INVESTIGATING TROPHIC INTERACTIONS BETWEEN PARASITES AND THEIR MARINE FISH HOSTS USING STABLE ISOTOPE ANALYSIS

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Declaration

I, **Mark Jonathan Weston**, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university

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Abstract

Parasitism is accepted as being an essential driver in the organization of biological communities. However, although there are estimated to be more parasitic than free-living organisms, parasites have been largely neglected from ecological studies and we have a relatively limited understanding of their trophic ecology. Hence, in order to incorporate parasites into food-web models and ecological studies, basic trophic interactions between parasites and their hosts need to be assessed. Here, I investigated host-parasite interactions using stable isotope analyses on a broad range of marine fish hosts and their associated copepod and monogenean gill parasites. The study also provides insight into the gill parasite diversity of a number of host species from South African marine waters.

Seventeen species of fish hosts, ranging from lower to upper trophic levels, were collected from South African waters in 2015 and 2016 during surveys conducted by the Department of Agriculture, Forestry and Fisheries from South African waters. These host species were; *Thunnus albacares, Thunnus maccoyii, Thunnus alalunga, Thunnus obesus, Isurus oxyrinchus, Prionace glauca, Xiphius gladius, Brama brama, Thyrsites atun, Seriola lalandi, Sarda sarda, Genypterus capensis, Merluccius paradoxus, Merluccius capensis, Lampanyctodes hectoris, Sardinops sagax* and *Trachurus capensis.* The head region, gills and operculae from a total of 1513 fish were examined and any ectoparasites removed, identified down to the lowest taxonomic level possible, and counted. A total of 32 parasite taxa, comprising 20 copepods and 12 monogenean species, were recorded. Two new host records and 15 new geographic records for South Africa were catalogued. The new host records are *Mazocraes* sp. infecting *T. atun,* and *Caligus dakari* infecting *T. capensis.* The new locality records consist of *Euryphorus brachypterus* recovered from *T. alalunga* and *T. obesus, Pseudocycnus appendiculatus* recovered from *T. albacares, T. alalunga* and *T. obesus, Hexostoma* sp. recovered from *T. albacares* and *T. obesus, Nasicola klawei* recovered from *T. albacares* and *T. obesus, Tristoma*

adcoccineum recovered from *X. gladius, Eobrachiella elegans* and two species from the genus *Parabrachiella* recovered from *S. lalandi* and *Anthocotyle merlucci* recovered from *M. paradoxus* and *M. capensis*. These new records have substantially contributed to the known parasite biodiversity within the South African marine environment.

Host-parasite interactions between 15 species of fish host and their copepod and monogenean gill parasites were investigated by means of stable isotope analysis. Host white muscle tissue, host gill tissue and parasite samples were analysed for their carbon and nitrogen isotopic signatures. Results indicated that $\delta^{15}N$ is tissue-specific, with host white muscle tissue showing significantly greater enrichment in ^{15}N compared to host gill tissue ($n = 60$; $Z = 5.66843$; $p < 0.00001$), and as gill tissue is what the parasites are presumably feeding on, host gill tissue was therefore selected as the most appropriate proxy. Comparisons of the $\delta^{15}N$ of the parasites and their associated host gill indicated that gill parasites in general are significantly enriched in ^{15}N compared to their associated host gill tissue ($n = 144$; $Z = 9.767249$; $p < 0.00001$), and that copepods show a significantly higher enrichment compared to monogeneans ($U = 987$; $n_{copepods} = 117$; $n_{monogeneans} = 28$; $p = 0.001121$). Further analysis of the effect of the diet and feeding mode on the trophic fractionation of $\delta^{15}N$ within the monogeneans showed that tissue-scraping monopisthocotyleans are significantly more enriched in ^{15}N compared to blood-feeding polyopisthocotyleans ($U = 22.00$; $n_{monopisthocotyleans} = 5$; $n_{polyopisthocotyleans} = 23$; $p = 0.035776$). A lack of definitive knowledge about the feeding modes used by the various copepod gill parasites precluded similar analysis for this group. At a species specific level, 13 out of 22 host-parasite interactions examined showed an enrichment of ^{15}N in the parasites compared to their associated host gill tissue with the majority of these comprising copepod-host interactions. A comparison between the $\delta^{15}N$ of the parasites and the estimated $\delta^{15}N$ of host blood resulted in 11 out of 18 host-parasite interactions showing significant enrichment in ^{15}N, of which 10 were copepod-host interactions. Mathematical corrections of $\delta^{13}C$ for samples with high C:N ratios appeared

successful for the majority of samples analysed. However some parasites showed very high C:N ratios, indicative of high lipid content, and the lack of lipid-extracted samples with which to compare the mathematically corrected samples prohibited any further biological inferences from being drawn and highlighting the necessity for the manual lipid extraction of such samples.

The present study has indicated a general enrichment in ^{15}N of parasites compared to their host gill and blood tissue. However there are variations in trophic fractionation according to parasite type, diet and feeding mode. Additionally, enrichment in ^{15}N of parasites appeared to be linked to the relative trophic level of their host, being higher for lower trophic level hosts and lower for higher trophic level hosts. This supports the hypothesis that trophic fractionation if nitrogen isotopes in food-webs is not linearly related to trophic level. This study provides further insight into the trophic interactions between parasites and their hosts which lends itself toward a more comprehensive understanding of trophic interactions occurring within ecological communities.

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“It is common to find parasites referred to as if they were in some way more morally oblique in their habits than other animals, as if they were taking some unfair and mean advantage of their hosts. If we once start working out such ‘responsibilities’ we find that the whole animal kingdom lives on the spare energy of other species or upon plants, while the latter depend upon the radiant energy of the sun. If parasites are to occupy a special place in this scheme we must, to be consistent, accuse cows of petty larceny against grass, and cactuses of cruelty to the Sun.”

-Charles Elton, 1927

Elton, C.S. 1927. *Animal Ecology*. University of Chicago Press: Chicago. pp. 75

Chapter 1: Introduction

1.1- Food-webs

The study of ecological networks has provided an influential tool for ecologists to further understand the dynamics and organization of organisms within a community (Dunne *et al.*, 2013). Of these network studies, a major focus has been on the study of trophic interactions (Table 1.1) and how communities are structured on a trophic basis (Paine, 1980). At the base of all trophic interactions are the producers (autotrophs) (Table 1.1). These organisms are able to synthesize organic molecules from inorganic sources through processes such as photosynthesis, where phytoplankton, land plants and algae are able to synthesize carbohydrates using light energy from the sun, carbon dioxide and water (Freeman, 2011). The synthesis of organic molecules from inorganic sources forms the basic mechanism by which energy enters an ecosystem (Freeman, 2011). Heterotrophic organisms (Table 1.1) are unable to synthesize their own organic molecules, and thus rely on feeding off other organisms (Freeman, 2011). Hence, energy is initially passed from the autotrophs to the herbivores and planktivores, which are then fed on by other species which in turn are the prey of larger predatory species (Figures 1.1 & 1.2) (Freeman, 2011). Organisms that are labeled consumers are one trophic level (Table 1.1) above the organisms on which they feed, while organisms that acquire their energy from the same general source, all belong to the same trophic level (Figure 1.2) (Lafferty *et al.,* 2008; Freeman, 2011). According to their trophic position, organisms can be grouped into various categories; apex predators exist at the top of the food-web with very few predators of their own; intermediate organisms have predators but also prey upon lower trophic levels; and basal organisms are the producers (photo- or chemosynthetic organisms) which are fed upon by a variety of predators but have no prey themselves (Thompson *et al.,* 2004).

These interactions are neatly illustrated in food-webs (Table 1.1; Figures 1.1 & 1.2) which provide biologists a simplified, visual outline of organism interactions within a community or ecosystem on a trophic basis (Paine, 1980; Pimm, 2002; Elser and Hessen, 2005; Freeman, 2011). Arrows on a food-web indicate the direction of nutrient and energy flow, where the tail of the arrow indicates the resource and the head indicates the consumer (Figure 1.1) (Paine, 1980; Pimm, 2002; Elser and Hessen, 2005; Freeman, 2011). Some systems are more efficient at transferring energy from producers at the base of the food-web to predators at higher trophic levels, with more productive ecosystems having a higher trophic efficiency (Table 1.1) resulting in their being able to support a larger number of trophic levels (Elser and Hessen, 2005).

Using the trophic links between species, ecologists are able to calculate food-web metrics to enable further understanding of community structure and interactions. For example, by calculating the average number of trophic interactions between species within a food-web, ecologists are able to calculate the link density (Table 1.1) (Dunne *et al*, 2013). As each trophic interaction is seen as a link between two species, communities which have a greater number of species interacting with each other will have a higher link density than those where only few species are present with limited trophic interactions (Dunne *et al.*, 2013). These trophic links are however only the observed trophic links within the community, but these may not be all of the trophic links that could possibly exist between species (Dunne *et al.*, 2013). Thus by comparing the total number of actual trophic links observed within a community to the number of trophic links that could theoretically exist in an ideal situation with no other influences, ecologists are able to calculate the food-web connectance (Table 1.1) (Dunne *et al.*, 2013). Food-webs which have very few realized trophic links compared to possible trophic links have a lower connectance compared to communities where the number of observed links approaches the number of possible links that could occur. There are also a number of other food-web metrics pertaining to the finer intricacies of food-webs themselves (Dunne *et al.*, 2013), which will not be covered here.

Table 1.1: A glossary of trophic terms used in the present study (Freeman, 2011; Dunne *et al.*, 2013)

Term	Definition
Trophic Interaction	The interaction between two organisms where one feeds off of the other in order to obtain energy and nutrients
Producer (Autotroph)	A organism that is able to synthesize organic molecules from inorganic molecules
Consumer (Heterotroph)	An organism that obtains nutrients and energy by feeding off other organisms
Trophic Level	A feeding level within an community, where organisms of the same trophic level acquire their nutrients and energy from the same general source
Food-Web	A network of trophic interactions between organisms within a community
Trophic Efficiency	The proportion of energy (or carbon produced) transferred from one trophic level to the next
Link Density	The average number of trophic interactions between species in a food-web
Connectance	The proportion of actual trophic links that exist within a food-web compared to the total number of possible links that can exist
Species Diversity	The total number of species within a community

Food-webs range in structure from simple (Figure 1.1) to highly complex (Figure 1.2), depending on the communities that they depict. One of the primary drivers behind food-web complexity is the diversity of species (Table 1.1) within the community (Molles, 2008; Krebs, 2009). As the species diversity increases, so does the number of potential trophic interactions (Krebs, 2009). Therefore, in communities that have low species diversity, such as the river system depicted in Figure 1.2, there are very few trophic links thus resulting in a relatively simple food-web (Power, 1990). However, in communities with a high species diversity, as found in Lake Victoria (Figure 1.2), there is a major increase in the number of possible trophic links, thus resulting in a highly complex food-web (Nyamweya *et al.*, 2016). Many larger, more complex food-webs are difficult to illustrate due to their high species diversity and large number of trophic interactions, thus ecologists have leaned towards computer modeling of trophic interactions to produce food-web outputs similar to that shown in Figure 1.2.

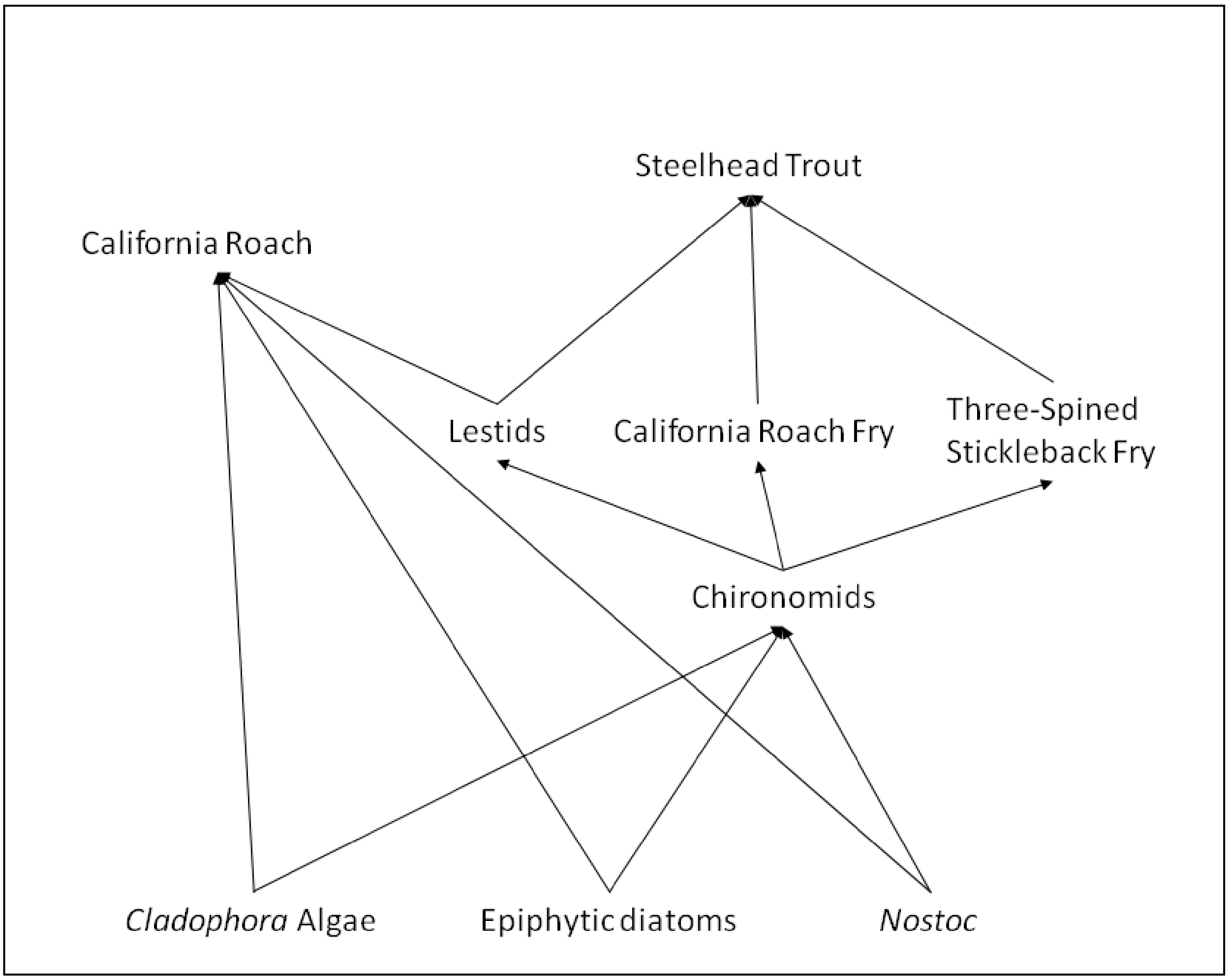

Figure 1.1: The food-web of the Eel River, California, during summer months (adapted after Power, 1990)

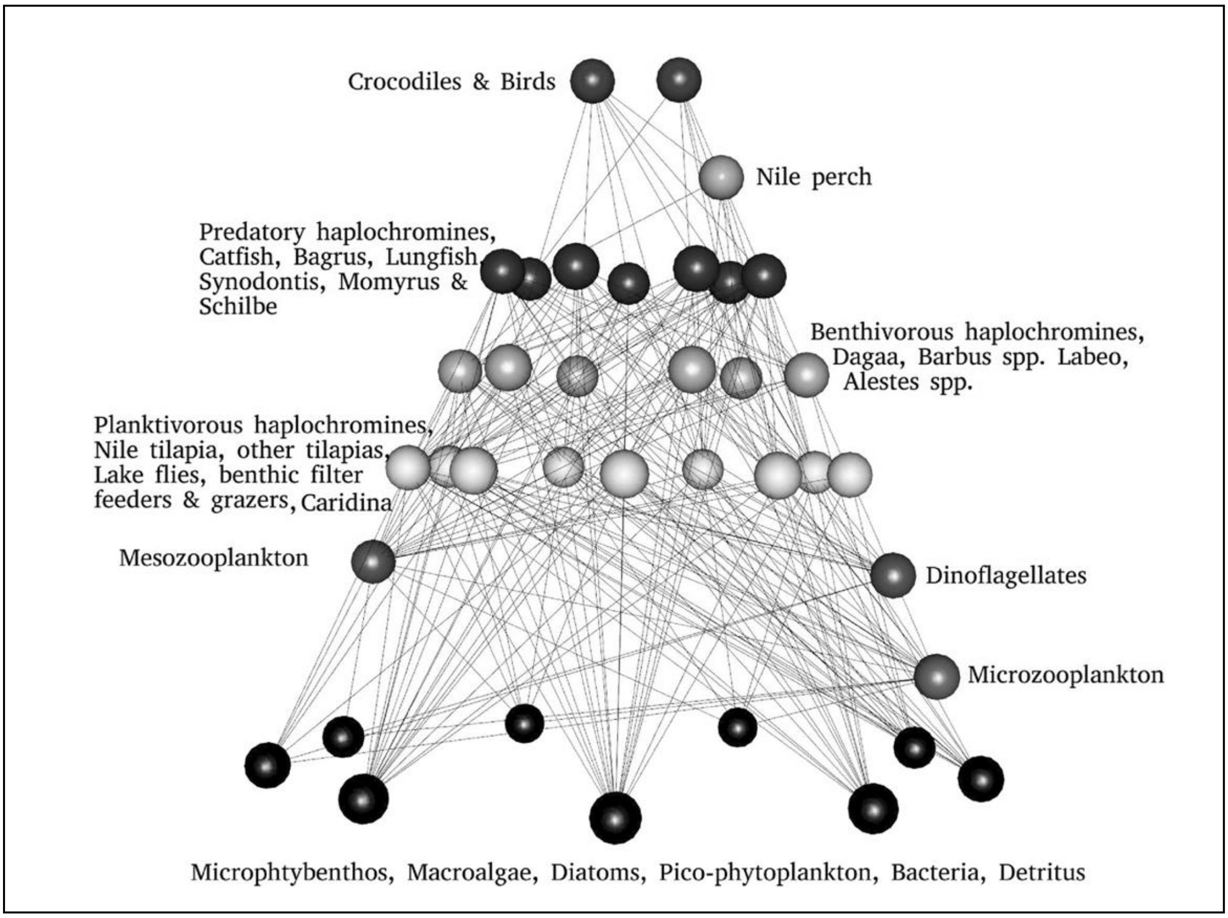

Figure 1.2: The food-web of Lake Victoria (adapted after Nyamweya *et al.*, 2016).

