Parasites of Kunene horse mackerel *Trachurus trecae* (Smith-Vaniz, 1986) with a comparison of parasites of Cape horse mackerel *T. capensis* (Castelnau, 1861) in the northern Benguela.

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Abstract

Two species of horse mackerel (T*rachurus trecae* and *Trachurus capensis*) reside in the northern Benguela ecosystem. Both are important economic commodities for the Angolan and Namibian fisheries and therefore need to be managed appropriately. Although the two species of horse mackerel share similar morphological characteristics and co-occur in Namibian waters in the northern Benguela, few studies have compared their parasite assemblages. To date there are no studies regarding the parasite profile of *T. trecae*. This study is the first to identify and document the parasite assemblage of *T. trecae* from the northern Benguela and forms the only parasite profile for this horse mackerel species. This study also assesses the effects of fish size and fish sex on the parasite assemblage of *T. trecae*, and compares the parasite assemblage of this species with that of *T. capensis* from the northern Benguela. Results indicate that the largest significant difference in parasite assemblage is between the two horse mackerel species (by convention $p<0.01$), but that significant differences are also found between small and large *T. trecae* and between immature, male and female *T. trecae*. The coccidian *Goussia cruciata* was found to have the strongest discriminatory power in all comparisons, and therefore serves as a potential indicator parasite or biotag for discriminating between different stocks of *T. trecae* and between *T. trecae* and *T. capensis* in the northern Benguela. Due to the lack of literature regarding the life history of *T. trecae* it is difficult to assess why there are sex effects on parasites infecting this species, as well as whether the interspecific difference in parasite assemblage is due to environmental conditions or species-specific relationships. Further investigations regarding the life history of *T. trecae* would assist interpretation of the results obtained here. This study provides a comprehensive knowledge of the parasite assemblages infecting *T. trecae* and thus lends to possible future studies regarding *T. trecae* stock structure. It also provides a starting point for conducting studies of the parasite assemblages of other fish in the northern Benguela.

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Introduction

The Benguela Ecosystem

The Benguela ecosystem is one of the four major eastern boundary upwelling systems of the world (Hutchings et al., 2009; Roux and Shannon, 2010) and occurs between the warm Agulhas Current in the south, and the subtropical Angola Current in the north (Figure 1, Roux et al., 2013). The system is characteristic of wind-driven upwelling and therefore high productivity, and is divided into a northern and southern part by the perennial upwelling Luderitz cell at about 26° S (Bianchi, 1992; Ekau and Verheye, 2005; Verheye and Ekau, 2005). Due to the high productivity in the area it supports large commercial fisheries for species such as anchovy, sardine, Cape hake and horse mackerel.

The northern Benguela is bounded to the north by a frontal region known as the Angola Benguela Front. This is a permanent feature established down to 200m in the ocean and moves seasonally between 14°-17°S depending on the strength of the Angola and Benguela currents. This front is thought to act as a semi-permanent barrier for fish movement (Boyer and Hampton, 2001). The southern Benguela is bounded in the south by the Agulhas Current retroflection area between 36°S and 37°S (Verheye and Ekau 2005). It is divided into two regions; the west coast upwelling and temperate shelf areas. Both these regions have seasonal wind driven upwelling and therefore moderate productivity that also supports fisheries (Hutchings et al., 2009; Roux et al., 2013).

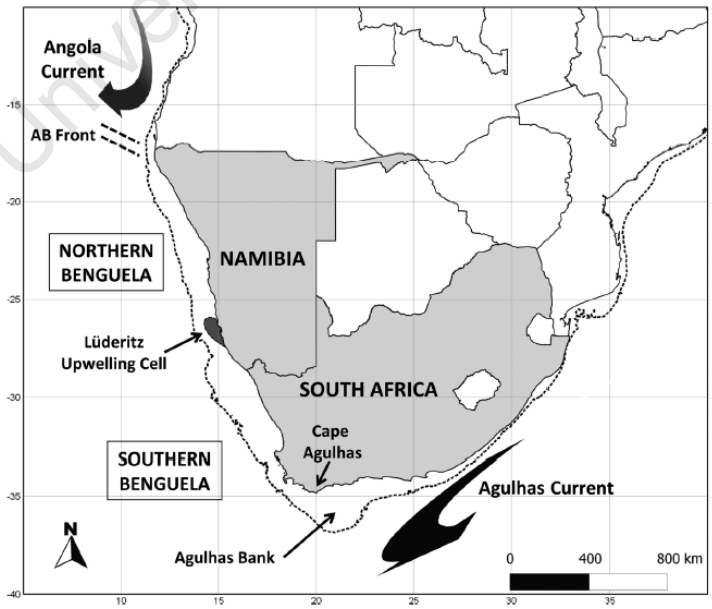

Figure 1: Map of the Benguela ecosystem showing the 500m depth contour (dashed line) and locations of the Angola Benguela Front, Luderitz Upwelling cell and Angola and Agulhas Currents from Roux et al. (2013).

Horse mackerel in the Benguela

Two species of horse mackerel are affected by the Benguela ecosystem, namely the Kunene horse mackerel (*T. trecae*) and the Cape horse mackerel (*T. capensis*) (Figure 2). Both species are very similar in appearance except for the dorsal accessory lateral line, which is larger in width in *T. capensis* (FAO, 2013a; FAO, 2013b). Both species have a fairly compressed elongate body with a large head and projected lower jaw, as well as a distinctive small, black spot on the upper edge of the opercula. Their upper body is a grayish, black colour while the lower two-thirds is paler, whitish silver (FAO, 2013a; FAO, 2013b). Of the *Trachurus* genus, *T. trecae* are known to have the fastest growth rate. Their maximum total length is recorded to be 35cm, while *T. capensis* are known to have a slightly smaller common length of 30cm.

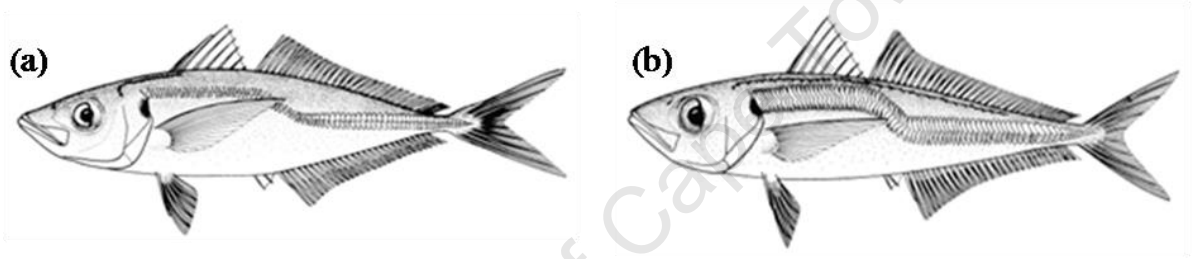

Figure 2: Line drawings of (a) Kunene horse mackerel *Trachurus trecae* and (b) Cape horse mackerel *Trachurus capensis* (both species known to have a common size of about 30cm fork length) (FAO, 2013a; FAO, 2013b).

T. trecae are a benthopelagic species distributed along the central eastern and central Atlantic along the African coastline from Mauritania to Angola/ Namibia (15°-22°S) (Figure 3a, FAO, 2013a). It usually occurs at depths of 20-100m along the coast and shelf and spawn in warm water regions off the coast of Mauritania and Moroccan Sahara (Arkhipov, 2009). Juveniles are found in subtropical and tropical waters, while adults are usually seen off northern Namibia (Boyer and Hampton, 2001). The species' diet consists of fish, squid and crustaceans (FAO, 2013a).

The other species of horse mackerel, *T. capensis* is found along the South African and Namibian coasts from Port Alfred (South Africa) to the northern border of the Benguela (Figure 3b, FAO, 2013b; Le Roux, 2013). Adults and juveniles occupy different habitats and show a clear eastward shift off the South African coast with age/size (McLaverty, 2012). This distribution shifts seasonally with both life stages moving south and being more wide spread

in the summer months, while becoming more congested in the winter months. In Namibia the smaller sized fish are found further north near the Kunene River, while the adults are situated in deeper waters of about 200-500m. The diet of *T. capensis* is more diverse than that of *T. trecae* and consists of a variety of crustaceans such as euphausiids, polychaetes, chaetognaths, as well as other fish and squid (Boyer et al., 2010).

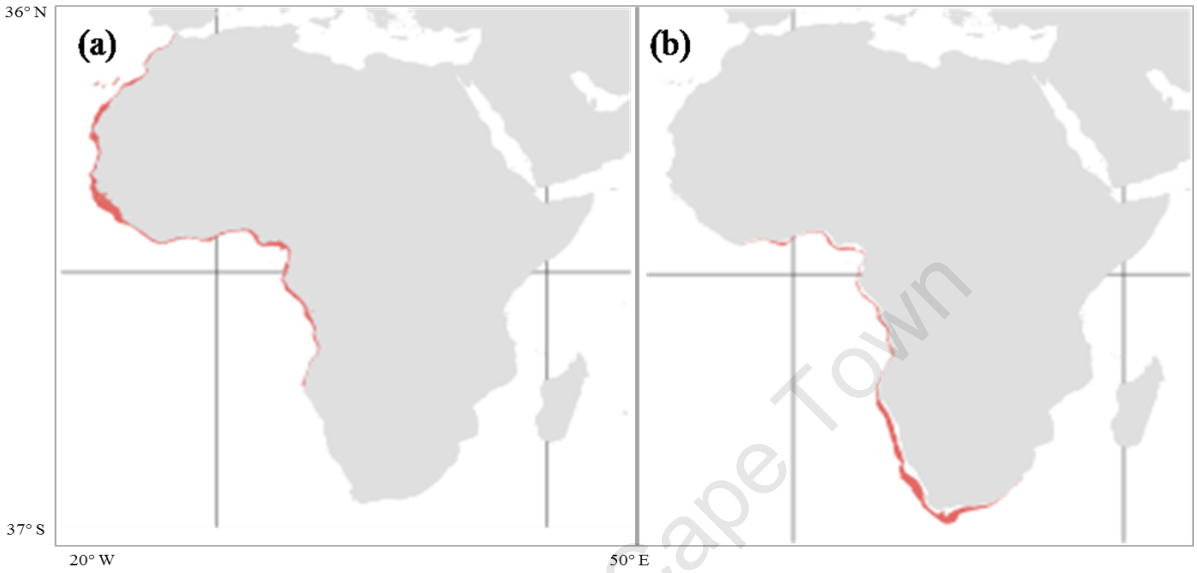

Figure 3: Global geographical distributions of (a) *Trachurus trecae* and (b) *Trachurus capensis* found along the coast of Africa, (area covered by map (FAO, 2013a; FAO, 2013b).

Both of these species are marketed as horse mackerel. Horse mackerel markets are mainly situated locally in Africa, especially central Africa and Zambia, while a small portion is exported to the Caribbean region. Commercially, horse mackerel are caught by three fishing methods dependent on the age of the fish. Juveniles are caught by purse-seine methods while mid-water and demersal trawlers target adult fish. Global catches of *T. capensis* are generally much higher than that of *T. trecae.* In 1999 the global catch of *T. trecae* was estimated to be about 81 000 tonnes (Figure 4a, FAO, 2013a) while *T. capensis* catch was estimated to be 386 361 tonnes at this time (Figure 4b, FAO, 2013b), almost five times the amount of *T. trecae.*

Both species are important economic commodities for the Angolan, Namibian and South African fisheries. They are sold frozen, dried, salted, smoked or canned and relied upon as a cheap source of protein for developing countries. In Namibian waters, *T. capensis* serves as the highest catch data of all fish species, but because of its low market value it is the second highest economic contributor to the fishing industry (Figure 5, Mundjulu, 2009; Kirchner et al., 2010; Roux et al., 2013).

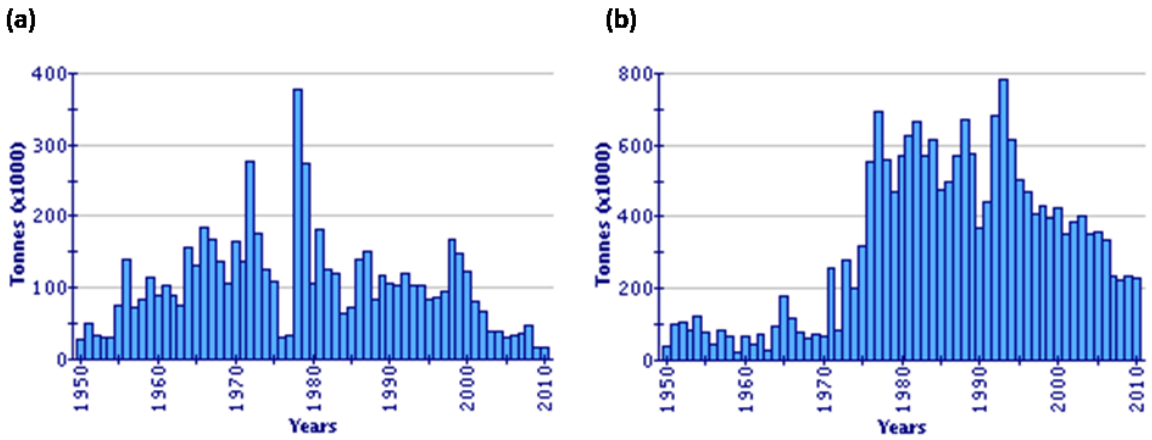

Figure 4: Global capture production (per 1000 tonnes) of (a) *Trachurus trecae* and (b) *Trachurus capensis* (FAO, 2013a; FAO, 2013b).

