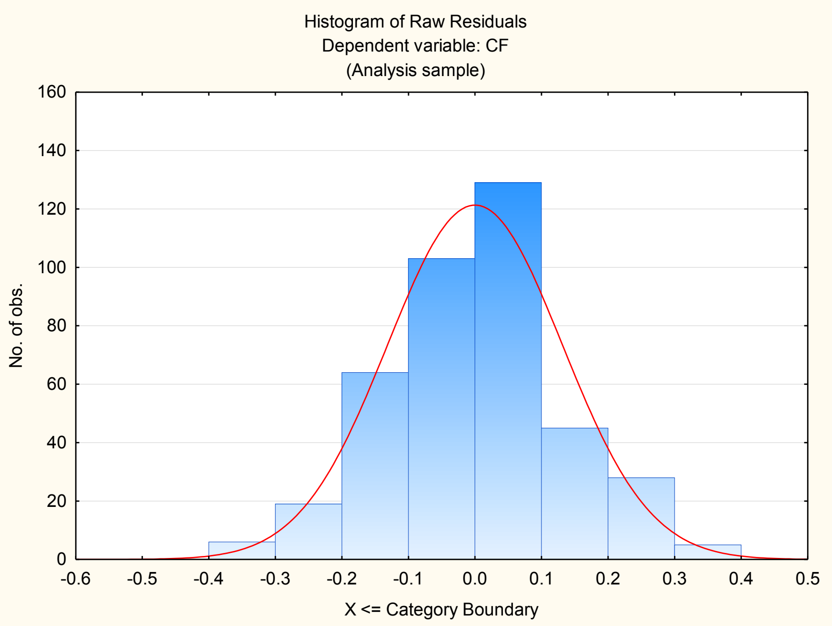
High and severe infection intensities of *E. sardinae* in *S. sagax* could lead to parasitic castration. However, it is unlikely that the infection could be a serious concern for fisheries management since highly infected fish constitute only 9% of the fish examined. Additionally, it is most likely that these infection levels are not new and *S. sagax* has always experienced them without a significant reduction in sardine population. But if the infection levels increase significantly due to sub-lethal effects like pollution, which can reduce sardine immunity, this could possibly become a serious concern for fisheries management.

Although the findings are interesting, the sample size used, especially for the Namibian and east coast regions, was not sufficient. Furthermore, samples used were not evenly spread throughout the different seasons of the year which would be required to rule out the potential seasonal variability effect on *E. sardinae* infection. Lastly, the method of identifying the oocysts in the infected testes tissues may misrepresent the actual number of infection, since it relies only on the sections cut from the gonads, and testes with low infection levels may not be identified. Future work therefore, should, among others, consider increasing the sample size, and examining seasonal variation in *E. sardinae* infection prevalence and infection intensity. Effects of the parasite on the host could further be assessed by fixing infected tissues for histology.

APPENDIX I

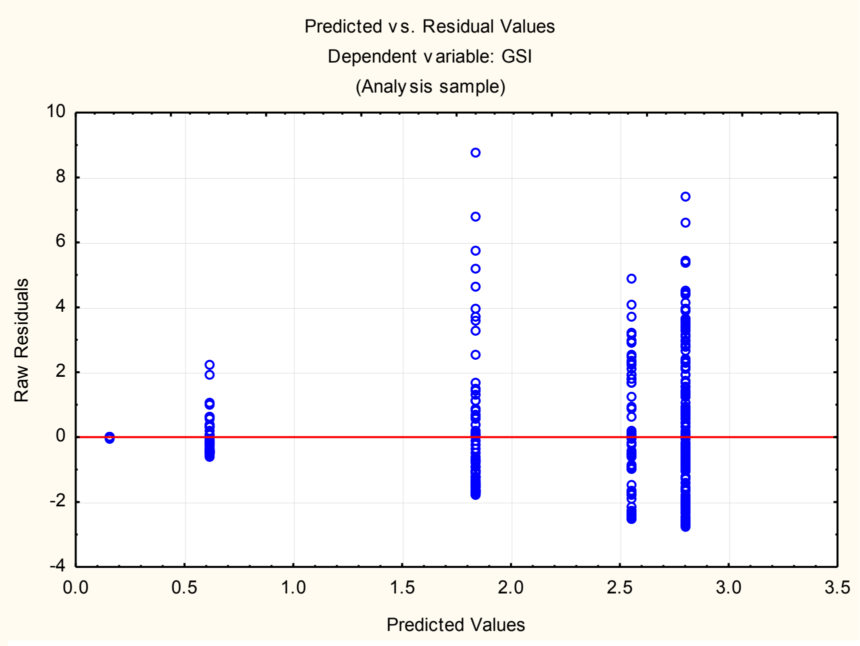


A: Histogram for residuals of data from the South African south coast and west coast showing normal distribution, where GSI is the dependent variable.

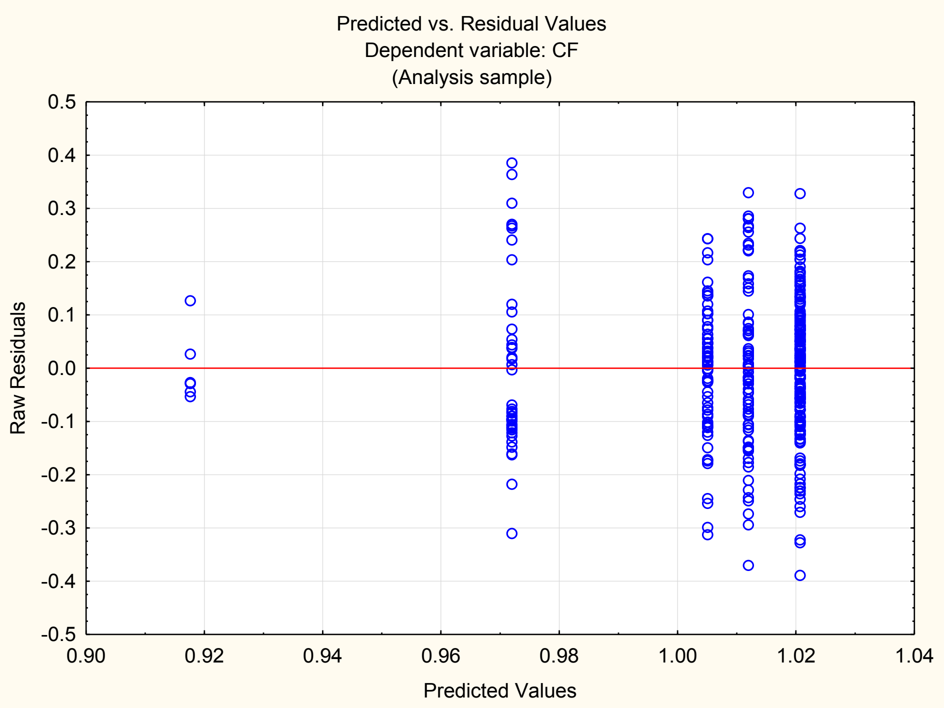


B: Histogram for residuals showing normal distribution for data from the South African south coast and west coast, where CF is the dependent variable.

APPENDIX II



A: Plot of residuals against predicted values for data from the South African south coast and west coast, where GSI is the dependent variable.



B: Plot of residuals against predicted values for data from the South African south coast and west coast, where CF is the dependent variable.

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