1.2- Parasitism

Parasitism is defined as a way of life whereby an organism draws its resources, such as nutrients, from another host organism, resulting in a relationship which is beneficial to the parasitic organism and detrimental to the host organism (Bush *et al.*, 2001; Freeman, 2011). Parasites are generally separated into macro- and microparasites (Marcogliese, 2004). Macroparasites are typically multicellular organisms that undergo sexual reproduction, with many utilizing multiple hosts in order to complete their life cycles and with these cycles often having intermittent free-living stages (Marcogliese, 2004). Microparasites, in contrast, are primarily unicellular, multiplying asexually and usually having shorter life spans (Marcogliese, 2004). Within these two divisions of parasites there are multiple types; ectoparasites are parasites which occur on the external surfaces of their hosts whereas endoparasites occur within the host; obligate parasites depend on their hosts for survival whereas facultative parasites are able to exist as free-living species but also have the ability to parasitize organisms; and finally temporary parasites only parasitize their hosts for short periods of time as opposed to permanent parasites which, as their name suggests, parasitize their hosts for their entire lifetime (Rohde, 2005). Likewise, hosts can be divided into various types; intermediate hosts possess developing stages of the parasite's life cycle, definitive hosts possess the final mature stages, and paratenic hosts are transport hosts within which the parasite does not undergo any form of development and serve as transmission pathways to the definitive host (Rohde, 2005).

In addition to these divisions there are two different types of parasitic life cycle, a direct life cycle and an indirect life cycle (Rohde, 2005). Direct life cycles involve only a single host organism. An example is the monogenean parasite *Polylabroides multispinosus* (Figure 1.3), adults of which parasitize the gills of the yellowfin bream, *Acanthopagrus australis,* and lay eggs which sink to the sea floor (Roubal and Diggles, 1993). The eggs hatch into a free-living oncomiracidium stage which then finds and attaches to the bream's gills where they mature (Roubal and Diggles, 1993). Indirect life cycles, on the other hand, are highly complicated involving multiple host species ranging over multiple trophic levels (Rohde, 2005). For example, adults of the thorny-headed worm (Acanthocephala) *Corynosoma cetaceum* (Figure 1.4) mature and reproduce within cetaceans such as whales and dolphins, and the parasite's eggs are released into the environment in the faeces (Taraschewski, 2005). These eggs are then ingested by small amphipods, within which the eggs hatch and encyst, and these amphipods are then fed on by fish which act as paratenic hosts (Taraschewski, 2005). The definitive hosts, cetaceans, then feed on the fish and thus become infected with the parasite. The adult Acanthocephala then attaches itself to the intestinal wall with its spined proboscis where it matures and reproduces, continuing the cycle (Taraschewski, 2005). Another example of a highly complex life cycle is that of the ascarid worm, *Pseudoterranova decipiens,* in which the adult matures and reproduces in seals whilst intermediate stages are found in crustaceans and a variety of fish species (McClelland, 2002; 2005).

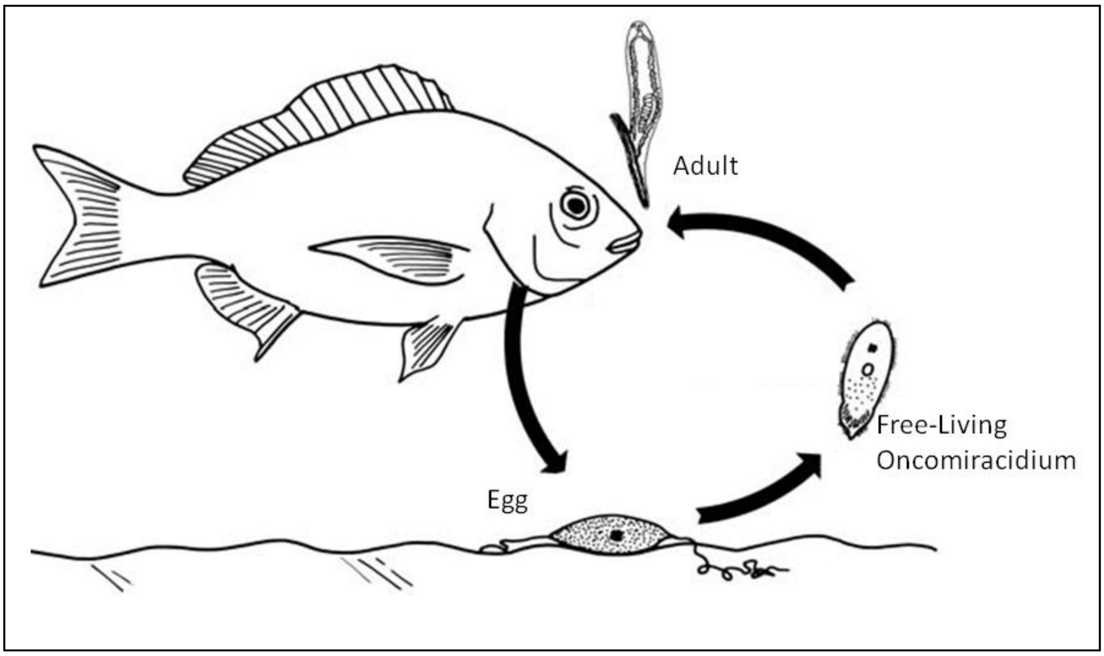

Figure 1.3: The life cycle of the monogenean *Polylabroides multispinosus* (adapted after Hayward, 2005)

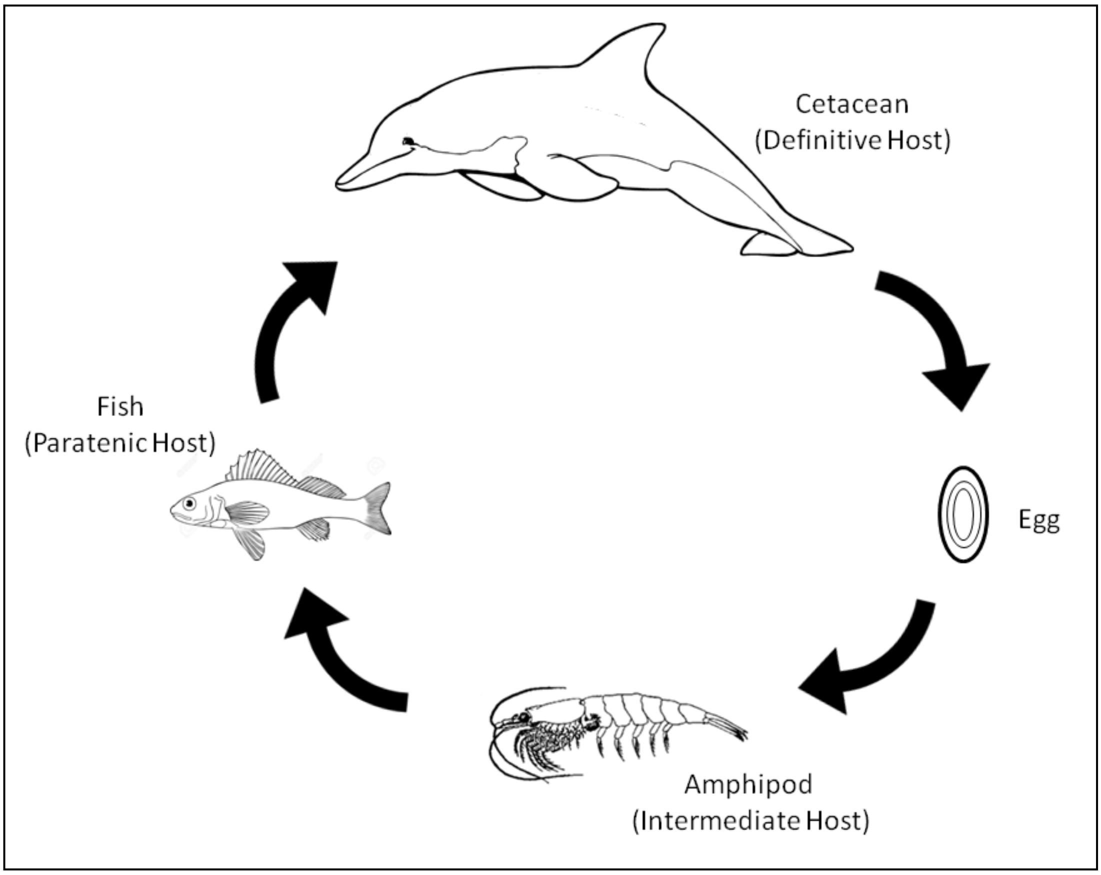

Figure 1.4: The life cycle of the acanthocephalan *Corynosoma cetaceum* (after Taraschewski, 2005)

It has been estimated that there are more parasitic species than free-living species in the biosphere (Sukhdeo, 2012), and thus a major proportion of organisms within an ecosystem are parasitic (Marcogliese, 2004). This means that the majority of trophic interactions found within an ecosystem occur between parasites and their hosts, and not between predators and their prey (Lafferty *et al.,* 2008). Parasitism, however, differs from predation events on which so many food-web and trophic studies have been based. A predator, throughout its lifetime, will kill and feed off a number of prey items whereas parasites will generally obtain nutrients from a single host during a single life stage but will only have a small impact on the host's fitness (Lafferty and Kuris, 2002; Lafferty *et al.,* 2008). The trophic strategies which have attributes of both predators and parasites are the parasitoids and micropredators (Lafferty and Kuris, 2002). Parasitoids are similar to parasites in that they only infect a single host victim during a single life stage, but in doing so, kill the host or reduce the host's fitness to zero (Lafferty and Kuris, 2002). Micropredators, alternatively, are similar to predators in that they will feed off of a number of victims during a single life stage but, like parasites, may only have a small or negligible impact on the host's fitness (Lafferty and Kuris, 2002).

Both predation and parasitism can be considered to regulate the populations of prey and host species as well altering the community structure of their ecosystem by regulating the abundance of organisms, preventing a single species from dominating (Dobson *et al.,* 2006; Watson, 2013). One such example of host regulation through parasitism is that of the rinderpest virus, *Rinderpest morbillivirus***,** which entered Africa via Sudan and Ethiopia in 1890 and within six years spread to the Cape regions, decimating ruminant populations along its path (Sinclair *et al.,* 2007). The major decline in ruminant populations resulted in a huge decline in grazing pressure, which in turn increased the fuel load resulting in an increase in fire frequency and severity (O'Connor *et al.,* 2014). This in turn resulted in vast areas undergoing bush encroachment, thus changing the habitat and its associated community (O’Connor *et al.*, 1993; Prins and van der Jeugd., 1993). Such parasites, termed ‘keystone parasites’, are those which parasitize dominant species within ecosystems (Marcogliese and Cone, 1997). The infection of these hosts results in a reduction in their competitive ability thus allowing for other, less-competitive species to have a larger influence on the ecosystem, which alters the community structure (Marcogliese and Cone, 1997). This was demonstrated through a study by Wood *et al.* (2007), who investigated the effects of the digenean trematode *Cryptocotyle lingua* parasitizing the invasive snail, *Littorina littorea*, which dominates the intertidal zones of north-western Atlantic coastlines. It was found that the infection of *L. littorea* by the digenean resulted in a significant reduction of grazing pressure by infected snails, thus allowing for an increase in algal biomass and affecting the composition of the algal community which in turn shifted the composition toward more ephemeral species (Wood *et al.*, 2007).

1.3- Incorporating Parasites into Food-Webs

Despite the fact that parasites have significant impacts on communities (Byers, 2009; Dunne *et al.*, 2013; Watson, 2013) and have been widely accepted as being essential in structuring biological communities (Mouritsen and Poulin, 2005), their presence and role have mostly been neglected in ecosystem and community studies (Marcogliese, 2004). Currently, only a few studies have attempted to incorporate parasites into food-webs (*e.g.* Paine, 1980; Huxham *et al.*, 1995; Marcogliese and Cone, 1997 and references therein; Marcogliese, 2002; Mouritsen and Poulin, 2002; Thompson *et al.*, 2004; Lafferty *et al.*, 2008; Dunne *et al.*, 2013). Hence whereas the majority of food-web studies provide a good representation of free-living species they miss a major proportion of organisms found within the ecosystem (Huxham *et al.*, 1995; Dobson *et al.*, 2006; Lafferty *et al.*, 2006a; Lafferty *et al.*, 2006b; Lafferty *et al.*, 2008). This may be ascribed to the majority of food-web and trophic ecologists focusing on free-living species and not parasitic organisms, as researchers have leaned toward collecting samples which are easily obtained and easily incorporated into studies (Lafferty *et al.,* 2008). Smaller, parasitic species pose a much greater challenge as many of these organisms have multiple life stages, and this is often confounded by incomplete and insufficient knowledge of their individual and frequently complex life cycles (Lafferty *et al.,* 2008). Parasites with complex life cycles are very difficult to integrate into food-webs because they parasitize multiple trophic levels over multiple separate life stages within the parasite's life cycle (Marcogliese and Cone, 1997; Lafferty *et al.,* 2008). This, along with many ecologists not having the necessary skills and taxonomic expertise to identify parasites, has led to an inadequate representation of parasite diversity in food-webs, with parasites often being grouped together or omitted entirely (Sukhdeo, 2012).

Marcogliese and Cone (1997) confronted the issue of parasite exclusion in many food-web studies and argued for their inclusion. Their justification for the inclusion of parasites into such studies was that the majority of known species, both marine and terrestrial, have associated parasites and that these parasites, when included into food-web studies, more completely explain trophic interactions and relationships occurring within a community as well as providing a true representation of the community's organism assemblage (Marcogliese and Cone, 1997). As many of these parasites have multiple life stages often pertaining to different hosts at different periods, the occurrence of the parasite in a host can also indicate the presence of the other hosts within the community (Marcogliese and Cone, 1997). Therefore, parasites can be used as a tool for ecologists to comprehensively understand the organisms inhabiting a community, even if those organisms are elusive. In addition, parasites provide a biological indication of trophic interactions between organisms since many parasitic species require multiple hosts in order to complete their life cycles and many tend to utilize trophic links between hosts as their transmission pathway (Marcogliese and Cone, 1997, Lafferty *et al.,* 2008). These parasites which utilize trophic links typically reside within or on their hosts for significant periods of time and can thus provide a long-term record of trophic links (Marcogliese and Cone, 1997; Lafferty *et al.,* 2008).

In order to gain insight into the diet of an organism, ecologists have leant toward analyzing the gut contents as this is a direct indication of what the organism has recently fed upon (Sheppard and Harwood, 2005). Gut contents, however, remain within the organism for relatively short periods of time and thus they merely provide a snapshot of the organism's diet (Marcogliese and Cone, 1997; Lafferty *et al.,* 2008). Parasites, conversely, provide a useful tool for ecologist to determine potential diet sources of an organism as the occurrence of particular parasites within the predator indicates that it must have previously fed upon certain prey species in order to become infected (Marcogliese and Cone, 1997, Lafferty *et al.,* 2008). Therefore, even if the prey item has since been digested and has no trace within the gut, the presence of the parasite indicates that the particular prey species does constitute a portion of the predator's diet. Gut content analysis is furthermore biased toward hard-bodied organisms, since the soft-bodied organisms tend to be digested at a faster rate and thus are difficult to identify, as well as being biased toward larger prey items as smaller prey items are digested more rapidly due to their larger surface area to volume ratio, increasing their exposure to digestive enzymes which digest from the outside in (Legler *et al.,* 2010).

Along with the incorporation of parasites into food-webs as a tool for parasitologists to further understand life-cycles and transmission pathways, it is important to note that parasites have the ability to alter the food-web structure and its dynamics. Incorporating parasites into food-webs has been found to increase species diversity, link density and connectance (Table 1.1) (Arias-González and Morand, 2006, Dobson *et al.,* 2006, Sukhdeo, 2012; Chase, 2013). The increases in these food-web elements occurs by the simple act of adding another organism to the food-web, which, regardless of whether it is a parasite or not, will alter these aspects (Arias-González and Morand, 2006, Dobson *et al.,* 2006, Sukhdeo, 2012; Chase, 2013). A study by Thompson *et* *al*. (2004) that modelled the food-web of a mudflat community in New Zealand found that the addition of parasites into the food-web matrix unexpectedly resulted in a slight decrease in connectance. However this anomalous result was accounted for by Lafferty *et al.* (2006a), who explained that many previous studies had incorrectly calculated the connectance matrix and thus underestimated the number of actual trophic links. Parasites uniquely alter food-web structure by forming new, higher trophic levels and increasing chain length (Huxham *et al.,* 1995; Arias-González and Morand, 2006; Dobson *et al.,* 2006; Lafferty *et al.,* 2006a; Lafferty *et al.,* 2006b; Sukhdeo, 2012; Dunne *et al.,* 2013). When parasites are incorporated into food-webs, the apex predators, which previously were considered to have no predators, now become the parasites' "prey", resulting in these top predators being reduced to intermediate organisms and the parasites forming a new, higher trophic level (Thompson *et al.,* 2004; Arias-González and Morand, 2006). As parasites now form the highest trophic level there is an increase in food chain length, which thus affects the basic food-web topology (Huxham *et al.,* 1995; Arias-González and Morand, 2006).

The changes in food-web structure and complexity following the incorporation of parasites were illustrated in a study by Dunne *et al.* (2013), who modelled the food-web of Estero de Punta Banda in Baja California. They initially modelled the food-web by only incorporating the free-living species from basal producers to apex predators (Figure 1.5A), and the model was then re-run and the known metazoan parasites of the free-living species included (Figure 1.5B). The addition of the parasites vastly increased the species diversity, resulting in an increase of the food-web connectance and an increase in the number of concomitant links between parasites and their hosts (Dunne *et al.,* 2013).