At the onset of pelagic fishery development in South Africa (early 1950s and 1960s), adult horse mackerel were the target of local and foreign demersal trawlers (Figure 5, Roux et al., 2013). By the 1970s however, horse mackerel catches declined, forming a large portion of by-catch in pelagic and demersal fisheries instead (Mukumangeni, 2006). Ten years later the sardine fishery collapsed, causing fisheries to introduce smaller mesh sizes and consequently causing horse mackerel catches to increase and become the main target of mid-water trawl. Similarly at this time, catches in the northern Benguela soared to excesses of 100 000 tons per year (Roux et al., 2013) and has continued to be the target of mid-water fisheries since, dominating Namibian landings in the past three decades.

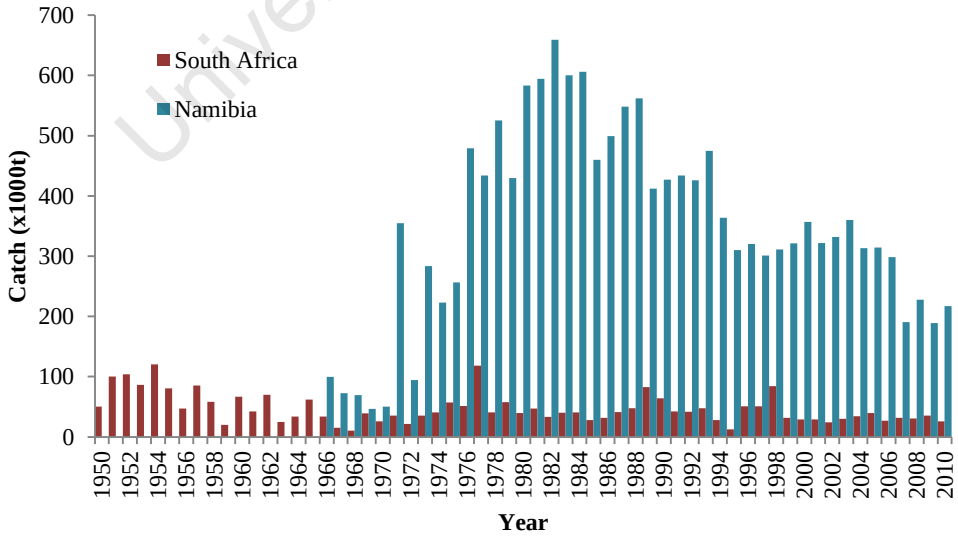

Figure 5: Catches (per 1000 tonnes) of horse mackerel (*Trachurus trecae* and *Trachurus capensis*) from 1950 to 2010 from South Africa and Namibia (redrawn using data from Roux et al., 2013).

Population structure of *Trachurus trecae* and *Trachurus capensis*

Although both *T. trecae* and *T. capensis* co-occur in Namibian waters, few studies have examined their population structure. To stay genetically and phenotypically different, populations have to be geographically or reproductively isolated (Nei, 1975). Two sub populations/stocks of *T. capensis* are currently recognized within the Benguela ecosystem, namely the northern Benguela stock and the southern Benguela stock (Naish, 1990). Although scant data exists for *T. trecae;* it is currently considered a single stock in the Eastern central Atlantic from Cape Bojador (26°N) to the south of Angola (FAO, 2013a). In 2002 however, Sardinha and Naevdal showed that *T. trecae* may consist of two different stocks, one in the northern tropical waters and the other in the southern cooler Benguela upwelling waters (Figure 6). Due to the high salinity and low temperatures south of the Angola Benguela front, a barrier to gene flow is thought to exist and cause the emergence of these two stocks of *T. trecae.*

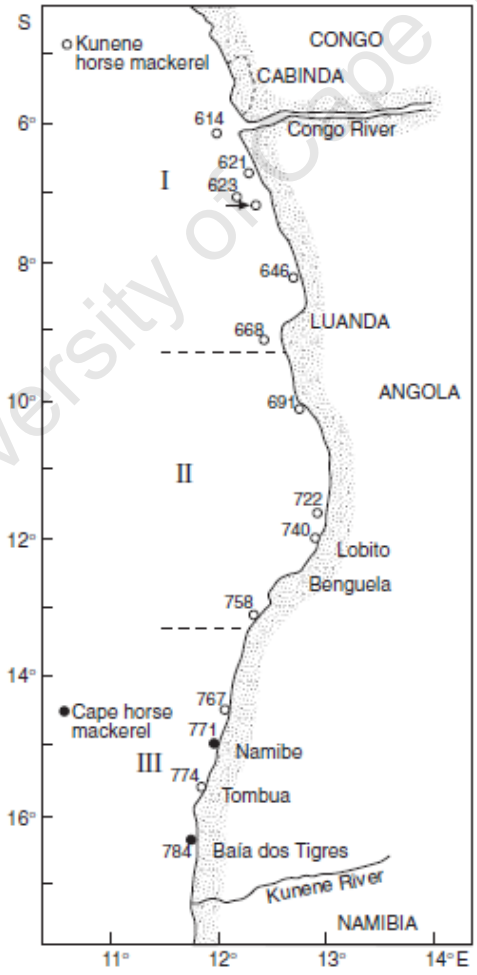

Figure 6: Locations of *Trachurus trecae* (Kunene horse mackerel) and *Trachurus capensis* (Cape horse mackerel) sampled off the Angolan coast in a study by Sardinha and Naevdal (2002).

A ‘stock’ is a term for a subpopulation of fish that are either genetically or phenotypically different or have different life history traits than other groups of fish from the same species (Begg and Waldmann, 1999; Waldman, 2005). In areas where two stocks mix, it is beneficial for fisheries to be able to distinguish between two stocks and base catches on this, so that one stock is not under fished while the other is over fished. There are many examples where overexploitation has resulted in the depletion of fish stocks, the most famous being the collapse of the Atlantic northwest cod fishery (Begg and Waldmann, 1999). This is why stock identification is a critical issue for the rational management of marine resources.

There is no single approach for defining stock structure; however an integration of several techniques is considered the best option (Begg and Waldmann 1999; Baldwin et al., 2011). Some techniques include fish morphometrics, artificial tags and fish genetics (Mackenzie and Abaunza, 1998). Artificial tagging is costly, is thought to cause behavioral changes and is usually restricted to larger fish sizes as it causes a high mortality in smaller fish. The tagging process requires a significant handling process, causing the fish to become stressed and less able to survive when released (Mosquera et al., 2003). Fish genetics using mitochondrial DNA is sometimes problematic when subpopulations have recently diverged or have complicated population dynamics (Baldwin et al., 2011).

Parasites as biotags

One method of examining stock structure that has been used successfully for over 60 years is using parasites as biological tags (Herrington, 1939; Mackenzie et al., 2002). The use of parasites for stock identification has become very popular in recent years and the increasing number of publications referring to the use of marine parasites as biological tags shows how valuable this method is (Mackenzie et al., 2008). It is very low cost, has simple sampling procedures and is suitable for any size or type of species. Before this method can be applied however, a comprehensive knowledge of the parasite assemblage of the species in question is required. The theory behind the use of parasites as biological tags is that subpopulations of fish are spatially separated over space or time; hence they are exposed to different parasite species endemic to different environmental regions. Thus, the qualitative or quantitative changes in parasite assemblages infecting fish should naturally be due to these fish being from different stocks and/or being in different areas (Mackenzie and Abaunza 1998; Begg and Waldman 1999; Mackenzie et al., 2008).

Little is known about the life cycles of most parasites species as they are very complex, often involving more than one host. These hosts need to co-occur in a specific environment so that the parasite can be successfully transmitted from one host to the next. Parasite assemblages infecting fish hosts can provide insights into the ecological, migratory and foraging history of the fish (Baldwin et al., 2011). Besides the host's geographical distribution, it's trophic position and feeding behavior can also be shown (Marcogliese, 2005). The largest factors affecting parasite assemblages in their hosts are temperature and salinity. This is because marine parasite distributions are determined mainly by temperature-salinity profiles (Esch and Fernandez, 1993). Not only does temperature distinguish where parasites are found, but it also affects host behavior. Temperature changes the way that a host feeds, what it feeds on, its ecology, resistance and distribution. It can affect the parasite by enhancing or slowing the development of eggs and time to mature (Marcogliese, 2005; Timi, 2007).

Three indices are commonly used to quantify parasite infection: the number of parasite individuals found within a host sample (abundance), the number of parasite individuals found infecting a single host (intensity) and the proportion of the host population infected with a particular parasite species (prevalence) (Bush et al., 1997). Entire parasite communities are also looked at when searching for a potential parasite as a biotag (Bush et al., 1997). This is because usually more than one parasite species live together in the same fish host.

In 2000/2001 the Horse Mackerel Stock Identification Research (HOMSIR) project was initiated to examine the stock structure of *T. trachurus* in the northeast Atlantic and Mediterranean Sea using a multidisciplinary approach (Abaunza et al., 2008). Even though a combination of several techniques was used, using parasites as biological tags provided the strongest indication indicated the strongest presence of separate stocks within the Atlantic Ocean and North Sea (Mackenzie et al., 2008). Among the parasite species that have been used for stock discrimination of horse mackerel, the HOMSIR project and other studies showed that nematodes from the genus *Anisakis* proved most useful in the north Atlantic (Matiucci et al., 2007), indicating that it could be a valuable biological tag.

Although numerous studies on the parasite assemblages infecting members of the *Trachurus* genus have been done, few studies have investigated parasites of *T. capensis* and none of *T. trecae*. Le Roux (2013) compared parasite assemblages between two size classes of *T. capensis* from the southern and northern Benguela and found significant differences in

parasite assemblages between size classes and between subsystems, indicating spatial and size differences in parasite fauna and supporting the hypotheses of discrete stocks in the southern and northern Benguela. Generally, *T. capensis* from the southern Benguela had higher prevalence values for *Gastrocotyle trachuri* (Monogenea), *Ceratomyxa australis* (Myxozoa) and *Anisakis* sp. (Nematoda) compared to fish from the northern Benguela. Northern Benguela *T. capensis* had higher prevalence values for *Goussia cruciata* (Coccidea). A large size effect was found for fish from both regions with regards to parasite community structure, infection intensity and parasite abundance. Small fish in the northern Benguela had significantly higher abundances of *G. cruciata* and *Anisakis* sp. than small fish from the southern Benguela. Large fish from the northern Benguela only had a higher abundance of *G. trachuri* than large fish from the southern Benguela, as large fish from the southern Benguela had significantly higher prevalence values for *Anisakis* sp., *C. australis* and *G. trachuri* (Le Roux, 2013). Results indicated that *Anisakis* sp. was most likely the best parasite for stock discrimination due to its large significance in discriminating between *T. capensis* found in the northern and southern Benguela. These results reinforced the same suggestion as the HOMSIR project.

This study is the first to examine parasite assemblages of *T. trecae* in the northern Benguela. It provides new data on the parasite fauna infecting *T. trecae* and acts as an important parasite profile for the species. It also provides information on the effect of fish size and sex on parasite assemblages, as well as which parasites could be used in future stock structure studies for *T. trecae* and discriminating between *T. trecae* and *T. capensis* in the northern Benguela.

The aims of this study were to document the parasitic assemblage of *T. trecae* in the northern Benguela and assess whether there are fish size affects (as seen in *T. capensis*) as well as fish sex effects on the parasite assemblage of *T. trecae*. In addition, the parasite assemblage of *T. trecae* was compared to that of previously collected *T. capensis* (Le Roux, 2013) from the northern Benguela. We expected a size and sex effect on the parasite assemblage of *T. trecae*, as well as differences in parasite assemblage between *T. trecae* and *T. capensis*.

Materials and Methods

Study site

Samples of *T. trecae* were caught off Southern Angola from three different stations by the Norwegian research vessel Dr Fridtjof Nansen during 2011 (Figure 7, Table 1).