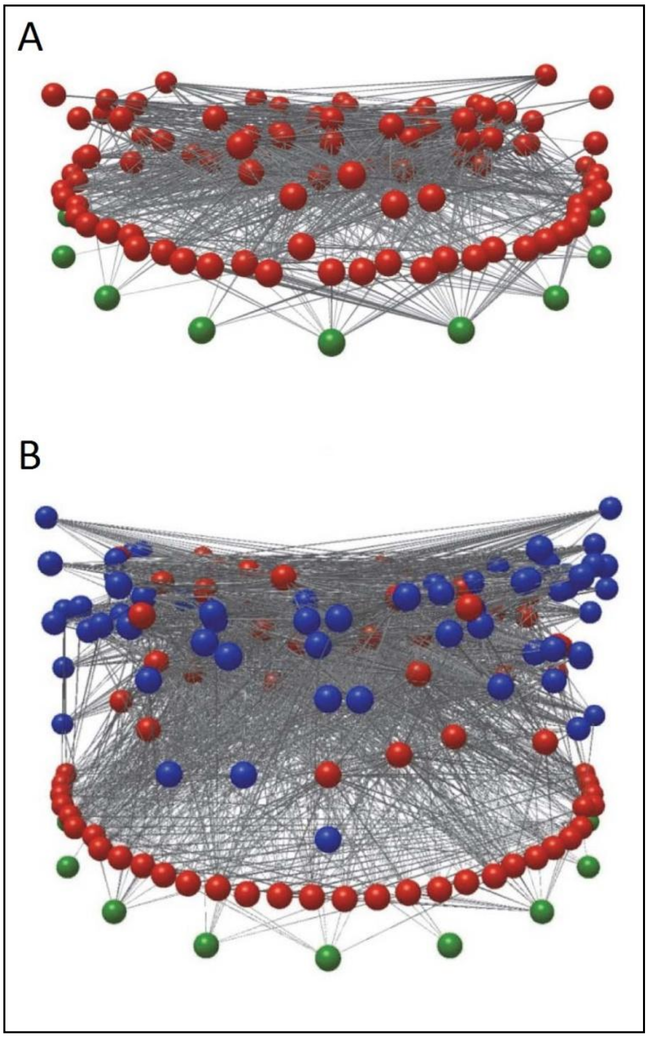

Figure 1.5: Diagrams of two modelled food-webs from Estero de Punta Banda, Baja, California showing A: the food-web incorporating only free-living species, and B: the food-web incorporating both free-living and parasite species. Green indicates producers, red indicates free-living organisms and blue indicates the parasites (after Dunne *et al.*, 2013).

Finally, the interaction between a parasite and its host is much like a typical consumer-resource interaction between a predator and its prey in terms of the transfer of resources, such as nutrients and energy, from one organism to the next (Lafferty *et al.,* 2008). The addition of parasites to food-webs alters the energy flow from producers to apex predators and in so doing, affects the trophic efficiency (Table 1.1) (Arias-González and Morand, 2006). A comprehensive study by Niquil *et al*. (1999) on a planktonic ecosystem found that the highest trophic efficiency occurred between the first and second trophic levels, *i.e.* between the producers and herbivores (as well as between detritus and detritivores), and that trophic efficiency decreased moving up the food chain. However, when parasites were included into food-web models it was found that trophic efficiency did not decrease as one moved up trophic levels (Arias- González and Morand, 2006). As parasites are added into food-webs, they increase the proportion of carnivores in a system, thus the system is no longer dominated by primary consumers and detritivores (Arias-González and Morand, 2006). This results in a larger proportion of autotrophic production reaching the higher trophic levels and hence the increase in trophic efficiency (Arias-González and Morand, 2006). Parasites are also able to influence trophic links and energy flow through a system by inducing behavioral changes in the host, thus increasing the consumption rate by predators on parasitized prey items (Poulin, 1994; Lafferty and Morris, 1996; Thomas *et al.,* 1998; Seppälä *et al.,* 2004). For example, Levri and Lively (1996) investigated the behavioural changes of a freshwater snail, *Potamopyrgus antipodarum*, arising from infection by the digenean trematode parasite, *Microphallus* sp. Those authors observed that the behavior of infected snails differed significantly from that of uninfected snails, where the infected snails spent a greater length of time during the morning on top of rocks. This manipulation of host behavior by the parasite greatly increased the probability of the snail being preyed upon by waterfowl, the final host organism of *Microphallus* sp., thus facilitating the parasite's life cycle (Levri and Lively, 1996).

Overall, the incorporation of parasites into trophic and food-web studies has been shown to be highly necessary in order to gain further and more comprehensive insight into community interactions and trophic relations. This is because parasites form such a large proportion of the number of organisms within a community (Marcogliese and Cone, 1997). However, as mentioned previously, there are very few studies that have attempted to include parasites into trophic and food-web analyses (Paine, 1980; Huxham *et al.,* 1995; Marcogliese and Cone, 1997 and references therein; Lafferty *et al.,* 2008; Dunne *et al.,* 2013) and even fewer studies have provided a comprehensive assessment of parasites found within a given community (Huxham *et al.,* 1995; Dunne *et al.,* 2013). Hence, our knowledge of how parasites fit into food-webs and the trophic levels they occupy in comparison to their hosts is still relatively limited. For that reason and in order to fully incorporate parasites into food-web models, we need to assess and better understand the basic trophic interactions between parasites and their hosts.

1.4- Methods for Understanding Trophic Interactions

There are a number of key methods that ecologists have used to gain further insight into trophic interactions between organisms within a community. These have included methods such as visual observations, gut content and faecal analyses, DNA-based molecular techniques, fatty acid analysis and stable isotope analysis.

The most basic of these methods is through visual observation of predatory events which, with modern-day technology and the use of video cameras, has become increasingly sophisticated (Merfield *et al.*, 2004). However, these visual observations are based on the chance that these interactions will occur within the observer's field of view, providing information on only a very small proportion of actual predatory events (Sheppard and Harwood, 2005). The analyses of gut contents and faecal matter have provided a direct method to ascertain what constitutes an organism's diet (Sheppard and Harwood, 2005). Gut content analysis, as discussed previously, has considerable bias toward hard-bodied organisms and larger prey items and the analysis of faecal matter is biased toward organisms containing indigestible material such as exoskeletons and bone, which are often difficult to identify (Sheppard and Harwood, 2005). Nevertheless, if combined with the use of DNA-based molecular techniques which utilize polymerase chain reaction (PCR) to amplify genetic material within the gut or faecal matter, one can begin to distinguish between prey species even if the prey isn't visually identifiable (Farrell *et al.*, 2000; Symondson, 2002). Although the use of DNA is a very promising technique it is still very expensive to run (Symondson, 2002), and there are other biochemical options available to ecologists including the analyses of fatty acids and stable isotopes. When a predator consumes a prey item, the fatty acids from the prey are deposited into the fat tissue of the predator with very little or no molecular alteration (Budge *et al.*, 2006). This makes it possible to trace back the possible prey items of a predator by analyzing its fatty acid composition (Budge *et al.*, 2006). This, however, may be difficult in terrestrial and marine environments as the fatty acids present within these environments are relatively common thus making it difficult to discern between possible diet sources (Budge *et al.*, 2006). The use of another biochemical tool for investigating trophic interactions, stable isotope analysis, is discussed in more detail below.

1.4.1- Stable Isotope Analysis

One method that is becoming increasingly popular amongst trophic ecologists is stable isotope analysis (SIA), which has a variety of applications, from understanding nutrient and elemental fluxes through ecosystems (Miranda *et al.*, 1997; Ferretti *et al.*, 2003) to describing migratory patterns of wildlife (Hobson, 1999; Rubenstein and Hobson, 2004).

Stable isotopes are atoms of the same element that are differentiated by differences in the number of neutrons and are the group of isotopes which do not undergo radioactive decay (Fry, 2006). The addition of neutrons to the nucleus of an atom does not alter the atom's chemistry but alters its atomic weight, thus resulting in heavier isotopes having more neutrons compared to lighter isotopes (Fry, 2006). These stable isotopes form less than 10% of known isotopes, as all known elements and those expected to exist occur in at least two isotopic forms, however the vast majority radioactively decay at a rapid rate and thus are not considered to be stable (Fry, 2006).

Trophic ecologists have been able to utilize stable isotopes to investigate trophic interactions because isotopic compositions change in a predictable manner as these elements are cycled through the food-web (Peterson and Fry, 1987). This is due to lighter isotopes reacting at a faster rate compared to heavier isotopes, hence the former are more readily incorporated into organic molecules and utilized for metabolic processes than the latter and this difference in the rate of reactions between the heavy and light isotopes is termed 'fractionation' (Fry, 2006). The heavier isotopes, as a result, are sequestered by the organism within its tissues and the lighter isotopes preferentially utilized (Fry, 2006). Consequently, as one moves up the food chain, the ratio of heavy to light isotope increases from prey to predator due to the persistence of the heavier isotopes (Peterson and Fry, 1984; Fry, 2006). There are numerous biological processes that are responsible for the isotopic fractionation, including the sequestration of the heavier isotopes during the assimilation of digested prey and the loss of lighter isotopes during excretion and respiration (Peterson and Fry, 1987; Boag *et al.,* 1998; Fry, 2006). When a sample has a higher isotopic ratio, and thus more of the heavier isotope, compared to another sample, it is said to be 'enriched'; in contrast, when it has less of the heavier isotope it is 'depleted' (Lajtha and Michener, 1994). By calculating the difference in isotopic ratios between predators and their prey, ecologists are able to determine the trophic position of organisms as well as gain further insight into the trophic relationships between organisms within a community (Post, 2002; Fry, 2006; Boecklen *et al.,* 2011).

Within the field of trophic ecology, stable isotope analyses are used to show how elements are cycled through food-webs using elements which are tightly coupled with organic molecules, more specifically, carbon and nitrogen (Fry, 2006). These isotopes form tracers which can be traced through trophic linkages and food-webs (Fry, 2006).

1.4.2- Nitrogen Isotopes

Nitrogen has two known stable isotopes, ^{14}N and ^{15}N, which occur in natural abundances of 99.64% and 0.36%, respectively (De Laeter *et al.,* 2003). Consumers obtain the majority of their nitrogen from the ingestion of proteins from their prey (Alberts *et al.,* 2002, O'Brien, 2015). When comparing the ratio of ^{15}N to ^{14}N (referred to as $\delta^{15}N$ and having units of ‰) of a consumer to the $\delta^{15}N$ of its diet, there is an average enrichment in ^{15}N of approximately +3.4 ± 1.1‰ (Minagawa and Wada, 1984). Using the enrichment pattern of ^{15}N, trophic ecologists are able to utilize nitrogen stable isotope ratios to provide information on food-web structure and the trophic levels of organisms. Minagawa and Wada (1984) measured the $\delta^{15}N$ ratios of a number of trophic levels within a marine intertidal habitat ranging from particulate organic matter and primary producers to herbivores, secondary consumers and finally to tertiary consumers. Those authors found that the primary producers, which consisted of seaweeds, as well as the particulate organic matter, had similar nitrogen isotopic ratios of approximately 6.8‰. The isotopic ratios of the herbivores ranged from 8.4‰ to 9.5‰, the secondary consumers, 10.6 ‰ to 12.7‰ and finally the tertiary consumer (a seagull) had a ratio of 16.2 ‰, thus the $\delta^{15}N$ values conformed to the varying trophic levels (Minagawa and Wada, 1984). The enrichment value of 3.4‰ from one trophic level to the next is however just an average value. Hussey *et al.* (2014) investigated the trophic positioning of organisms relative to their $\delta^{15}N$ through modeling, and found that the enrichment of ^{15}N does not increase at 3.4‰ increments in a linear fashion but is rather scaled relative to the trophic position of the animal, with enrichment between the lower trophic levels greater than 3.4‰ and the enrichment between organisms at higher trophic levels less than 3.4‰ (Figure 1.6).

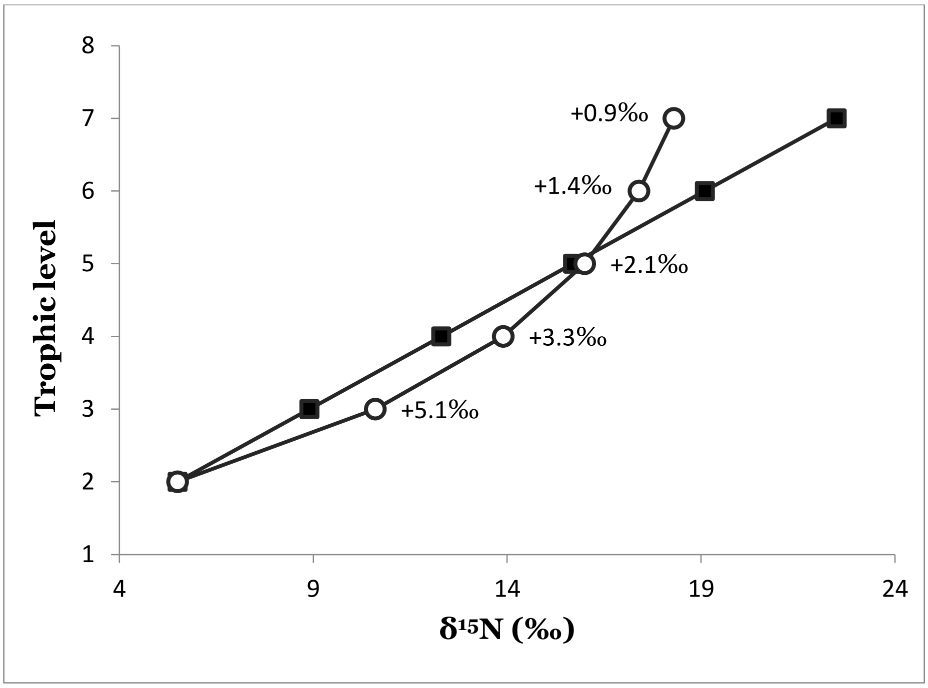

Figure 1.6: The modelled nitrogen enrichment across trophic levels of the KwaZulu-Natal coastal shelf food-web. (-■-) indicates the assumed 3.4‰ enrichment of ^{15}N between trophic levels; (-○-) indicates the scaled ^{15}N enrichment relative to trophic level. The values on the graph indicate the enrichment increments of ^{15}N between successive trophic levels (after Hussey *et al.*, 2014).

1.4.3- Carbon Isotopes

Carbon also has two known stable isotopes, ^{12}C and ^{13}C, with their natural abundances being 98.93% and 1.07%, respectively, with other isotopic forms being radioactive and only occurring in trace amounts (De Laeter *et al.*, 2003). When comparing the ratio of ^{13}C to ^{12}C (referred to as δ^{13}C and having units of ‰) of a consumer to its diet it has been found that there is only a slight enrichment of the heavy isotope, approximately 0.5-1‰ (DeNiro and Epstein, 1978; Michener and Schell, 1994). Ecologists have used this slight enrichment pattern of an organism in relation to its diet to trace back to its source production (DeNiro and Epstein, 1978). This is achieved by initially estimating the δ^{13}C of the diet in relation to the δ^{13}C of the animal and then determining the contribution of potential diet sources of which the δ^{13}C values are known, to generate the δ^{13}C of the initial dietary estimation (DeNiro and Epstein, 1978). This is especially possible in ecosystems where the potential diet sources have very little to no δ^{13}C overlap (DeNiro and Epstein, 1978; Michener and Schell, 1994), for example in grassland systems containing C_3 and C_4 grasses which range in δ^{13}C values from -24‰ to -34‰ and -6‰ to - 19‰, respectively, as

C_4 grasses have a CO_2 concentrating mechanism reducing water loss in arid climates which has resulted in an enrichment of ^{13}C (Smith and Epstein, 1971; O'Brien, 2015). Identifying source production is also possible in near-shore marine environments where the $\delta^{13}C$ of aquatic and terrestrial C_3 plants shows little to no overlap (Schoeninger and DeNiro, 1984). Marine plants, including some phytoplankton, have elevated $\delta^{13}C$ values, ranging from -17‰ to -20‰, compared to C_3 land plants, as many take up ^{13}C enriched bicarbonate from the surrounding sea water during photosynthesis as opposed to terrestrial plants which utilize atmospheric carbon dioxide (O'Brien, 2015). The dissolution process by which CO_2 dissolves into seawater forming bicarbonate favours the heavier carbon isotope, resulting in the bicarbonate in the sea water having enriched $\delta^{13}C$ compared to atmospheric CO_2, which consequently leads to an increased $\delta^{13}C$ values in aquatic marine plants compared to terrestrial C_3 plants (O'Brien, 2015).

1.4.4- Using Stable Isotopes to Investigate Host-Parasite Interactions

There have been only a small number of studies which have investigated the trophic relationships between parasites and their hosts through the use of SIA (Doucett *et al.,* 1999; Iken *et al.,* 2001; Pinnegar *et al.,* 2001; Deudero *et al.,* 2002; Neilson *et al.,* 2005; Demopoulos and Sikkel, 2015). These studies have shown contrasting results, with some host-parasite interactions showing the characteristic enrichment pattern as observed between a typical predator and prey (Doucett *et al.,* 1999; Iken *et al.,* 2001; Deudero *et al.,* 2002; Neilson *et al.,* 2005), whereas others show either no significant differences in isotopic ratios (Iken *et al.,* 2001; Demopoulos and Sikkel, 2015), or in some cases, even depletion (Iken *et al.,* 2001; Pinnegar *et al.,*2001; Deudero *et al.,* 2002). This is further complicated as some parasites have an impact on host energetics, such as migration and foraging patterns, as well as on the host's physiological condition which in turn can influence the host's isotopic signature. This was shown by Welicky *et al.* (2017), whereby infection by the parasitic isopod *Anilocra haemuli,* on the French Grunt *Haemulon flavolineatum*, reduced host condition, increased energetic demands and reduced foraging effort, resulting in enrichment in the host $\delta^{15}N$ and $\delta^{13}C$ compared to uninfected individuals.

This study therefore aims to further investigate these conflicting patterns by analysing the stable isotope ratios of parasites and their hosts from a wide range of South African marine fish species covering multiple trophic levels. The parasites on which this study is based are copepod and monogenean gill ectoparasites, as these are commonly found parasitizing the gills of fish from varying trophic levels in relatively high abundances and thus provide an ideal group of parasites in order to investigate host-parasite trophic interactions

1.5- Feeding Morphology of Gill Parasites

The gills in teleost fish are found within the opercular chambers which are situated posteriorly from the buccal cavity or mouth region (Figure 1.7; Moyes and Schulte, 2008). The gills consist of a number of bony gill arches which support two rows of gill filaments (Figure 1.7; Campbell and Reece, 2005). Each filament has a large number of lamellae which are small folds running perpendicularly to the filament (Figure 1.7; Moyes and Schulte, 2008). These filaments and lamellae greatly increase the surface area of the gills which increases the efficiency of gaseous exchange and oxygen uptake (Moyes and Schulte, 2008). The lamellae are coated in a thin layer of epithelial cells and contain a large network of capillaries (Freeman, 2011). It is on this thin epithelial covering as well as the blood within the network of capillaries, that the majority of gill parasites feed (Boxshall, 2005; Hayward, 2005; Whittington, 2005). This, along with the opercular chambers which provide a protected niche space and the flow of water through the gills which facilitates parasite exposure to the gills and their settlement (Mikheev *et al.*, 2014), provides a suitable microhabitat for parasites to inhabit and establish themselves.

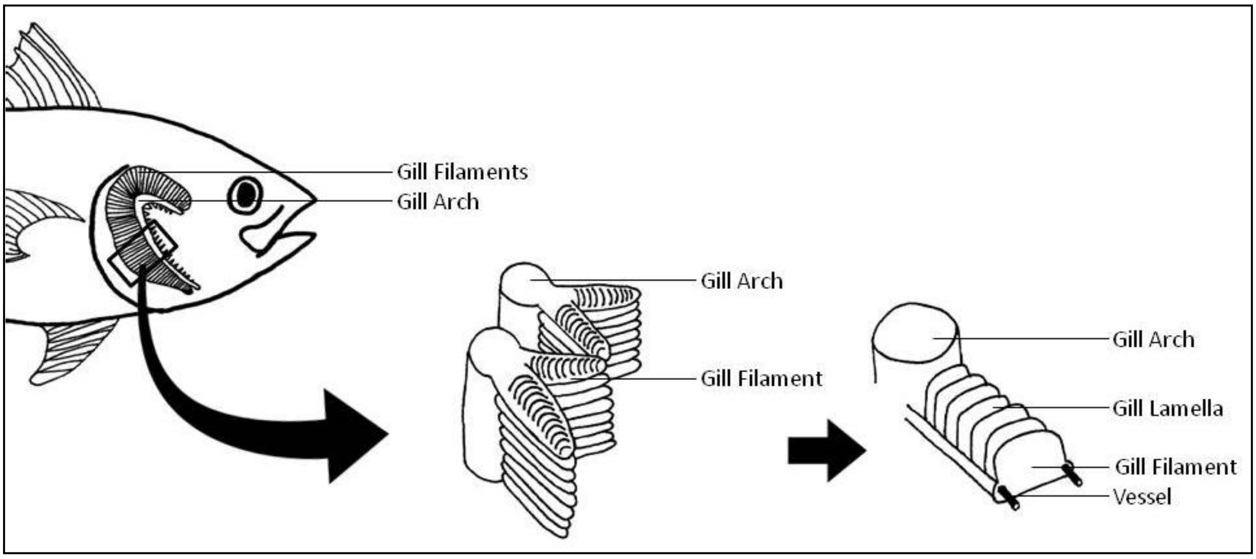

Figure 1.7: Diagram showing the macro- and microstructures of the teleost fish gill (redrawn after Moyes and Schulte, 2008).

There have been a large number of studies documenting gill parasites in teleost fish (*e.g.* Hanek and Fernando, 1978; Ramasamy *et al.*, 1985; Lo and Morand, 2000; Johnson *et al.,* 2004). These studies have investigated various aspects of their biology, ecology and microhabitat preferences (Ramasamy *et al.,* 1985; Lo and Morand, 2000). Of all the gill metazoan parasites infecting marine fish, the copepods and monogeneans are the most speciose groups (Rohde, 2005). Thus, due to their high prevalence, this study focuses on copepod and monogenean metazoan gill parasites and their associated marine fish hosts.

1.5.1- Copepods

Copepods are one of the most abundant invertebrate groups found in both marine and freshwater habitats (Humes, 1994), with approximately 11 500 accepted species of which nearly half are associated in symbiotic or parasitic relationships with host organisms (Boxshall, 2005). Of the parasitic copepods, approximately 75% are placed in the Order Siphonostomatoida Thorell, 1859 (Gunn and Pitt, 2012). The digestive system of the siphonostomatoids is similar to the majority of other copepods and comprises a foregut, midgut and hindgut which terminates at an anus (Goater *et al.,* 2014). Although there is very little literature available on the actual feeding mechanism of many copepod species, siphonostomatoid gill parasites generally feed off one of two host tissues; either the epithelium of the gill lamellae or the host's blood (Pillai, 1985). These feeding activities may have minor pathological effects on the hosts through the damage to gill lamellae (Pillai, 1985). Andrews *et al.* (2010) investigated the pathological effects of *Chondrocanthus goldsmiddi* parasitizing the gills of the striped trumpeter *Latris lineata,* and found that parasite infestation resulted in tissue hyperplasia and necrosis at the site of attachment and feeding. This in turn may lead to a decrease in respiratory efficiency due to the decrease in respiratory surface area (Bennett and Bennett, 1994).

There are a number of copepod species that graze and feed off of the thin epithelium of the host's gills (Kabata, 1974; Benz and Adamson, 1989; Boxshall, 1990; Chandran, 1994). The mechanism of feeding is similar between species and involves the scraping off of pieces of epithelium from the gill and drawing it back into the digestive tract (Pillai, 1985). This is achieved through raking the gill lamellae with their mandibles and funneling the fragments back into the buccal cavity (Benz and Adamson, 1989; Chandran, 1994). However, species within the Family Caligidae differ slightly in that tissue is scraped off using a toothed appendage at the tip of the labium called the strigil and the mandibles are then used to move the fragments of tissue into the buccal cavity (Figure 1.8A and 1.8B; Kabata 1974; Boxshall, 1990).

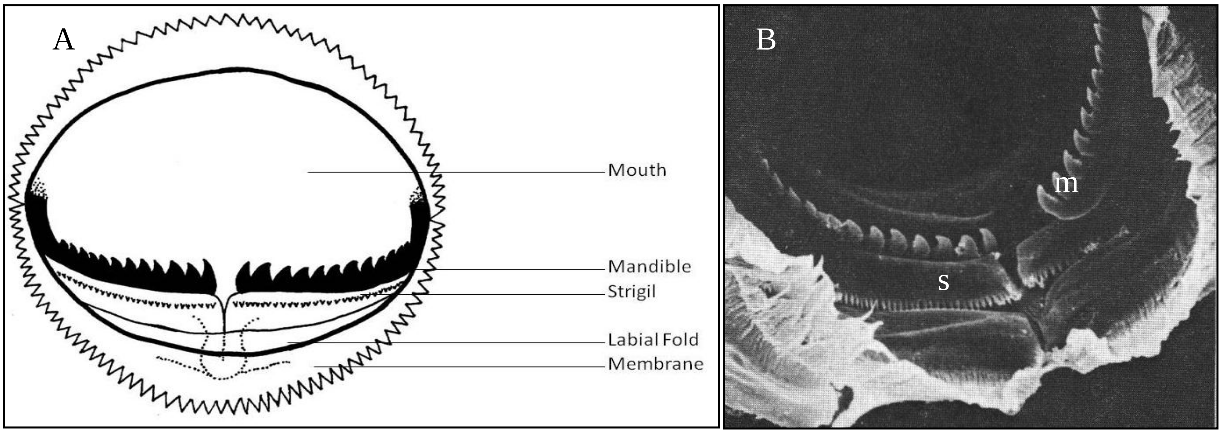

Figure 1.8: Diagrams showing A: The typical mouthpart morphology of a caligid copepod indicating the strigil and mandibles (redrawn from Kabata, 1974) B: A scanning electron micrograph of the mouth parts of *Lepeoptheirus salmonis* indicating the strigil (s) and mandible (m) (adapted after Kabata, 1974).

The blood-feeding Siphonostomatoida include species in the Pseudocynidae and Kroyeriidae and may include the family Hatschekiidae amongst others (Natarajan and Nair, 1972; Benz and Dupre, 1986; Scott-Holland *et al.,* 2006). In the family Pseudocynidae, the mandibles are used to lacerate the gill lamellae to produce a wound and the mouth cone is then used to suck the blood into the digestive tract with the assistance of muscles positioned within the mouth cone itself (Natarajan and Nair, 1972). There is very limited knowledge and literature regarding the feeding mechanism and diet of the family Kroyeriidae. Benz and Dupre (1986) investigated the copepod *Kroyeria carchariaeglauci* on the gills of the blue shark *Prionace glauca,* and postulated that the site of attachment is highly vascularized and the reddish colour of the female genital complex suggests that this parasite feeds at least to some extent on host blood, although the mechanism remains unclear. There is also very little known about feeding mechanisms of the family Hatschekiidae (Scott-Holland *et al.*, 2006). The lack of information may be as a result of the characteristics of the mouthparts not being deemed necessary for taxonomic identification (Jones, 1985), as the distinguishing characteristics of the genus *Hatschekia* are their elongated genital segment and the shape of the cephalothorax (Oldewage, 1993).

1.5.2- Monogeneans

Monogeneans are a class of ectoparasitic flatworms within the phylum Platyhelminthes which are characterized by the presence of an attachment organ or ‘haptor’ (Buchmann and Lindenstrøm, 2002). The structure of this organ further divides monogeneans into two subclasses, the Polyopisthocotylea and the Monopisthocotylea (Buchmann and Lindenstrøm, 2002). The posterior attachment organ (opisthaptor) of the Polyopisthocotylea is made up of a number of clamp-like structures which close over the host epithelium and attach the parasite to the host (Figure 1.9A) (Hayward, 2005). The attachment organ of a typical monopisthocotylean is made up of a single opisthaptor which, depending on the family, has large hook-like structures (hamuli) or smaller hooklets within the haptor itself (Figure 1.9B) (Whittington, 2005).

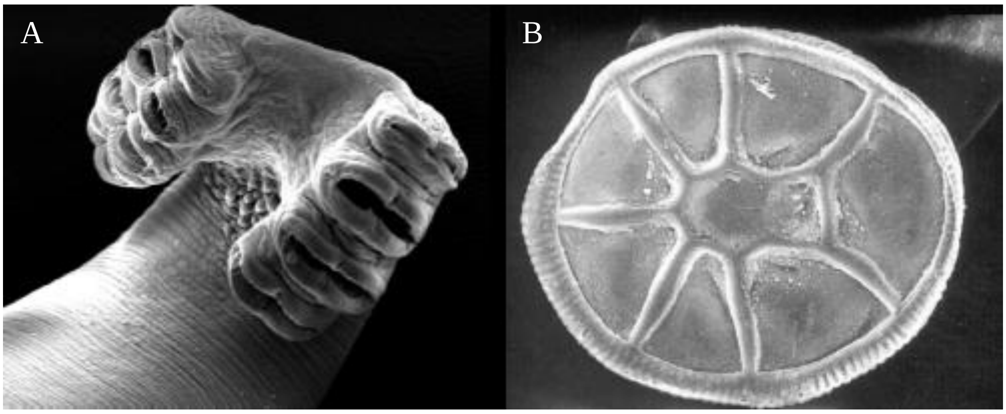

Figure 1.9: Scanning electron micrographs showing A: The ventral view of the clamp-like haptors of the polyopisthocotylean monogenean, *Discocotyle sagittata* and B: The ventral view of the opishaptor of a monopisthocotylean monogenean, *Capsala* sp. (adapted after Rubio-Godoy, 2007).

The digestive systems of both monogenean subclasses are similar in their general structure and consist of a mouth, buccal funnel, pharynx and oesophagus, and both lack an anus (Goater *et al.,* 2014, Buchman *et al.,* 2004). The subclasses differ in their diets, however, with polyopisthocotylean monogeneans feeding directly off host blood whereas monopisthocotylean monogeneans scrape off and consume the epithelium and mucous found on the surface of the attachment site (Buchmann and Lindenstrøm, 2002; Buchmann *et al.,* 2004; Hayward, 2005; Whittington, 2005; Rubio-Godoy, 2007; Goater *et al.,* 2014). Polyopisthocotyleans feed by gently lacerating the epithelial cells and ingesting the blood from within the vessels beneath (Wootten, 2012). This blood is then haemolysed within the gut and the globin component of the haemoglobin absorbed as this constitutes the basic nutrient for the monogeneans (Llewellyn, 1954). Feeding by polyopisthocotyleans is considered to be benign in wild populations, but major complications occur in cultured fish because individuals are living in higher densities and the parasites have a better chance of proliferating (Whittington, 2005). Feeding by the monopisthocotyleans is achieved through pharangeal secretions, including enzymes, which digest and separate epithelial cells, which are then sucked in through the pharynx or consumed through the inversion of the pharynx itself (Whittington, 2005). The ingested material passes through the pharynx and into the intestine where it is digested internally (Hickman *et al.*, 2012). The thinning of the epithelium through the scraping and consumption of the epithelial tissue may result in secondary complications and infections (Rubio-Godoy, 2007), especially in cases where there is a large abundance of parasites which damage the tissue at a faster rate than it is able to regenerate (Whittington, 2005).

1.6- Thesis Aims and Objectives

The main objective of this study is to further investigate trophic interactions between gill parasites and their marine fish hosts using stable isotope analysis for a broad trophic range of host species from zooplanktivorous small pelagic fish to apex fish predators. This is the first study to apply SIA to elucidate such interactions in South African marine fish, hence in addition to investigating the trophic interactions between parasites and their hosts, and because a wide range of fish hosts were examined, it also provides insight into the gill parasite diversity of South African marine fish.

The specific aims of this study which will be elaborated in subsequent chapters are as follows:

1. **To document and catalogue the copepod and monogenean head ectoparasite assemblages of several species of marine fish in South African waters, cataloguing any new host and locality records**.
2. **To investigate the trophic relationships between parasites and their hosts through stable isotope analysis, to;**
    a. Assess whether the host gill tissue on which the parasite feeds differs in its isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) compared to host muscle tissue, typically sampled in stable isotope studies, in order to select the most appropriate host tissue proxy with which to compare the parasite isotope ratios.

b. Asses if gill parasites in general are enriched (in both ^{13}C and ^{15}N) compared to their hosts.

c. Assess if copepod and monogenean gill parasites show different levels of trophic fractionation (for both $\delta^{13}C$ and $\delta^{15}N$) compared to their associated hosts.

d. Assess if trophic fractionation (for both $\delta^{13}C$ and $\delta^{15}N$) of monogenean gill parasites is affected by their feeding mode and different food sources.

e. To assess whether individual parasite taxa or species are enriched (in both ^{13}C and ^{15}N) compared to their hosts.

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Chapter 2: Gill Parasites of some South African Marine Fishes

2.1-Introduction

2.1.1- South African Marine Environment

The coastal marine environment of South Africa is known to be one of the most complex and diverse ecosystems in the world (Lutjeharms *et al.,* 2001), and contains over 12 000 species of marine flora and fauna of which approximately one third is endemic to South Africa (Branch *et al.,* 2016). The main contributing factor to this rich diversity is the presence of two very different oceanic currents and water masses surrounding the coastline; the warmer Agulhas Current of the Indian Ocean on the eastern seaboard, and the colder, nutrient-rich Benguela Current of the Atlantic Ocean on the western seaboard (Figures 2.1 & 2.2; Lutjeharms *et al.,* 2001; Heemstra and Heemstra, 2004).

The Agulhas Current is a western boundary current that flows down the eastern coastline of southern Africa from approximately 27°S to 40°S (Barale, 2014). The current transports warm tropical and subtropical water, sourced predominantly from the South-West Indian Ocean Sub-Gyre as well as the Mozambican and East Madagascan currents, southward at speeds around 2m.s^{-1} (Lutjeharms *et al.,* 2001, Lutjeharms, 2006). At approximately 36°S, it leaves the coastal shelf and projects offshore along the Agulhas Bank where it comes into contact with the strong flowing Antarctic Circumpolar Current (Barale, 2014). At this point, the majority of the water mass is retroflected back to form the Agulhas Return Current (Figures 2.1 & 2.2), however a small proportion of the warmer water is ‘leaked’ into the South Atlantic forming warm core eddies called Agulhas Rings (Barale, 2014). In the area where the Agulhas Current meets the Benguela Current there is large-scale mixing and upwelling, making this area highly productive (Barale, 2014). There are also a number of smaller upwelling areas along the southern and eastern coastlines. These occur at the widening of the coastal shelf off of Port Alfred forming the

Port Alfred Upwelling Cell (Figures 2.1 & 2.2), and between Durban and Richards Bay, forming the Natal Bight (Lutjeharms *et al.,* 2000; Lutjeharms, 2006) as well as a number of minor upwelling areas as a result of shear-edge eddies and Ekman transport (Schumann, 1986; Lutjeharms *et al.,* 2000; Lutjeharms *et al.,* 2003; Lutjeharms, 2006)

The Benguela Current is the north-flowing current on the western coastline of southern Africa from 37°S to 5°S and forms the eastern section of the South Atlantic Gyre (Shillington, 2006; Barale, 2014). Along with the Canary, Humboldt and California Currents, it forms one of the four major Eastern Boundary Upwelling Ecosystems in the world's oceans (Fréon *et al.,* 2009). The Benguela Current ecosystem is split into four major components; the Angolan subtropical zone, the northern Benguela, the Lüderitz upwelling zone and the southern Benguela (Figure 2.1) (Hutchings *et al.,* 2009). The southern Benguela is further sub-divided into the western coastline upwelling region comprising of seasonal wind-driven upwelling events, and the Agulhas Bank, which is a shallow shelf system characterized by having a combination of upwelling and seasonal occurrences of mixing and stratification (Hutchings *et al.,* 2009). The Benguela upwelling region stretches along the coastline from Cape Agulhas (20°E), South Africa's most southerly tip, to Cape Frio in northern Namibia, where the northern boundary is determined by the southward flowing Angola Current where it forms the Angola-Benguela Front at approximately 17°S (Figure 2.1; Hutchings *et al.,* 2009). Within this stretch, there are areas of major upwelling located at Lüderitz (24°S) and Cape Frio (17°S), with the Lüderitz cell separating the system into the northern and southern Benguela (Figures 2.1 & 2.2; Hutchings *et al.,* 2009). The drivers behind these major upwelling phenomena are the southeasterly trade-winds that result in offshore Ekman transport, with changes in shelf width and the topography of the coastline forming multiple minor upwelling cells which can vary seasonally due to the movements of the South Atlantic and South Indian Anticyclones (Chavez and Messié, 2009; Hutchings *et al.,* 2009).