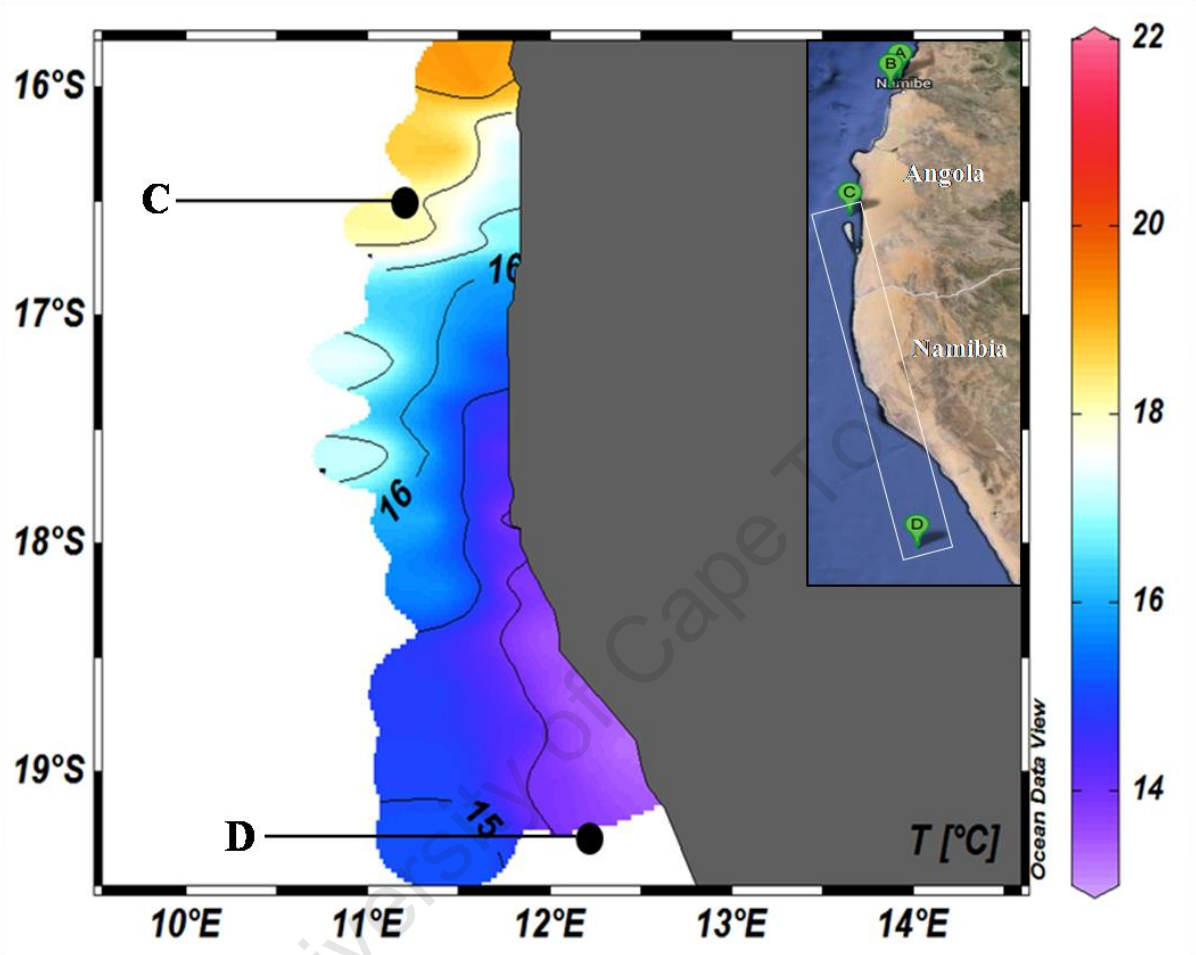

Figure 7: Map showing SST (contours) and the sample locations of *Trachurus trecae* (stations A-C) and *Trachurus capensis* (station D, Le Roux, 2013) off southern Angola during a cruise by the Norwegian research vessel Dr. Fridtjof Nansen (Zaera et al., 2011). Station C and D are located above, however stations A and B are further north. Note that position of station 4 has been slightly moved to fit onto the figure.

Table 1: Sample locations of *Trachurus trecae* and *Trachurus capensis* caught in the northern Benguela in 2011 by Norwegian research vessel Dr. Fridtjof Nansen.

Station number	Longitude	Latitude	Date caught	Species caught	Number of fish per sample (N)
A	12°12' E	15°03' S	09-Aug-2011	*T. trecae*	23
B	12°04' E	15°15' S	09-Aug-2011	*T. trecae*	24
C	11°70' E	16°45' S	12-Aug-2011	*T. trecae*	25
D	12°26' E	19°77' S	24-Aug-2011	*T. capensis*	43 (data from Le Roux, 2013)

Sample collection and processing

Fish were individually packaged at sea, labelled, frozen and shipped to the Department of Biological Sciences, University of Cape Town to be stored in freezers until dissection. Freezing the hosts would have no effect on the number of parasites found (Llewellyn 1962). Before a full external parasitological examination was performed, each fish was thawed and its caudal length to the nearest 0.1cm measured and weight to the nearest 0.5g recorded. The external parasitological examination was performed by looking over the skin of the fish, under its fins and in its mouth for any external parasites. Both opercula, eyes and gills were removed and examined for parasites under a dissecting microscope (Leica EZ34) at magnifications of 10x-63x (Figure 8a,c). Subsequently, the fish were cut open along the ventral line and internal organs removed for parasitological examination. Fish were sexed and the body cavity examined for any macroscopic parasites, and then wet mounts of equal sized (0.5cm x 0.5cm) pieces of tissue from the gonads, kidney, spleen, liver and muscle were examined under a compound microscope (Leica DM750) at 400x magnification. The viscera (stomach, intestine and pyloric ceacae) as well as their contents were also examined for parasites using the dissecting microscope (Leica EZ34) (Figure 8b). All parasitic species found were recorded and subsequently preserved in 70% alcohol. The site of infection and number of individuals of each parasite taxa found was recorded for each host. A categorical scale was used to determine the relative estimates of parasites found in the wet mounts under the compound microscope (Table 2). Parasites were identified with the help of Dr. Cecile Reed (University of Cape Town) and using micrographs in Le Roux (2013).

Table 2: Categorical scale used to indicate the relative estimate of parasites found.

Scale	Number of parasites in field of view
x0	0
x1	1 to 10
x10	11 to 100
x100	101 to 1000

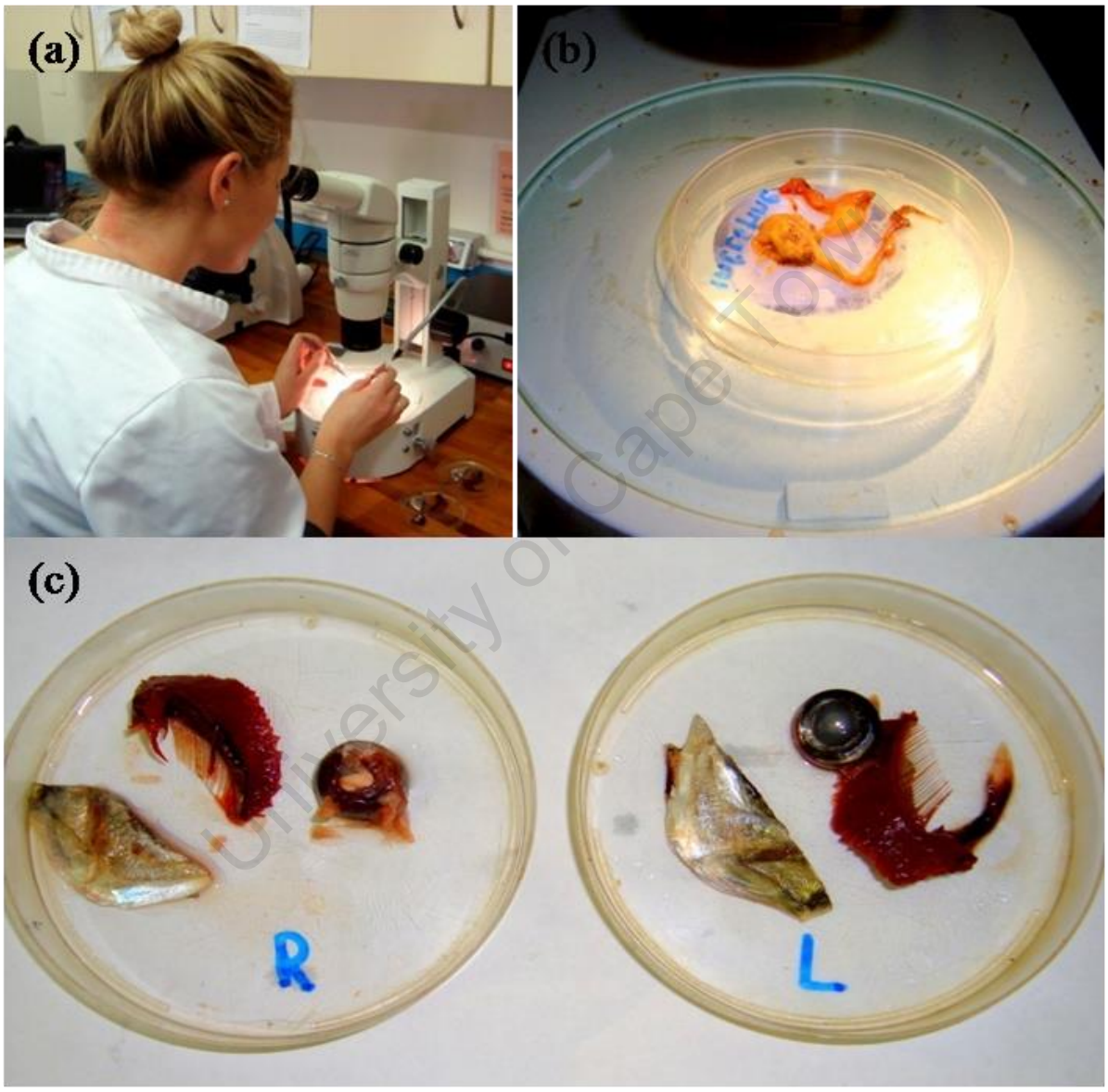

Figure 8: (a) Processing *Trachurus trecae* for parasites under a dissecting microscope; (b) the stomach and intestine under the dissecting microscope; (c) the right and left opercula, eyes and gills prior to examination.

Data analysis

Three measures of parasitic infection were used as described by Bush et al. (1997); the proportion of the host population infected with a particular parasite species (prevalence), the mean number of parasite individuals found infecting a single host (mean intensity) and the mean number of parasite individuals found within a host sample (mean abundance). These were calculated for each parasitic taxa found. All data was recorded in Microsoft Office Excel 2007.

$$Prevalence = \frac{Number\ of\ infected\ fish}{Total\ number\ of\ fish} \times 100\%$$

$$Mean\ intensity = \frac{Total\ number\ of\ parasites}{Number\ of\ infected\ fish}$$

$$Mean\ abundance = \frac{Total\ number\ of\ parasites}{Total\ number\ of\ fish}$$

The entire parasite community infecting *T. trecae* was analysed in terms of prevalence, mean infection intensity and mean infection abundance values. A comparison of prevalence values between two size classes of *T. trecae* in the northern Benguela were made as two groups of different sized fish were available (see results). A comparison of prevalence values between immature, male and female *T. trecae* was also made. In addition, prevalence values of the same size class *T. trecae* and *T. capensis* from the northern Benguela were compared.

Differences in parasite assemblages infecting different size classes and genders of *T. trecae* and similar size classes of *T. capensis* were analysed using the Primer 6, version 6.1.5 software package. A square root transformation of the data was used to reduce the effect of larger abundances and a Bray-Curtis similarity coefficient was used to measure resemblance. The differences in parasite assemblages between small and large *T. trecae* were tested using a one-way ANOSIM. This was then repeated for differences in parasite assemblages between male, female and immature *T. trecae*. The data were analysed in terms of a group average hierarchical cluster analysis and dendrograms for each factor (size and sex) were plotted. A non-metric Multi-Dimensional Scaling ordination was performed and configuration plots for each factor were plotted. A one-way SIMPER analysis based on the Bray-Curtis similarity coefficient was then used to select the parasite species responsible for the clustering of

samples. This data analysis method was then repeated for the comparison of parasite assemblages between large *T. trecae* and large *T. capensis*; however a fourth root transformation was used and sex was not included.

A discriminate function analysis (DFA) was used to analyse the abundance values of infecting parasites of small *T. trecae*, large *T. trecae* and large *T. capensis*. A standard DFA as used by Melendy et al. (2005), McClelland and Melendy (2011) and Le Roux (2013) was used to similarly select the parasite species responsible for distinguishing between different sizes and species of horse mackerel in the northern Benguela. This was performed using the Statistica 10 (StatsSoft 2011) software package. All statistical tests were considered significant at $p<0.05$.

Results

A total of 72 *T. trecae* from three sampling stations in the northern Benguela were examined (Figure 7, Table 1). Fish ranged in size from 9-22cm in caudal length and showed a clear bimodality at 14cm in their size distribution (Figure 9). For this reason, fish were divided into two size classes; a small size class of 9-14cm and a large size class of 16-22cm. Of the 16-22cm size class, 48 *T. trecae* were compared to 24 *T. capensis* sampled in the northern Benguela by Le Roux (2013). These *T. capensis* were sampled during the same survey that collected the *T. trecae* species (Table 1).

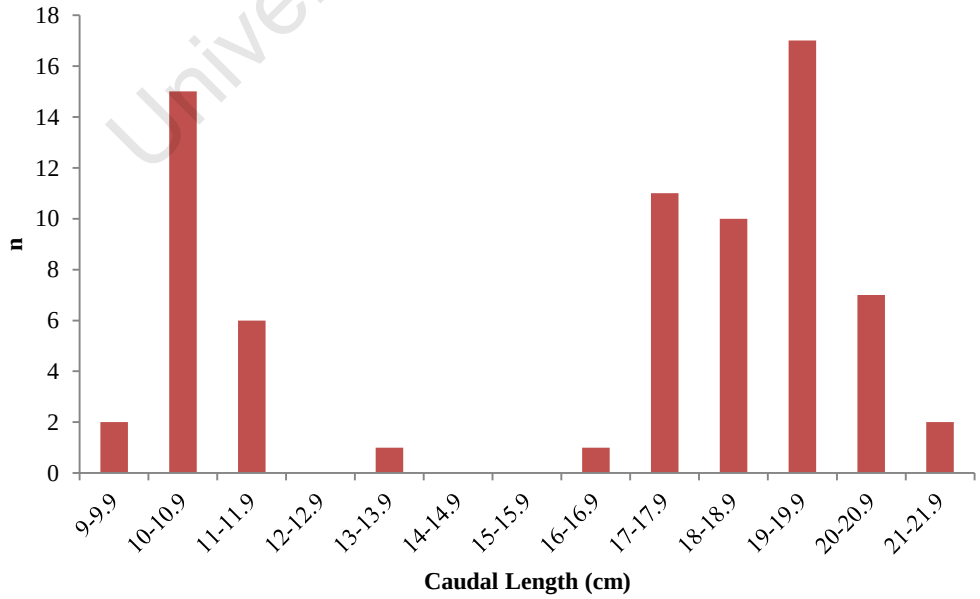

Figure 9: Length frequency distribution of *Trachurus trecae* (n=72) from the northern Benguela sampled in August 2011 by the research vessel Dr Fridjof Nansen.