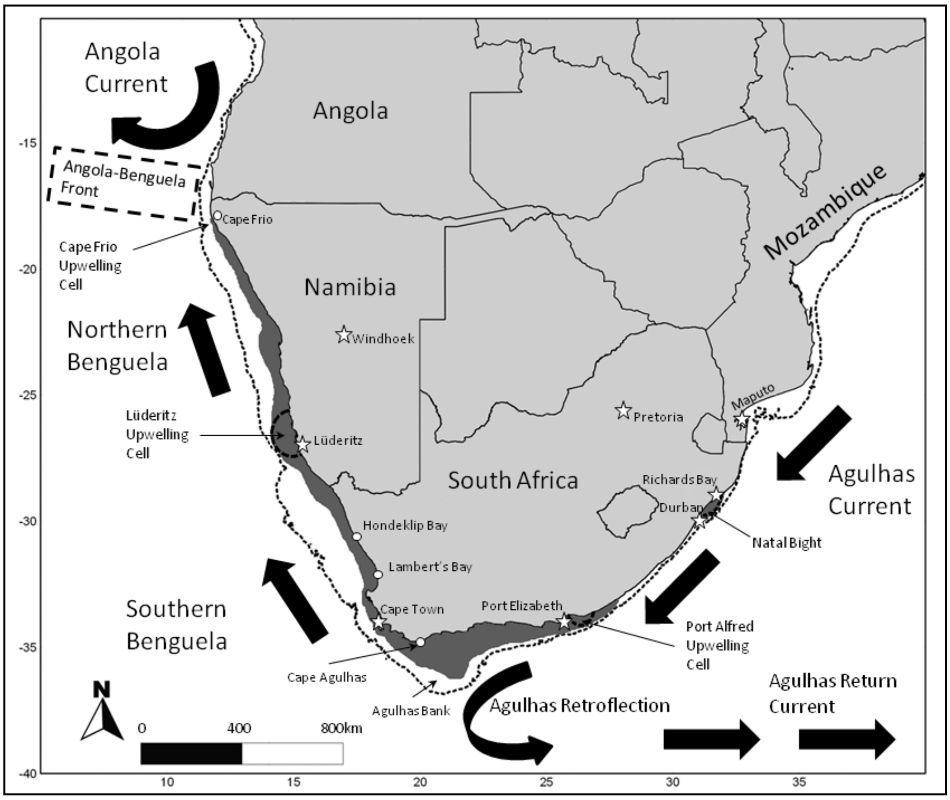


Figure 2.1: Map showing the oceanic currents around southern Africa, indicating the southerly flowing Agulhas Current, The Natal Bight, the Port Alfred Upwelling Cell, the Agulhas Bank, the Agulhas Retroflection, the northerly flowing Benguela Current, the Lüderitz Upwelling Cell, the Cape Frio Upwelling Cell and the Angola-Benguela Front. Dark grey shading indicates areas of upwelling (adapted after Roux *et al.*, 2013 with information from Lutjeharms *et al.*, 2000; Lutjeharms, 2006; Shillington *et al.*, 2006; Hutchings *et al.*, 2009; Barale, 2014).

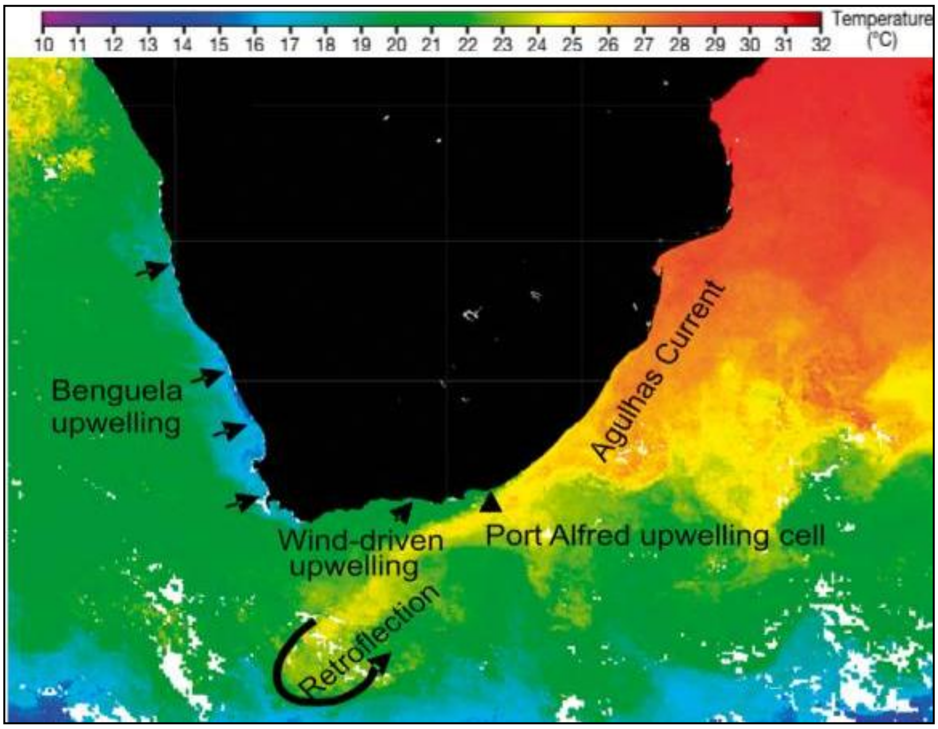


Figure 2.2: Image showing the sea surface temperatures of the Benguela and Agulhas current systems during February 2009. Arrows indicate the upwelling cells on the southern and western coastlines as well as the Agulhas Retroflection (adapted after James *et al.*, 2013).

Upwelling draws nutrient-rich water from the depths to the surface and in doing so fuels high levels of productivity (Hutchings *et al.,* 2009). Blooms in productivity attract predators from multiple trophic levels, vastly increasing the biomass (Chavez and Messié, 2009). As a result, the majority of South African fisheries are situated on the West and South Coasts with the entire Benguela ecosystem providing a fishery catch size of approximately 1.3 million tons annually (Fréon *et al.*, 2009).

2.1.2- Marine Fish Parasitology in Southern Africa

The majority of marine biological research within South Africa has focused on larger, more charismatic classes and orders including marine mammals, birds, reptiles and fishes, but our state of knowledge of many marine invertebrates, let alone parasites, is fairly limited (Griffiths *et al.,* 2010). Consequently, marine fish parasites are relatively understudied within the South African marine environment. There has, however, been a growing interest within the field with a number of recently completed as well as ongoing studies focusing on assessing the parasite assemblages of economically important and other fish species, predominantly underway at the University of Cape Town (*e.g.* Yeld, 2009; Bih, 2012; Reed *et al.*, 2012; Bowker, 2013; Le Roux, 2013; Ssempa, 2013; Bartošová *et al.,* 2015; Poddubnaya *et al.,* 2015; Hendricks, 2016; Mackintosh, 2016; Malongweni, 2016; Nunkoo *et al.*, 2016; Sibanda, 2016; Poddubnaya *et al.*, 2017) as well as by Nico Smit and colleagues at North West University (*e.g.* Smit and Davies, 1999; Smit *et al*., 2013; Smit *et al.*, 2014; Smit and Hadfield, 2015; Welicky *et al.*, 2017) and Susan Dippenaar and colleagues at the University of Limpopo (*e.g.* Dippenaar, 2004; Dippenaar and Jordaan 2008; Dippenaar et al 2010; Dippenaar, 2012; Dippenaar and Lebepe, 2013). Some studies have utilized the assemblage data and applied it to various other aspects of marine science, such as stock discrimination and pollution bio-indicators (*e.g.* Weston *et al.,* 2015; Morris *et al.*, 2016).

2.1.3- South African Fish Species Examined

The present study focuses primarily on fish species that are of major economic importance in South Africa, but a number of species which are of less economic importance are also included. The most valuable South African marine fishery is the hake fishery, providing upwards of 30 000 jobs and bringing in R 5.2 billion annually (DAFF, 2014). Of the two species of hake found in South African waters, the shallow-water hake, *Merluccius capensis*, contributes 70% of the hake caught on the south coast, whereas the deep-water hake, *Merluccius paradoxus*, contributes 90% of the hake catch on the west coast (DAFF, 2014). Second to the hake fishery in terms of its value is the purse-seine fishery targeting small pelagic shoaling species including sardine, *Sardinops sagax* (DAFF, 2014). Together with anchovy *Engraulis encrasicolus* and West Coast round herring *Etrumeus whiteheadi*, these small pelagic fishes contribute approximately 90% of the purse-seine fishery catch with a total landing of 485 000 tonnes in 2012 (DAFF, 2014). Cape horse mackerel, *Trachurus capensis*, is targeted by mid-water trawl vessels with the *FV Dessert Diamond* being the only large mid-water trawler targeting *T. capensis* in South Africa but a small number of hake trawlers which catch the species on an opportunistic basis (Smith and Cochrane, 2016). Within the linefish sector, the predominantly targeted species are the snoek, *Thyrsites atun*, and the yellowtail amberjack, *Seriola lalandi*, with annual landings of 6809 and 382 tonnes respectively, during 2012 (DAFF, 2014). The pole and line fishery targets larger predatory species, including albacore tuna, *Thunnus alalunga* (DAFF, 2014). Other species of tuna, including the yellowfin tuna, *Thunnus albacares*, and bigeye tuna, *Thunnus obesus*, are mainly targeted in the longline fisheries with the southern bluefin tuna, *Thunnus maccoyii* only contributing a small proportion due to its strict quota and limited abundance (DAFF, 2014). These longline fisheries also target billfish such as the swordfish, *Xiphias gladius*, as well as sharks including the shortfin mako, *Isurus oxyrinchus*, and blue shark, *Prionace glauca,* which in 2011 had recorded landings of 644.4 and 543.4 tonnes, respectively (DAFF, 2014).

The species which are of less economic importance include the Atlantic bonito (*Sarda sarda*), kingklip (*Genypterus capensis*) which although is an economically valuable species, is mostly caught as by-catch, Atlantic pomfret (*Brama brama*) and Hector's lantern fish (*Lampanyctodes hectoris*). Of these 17 species, the majority have a cosmopolitan distribution whereas others (particularly the demersal species) are more regionally distributed (Table 2.1).

Table 2.1: The distribution of fish species examined in the present study (after Froese and Pauly, 2017; Heemstra and Heemstra, 2014; and van der Elst, 1993; Distribution maps after www.aquamaps.org, version of Aug. 2016).

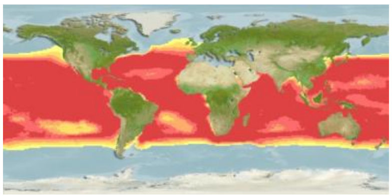
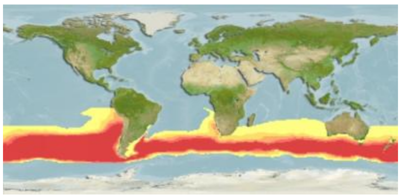
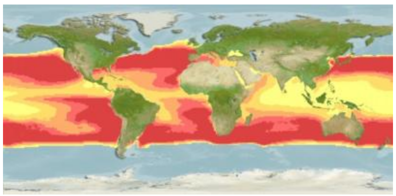
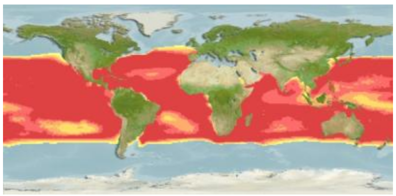
Host Species	Distribution	Distribution Map
Large Pelagics		
Yellowfin tuna *Thunnus albacares*	Circumglobal- inhabiting warm-temperate regions	
Southern bluefin tuna *Thunnus maccoyii*	Circumglobal- inhabiting temperate-cold regions in the Southern Hemisphere	
Albacore tuna *Thunnus alalunga*	Circumglobal- inhabiting warm-temperate regions	
Bigeye tuna *Thunnus obesus*	Circumglobal- inhabiting warm-temperate regions	

Table 2.1: (Continued)

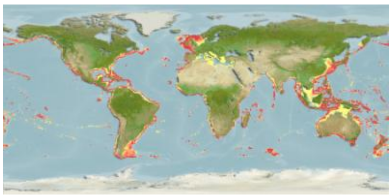
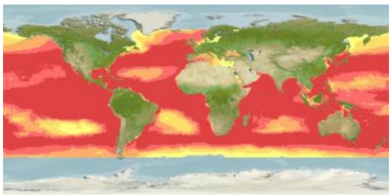
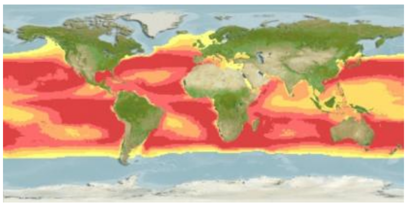
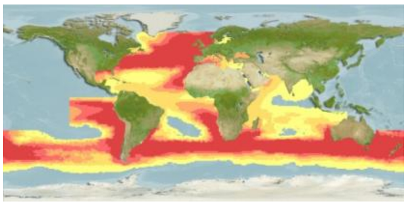
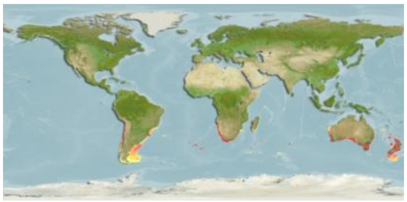
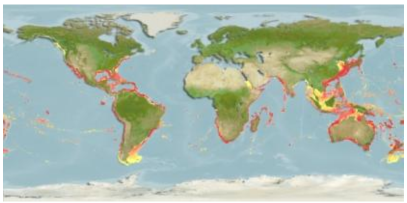
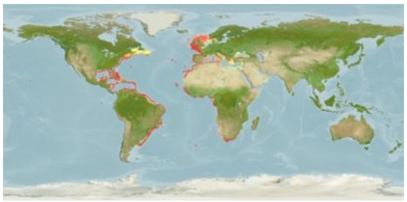
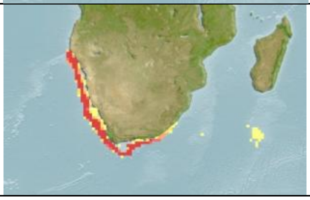
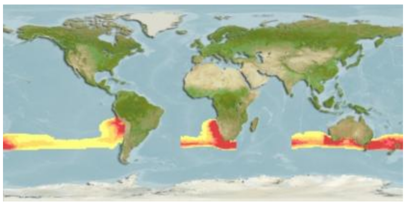
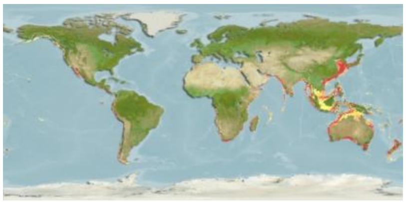
Host Species	Distribution	Distribution Map
Shortfin mako shark *Isurus oxyrinchus*	Circumglobal- inhabiting warm-temperate regions	
Blue shark *Prionace glauca*	Circumglobal- inhabiting warm-temperate regions	
Swordfish *Xiphias gladius*	Circumglobal- inhabiting warm-temperate regions	
Middle-Sized Pelagics		
Atlantic pomfret *Brama brama*	Circumglobal- inhabiting warm-temperate regions	
Snoek *Thyrsites atun*	Circumglobal- temperate waters of the Southern Hemisphere	
Yellowtail amberjack *Seriola lalandi*	Circumglobal- inhabiting warm-temperate regions	
Atlantic bonito *Sarda sarda*	Warm- temperate regions of Atlantic Ocean, Mediterranean and Black Sea	

Table 2.1: (Continued)

Demersal		
Kingklip *Genypterus capensis*	Regional (Endemic)-West Coast to South Coast	
Deep-water hake *Merluccius paradoxus*	Regional (Endemic)-West and South Coasts	
Shallow-water hake *Merluccius capensis*	Regional (Endemic)-West and South Coasts	
Small Pelagics		
Hector's lanternfish *Lampanyctodes hectoris*	Circumglobal- colder waters of the Southern Hemisphere	
Sardine *Sardinops sagax*	Circumglobal- Indo-Pacific regions	
Horse mackerel *Trachurus capensis*	Regional – West Coast spreading to the South Coast	

There is a fair amount knowledge on the biology and ecology of these species (van der Elst, 1993; Heemstra and Heemstra, 2004; Froese and Pauly, 2017), especially as the majority are economically valuable and targeted by fisheries. However, since marine parasitology within South Africa is understudied, very little literature exists on parasite assemblages of these fish species in South African waters.

2.1.4- A Review of Copepod and Monogenean Parasite Diversity in Selected South African Marine Fish.

Two of the most diverse groups of ectoparasites infecting fish are the copepods and monogeneans (Rohde, 2005), which are often found parasitizing the exterior surfaces, gill and buccal regions of their hosts. Table 2.2 consolidates a comprehensive search of literature investigating copepod and monogenean parasite assemblages of the 17 aforementioned species within South African waters.

Table 2.2: Known copepod and monogenean parasites of the fish species examined in this study from South African waters (1: Oldewage and van As, 1989, 2: Kensley and Grindley, 1973, 3: Cressey and Cressey, 1980, 4: Oldewage, 1995, 5: Dippenaar and Jordaan, 2007, 6: Oldewage and Smale, 1993, 7: Cressey, 1967, 8: Barnard, 1948, 9: Oldewage, 1993a, 10: Mackintosh, 2016, 11: Nunkoo *et al.,* 2016, 12: Barnard, 1955b, 13: Barnard, 1955a, 14: Sibanda, 2016, 15: Payne, 1986, 16: Botha, 1986, 17: Oldewage, 1992, 18: Wilson, 1923, 19: Oldewage, 1993b, 20: Reed *et al.,* 2012, 21: Le Roux, 2013)

Host Species	Parasite Taxa	Parasite Type	Reference
Large Pelagics			
Thunnus albacares	*Caligus productus*	Copepod	1
	Euryphorus brachypterus	Copepod	2
	Pennella filosa	Copepod	2
Thunnus maccoyii	*Euryphorus brachypterus**	Copepod	2, 3
Thunnus alalunga	No studies		
Thunnus obesus	*Caligus coryphaenae*	Copepod	2
Isurus oxyrinchus	*Alebion carchariae*	Copepod	4
	Anthosoma crassum	Copepod	5
	Dinemoura latifolia	Copepod	6, 7
	Echthrogaleus denticulatus	Copepod	6
	Nemesis lamna lamna	Copepod	6
	Pandarus smithii	Copepod	2, 6
	Phylothereus cornutus	Copepod	5
Prionace glauca	*Anthosoma crassum*	Copepod	2
	Dinemoura latifolia	Copepod	2
	Echthrogaleus coleoptratus	Copepod	2
	Kroyeria carchariaeglauci	Copepod	2
	Nemesis robusta	Copepod	2
	Pandarus smithii	Copepod	2
Xiphias gladius	No studies		
Middle-Sized Pelagics			
Brama brama	*Hatschekia conifera*	Copepod	8, 9, 10
Thyrsites atun	*Caligus coryphaenae*	Copepod	11
	Caligus dakari	Copepod	11
	Caligus pelamydis	Copepod	3, 12
	Caligus zei	Copepod	11, 12
	Hatschekia conifera	Copepod	11
	Nothobomolochus fradei	Copepod	11

*Kensley and Grindley (1973) and Cressey and Cressey (1980) recorded *Euryphorus brachypterus* on *Thunnus thynnus* and not *Thunnus maccoyii*

Table 2.2: (Continued)

Host Species	Parasite Taxa	Parasite Type	Reference
Seriola lalandi	*Caligus aesopus*	Copepod	2
	Caligus lalandei	Copepod	8
	Caligus lunatus	Copepod	12
	Lepeoptheirus lalandi	Copepod	2
	Lernanthropus ecclesi	Copepod	2
Sarda sarda	*Caligus biseriodentatus*	Copepod	1
	Caligus bonito	Copepod	2, 12
	Caligus pelamydis	Copepod	13
Demersal			
Genypterus capensis	*Chondracanthus colligens*	Copepod	2
	Chondracanthus sp.	Copepod	14
	Parabrachiella supplicans	Copepod	12
	Sphyrion laevigatum	Copepod	2, 13, 15
Merluccius paradoxus	*Parabrachiella insidiosa*	Copepod	16
	Parabrachiella supplicans	Copepod	17
Merluccius capensis	*Caligus elongatus*	Copepod	12
	Caligus rapax	Copepod	12
	Chondracanthus merlucci	Copepod	13, 16
	Parabrachiella insidiosa	Copepod	12, 13, 16, 18
	Parabrachiella supplicans	Copepod	19, 17
Small Pelagics			
Lampanyctodes hectoris	*Cardiodectes medusaeus*	Copepod	2, 12
Sardinops sagax	*Clavellisa ilishae*	Copepod	2, 12
	Mazocraes sardinopsi	Monogenean	20
	Nothobomolochus fradei	Copepod	20
Trachurus capensis	*Caligus elongatus*	Copepod	12
	Caligus rapax	Copepod	12**
	Caligus sp.	Copepod	21
	Gastrocotyle trachuri	Monogenean	21
	Lernathropus trachuri	Copepod	21

**Barnard (1955b) recorded *Caligus rapax* on *Trachurus trachurus*

2.1.5- Aim and Objective

This study aims to document some copepod and monogenean ectoparasites infecting the head region of several species of marine fishes from a range of trophic levels in South African waters, cataloguing any new host and locality records. It must be noted that this study is not exhaustive regarding the gill parasite faunas of the host species examined as many specimens were obtained on an opportunistic basis.

2.2- Methods

2.2.1- Sampling Protocol

Fish specimens were obtained from the West and South Coasts of South Africa during 2015 and 2016 from research surveys conducted by the Department of Agriculture, Forestry and Fisheries (DAFF), as well as from local fishermen and fishing competitions (Figure 2.3).

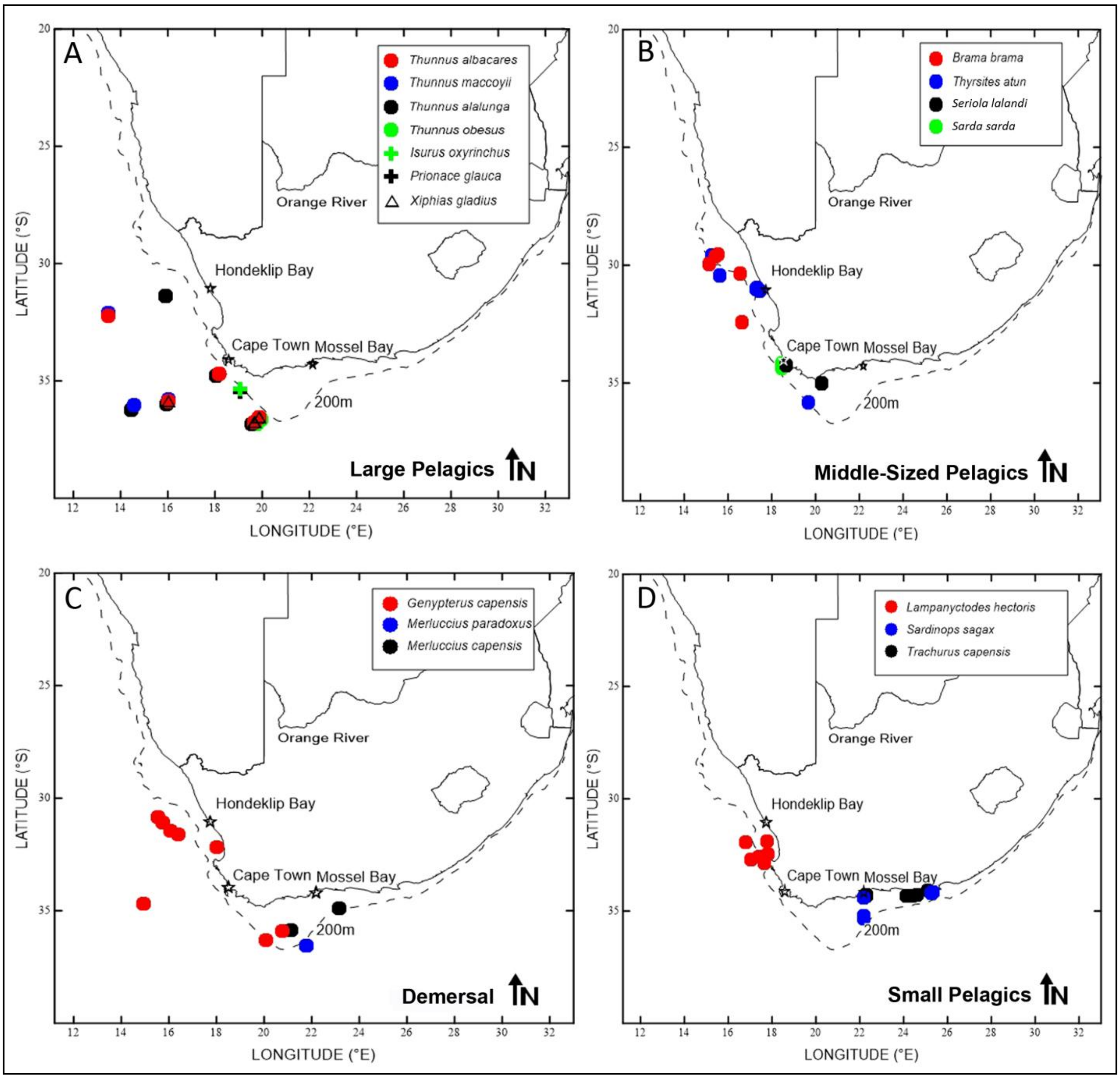


Figure 2.3: Showing the catch localities of the 17 species of fish in South African waters. Species were grouped according to their habitat and size. A: Large pelagic species; B: Middle-sized pelagic species; C: Demersal species; D: Small pelagic species.

Collected samples, comprising of only heads for the majority of the large pelagic species but whole bodies for the smaller species, were frozen at -20°C until dissections were due to commence, whereby individual samples were thawed to room temperature prior to dissection.

The head region and operculae were screened for ectoparasites and any parasites found were removed using fine-tipped forceps and placed in a Petri-dish containing freshwater until further processing after the dissection was completed. Gills were manually excised from heads and separated into individual gill arches which were then individually examined and each gill filament was inspected for the presence of parasites. In the case of the smaller host species (*S. sagax, T. capensis*), gills were examined using a dissecting microscope (Leica EZ4).

Two or three individuals of each parasite species found were fixed and preserved in 70% ethanol as type specimens for further identification. Images of the most prevalent copepod species were taken using a Canon EOS-650D camera attached to a stereoscopic zoom microscope (Nikon SMZ800) illuminated with fibreoptics (Fibreoptic-Heim LQ 1100). As the sampled material was frozen and then thawed for dissection, the samples of the monogenean parasites lost integrity and thus could not be photographed.

2.2.2- Species Identification

Copepods

Preserved individual copepod parasites were identified to the lowest level of classification possible. When required, specimens were placed in 90% DL-lactic acid and stained with Mexican Red dye for 2-3 minutes to better distinguish characteristic features and were observed under either a dissecting microscope (Leica EZ4) or a compound microscope (Leica ICC50) depending on the size of the parasite. Copepods were identified from Barnard (1955a), Barnard (1955b), Perkins (1983), Ho and Do (1984), Pillai (1985), Jones (1988), Kabata (1988), Izawa

(2008), Boxshall and El-Rashidy (2009) and El-Rashidy and Boxshall (2010) (see Appendix 1A). Nomenclature follows the World Register of Marine Species (WoRMS).

Monogeneans

Monogeneans were identified to lowest possible level of classification using Jones (1933), Chauhan (1950), Price (1961), Kohn *et al.* (2004), Chisholm and Whittington (2007), and Akmirza (2013) (see Appendix 1B). Taxa that could not be identified morphologically were grouped into morphospecies and the taxonomic expertise of Dr. Kevin Christison (Department of Agriculture, Forestry and Fisheries) was called upon to separate the morphospecies into monopisthocotylean and polyopisthocotylean subclasses.

2.2.2- Data Analysis

The parasite assemblages were analysed using the following quantitative indices; prevalence, mean intensity of infection and mean abundance, in accordance with Bush *et al.* (1997).

Prevalence was calculated as the number of individual hosts of a particular species infected with the parasite regardless of the number of parasite individuals, divided by the total number of hosts of that particular species examined, expressed as a percentage (Equation 2.1).

(2.1) $Prevalence = \frac{}{}$

Mean intensity of infection is the average number of a single parasite species found infecting hosts of a single species. It was calculated by dividing the total number of parasites of a particular species found on a single host species by the total number of infected hosts, expressed as parasites per infected fish (parasites.infected fish^{-1}) (Equation 2.2).

(2.2) $\frac{}{}$

Mean parasite abundance is the average number of parasites of a particular species occurring on the host species, regardless of whether an individual host is infected with the parasite or not. This indicates the average number of a particular parasite species within the host population. This was calculated by dividing the total number of parasites of a particular species found on a single host species by the total number of examined hosts of that species, expressed as parasites per fish (parasites.fish^{-1}) (Equation 2.3).

(2.3)

2.3- Results

A total of 1513 individual fish from 17 host species comprising two species of elasmobranchs and fifteen species of teleosts from varying trophic levels were examined (Table 2.3).

Table 2.3: Summary of the characteristics of the samples examined including host species, common names, sample sizes, types of specimen dissected and mean head/fork length (±SD).

Host Species	Common Name	n	Type of Specimen	Mean Head/Fork Length (cm) (±SD)
Large Pelagics				
Thunnus albacares (Bonnaterre, 1788)	Yellowfin tuna	44	Head	29.53 ± 3.51
Thunnus maccoyii (Castelnau, 1872)	Southern bluefin tuna	4	Head	45.35 ± 7.35
Thunnus alalunga (Bonnaterre, 1788)	Albacore tuna	42	Head & Whole	84.38 ± 6.67*
Thunnus obesus (Lowe, 1839)	Bigeye tuna	15	Head	35.87 ± 5.04
Isurus oxyrinchus (Rafinesque, 1810)	Shortfin mako shark	2	Whole	129.10 ± 8.63
Prionace glauca (Linnaeus, 1758)	Blue shark	2	Whole	134.25 ± 25.81
Xiphias gladius (Linnaeus, 1758)	Swordfish	9	Head	38.04 ± 7.87**
Middle-Sized Pelagics				
Brama brama (Bonnaterre, 1788)	Atlantic pomfret	11	Whole	41.62 ± 3.57
Thyrsites atun (Euphrasen, 1791)	Snoek	35	Head	19.33 ± 2.24
Seriola lalandi (Valenciennes, 1833)	Yellowtail amberjack	45	Head	17.80 ± 1.85
Sarda sarda (Bloch, 1793)	Atlantic bonito	16	Whole	50.54 ± 6.14
Demersal				
Genypterus capensis (Smith, 1847)	Kingklip	22	Whole	72.08 ± 12.16
Merluccius paradoxus (Franca, 1960)	Deep-water hake	16	Whole	49.74 ± 13.17
Merluccius capensis (Castelnau, 1861)	Shallow-water hake	10	Whole	40.40 ± 7.26
Small Pelagics				
Lampanyctodes hectoris (Günther, 1876)	Hector's lanternfish	892	Whole	N/A
Sardinops sagax (Jenyns, 1842)	Sardine	156	Whole	18.43 ± 1.85***
Trachurus capensis (Castelnau, 1861)	Cape horse mackerel	192	Whole	20.61 ± 5.07

*Mean fork length of specimens for which data was available (see Appendix 2C)
**Mean lower jaw length used as proxy
***Standard length transformed to fork length using length-length relationships provided by FishBase (Froese and Pauly, 2017)

A total of 32 parasite taxa comprising 20 copepod and 12 monogenean species were recorded from the 17 fish species examined (Table 2.4; Appendix 2). Utilizing taxonomic keys and various publications, three copepods and two monogeneans were identified to genus level and 12 copepods and four monogeneans were identified to species level. The remaining specimens (five copepods and six monogeneans) were not completely identified as they comprised a very small portion of the total parasite assemblage and required specialist taxonomic knowledge.

Table 2.4: The parasite assemblage of the 17 species of fish hosts sampled. Table indicates the parasite taxa, type (C: Copepod, M: Monogenean), site of infection, prevalence (%), mean infection intensity (±SD) and the mean parasite abundance (±SD) per host. * denotes a new geographic record and # denotes a new host record.

Host	**Parasite Taxa**	**Type**	**Site of Infection**	**Prevalence (%)**	**Mean Infection Intensity ± SD (parasite.infected fish $^{-1}$)**	**Mean Parasite Abundance ± SD (parasites.fish^{-1})**
Large Pelagics						
Thunnus albacares (n=44)	*Euryphorus brachypterus*	C	Gill	51.2	4.8 ± 4.9	2.4 ± 4.2
	Pseudocycnus appendiculatus *	C	Gill	51.2	3.0 ± 2.5	1.6 ± 2.3
	Caligus sp. 1	C	Gill	2.3	1	0.02 ± 0.2
	Hexostoma sp. *	M	Gill	4.7	2.5 ± 2.1	0.1 ± 0.6
	Nasicola klawei *	M	Nares	32.6	2.2 ± 1.3	0.7 ± 1.3
	Monogenean sp. 1	M	Gill	9.3	1.8 ± 1.7	0.2 ± 0.7
	Monogenean sp. 2	M	Gill	7	1.3 ± 0.6	0.1 ± 0.4
Thunnus maccoyii (n=4)	*Euryphorus brachypterus*	C	Gill	100	24.3 ± 30.9	24.3 ± 30.9
Thunnus alalunga (n=42)	*Euryphorus brachypterus* *	C	Gill	46.5	3.9 ± 4.1	1.8 ± 3.4
	Pseudocycnus appendiculatus *	C	Gill	65.1	4.3 ± 3.5	2.8 ± 3.5
	Copepod sp. 1	C	Gill	2.3	1	0.02 ± 0.2
Thunnus obesus (n=15)	*Euryphorus brachypterus**	C	Gill	86.7	16.6 ± 16.7	14.4 ± 16.6
	Pseudocycnus appendiculatus *	C	Gill	13.3	1.5 ± 0.7	0.2 ± 0.6
	Hexostoma sp.*	M	Gill	60	4.2 ± 3.2	2.5 ± 3.2
	Nasicola klawei *	M	Nares	6.7	2	0.3 ± 0.5
	Monogenean sp. 1	M	Gill	6.7	3	0.2 ± 0.8
Isurus oxyrinchus (n=2)	*Nemesis lamna lamna*	C	Gill	100	47.0 ± 17.0	47.0 ± 17.0
Prionace glauca (n=2)	*Kroyeria carchariaeglauci*	C	Gill	100	153.0 ± 45.3	153.0 ± 45.3
	Copepod sp. 2	C	Gill	50	1	0.5 ± 0.7
	Copepod sp. 3	C	Gill	50	1	0.5 ± 0.7
	Copepod sp. 4	C	Gill	50	2	1 ± 1.4

Table 2.4: (Continued)

Host	**Parasite Taxa**	**Type**	**Site of Infection**	**Prevalence (%)**	**Mean Infection Intensity ± SD (parasite.infected fish $^{-1}$)**	**Mean Parasite Abundance ± SD (parasites.fish^{-1})**
Xiphias gladius (n=9)	*Tristoma adcoccineum* *	M	Gill	77.8	4.0 ± 2.5	3.1 ± 2.8
	Monogenean sp. 3	M	Gill	11.1	1	0.1 ± 0.3
	Monogenean sp. 4	M	Gill	11.1	1	0.1 ± 0.3
	Monogenean sp. 5	M	Gill	11.1	1	0.1 ± 0.3
Middle-Sized Pelagics						
Brama brama (n=11)	*Hatschekia conifera*	C	Gill	100	165.9 ± 154.3	165.9 ± 154.3
	Monogenean sp. 6	M	Gill	81.8	3.8 ± 2.7	3 ± 2.9
Thyrsites atun (n=35)	*Caligus dakari*	C	Gill	34.3	3.3 ± 2.0	1.1 ± 1.9
	Hatschekia conifera	C	Gill	54.3	5.8 ± 6.9	3.1 ± 5.8
	Mazocraes sp.#	M	Gill	20	1.4 ± 1.1	0.3 ± 0.8
Seriola lalandi (n=45)	*Caligus aesopus*	C	Gill	2.2	3	0.1 ± 0.4
	Eobrachiella elegans *	C	Buccal Folds	6.7	2.0 ± 0.6	0.1 ± 0.5
	Parabrachiella sp. 1 *	C	Gill	22.2	3.1 ± 2.5	0.7 ± 1.7
	Parabrachiella sp. 2 *	C	Nares	11.1	1	0.1 ± 0.3
	Copepod sp. 5	C	Gill	2.2	1.75 ± 0.1	0.02 ± 0.6
Sarda sarda (n=16)	*Caligus bonito*	C	Gill and Buccal Cavity	75	3.0 ± 2.4	2.3 ± 2.5
Demersal						
Genypterus capensis (n=22)	*Chondracanthus colligens*	C	Gill	18.2	2.0 ± 1.2	0.4 ± 0.9
Merluccius paradoxus (n=16)	*Anthocotyle merlucci**	M	Gill	25	1.5 ± 0.6	0.4 ± 0.7
Merluccius capensis (n=10)	*Anthocotyle merlucci**	M	Gill	70	2.0 ± 1.2	1.4 ± 1.3
Small Pelagics						
Lampanyctodes hectoris (n=892)	*Cardiodectes medusaeus*	C	Heart	19.1	N/D	N/D
Sardinops sagax (n=156)	*Clavellisa ilishae*	C	Gill	2.6	3.0 ± 3.7	0.08 ± 0.7
	Nothobomolochus fradei	C	Gill	1.3	1	0.01 ± 0.1
Trachurus capensis (n=192)	*Caligus dakari* #	C	Gill	1.0	1	0.01 ± 0.1
	Gastrocotyle trachuri	M	Gill	72.9	3.2 ± 2.4	2.3 ± 2.5

Within the group of large pelagics, the species within the genus *Thunnus* showed very similar copepod parasite assemblages. *Euryphorus brachypterus* was found on all four of the examined species with prevalences of 46.5% (n=42) on *T. alalunga*, 51.2% (n=44) on *T. albacares*, 86.7% (n=15) on *T. obesus* and 100% (n=4) on *T. maccoyii*. *Pseudocycnus appendiculatus* was recorded on three of the four *Thunnus* species examined with prevalences of 13.3% (n=15) on *T. obesus,* 51.2% (n=44) on *T. albacares* and 65.1% (n=42) on *T. alalunga,* but none were recovered from *T. maccoyii.* Only two parasite species were recorded from the two elasmobranch species examined; *Nemesis lamna lamna* parasitizing the gills of *Isurus oxyrinchus,* and *Kroyeria carchariaeglauci* parasitizing the gills of *Prionace glauca*. Both species showed 100% prevalence and very high infection intensities on their respective hosts, with *N. lamna lamna* having a mean infection intensity of 47 ± 12 parasites per infected fish and *K. carchariaeglauci* having a mean infection intensity of 153 ± 32 parasites per infected fish. *Tristoma adcoccineum* had the highest prevalence on the gills of *Xiphias gladius*, occurring in 77.8% (n=9) of examined specimens with a mean infection intensity of 4 ± 2.5 parasites per infected fish.