Parasite assemblages

A total of six parasite taxa were recorded for *T. trecae*, of which four were identifiable to species, two to genus and one to class level (Table 3, Figure 10). The most prevalent parasitic species infecting *T. trecae* was the coccidian *Goussia cruciata* (68.1%) infecting the liver followed by the monogenean *Gastrocotyle trachuri* (45.8%) infecting the gills and lastly the unidentified *digenean* (25.0%) infecting the pyloric ceacae, intestine, gills and stomach. All other parasitic species infecting *T. trecae* showed much lower prevalence.

Large *T. trecae* had higher prevalence values compared to small *T. trecae* for all parasitic taxa, except for the myxozoan *Davisia donecae* which infected the kidney and spleen of 2.2% of large *T. trecae* and 12.5% of small *T. trecae* (Figure 11). The largest difference in prevalence between the two size classes was the coccidian *G. cruciata* which infected the livers of 85.4% of large *T. trecae* and 29.2% of small *T. trecae*. The second largest difference was the unidentified *digenean*, which infected organs of 35.4% of large *T. trecae* and 4.2% of small *T. trecae*.

Male *T. trecae* had higher prevalence values compared to female and immature *T. trecae* for all parasitic taxa, except for the coccidian *G. cruciata* which infected 83.3% of females, 65.2% of males and 35.5% of immature *T. trecae*, as well as the myxozoan *D. donecae* which infected 11.1% of females, 8.7% of males and no immature *T. trecae* (Figure 12). There were no large differences in prevalence values between male and female *T. trecae*, but large differences between immature and male *T. trecae*, and immature and female *T. trecae* respectively. Only two parasite species infected immature *T. trecae*; *G. cruciata* as already mentioned and *G. trachuri* with a prevalence of 19.4%.

Table 3: Parasites infecting *Trachurus trecae* (Smith-Vaniz, 1986) in the northern Benguela in terms of prevalence, mean infection intensity and mean infection abundance values.

Class	**Species**	**Site**	**Prevalence (%)**	**Mean infection intensity**	**Mean parasite abundance**
Coccidea	*Goussia cruciata*	Liver	68.1	x10	x10
Copepoda	Unidentified sp.	Gills	4.2	1.0 (±0)	0.0 (±0.0237)
Trematoda	Unidentified *digenea*	Pyloric Ceacae, intestine, gills, stomach	25.0	3.1 (±0.6949)	0.8 (±0.2334)
Monogenea	*Gastrocotyle trachuri*	Gills	45.8	3.0 (±0.3536)	1.4 (±0.2394)
Myxozoa	*Ceratomyxa australis*	Gall bladder	4.2	1.0 (±0)	0.0 (±0.0237)
	Davisia donecae	Gall bladder, liver, kidney	5.6	1.0 (±0)	0.1 (±0.0272)
Nematoda	*Anisakis* sp.	Intestine, pyloric ceacae	8.3	1.5 (±0.3416)	0.1 (±0.0557)

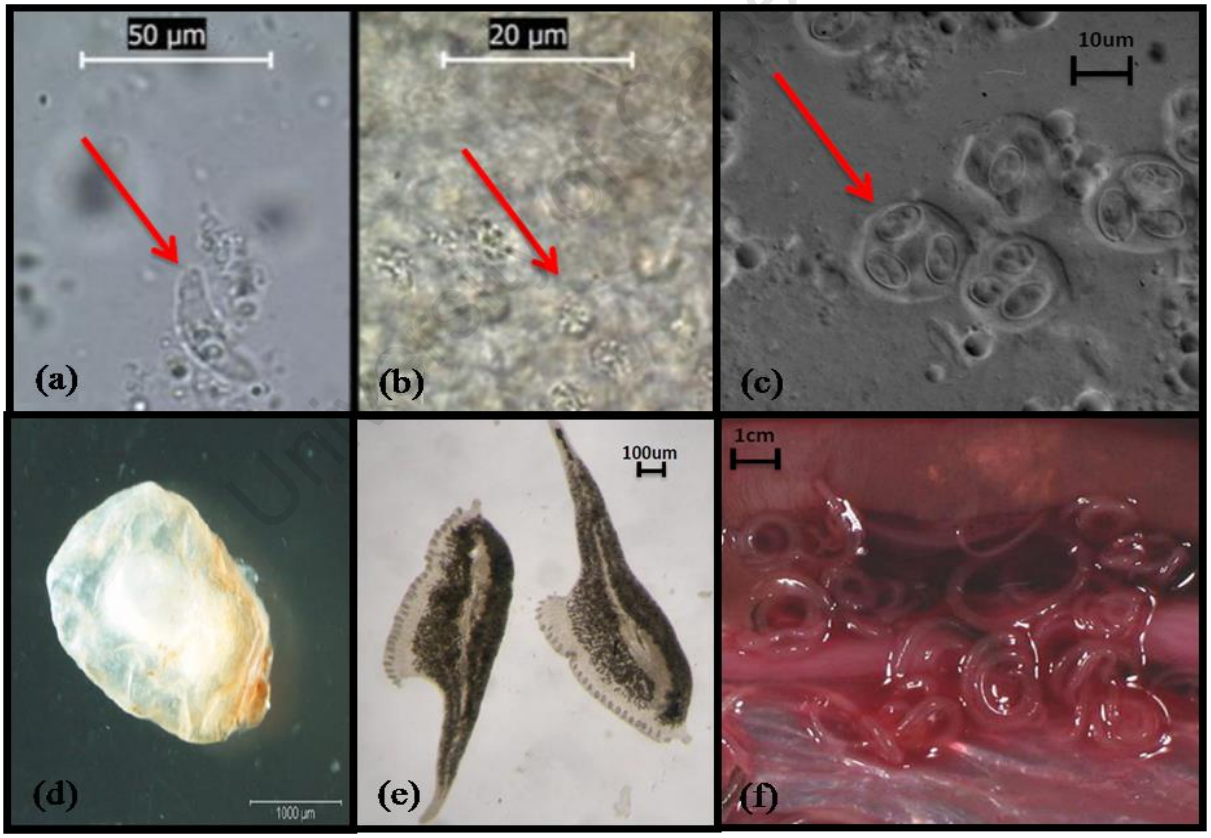

Figure 10: Parasite species found in *Trachurus trecae* and *Trachurus capensis* from the northern Benguela; (a) myxozoan *Ceratomyxa australis* found in the gall bladder, (b) myxozoan *Davisia donecae* found in the liver, kidney and spleen, (c) coccidian *Goussia cruciata* found in the liver and gall bladder, (d) unidentified *digenea* sp. found in the pyloric ceacae, intestine, gills and stomach, (e) monogenea *Gastrocotyle trachuri* found in the gills and (f) nematoda *Anisakis* sp. found in the pyloric ceacae, gonads, stomach, intestine and muscle. All photographs were taken from Le Roux (2013).

A total of six parasite taxa were recorded for *T. capensis*, of which six were identifiable to species and two to genus level (Table 4, Le Roux, 2013). Similar parasite assemblages were found in both *T. trecae* and *T. capensis* with the exception of an identified copepod found only on the gills of *T. trecae* with a prevalence value of 6.3% and the trematode *Ectenurus lepidus* found only in the gut of *T. capensis* (50.0%). The coccidian *G. cruciata* was the most prevalent (100.0%) parasitic species infecting the liver of *T. capensis* (Table 3). This was followed by a nematode from the genus *Anisakis* sp. (75.0%) infecting the viscera, which interestingly showed the lower infection prevalence in *T. trecae* (8.3%). Mean infection abundances and intensities were similar for all common parasitic species infecting both *T. trecae* and *T. capensis* except the coccidian *G. cruciata*. Sites of infection differed slightly for common parasitic species infecting *T. trecae* and *T. capensis* such as *G. trachuri* which infected the gills of *T. trecae*, but infected the stomachs of *T. capensis*.

Table 4: Parasites infecting *Trachurus capensis* (Castelnau, 1861) in the northern Benguela in terms of prevalence, mean infection intensity and mean infection abundance values from Le Roux, 2013).

Class	**Species**	**Site**	**Prevalence (%)**	**Mean infection intensity**	**Mean parasite abundance**
Coccidea	*Goussia cruciata*	Liver, gall bladder	100.0	x1000	x1000
Copepoda	*Lernanthropus trachuri*	Gill	50.0	1.0 (±0.2876)	0.0 (±0.2444)
Trematoda	*Digenea* sp.	Gill, intestine	20.8	3.1 (±0.5477)	0.8 (±0.1989)
	Ectenurus lepidus	Intestine	4.2	1.0 (±0)	0.0 (±0.0417)
Monogenea	*Gastrocotyle trachuri*	Stomach	4.2	3.0 (±0)	1.4 (±0.0417)
Myxozoa	*Ceratomyxa australis*	Gall bladder	16.7	1.0 (±0)	0.2 (±0.0777)
	Davisia donecae	Kidney, spleen	33.3	1.0 (±119.2499)	0.1 (±0.0272)
Nematoda	*Anisakis* sp.	Pyloric ceacae, gonads, stomach, intestine, muscle	75.0	1.5 (±6.4185)	0.1 (±0.0557)

Of the entire parasite assemblage infecting both horse mackerel species, four parasitic species (*G. cruciata, C. australis, D. donecae,* and *Anisakis* sp.) infected a larger proportion of *T. capensis* than *T. trecae* while an unidentified copepod, unidentified *digenea*. and *G. trachuri* had higher prevalence values in *T. trecae* (Figure 13). The largest difference in prevalence between *T. trecae* and *T. capensis* of 16-22cm was a nematode from the genus *Anisakis* sp. infecting the viscera with a prevalence of only 10.4% in *T. trecae* and 75.0% in *T. capensis*. The second largest difference was the infection prevalence of a monogenean *G. trachuri*, which infected a larger portion of *T. trecae* (52.0%) than *T. capensis* (4.2%). The coccidian

G. cruciata showed high prevalence values in both species of horse mackerel with all the livers of *T. capensis* being infected (100.0%) and 85.4% of all the livers of *T. trecae*.

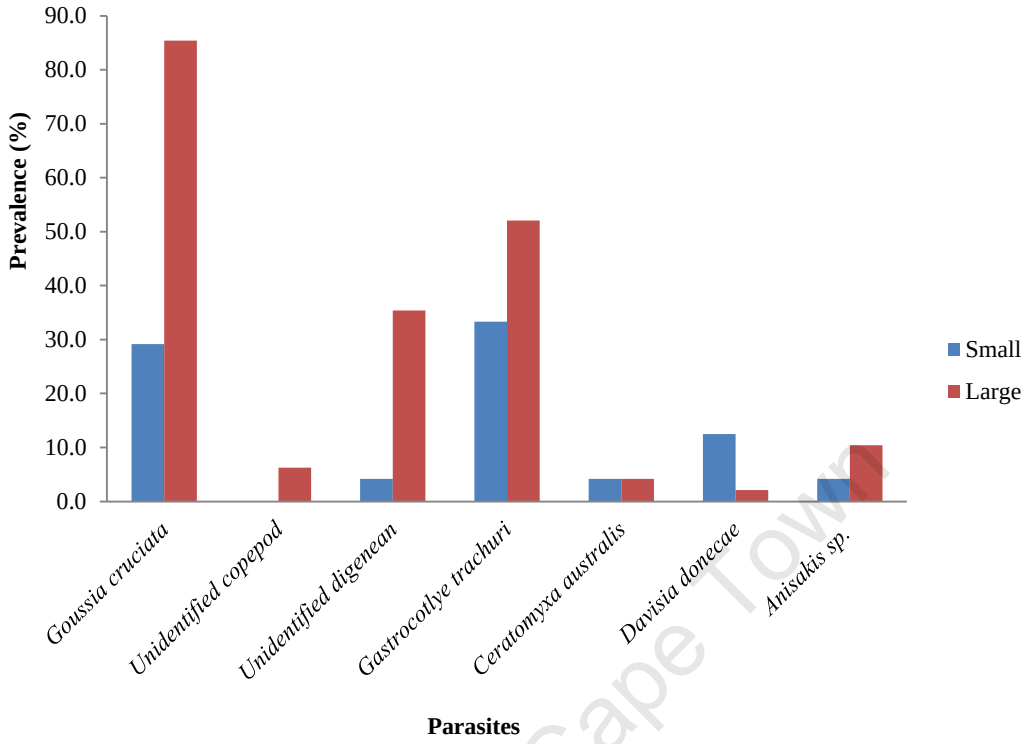

Figure 11: Comparison of prevalence values for common parasite taxa infecting small (9-14cm) and large (16-22cm) *Trachurus trecae* (n=72) in the northern Benguela