Within the group of middle-sized pelagics, both *Hatschekia conifera* and *Caligus dakari* were highly prevalent on *Thyrsites atun* and occurred on 54.3% (n=35) and 34.3% (n=35), respectively, of the individuals examined. *Hatschekia conifera* was also highly prevalent (100%; n=11) on *Brama brama* and was found with a mean infection intensity of 145 ± 46.5 parasites per infected fish. *Caligus bonito* was the only copepod parasite found on the gills and buccal cavity of *Sarda sarda* in the present study with a relatively high prevalence of 75% (n=16) and a mean infection intensity of 3 ± 0.7 parasites per infected fish.

Only a few demersal species were examined for parasite infections during this study (Table 2.3). *Genypterus capensis* was found to be parasitized by one copepod, *Chondracanthus colligens* with a low prevalence of 18.2% (n=22) and a mean infection intensity of 2.0 ± 0.6 parasites per infected fish. The two South African hake species, *Merluccius capensis* and *M. paradoxus,* were both parasitized by the monogenean *Anthocotyle merlucci*. This parasite was more prevalent on *M. capensis*, occurring on 70% (n=10) of the specimens dissected with a mean infection intensity of 2.0 ± 0.4 parasites per infected fish compared to *M. paradoxus* where it occurred on only 25% (n=16) of specimens with a mean infection intensity of 1.5 ± 0.3 parasites per infected fish.

Amongst the small pelagic species, *Cardiodectes medusaeus* was the only parasite found infecting *Lampanyctodes hectoris* with a prevalence of 19.1% (n=892). *Clavellisa ilishae* and *Nothobomolochus fradei* were found parasitizing the gills of *Sardinops sagax,* both occurring at very low prevalence levels with *C. ilishae* being found on 2.6% (n=156) of fish with a mean infection intensity of 3 ± 1.7 parasites per infected fish, and *N. fradei* found on only 1.3% of fish with a mean infection intensity of 1 parasite per infected fish. Of the 192 specimens of *Trachurus capensis* dissected, the monogenean parasite *Gastrocotyle trachuri* was found on 140 individuals with a prevalence of 72.9% and a mean infection intensity of 3.2 ± 0.2 parasites per infected fish. *Caligus dakari* was the only copepod found to infect *T. capensis* but had a very low prevalence (1%) and mean infection intensity of 1 parasite per infected fish. Figures 2.4 (A-D) and 2.5 (A-H) show the copepod parasites which have the highest prevalences and abundances on their associated hosts.

Two new host records were found in the present study which comprised the monogenean, *Mazocraes* sp. found parasitizing the gills of *Thyrsites atun*, and the copepod¸ *Caligus dakari* parasitizing *Trachurus capensis* (Figure 2.5G; Table 2.5). The present study also recorded 15 new geographic records for South Africa (Table 2.5). These new locality records are for *Euryphorus brachypterus* (Figure 2.4A) recovered from *Thunnus alalunga* and *T. obesus; Pseudocycnus appendiculatus* (Figure 2.4B) recovered from *T. albacares, T. alalunga* and *T. obesus; Nasicola klawei* recovered from *T. albacares* and *T. obesus; Hexostoma* sp. recovered from *T. albacares* and *T. obesus; Tristoma adcoccineum* recovered from *Xiphias gladius; Eobrachiella elegans* (Figure 2.5D) recovered from *Seriola lalandi*; two species within the genus *Parabrachiella* (Figure 2.5B & 2.5C) recovered from *S. lalandi,* and *Anthocotyle merlucci* recovered from *Merluccius paradoxus* and *M. capensis* (Table 2.5).

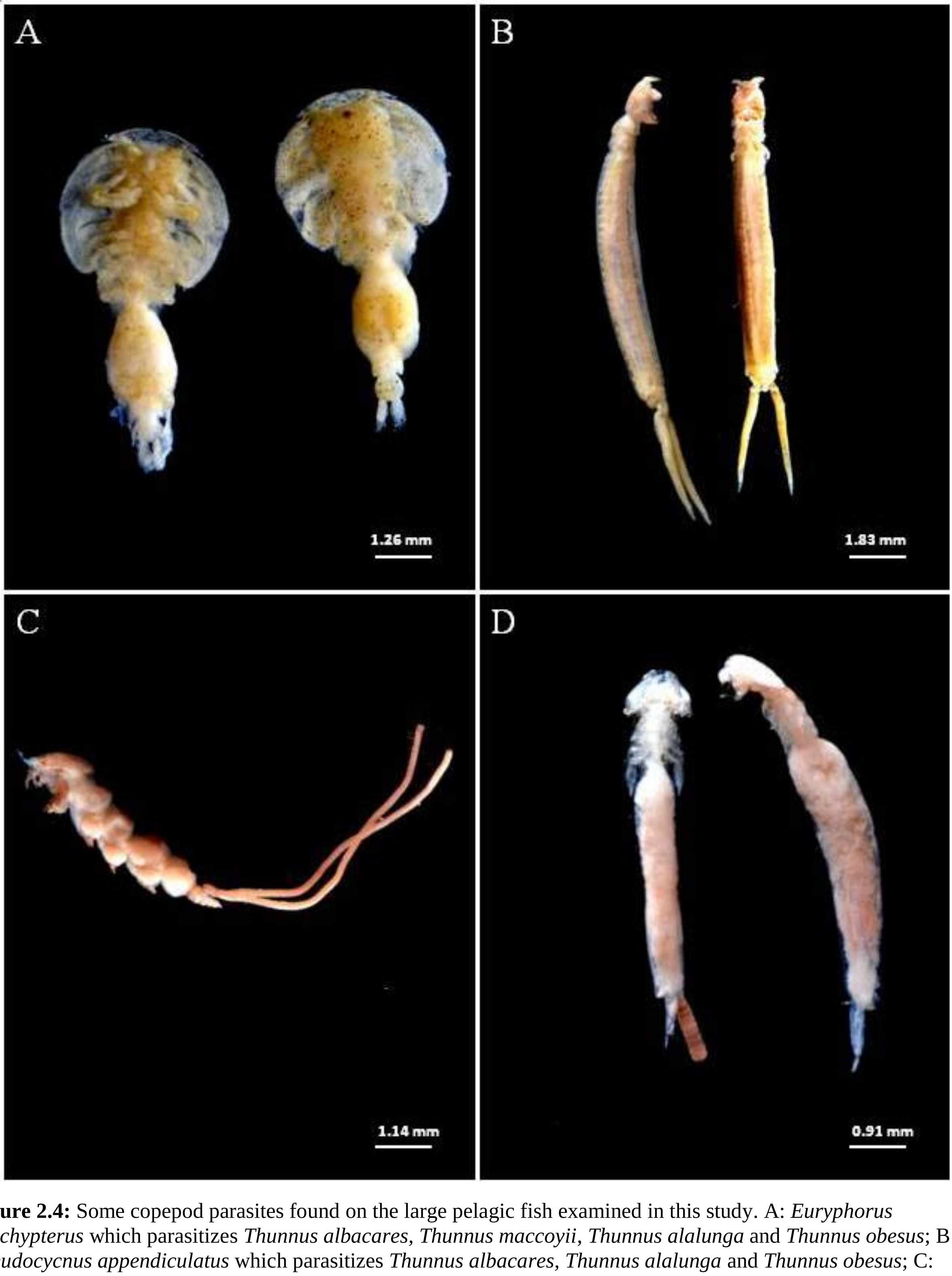


Figure 2.4: Some copepod parasites found on the large pelagic fish examined in this study. A: *Euryphorus brachypterus* which parasitizes *Thunnus albacares, Thunnus maccoyii, Thunnus alalunga* and *Thunnus obesus*; B: *Pseudocycnus appendiculatus* which parasitizes *Thunnus albacares, Thunnus alalunga* and *Thunnus obesus*; C: *Nemesis lamna lamna* which parasitizes *Isurus oxyrinchus*; D: *Kroyeria carchariaeglauci* which parasitizes *Prionace glauca* (images courtesy of E/Prof. Charles Griffiths, UCT).

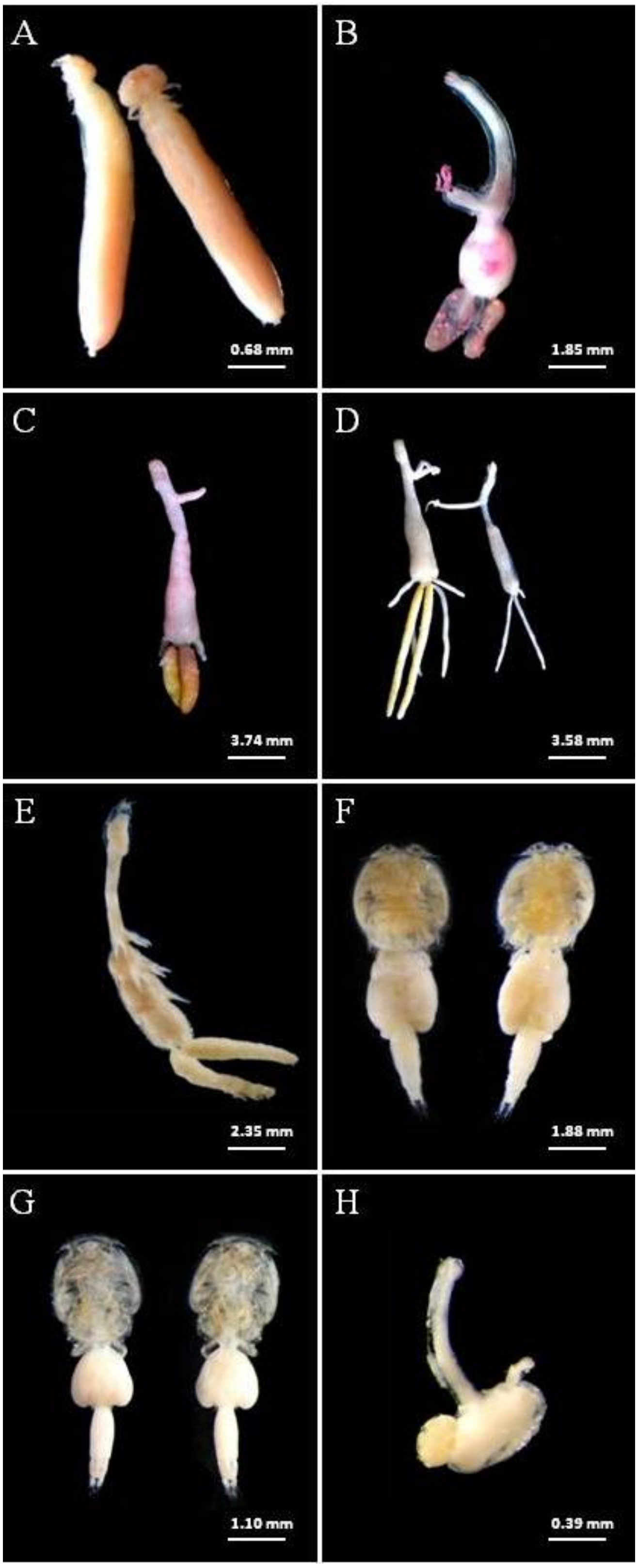


Figure 2.5: Some copepod parasites found on the middle-sized pelagic, demersal and small pelagic fish examined in this study. A: *Hatschekia conifera* which parasitizes *Brama brama* and *Thyrsites atun*; B: *Parabrachiella* sp. 1 which parasitizes *Seriola lalandi*; C: *Parabrachiella* sp. 2 which parasitizes *Seriola lalandi*; D: *Eobrachiella elegans* which parasitizes *Seriola lalandi*; E: *Chondracanthus colligens* which parasitizes *Genypterus capensis*; F: *Caligus bonito* which parasitizes *Sarda sarda*; G: *Caligus dakari* which parasitizes *Thyrsites atun* and *Trachurus capensis*; H: *Clavellisa ilishae* which parasitizes *Sardinops sagax* (images A-D, courtesy of E/Prof. Charles Griffiths, UCT; images E-H taken by author).

2.4- Discussion

A wide range of fish species were examined in the present study to provide further insight into the diversity of copepod and monogenean parasites 17 species of South African marine fishes.

Thirty-two parasite taxa (20 copepods and 12 monogeneans) were found to infect the head region (including the nares, buccal cavity, operculae and gills) or heart (in the case of *Lampanyctodes hectoris*) of the 17 fish host species examined. Two of these were new host records with the remaining species having previously been reported on their respective hosts. Fifteen new geographic records were also documented, illustrating that marine fish parasites are understudied within the South African marine environment. Some groups are better studied than others, and thanks to taxonomists such as Professor Susan Dippenaar and her collaborators at the University of Limpopo in South Africa, the marine siphonostomatoid copepods in particular have been well documented with multiple papers on taxonomy and host records as well as a number of checklists published (*e.g.* Dippenaar *et al.,* 2000; Dippenaar, 2004; Dippenaar and Jordaan, 2006). With regard to the study of monogenean parasites of fishes within South Africa there has been a fair amount of research conducted on freshwater systems (*e.g.* Crafford and Avenent-Oldewage, 2010; Madanire-Moyo *et al.,* 2011; Olivier *et al.,* 2009; Smit *et al.,* 2017), but very little on monogeneans parasitizing marine species with Kevin Christison (Department of Agriculture, Forestry and Fisheries) being one of the only experts in the field (Griffiths *et al.,* 2010).

In most cases, each host species showed relatively high prevalence and infection intensities of one or two parasite species (Table 2.4). However, although these parasites had relatively high prevalence amongst the host populations, the infection intensity varied from high to low between individual hosts. This likely reflects the natural aggregated distribution of parasites where certain parasites tend to occur in large numbers on some individuals and low numbers on other individuals, often termed a clumped distribution (Leung, 1998; Molles, 2008). There are a number of factors that cause aggregated distributions. Host age plays an important role, as hosts may accumulate parasites over their lifetimes, resulting in older individuals having a higher parasite abundance compared to younger individuals (Zelmer and Arai, 1988). Previously infected hosts may also have an increased susceptibility to further parasite infection and accumulation, as was shown in a study by Poulin and FitzGerald (1989) who investigated the aggregated distribution of *Argulus canadensis* on threespine sticklebacks, *Gasterosteus aculeatus*, and found that parasites infected previously-infected fish more often than previously-uninfected fish. Despite showing high overall prevalence of infection, the aggregated distribution found during this present study skewed the overall infection intensity and overall mean parasite abundance results across all host species, as a number of host individuals showed particularly high individual parasite abundances.