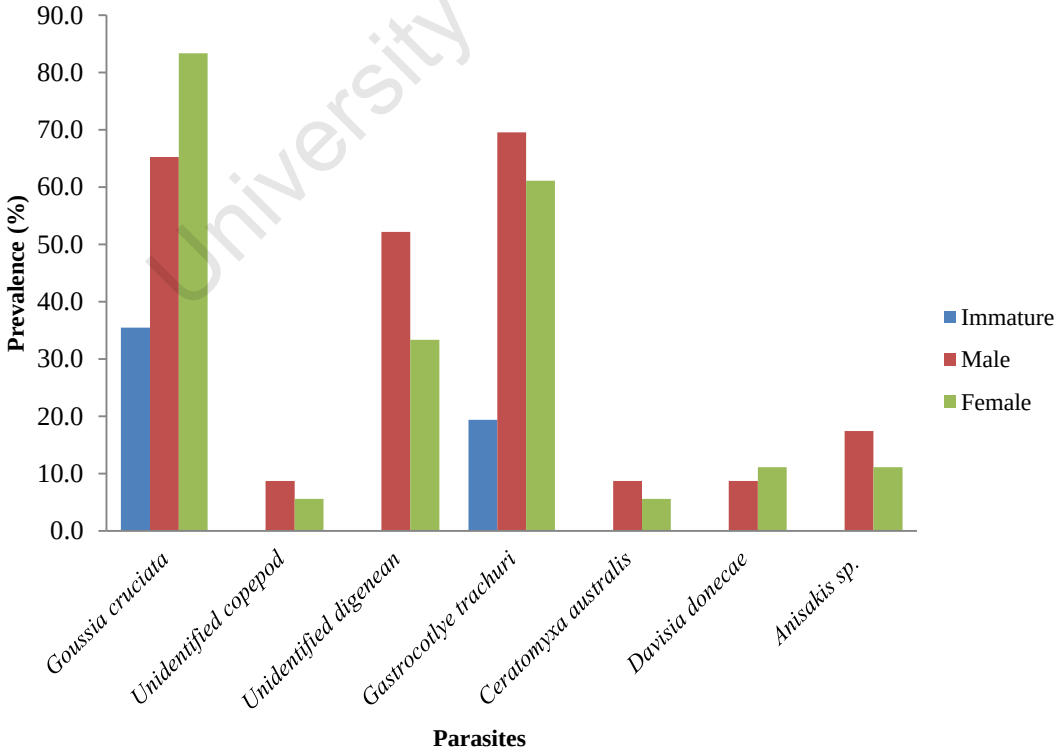

Figure 12: Comparison of prevalence values for common parasite taxa infecting immature (n=31), male (n=23) and female (n=18*) Trachurus trecae* in the northern Benguela.

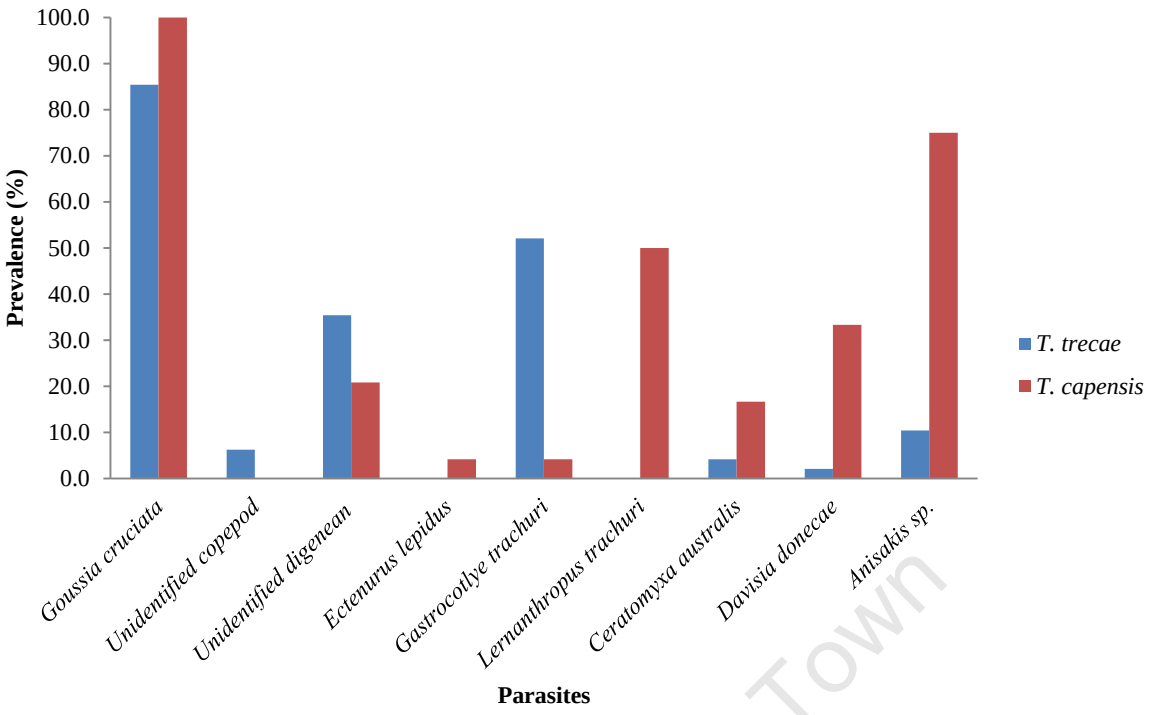

Figure 13: Comparison of prevalence values for common parasite taxa infecting same size class (16-22cm) *Trachurus trecae* (n=48) and *Trachurus capensis* (n=24) in the northern Benguela.

Statistical analyses

Size effect of parasite assemblages between small and large *Trachurus trecae*

Significant size separations were detected in *T. trecae* based on parasite assemblage (ANOSIM: R=0.293, p<0.001). The cluster MDS analysis revealed the existence of four main groups of *T. trecae* with similar parasite assemblages (Figure 14a, b). The SIMPER analysis indicated that *G. cruciata* contributed the most to the dissimilarities found between size classes with an average infection of 2.7 *G. cruciata* per small fish, 5.2 *G. cruciata* per large fish and a total dissimilarity contribution of 62.6% (Figure 17). This was followed by *G. trachuri* with an average infection of 0.9 *G. trachuri* per small fish, 0.8 *G. trachuri* per large fish and a total dissimilarity contribution of 17.4% (Figure 17).

Sex effect of parasite assemblages of *Trachurus trecae*

Significant gender separations were detected based on the parasite assemblage between immature, male and female *T. trecae* (ANOSIM: R=0.107, p<0.01). The cluster and MDS analysis revealed the existence of four main groups of *T. trecae* with similar parasite assemblages (Figure 15a, b). A greater separation based on parasite assemblage is seen between immature fish and male/female fish than between male and female fish respectively,

although there is a small distinction between males and females (Figure 15b). The SIMPER analysis indicated that *G. cruciata* contributed the most to dissimilarities found between sex aggregations contributing 56.0%, 62.9% and 62.3% for male and female, male and immature, and female and immature fish dissimilarities respectively (Figure 17). The second highest contributor to dissimilarity was the *G. trachuri* species.

Interspecific differences of parasite assemblages of *Trachurus trecae* and *Trachurus capensis*

Significant species separations were detected based on the parasite assemblage (ANOSIM: R=0.377, p<0.01). The cluster and MDS analysis revealed clear separation between *T. trecae* and *T. capensis* species with the exception of five *T. capensis* fish (Figure 16a, b). The SIMPER analysis indicated that *G. cruciata* contributed the most to the dissimilarities found between the two species of horse mackerel with an average infection of 1.8 *G. cruciata* per *T. trecae*, 4.1 *G. cruciata* per *T. capensis* and a total dissimilarity contribution of 41.0% (Figure 17). This was followed by *Anisakis* sp. with an average infection of 0.1 *Anisakis* sp. per *T. trecae*, 1.3 *Anisakis* sp. per *T. capensis* and total dissimilarity contribution of 59.0% (Figure 17).

Selected Parasite species

Mean infection abundance and mean infection intensity was compared between small and large *T. trecae*, and between large *T. trecae* and large *T. capensis* for six common parasites; (a) *Ceratomyxa australis*, (b) *Anisakis* sp., (c) *Davisia donecae*, (d) unidentified *digenea* sp., (e) *Gastrocotyle trachuri* and (f) *Goussia cruciata*.

The mean infection abundance of *C. australis* was highest in large *T. capensis* (approximately 0.16), did not infect small *T. trecae* and had a small infection abundance of 0.06 in large *T. trecae* (Figure 18a). Similarly, mean infection abundance of *Anisakis* sp. (12.0) and *D. Donecae* (60.0) were also highest in large *T. capensis*, with almost zero values for small and large *T. trecae* (Figure 18b, c). Conversely, unidentified *digenea* and *G. trachuri* mean infection abundance was highest in large *T. trecae* (1.2 and 1.8 respectively) (Figure 18d, e). *Goussia cruciata* was found to have a modal mean abundance infection of x1000 for large *T. capensis*, but zero infections for both small and large *T. trecae* (Figure 18f).

Ceratomyxa australis mean infection intensity was identical in both large *T. trecae* and large *T. capensis* while a zero mean intensity was found in small *T. trecae* (Figure 19a). *Anisakis* sp. and *D. donecae* both showed higher mean infection intensities in large *T. capensis* (approximately 15 and 200 respectively) compared to near zero intensities in small and large *T. trecae* (Figure 19b, c). Unidentified *digenea* sp. had the highest mean infection intensity of approximately 3.5 in large *T. trecae* compared to a value of two in large *T. capensis* and a value of zero in small *T. trecae* (Figure 19d). *Gastrocotyle trachuri* infection intensity was also highest in large *T. trecae* (approximately 3.5) with smaller intensities in both small *T. trecae* and large *T. capensis* (Figure 19e). The majority of individual small and large *T. trecae* had no or little infection by *G. cruciata* while there was conversely a very high mean infection intensity of x1000 found in *T. capensis* (Figure 19f).

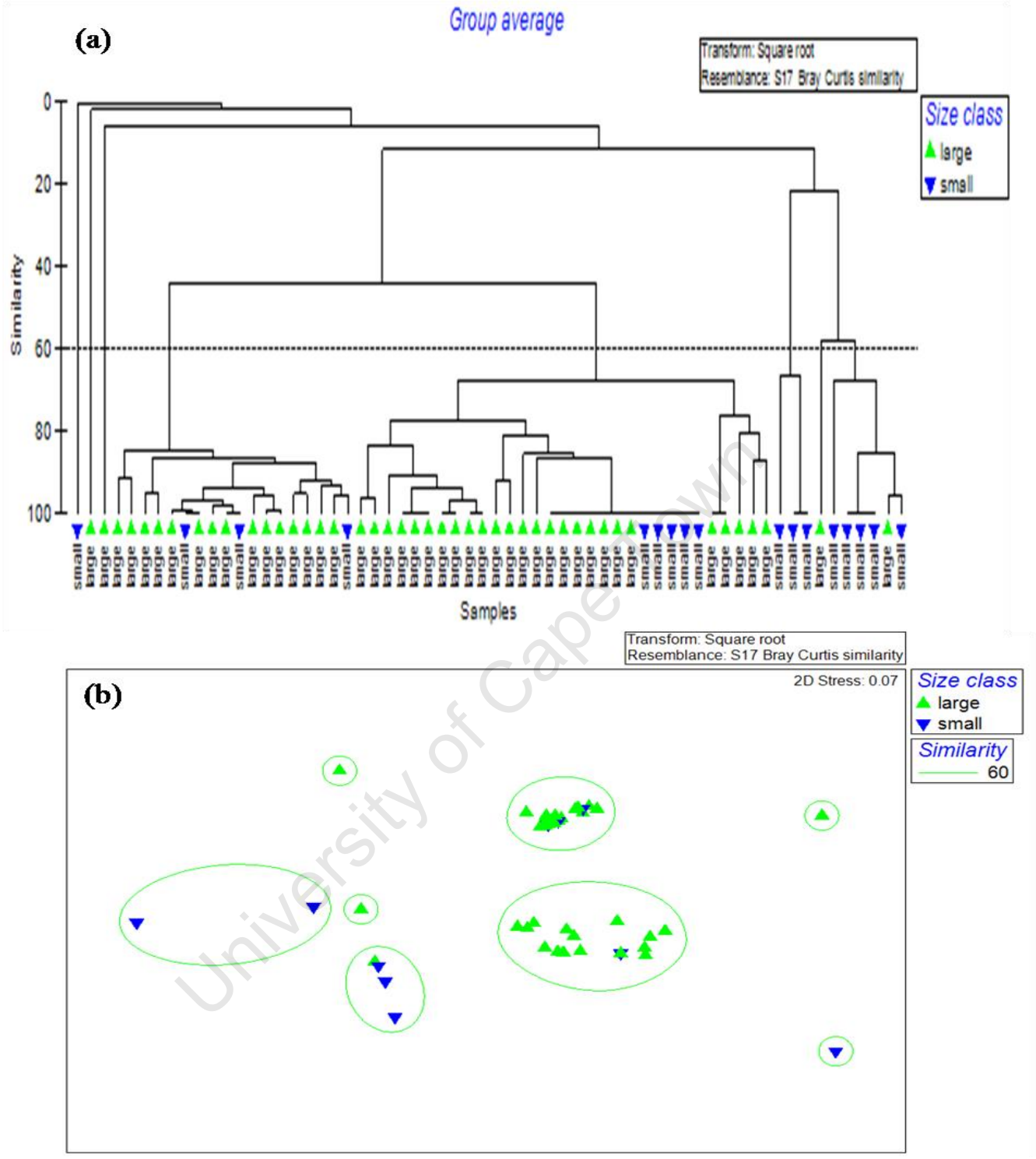

Figure 14: (a) Dendrogram and (b) MDS diagram of parasite species infecting small (n=24) and large *Trachurus trecae* (n=48) samples found in the northern Benguela.

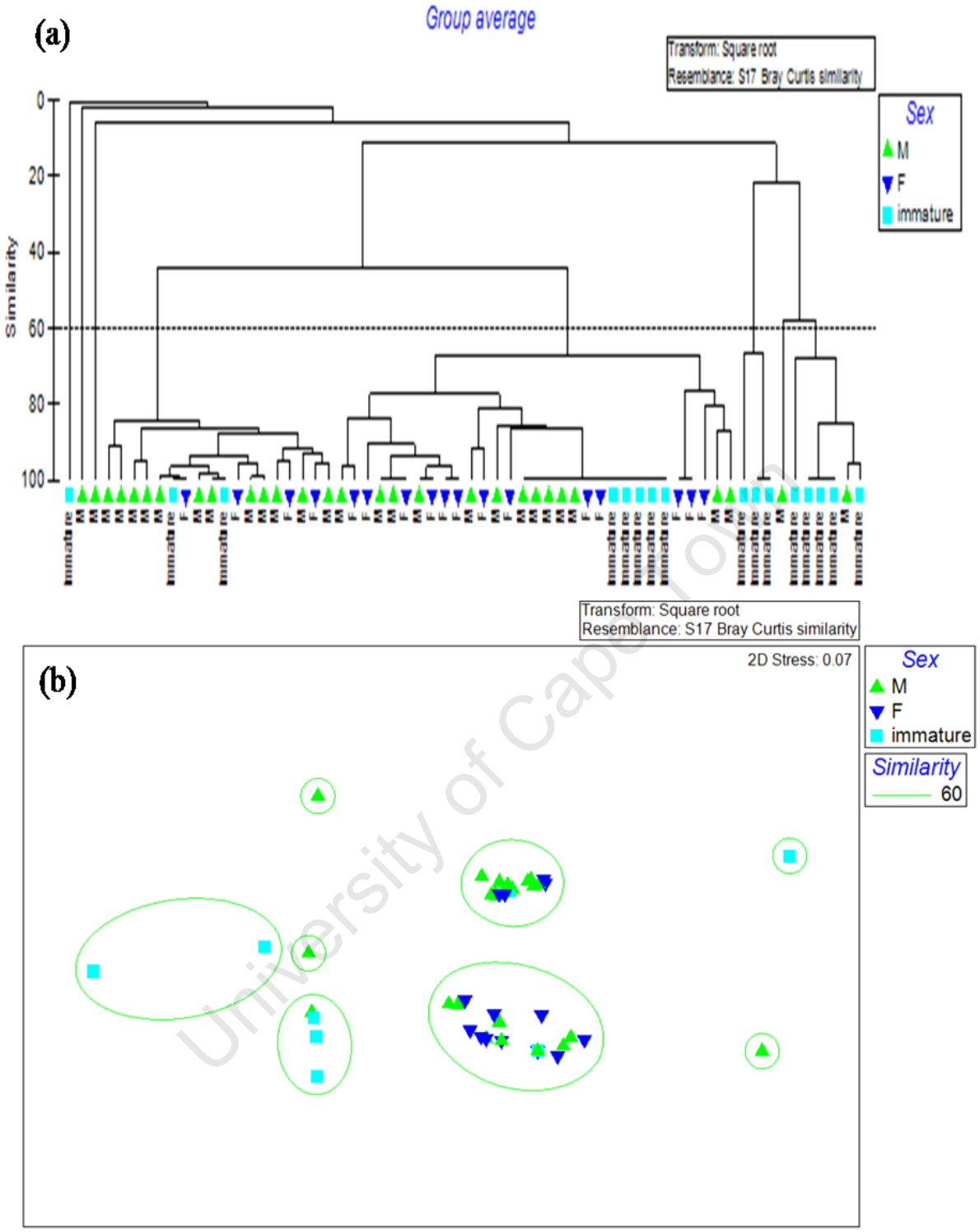

Figure 15: (a) Dendrogram and (b) MDS diagram of parasite species infecting male (n=23), female (n=18) and immature (n=31) *Trachurus trecae* samples found in the northern Benguela.

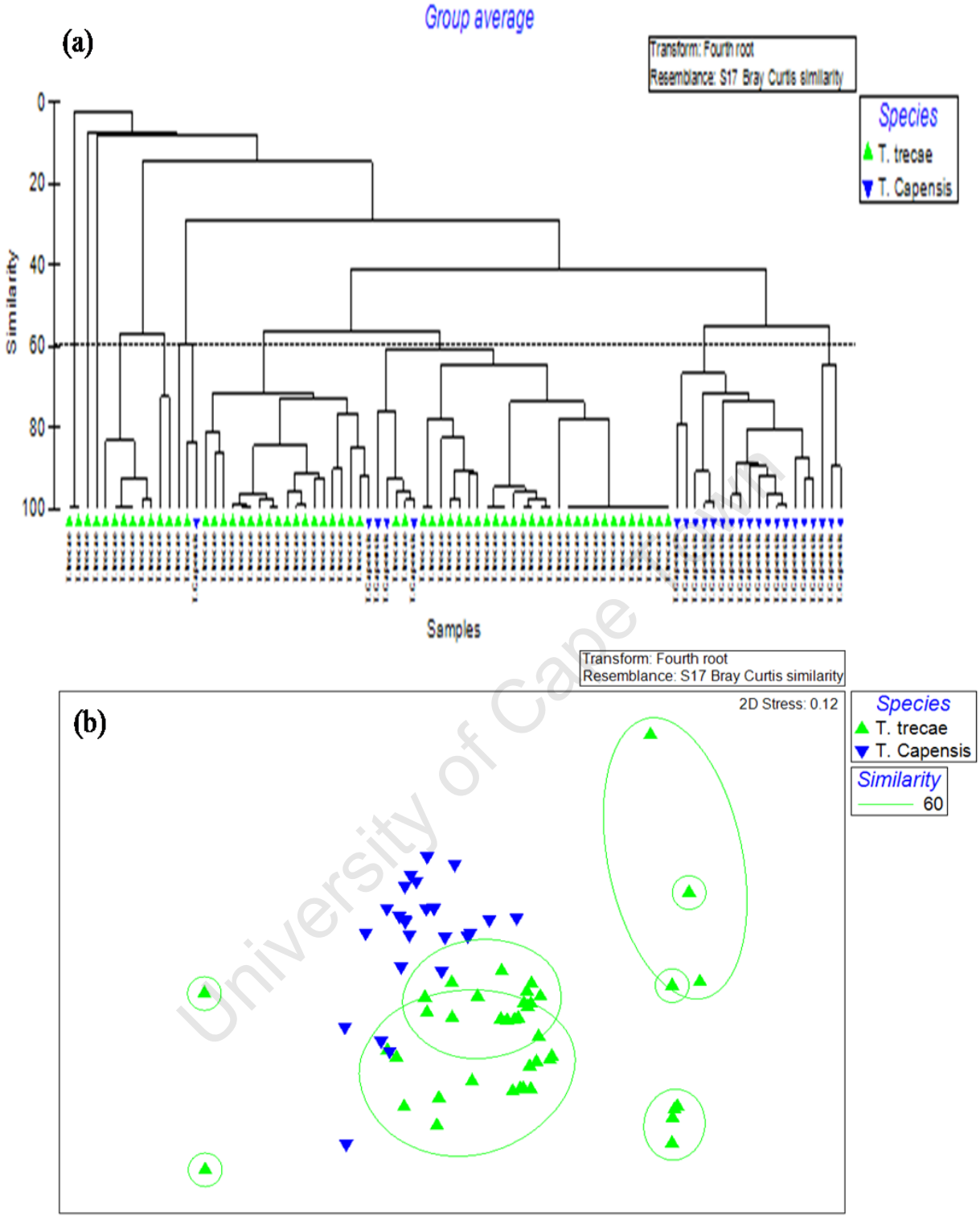

Figure 16 (a) Dendrogram and (b) MDS diagram of parasite species infecting same size class *Trachurus trecae* (n=48) and *Trachurus capensis* (n=24) samples found in the northern Benguela.

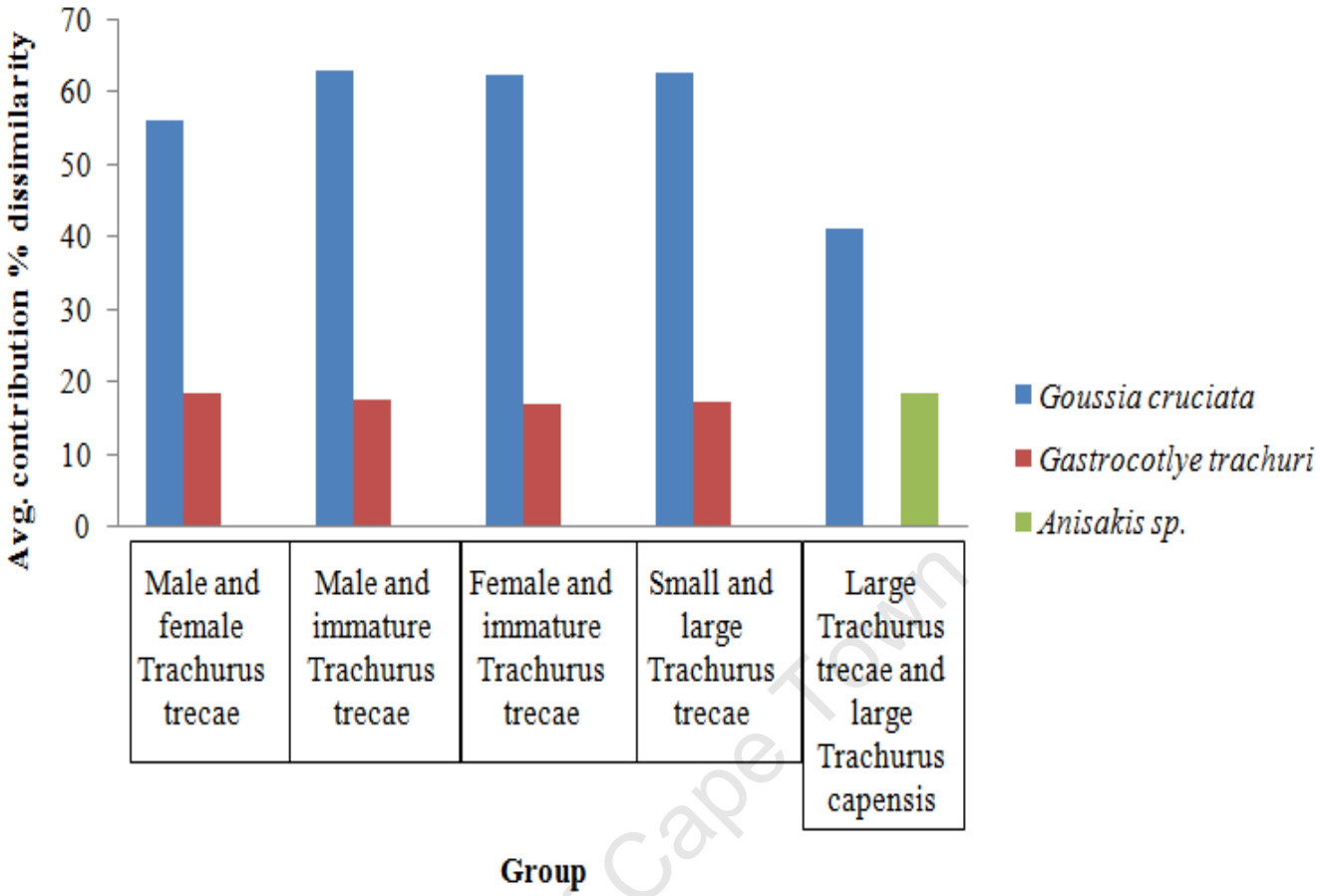

Figure 17: Bar graph showing the SIMPER Analysis of parasite species dissimilarity contributions between immature, male and female *Trachurus trecae* (n=72), small (n=24) and large *Trachurus trecae* (n=48) and between large *Trachurus trecae* (n=48) and large *Trachurus capensis* (n=24).

Discriminate Function Analysis (DFA)

Two separate standard DFA's were performed using the six selected parasitic species to assess whether (a) smaller and larger *T. trecae* could be distinguished using abundance data of the selected parasitic species and (b) whether *T. trecae* and *T. capensis* of similar sizes could be distinguished using abundance data of the selected parasitic species.

Discriminate analysis of Small and large *Trachurus trecae*

Despite significant differences found in the one-way ANOSIM, no parasites were found to be significant in discriminating between small and large *T. trecae* in the northern Benguela (Table 5). For this reason only 66.7% of *T. trecae* were correctly classified. All large *T. trecae* were correctly classified, but no small *T. trecae* were correctly classified.

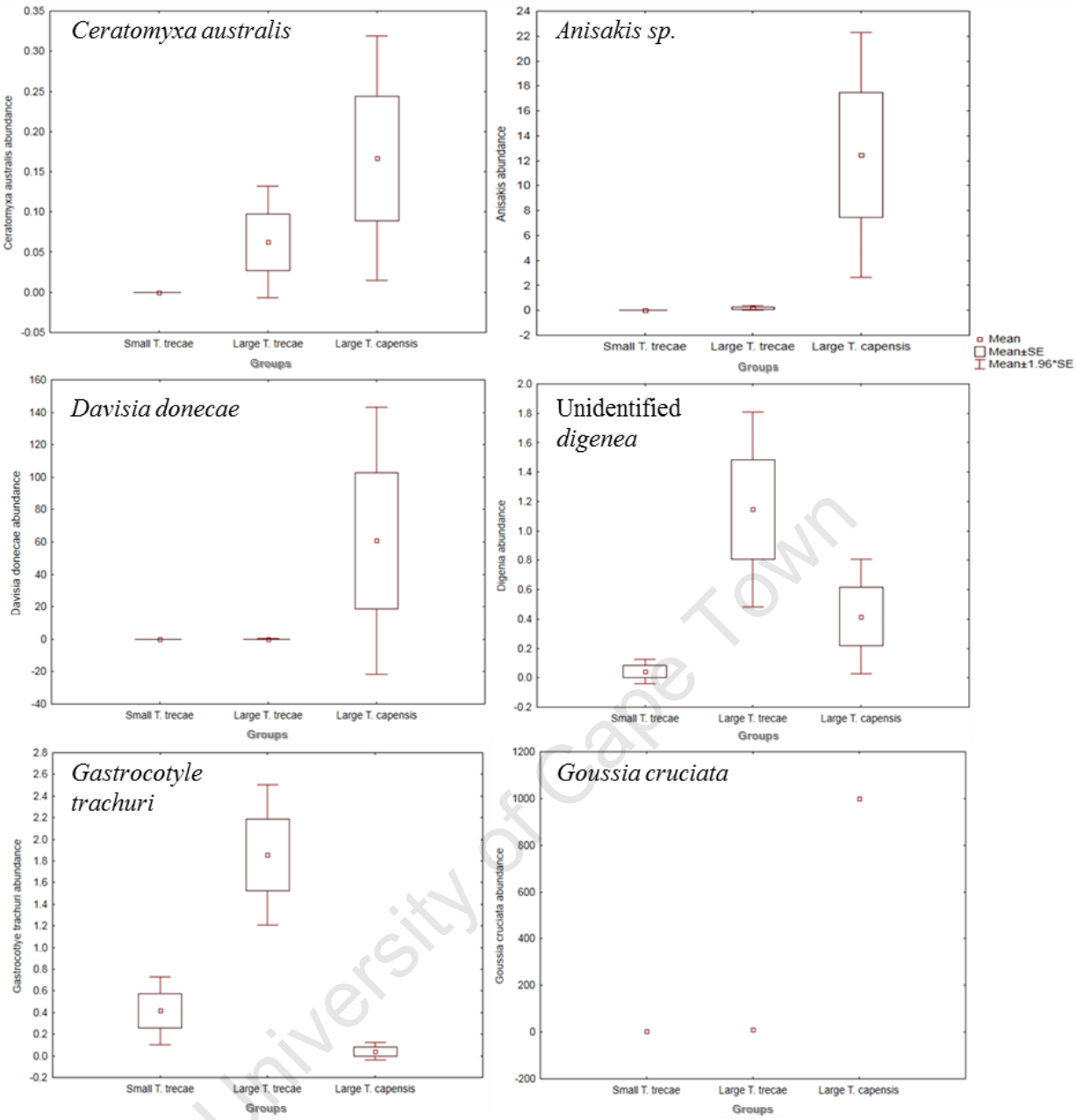

Figure 18: Box and whisker plots showing mean (*modal) infection abundance of six selected common parasitic species infecting small *Trachurus trecae* (n=24), large *Trachurus trecae* (n=48) and large *Trachurus capensis* (n=24) from the northern Benguela.

Discriminate analysis of *Trachurus trecae* and *Trachurus capensis*

Two parasitic species were found to be significant in discriminating between the different species of horse mackerel found in the northern Benguela, namely, *G. trachuri* (F=6.92, p<0.01) and *G. cruciata* (F=45.05, p<0.01) (Table 6). These parasites correctly classified 87.5% of horse mackerel (Table 7). All *T. trecae* were correctly classified, but only 2/3 of *T. capensis* were correctly classified.

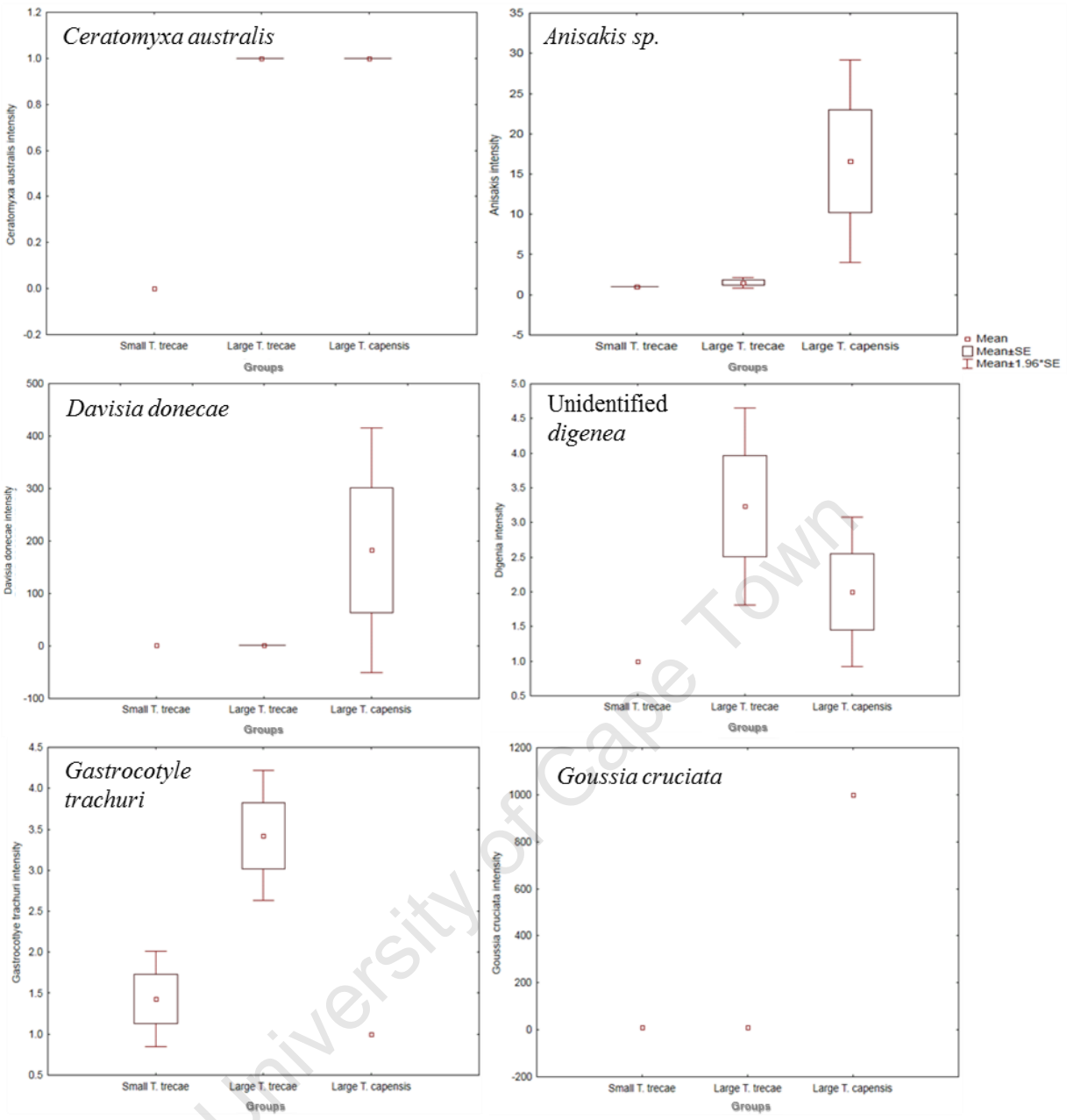

Figure 19: Box and whisker plots showing mean (*modal) infection intensity of six selected common parasitic species infecting small *Trachurus trecae* (n=24), large *Trachurus trecae* (n=48) and large *Trachurus capensis* (n=24) from the northern Benguela.

Table 5: Discriminate Function Analysis of parasite abundances revealing the classification of *Trachurus trecae* into small and large size classes in the northern Benguela. % correct indicates the percentage of the total sample correctly classified into small or large *Trachurus trecae.*

		Predicted		
		Small	Large	% correct
Actual	Small	0	24	0.00
	Large	0	48	100.00
	Total	0	72	66.67

Table 6: Discriminate Function Analysis results of parasite abundances infecting large *Trachurus trecae* and *Trachurus capensis* found in the northern Benguela. Parasitic species marked in bold indicate significant contributions to the DFA.

	F value	P value	Standardized coefficient of canonical variable
Unidentified *digenea*.	1.25	0.27	-0.53
Gastrocotyle trachuri	**6.92**	**0.01**	0.86
Ceratomyxa australis	0.19	0.66	0.15
Davisia donecae	0.01	0.92	0.03
Anisakis sp.	0.00	0.98	-0.01
Goussia cruciata	**45.05**	**0.00**	-0.92

Table 7: Discriminate Function Analysis of parasite abundances revealing the classification of horse mackerel into *Trachurus trecae* and *Trachurus capensis* species in the northern Benguela. % correct indicates the percentage of the total sample correctly classified into either species of horse mackerel.

		Predicted		
		Trachurus trecae	*Trachurus capensis*	% correct
Actual	*Trachurus trecae*	48	0	100.0
	Trachurus capensis	9	15	62.5
	Total	57	15	87.5

Discussion

Entire parasite assemblages and selected parasite taxa were used in statistical analyses (ANOSIM and DFA respectively) to determine whether the parasite assemblage of *T. trecae* is driven by intraspecific relationships (fish size and sex). In addition this parasite assemblage was compared to that of *T. capensis* to assess whether there is a species specific infection pattern.

Intraspecific comparisons

A significant size effect on parasite assemblages was found for *T. trecae* with the coccidian *G. cruciata* and the monogenean *G. trachuri* contributing the most to this discrimination. These results are consistent with the literature. Parasitic infections increase with size as older, larger fish have a longer time to accumulate parasites and larger internal and external space for parasite establishment. Larger fish also eat more parasitized prey, which causes them to have higher infections (Poulin, 2000; Abollo et al., 2001). In contrast, the myxozoan *D. donecae* had a higher prevalence in small *T. trecae* than large *T. trecae*. A similar result was found for *T. capensis* (Le Roux 2013) where the coccidian *G. cruciata* had a higher prevalence in small *T. capensis* than large *T. capensis*. In this study 24 small *T. trecae* were compared with 48 large *T. trecae*. These sample sizes are relatively small and could have affected the results. Larger samples of *T. trecae* and *T. capensis* could be used for better comparisons as well as including the putative 'northern' stock of *T. trecae* to test the two stock hypothesis of Sardinha and Naevdal (2002).

Although small, a significant sex effect based on parasite assemblage was found between immature, male and female *T. trecae*. Parasites that contributed significantly to this discrimination were the coccidian *G. cruciata* and the monogenean *G. trachuri.* Both parasitic species had higher average abundances in males compared to females and immature fish respectively. These results contradict those found in literature where parasitic infections are seen to have no difference in infection with host sex (Abollo et al., 2001); however these results reflect the size effect of parasite assemblage as immature fish are usually quite small and will therefore have fewer parasitic infections than larger males and females.

Interestingly, the Discriminate Function Analysis proved ineffective in finding significant parasitic species that could be used for discriminating between the two size classes of *T. trecae.* Although the ANOSIM and DFA both test statistically whether there is a significant difference between two or more groups of sampling units, the analyses have different assumptions. The DFA is said to be a more powerful analysis, but is quite sensitive to outliers. It also has assumptions of normal distribution and homogeneity of variances/covariances etc., which could weaken results if not met. ANOSIM assumes that all ranked dissimilarities within groups have equal medians and ranges and is said to be a more rhobust alternative. It has recently gained widespread use by marine ecologists because of the user friendly PRIMER software package (Anderson, 2001). Although it is difficult to state

which analysis should be used in this study, it would be unwise to only look at results of one analysis for fear of missing significant differences given in another. Since only common parasites infecting both horse mackerel were used in the DFA, parasitic species that were not present in both horse mackerel could have served as significant discriminates. Therefore if further DFA's are done, they should include all parasitic species.

Interspecific comparison of similar sizes

Differences in parasite assemblages were larger between *T. trecae* and *T. capensis* than between *T. trecae* of different sizes or gender. In both statistical analyses *G. cruciata* was the most significant parasite species in distinguishing between different species of horse mackerel in the northern Benguela and so possibly could be used as a biotag for discriminating between the two *Trachurus* species in the future. Using this parasitic species 87.5% of the total sample of horse mackerel was classified correctly into *T. trecae* and *T. capensis*. This is consistent with literature regarding the use of *G. cruciata* as a potential biotag (Abollo et al., 2001; Mackenzie et al., 2004; Gestal and Azevedo, 2005). Again however, differences between the ANOSIM and DFA analyses arose regarding the second highest contributor. The ANOSIM indicated *Anisakis* sp. to be the second highest contributor for species discrimination, whereas the DFA found *G. trachuri* to be the second highest contributor.

Anisakis sp. had a very high prevalence in *T. capensis*, but barely infected *T. trecae*. This could be due to only a small amount of *Anisakis* sp. being able to tolerate the warmer temperature of 18°C as opposed to 14°C at *T. capensis* sample location. Matiucci et al., (2008) found significant differences in proportions of infection by two species of *Anisakis* (*A. pegreffii* and *A. simplex*) in *T. trachurus* along the Iberian coast. These results indicated discrete sub populations of *T. trachurus* in the Mediterranean and Atlantic waters, confirming the use of *Anisakis* sp. as a good biotag in stock assessment. A similar use of *Anisakis* sp. was found in the HOMSIR project Mackenzie et al. (2002), (Mackenzie et al., 2007), as well as research done by Choua et al., (2011), and Le Roux (2013). Literature regarding the monogenean *G. trachuri* is sparse in comparison to the large number of studies dedicated to *G. cruciata* or *Anisakis* sp.

Parasite assemblages infecting *T. trecae* and *T. capensis* were similar with the exception of two parasitic taxa; Trematoda and Copepoda. The trematode *E. Lepidus* and Copepod *L.*

trachuri only infected *T. capensis*, however under further examination the unidentified copepod infecting *T. trecae* could have been *L. trachuri*. From literature, *L. trachuri* was found to occur in *T. capensis* residing in Namibian waters and not southern Benguela waters (Le Roux, 2013). *T. trecae* in the northern Benguela however, were not infected indicating a possible species specific infection pattern.

Only unidentified *digenea* and *G. trachuri* had higher infection prevalence's in *T. trecae*, while *G. cruciata*, *C. australis*, *D. donecae* and *Anisakis* sp. infected a larger proportion of *T. capensis*. Parasitic infections are known to vary inter annually due to seasonal conditions and therefore samples should not be taken across seasons (Gestal and Azevedo, 2005). In this study samples were taken at the same time of year, eliminating this possibility. Equal size class samples were compared between *T. trecae* and *T. capensis*, eliminating any size effect. Therefore environmental or species specific relationships are the only factors that could have caused the difference in parasite assemblages. Many more parasites, both in terms of taxa and numbers within a taxon were found in *T. capensis*. This could be due to the reader effect as Le Roux (2013) may have had a better ability to find parasites than me. Identifying parasites to their species level was also difficult and is a constraint as this project serves as the only parasite profile for *T. trecae.*

As early as 1962 Llewellyn discovered the importance of knowing the biology of parasite hosts, before any conclusions regarding parasite assemblages are made. These parasite assemblages are based on the diets of hosts due to their infected prey (intermediate hosts), as well as host life history traits (Begg and Waldman, 1999). The significant interspecific difference found in parasite assemblage between *T. capensis* and *T. trecae* could have been due to the difference in diets of the two horse mackerel species, as *T. capensis* seems to have a more diverse diet. However, as there is scant data on the life history and biology of *T. trecae*, host specific conclusions cannot be made.

Certain parasites can only tolerate and survive specific environmental conditions, as well as needing specific intermediate hosts to carry out their life cycle. Even though both species of horse mackerel were from the northern Benguela, the *T. trecae* and *T. capensis* samples were taken either side of the Angola-Benguela Front. As previously mentioned the Angola-Benguela Front is thought to act as a semi-permanent barrier to fish movement (Boyer and Hampton, 2001). This environmental difference, as well as the temperature differences found

north and south of the Angola-Benguela Front could have caused significant differences in parasite assemblages. Seasonality cannot be a factor in this discrimination as the fish were all sampled during the same research survey. In Future studies, samples of both horse mackerel species should be taken at closer proximities to each other so that if significant differences between parasite assemblages are still found, it can be concluded that these parasites have species specific relationships. The physiological effects of parasite infections on host fish were not examined in this study and could be included in future studies.

Conclusion

This study provides a comprehensive knowledge of the parasite assemblage infecting *T. trecae* and therefore serves as a basis for possible future population structure studies of this horse mackerel species. There is a significant intraspecific relationship of the parasite assemblage of *T. trecae* with regards to size and sex and a species specific relationship of the parasite assemblages between *T. trecae* and *T. capensis*. Reasons are unclear for the effect of fish sex on the parasite assemblage infecting *T. trecae*, but this study suggests that this merely reflects the size effect of the parasite assemblage infecting *T. trecae*. It was difficult to conclude whether the interspecific differences in parasite assemblage were due to species specific infection patterns or to environmental relationships due to the lack of literature regarding *T. trecae.* Even though literature (e.g. the HOMSIR Project) has shown *Anisakis* sp. to be the most prominent indicator species for the *Trachurus* genus, this study however, identifies the coccidian *G. cruciata* as a potential new indicator species for discrimination between morphologically similar *T. trecae* and *T. capensis*.

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Appendix

Appendix A: Raw data of horse mackerel *T. trecae* dissections (site of infection not included, *med=medium size class, *imm=immature sex).

Sample	Fish ID	Caudal Length (cm)	Size class	Weight (g)	Sex	Copepod	*Digenia*	*Gastrocotyle trachuri*	*Ceratomyxa australis*	*Davisia donecae*	*Anisakis*	*Goussia cruciata*
45	1	19.6	large	118	M	0	0	0	0	0	0	10
45	2	17.7	large	101	M	0	0	0	0	0	1	0
45	3	18.5	large	92.5	M	0	0	0	0	0	0	100
45	4	17.3	large	99	M	0	0	0	0	0	0	0
45	5	18.5	large	103.5	F	0	0	0	0	0	0	0
45	6	17	large	95.5	F	0	0	3	0	0	0	10
45	7	19.8	large	139.5	M	0	0	6	0	0	0	0
45	8	19.5	large	132.5	F	0	0	0	0	0	1	100
45	9	17.5	large	98	M	0	0	1	0	0	0	100
45	10	19.9	large	136	F	0	0	0	0	0	0	10
45	11	18	large	112.5	M	0	0	2	0	0	0	10
45	12	19.5	large	132.5	F	0	0	5	0	0	0	10
45	13	18.2	large	103.5	M	0	3	5	0	0	0	10
45	14	17.8	large	99	M	0	0	2	0	0	0	100
45	15	17.3	large	97	M	0	1	5	0	0	0	100
45	16	17.3	large	88	M	0	1	5	0	1	0	0
45	17	19.4	large	113.5	M	0	0	0	0	0	1	10
45	18	19.5	large	130	F	0	0	0	0	0	0	10
45	19	18.9	large	116.5	M	0	2	4	0	0	2	10
45	20	19.7	large	135	M	0	0	1	0	0	0	10
54	26	16.7	large	64.5	F	0	0	0	1	0	0	100
44	46	18.3	large	106	M	0	0	0	0	0	0	10
44	47	19.6	large	136	M	0	0	5	0	0	0	100
44	48	17.4	large	83	M	0	1	4	0	0	0	100
44	49	19.4	large	127	M	0	1	0	0	0	0	0
44	50	19.5	large	126.5	M	0	0	0	0	0	0	10
44	51	17.8	large	97	M	0	10	1	0	0	0	100
44	52	19	large	113.5	F	0	0	4	0	0	0	100
44	53	19.4	large	132	M	0	0	0	0	0	0	10
44	54	18.1	large	115.5	F	0	0	2	0	0	0	10
44	55	17.5	large	99.5	F	0	2	2	0	0	0	100
44	56	18.2	large	111	M	0	1	0	0	0	0	100
44	57	19.2	large	120.5	M	0	1	1	0	0	0	10
2	58	18.9	large	110	F	0	2	1	0	0	0	10
44	59	19.2	large	117.5	F	0	4	0	0	0	0	10
44	60	19.6	large	120.5	M	0	7	10	0	0	0	100
44	61	17.2	large	83.5	M	1	3	1	0	0	0	100
44	62	18.5	large	107	F	1	0	0	0	0	0	10

44	63	19.9	large	135.5	F	0	0	1	0	0	0	10
45	64	20.5	large	144	M	0	0	0	0	0	0	10
45	65	21.7	large	159.5	M	0	0	0	0	0	0	0
45	66	20	large	139.5	F	0	0	0	0	0	3	10
45	67	20.7	large	136	M	0	0	1	0	0	0	10
44	68	21	large	156	M	0	0	0	1	0	0	10
44	69	20.3	large	137.5	F	0	0	3	0	0	0	10
44	70	20.3	large	159	M	1	2	0	0	0	0	100
44	71	20.4	large	130	F	0	4	0	0	0	0	10
44	72	20.5	large	163.5	F	0	10	2	0	0	0	10
54	21	11	small	22	imm	0	0	3	0	0	0	0
54	22	10.3	small	19.5	imm	0	0	0	0	0	0	10
54	23	10.4	small	21	imm	0	0	0	0	0	0	10
54	24	10.7	small	21.5	imm	0	0	0	0	0	0	0
54	25	13.5	small	42.5	M	0	1	0	0	0	1	100
54	27	11	small	21	imm	0	0	0	0	1	0	0
54	28	10.5	small	20.5	imm	0	0	0	0	0	0	0
54	29	11.3	small	23.5	imm	0	0	2	0	0	0	100
54	30	11.5	small	27	imm	0	0	0	1	0	0	0
54	31	11	small	22	imm	0	0	0	0	0	0	10
54	32	10.5	small	19.5	imm	0	0	0	0	0	0	10
54	33	10.5	small	19	imm	0	0	3	0	0	0	0
54	34	9.8	small	17	imm	0	0	0	0	0	0	0
54	35	10	small	17	imm	0	0	1	0	1	0	0
54	36	11.3	small	22.5	imm	0	0	0	0	0	0	0
54	37	10.5	small	20	imm	0	0	3	0	0	0	0
54	38	10.8	small	19.5	imm	0	0	5	0	0	0	0
54	39	9.8	small	17	imm	0	0	0	0	1	0	0
54	40	10.3	small	17	imm	0	0	4	0	0	0	100
54	41	10.3	small	18	imm	0	0	1	0	0	0	0
54	42	10.5	small	18	imm	0	0	0	0	0	0	0
54	43	10.5	small	20	imm	0	0	0	0	0	0	0
54	44	10.3	small	20	imm	0	0	0	0	0	0	10
54	45	10.8	small	20.5	imm	0	0	0	0	0	0	0

Appendix B: Raw data of horse mackerel *T. capensis* dissections (site of infection not included, Le Roux, 2013).

Sample Area	Fish ID	Caudal Length (cm)	Size Class	Weight (g)	Sex	*Digenia*	*Lernanthropus trachuri*	*Gastrocotyle trachuri*	*Ceratomyxa australis*	*Davisia donecae*	*Anisakis*	*Goussia cruciata*	*Ectenurus lepidus*
NB	101	17.8	Small	65.69	F	0	0	0	0	0	0	1	0
NB	102	20.1	Small	92.22	M	0	0	0	0	0	0	10	0
NB	103	17.9	Small	64.89	F	0	0	0	0	0	0	10	0
NB	104	17.5	Small	69.61	F	0	0	0	0	0	0	11	0
NB	105	20.5	Small	92.84	F	0	0	0	0	0	0	100	0
NB	106	18.3	Small	65.26	F	0	0	0	0	0	0	100	0
NB	107	19.9	Small	69.8	F	0	0	0	0	0	1	100	0
NB	108	19.6	Small	88.3	M	0	0	0	0	0	2	100	0
NB	110	20	Small	119.08	F	0	0	0	0	0	2	100	0
NB	111	20.5	Small	87.25	M	0	0	0	0	0	4	1000	0
NB	112	18.4	Small	69	M	0	0	0	0	0	4	1000	0
NB	113	19.7	Small	92.81	M	0	0	0	0	0	5	1000	0
NB	114	20.4	Small	96.81	F	0	1	0	0	0	5	1000	0
NB	115	18.2	Small	69.46	F	0	1	0	0	0	5	1000	0
NB	116	18.7	Small	74.78	M	0	1	0	0	0	5	1000	0
NB	117	20.1	Small	96.96	M	0	1	0	0	0	6	1000	0
NB	118	21.7	Small	118.34	M	0	1	0	0	10	8	1000	0
NB	119	18.2	Small	69.4	F	0	2	0	0	10	9	1000	0
NB	120	18.9	Small	71.36	M	0	2	0	0	10	11	1000	0
NB	121	17.7	Small	59.4	F	1	2	0	0	20	14	1000	0
NB	122	19	Small	72.7	F	1	2	0	1	100	15	1000	0
NB	123	20.2	Small	95.28	F	2	3	0	1	110	25	1000	0
NB	124	21.3	Small	102.66	M	2	3	0	1	200	78	1000	0
NB	125	19	Small	82.02	M	4	4	1	1	1000	100	1010	1