Within the group of large pelagic fish, the four *Thunnus* species examined showed very similar copepod parasite assemblages. *Euryphorus brachypterus* was found on all four examined species (*T. albacares, T. maccoyii, T. alalunga,* and *T. obesus*), which is expected as the parasite species is commonly found parasitizing large tunas (Williams and Bunkley-Williams, 1996). The observations of *E. brachypterus* infecting *T. alalunga* and *T. obesus* provide two new geographic records, extending the distribution of this parasite to South Africa. *E. brachypterus* has previously been reported on *T. alalunga* elsewhere including New Zealand and Puerto Rico, as well as infecting *T. obesus* within the Atlantic Ocean (Hewitt and Hine, 1972; Williams and Bunkley-Willliams, 1996). *Pseudocycnus appendiculatus* was recorded on *T. albacares, T. alalunga* and *T. obesus* but was not found parasitizing the gills of *T. maccoyii*. The observations of *P. appendiculatus* infecting *T. albacares, T. alalunga* and *T. obesus* provide three new geographic records for South Africa. *P. appendiculatus* has previously been reported infecting *T. albacares* in other regions including Puerto Rico, Angola, from islands within the Indian Ocean, Somalia, Australia, Philippines, Indo-Pacific, Peru, Costa Rica, the Gulf of Guinea, and within the Benguela itself but the exact locality was not specified (Cressey and Cressey, 1980; Williams and Bunkley-Williams, 1996; Dippenaar, 2004). *P. appendiculatus* has also been reported on *T. alalunga* in the Mediterranean Sea, New Zealand, India and Puerto Rico (Hewitt and Hine, 1972; Pillai, 1985; Williams and Bunkley-Williams, 1996, Mele *et al.,* 2010), as well on *T. obesus* off of Namibia and Angola, India, the Puerto Rico, Juan Fernandez Islands in the South-West Pacific Ocean, and in the North Atlantic Ocean (Cressey and Cressey, 1980; Pillai, 1985; Williams and Williams-Bunkley, 1996; Dippenaar, 2004). *P. appendiculatus* has not yet been recorded parasitizing *T. maccoyii* in South Africa however it has previously been reported parasitizing *T. maccoyii* in Australia, with relatively high prevalence of 44.8% (Hayward *et al.,* 2007). The record of *Nasicola klawei* parasitizing the nares of *T. albacares* and *T. obesus* also provides two new geographic records for South Africa. *N. klawei* has previously been reported infecting *T. albacares* elsewhere including multiple regions of the Atlantic Ocean, off the coastline of Brazil, in the Gulf of Mexico and on the eastern coastlines of Australia, Peru, Puerto Rico and Hawaii (Hendrix, 1994; Williams and Williams-Bunkley, 1996; Kohn *et al.,* 2004; Chisholm and Whittington, 2007; Bullard *et al.,* 2011; Justo and Kohn, 2015) as well as infecting *T. obesus* within the Pacific and Atlantic Oceans (Williams and Williams-Bunkley, 1996). *Hexostoma* sp. recovered from *T. albacares* and *T. obesus*, although at a generic level, also provides two new geographic records with no other records of the genus having previously been reported from South Africa. Only two copepod parasite species were recorded from the two elasmobranch species examined; *Nemesis lamna lamna*, parasitizing the gills of *Isurus oxyrinchus,* and *Kroyeria carchariaeglauci,* parasitizing the gills of *Prionace glauca.* Both species showed 100% prevalence on their respective hosts and very high mean infection intensities, which seem to be a common attribute of these two parasite species as other studies have observed similar results (Benz and Dupre, 1987; Dippenaar *et al.,* 2000). Benz and Dupre (1987) investigated the distribution of *K. carchariaeglauci* on the gills of *P. glauca* and documented an infection intensity of 116 to 1250 parasites per individual shark. Dippenaar *et al.* (2008) studied the spatial distribution of *N. lamna lamna* on the white shark, *Carcharodon carcharias*, and found the parasite abundance ranged from four to 212 individuals per host. However, it must be noted that only two individuals of each host species were examined in the present study and therefore the prevalence and abundance of each parasite on its associated host may vary with increased sample sizes. *Tristoma adcoccineum* had the highest prevalence on *Xiphias gladius* and provides a new geographic record for South Africa, having previously been reported infecting *X. gladius* within the Pacific Ocean (Williams and Bunkley-Williams, 1996, Smith *et al.,* 2004).

Within the group of middle-sized pelagics, the record of *Mazocraes* sp. parasitizing the gills of *Thyrsites atun* and occurring on 20% of fish examined is a new host record. The only representative of the genus reported infecting fish within South African waters is *M. sardinopsi* recorded on the gills of *Sardinops sagax* (Reed *et al.,* 2012). *Hatschekia conifera* and *Caligus dakari* were also highly prevalent on *Thyrsites atun*. The results obtained in the present study are similar to those presented by Nunkoo *et al.* (2016) who examined the parasite assemblage of *T. atun* and recorded prevalences of *H. conifera* (54.6%) and *C. dakari* (37.9%) from individuals caught off the west coast of South Africa. *H. conifera* was also highly prevalent on the gills of *Brama brama* and occurred on all the individuals examined. Barnard (1948) originally described the parasite species from *B. brama* off the South African West coast as *H. acuta,* but gave a very brief description of the specimen (Oldewage, 1993a). Kabata (1981) recovered specimens of *H. conifera* from the gills of *Brama japonicus* and after further comparison of both *H. conifera* and *H. acuta,* identified them to be conspecific and has since transferred *H. acuta* to *H. conifera.* The prevalence of *H. conifera* from *B. brama* in the present study was in contrast to that of Paula *et al.* (2012) who documented multiple parasitic copepods from a range of fish species in the Argentinean Sea and found that of the four *B. brama* they examined, the prevalence of *H.*

conifera was only 50%, but their results may be a consequence of a very small sample size. Oliva *et al.* (2016) documented the parasite assemblage of *B. australis* off of the Chilean coastline and found a similar prevalence of *H. conifera* to that obtained in the present study but with a lower mean infection intensity.

Eobrachiella elegans was one of five copepod parasites found infecting *Seriola lalandi* and provides the first geographic record of this parasite for South Africa. *E. elegans* has previously been recorded parasitizing *S. lalandi* in Japan (Johnson *et al.,* 2004) as well as parasitizing *S. dumerili* off of Tunisia (Benmansour and Hassine, 1998). Although there have been multiple records of species within the genus *Parabrachiella* infecting other fish within South African waters (*e.g.* Oldewage, 1993b, Barnard, 1955b, Barnard, 1955a, Botha, 1986, Oldewage, 1992, Wilson, 1923), there have been no records of the genus infecting *S. lalandi* in South Africa. Therefore, although the specimens were only identified to a generic level, the two *Parabrachiella* species found infecting *S. lalandi* in the present study provide two new locality records.

Caligus bonito was the only copepod parasite recorded from *Sarda sarda* and was found infecting 75% of individual hosts examined. *C. bonito* has been known to infect a wide range of pelagic fish including *Thunnus thynnus, Auxis rochei* and *Coryphaena hippurus* (Williams and Bunkley-Williams, 1996). The prevalence of *C. bonito* parasitizing *S. sarda* in the present study was higher than that obtained by Alves and Luque (2006) from *S. sarda* caught in Brazilian waters, which only showed a prevalence of 30%. The result obtained in the present study may however be biased as only 16 specimens of *S. sarda* were examined compared to Alves and Luque (2006) who sampled 30 in total.

Within the group of demersal species examined, *Genypterus capensis* was found to be parasitized by only one copepod, *Chondracanthus colligens*. Sibanda (2016) recorded a

Chondracanthus species parasitizing *G. capensis* off South Africa with a slightly lower prevalence than observed in the present study. Although *Chondracanthus colligens* is the only known Chondracanthid to parasitize *G. capensis* (Kensley and Grindley, 1973), Sibanda (2016) did not identify the parasite to species level and the specimen could not be located; therefore a direct comparison could not be made.

Both South African hake species, *Merluccius capensis* and *M. paradoxus*, were parasitized by the monogenean *Anthocotyle merlucci* with a higher prevalence on *M. capensis* compared to *M. paradoxus*. *A. merlucci* has a cosmopolitan distribution and has been recorded parasitizing the gills of multiple species within the genus *Merluccius,* including *M. merluccius* and *M. gayi gayi* (Oliva and Ballón, 2002; Oguz and Bray, 2008; Akmirza, 2013). The observations of *A. merlucci* parasitizing *M. capensis* and *M. paradoxus* provides two new geographic records for South Africa with *A. merlucci* having previously been recorded parasitizing *M. capensis* and *M. paradoxus* off Namibia (Reimer, 1993).

Amongst the small pelagics, *Cardiodectes medusaeus* was found infecting 19.1% of *Lampanyctodes hectoris* individuals examined. This copepod species is globally distributed and is commonly found parasitizing multiple species of myctophid lanternfish by pushing its trunk and head regions into the heart of the fish (Perkins, 1983; Sakuma *et al.*, 1999; Catul *et al.*, 2011).

Clavellisa ilishae and *Nothobomolochus fradei* were the only two parasites found infecting the gills of *Sardinops sagax* and occurred at very low prevalences and infection intensities. These very low prevalences and infection intensities were also found by Reed *et al.* (2012), who studied the parasite assemblage of *S. sagax* in South African waters and found that both parasites occurred at a prevalence of 10% and an infection intensity of 1.2 parasites per infected fish.

Caligus dakari and *Gastrocotyle trachuri* were the only two parasite species found infecting the gills of *Trachurus capensis*. *C. dakari* recovered from *T. capensis* provides a new host record for the parasite species which, within a South African context, has previously been reported parasitizing *Thyrsites atun* and *Pomatomus saltatrix* from South Africa (Barnard, 1955a; Nunkoo *et al.*, 2016). Barnard (1955a) recorded the species as *C. mauritanicus* parasitizing *P. saltator*, but the parasite species has since been synonymized with *C. dakari* (Boxshall and El-Rashidy, 2009) and *P. saltator* was a misspelling of *P. saltatrix*. *G. trachuri* was found infecting 72.9 % of host individuals whereas only two *C. dakari* individuals were found on the 196 host individuals examined. The prevalence of *Gastrocotyle trachuri* found infecting *T. capensis* in the present study was higher than that (56.4%) recorded by Le Roux (2013) who investigated the parasite assemblage of *Trachurus capensis* in South African waters. This may be as a result of a smaller sample size (n=125) compared to the current study (n=192).

Conclusion

The present study is one of the first studies from South Africa to document the gill parasite assemblages of multiple hosts over multiple trophic levels. The marine ecosystem surrounding South Africa is hugely diverse but, as indicated by Griffiths *et al.* (2010), Smit and Hadfield (2015) and Dippenaar (2016), the field of parasitology within South Africa is still in its infancy compared to other, more advanced countries. This is also shown in the present study where of the 32 parasite taxa recorded, two were new host records and 15 were new geographic records. In addition, and as there have been no past records of monogenean species parasitizing the *Thunnus* species, *Xiphias gladius* and *Brama brama*, even the unidentified monogeneans in the present study could potentially provide new geographic records. There is therefore huge potential for further marine parasitological studies in South Africa including documenting the parasite assemblage of further marine fish species, taxonomy, host-parasite interactions, bio-tagging and the use of parasites as bio-indicators.

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Chapter 3: Investigating Fish Host-Parasite Interactions using Stable Isotopes.

3.1- Introduction

3.1.1- Stable Isotope Analysis

The analysis of stable isotopes as an affordable tool to explore trophic interactions and how nutrients and energy are cycled and recycled through food-webs has become increasingly popular in recent years (Michener and Schell, 1994; Post, 2002; Fry, 2006; Boecklen *et al.*, 2011). More specifically, the elements of nitrogen and carbon and their respective isotopes have aided ecologists to further understand the source production of food-webs, trophic positioning of organisms, and to gain further insight into community interactions and community structure (DeNiro and Epstein, 1978; Minagawa and Wada, 1984).

The ratios of heavy to light isotopes of nitrogen (^{15}N and ^{14}N) and carbon (^{13}C and ^{12}C) within an organism change in a predictable manner as one moves up trophic levels in a food-web (Peterson and Fry, 1987), with predators being typically enriched in the heavier isotope of these elements compared to their prey in typical predator-prey interactions (Pinnegar *et al.*, 2001). This enrichment of the heavier isotope occurs as a result of the lighter isotope being more readily utilized during metabolic activity and hence excreted, resulting in the heavier isotope being sequestered within the organism's tissues (Fry, 2006). Therefore, as one moves up trophic levels, the ratio of heavy to light isotopes, expressed as $\delta^{15}N$ for nitrogen and $\delta^{13}C$ for carbon, generally increases, a process known as trophic fractionation (Post, 2002; Fry, 2006). Trophic fractionation of nitrogen typically ranges from -0.7 to 9.2‰ (Vander Zanden and Rasmussen, 2001), depending on the ecosystem and the organisms involved (herbivores or carnivores), with an overall mean value of 3.4 ± 0.1‰ (Minagawa and Wada, 1984, Post, 2002). Trophic fractionation of carbon typically ranges from -3 to +4‰ with an overall mean value of 0.4 ±

1.3‰ (Post, 2002). Stable isotope ratios of nitrogen are utilized for trophic level positioning whereas those of carbon are used to identify source production (DeNiro and Epstein, 1978; Minagawa and Wada, 1984; Hussey *et al.,* 2014).

The majority of the nitrogen found within an organism's tissues is sourced directly from protein within their diets (Minagawa and Wada, 1984). Carbon, however, is sourced from a variety of different macromolecules such as carbohydrates, proteins and lipids, and the ratios of ^{13}C to ^{12}C of these macromolecules affect the overall δ^{13}C of the organism's tissues (DeNiro and Epstein, 1978). Lipids are generally significantly depleted in ^{13}C compared to the other macromolecules by approximately 6-8‰, and thus a high lipid content in a sample can skew the δ^{13}C signature (Post *et al.,* 2007). By dividing the total percentage of carbon by the total percentage of nitrogen of a sample one can derive the C:N ratio, which provides an indication of the amount of lipid present within the sample (Sweeting *et al.,* 2006). Pure protein has a C:N ratio of approximately 2.9 (McConnaughey and McRoy, 1979) and nitrogen is absent from lipids, thus any deviation in the C:N ratio from 2.9 indicates that lipids are present (Sweeting *et al.,* 2006). It is generally not necessary to account for lipids in samples that have a low lipid content, however any sample that has a C:N greater than 3.5 indicates high lipid content and these lipids need to be accounted for in order to obtain an accurate δ^{13}C signature (Post *et al.,* 2007). This can be achieved by either manually extracting the lipids from the sample or through mathematical corrections. Manual extraction methods involve utilizing solvents such as chloroform and methanol to dissolve and separate out the lipids, which can then be removed and the sample analysed (Folch *et al.,* 1957; Bligh and Dyer, 1959; de Lecea and de Charmoy, 2015). These methods however, are laborious and can be costly to perform, and lipid extraction may affect the δ^{15}N values (Sweeting *et al.,* 2006). As a result, in order to obtain accurate measures of both the δ^{13}C and δ^{15}N of a lipid-rich sample, analyses need to be conducted on both treated and untreated samples, respectively (Sweeting *et al.,* 2006). An alternate solution to account for lipids is to correct the δ^{13}C of lipid-rich samples mathematically and a number of equations have been proposed to achieve this (*e.g.* McConnaughey and McRoy, 1979; Kiljunen *et al.,* 2006; Sweeting *et al.,* 2006; Post *et al.,* 2007). However, many of these have been criticized due to multiple assumptions having to be made across species (de Lecea and de Charmoy, 2015).

3.1.2- Host-Parasite Interactions and Stable Isotope Analysis

Although being widely applied to understand trophic relations between free-living species, the use of stable isotope analyses to further our understanding of host-parasite interactions is relatively limited and studies that have attempted to investigate these interactions using stable isotopes have shown conflicting results. Many of these studies have found differing fractionation patterns at a host-parasite species level, with only a small number of these interactions showing the typical enrichment pattern for $\delta^{15}N$ (Boag, *et al.,* 1998; Doucett *et al.,* 1999; Iken *et al.,* 2001; Neilson *et al.,* 2005; Demopoulos and Sikkel, 2015), and the majority of interactions showing either no significant difference between $\delta^{15}N$ of the parasite compared to that of its host or showing a depletion pattern (Boag, *et al.,* 1998; Iken *et al.,* 2001; Pinnegar *et al.,* 2001; Deudero *et al.,* 2002; Doi *et al.,* 2010; Demopoulos and Sikkel, 2015).

Demopoulos and Sikkel (2015) investigated host-parasite trophic linkages in three common reef fish, the longfin damselfish (*Stegastus diencaeus*), the French grunt (*Haemulon flavolineatum*) and the squirrelfish (*Holocentrus adscenscionis*), and their associated parasites. These parasites were the gnathiid isopod *Gnathia marleyi*, including its multiple developmental stages, which parasitizes all three fish species, as well as two cymothoid isopods; *Anilocra haemuli,* that parasitizes *H. flavolineatum,* and *Anilocra holocentric* that parasitizes *H. adscenscionis.* All three species of parasite attach and infect the exterior surfaces of their fish hosts. When comparing the $\delta^{15}N$ of the hosts and their associated parasites, the only two cases of significant enrichment were between the 3rd praniza larval stage of the gnathiid and the blood of *H.*

flavolinaetum, and between *A. haemuli* and both the muscle tissue and blood of *H. flavolinaetum* (Demopoulos and Sikkel, 2015). The other interactions between *S. diencaeus* and *H. adscenscionis* and their associated parasites showed neither significant enrichment nor depletion in δ^{15}N (Demopoulos and Sikkel, 2015). The absence of significant enrichment in δ^{15}N of many of the parasites may also be as a result of the infection of the host leading to a reduction in host condition and an increase in energetic demands which can result in an enrichment of the host tissues (Welicky *et al.*, 2017). This parasite induced enrichment of the host tissue could then potentially obscure any enrichment pattern observed between parasite and host.

Similarly, a study by Pinnegar *et al.* (2001) investigated the trophic linkages between four common fish species, the sea bream (*Boops boops),* the whiting (*Merlangius merlangus*), the flounder (*Platichthys flesus*) and the stickleback (*Gasterosteus aculeatus*), and their individual associated parasites (nematoda, cestoda, copepoda and isopoda). It was found that the ectoparasites (copepoda and isopoda) showed no significant difference in δ^{15}N compared to their respective hosts' muscle tissue and that the endoparasites showed significant depletion in δ^{15}N compared to their respective hosts' muscle tissues (Pinnegar *et al.*, 2001). This depletion pattern was also observed in a study by Deudero *et al.* (2002), which investigated host-parasite interactions covering a broad range of fish host species and their associated parasites. Of the 10 host species examined and 29 host-parasite interactions analysed using stable isotopes, only three showed an enrichment of ^{15}N of the parasite compared to the host muscle tissue and of those three, one was not statistically significant and the others had too small sample size to be statistically confirmed (Deudero *et al.*, 2002). Of the remaining 26 interactions, seven showed significant depletion and the remainder were not significantly different, mainly due to small sample sizes, but showed a general depletion trend.

These studies have suggested a number of factors that have attributed to these confounding results. Firstly, some parasites are highly selective in their habitat choice and on the tissue on which they feed (Boag *et al.,* 1998; Iken *et al.,* 2001), with some parasites feeding directly on host tissue whereas others feed on digested material on which the host has previously fed (Lafferty *et al.,* 2008). This is further complicated by the fact that some parasites selectively absorb specific compounds such as specific amino acids from their food sources (Lafferty *et al.,* 2008), and these compounds can have varying isotopic ratios (Wolf *et al.,* 2009). One such example of this high selectivity commonly displayed by parasites is a study by Iken *et al.* (2001) which investigated the isotopic composition of the deep-sea benthic community, including some fish host-parasite interactions, in the Porcupine Abyssal Plain, North-East Atlantic. Those authors found that when comparing the $\delta^{15}N$ of trematode and nematode parasites to the $\delta^{15}N$ of their hosts, the grenadiers *Chalinura profundicola* and *C. leptolepis*, the trematodes showed no significant difference in $\delta^{15}N$ whilst the nematodes showed a significant depletion in ^{15}N (Iken *et al.,* 2001). This difference was attributed to the locations of the digestive tract inhabited by the two parasite taxa (Iken *et al.,* 2001). The trematodes inhabit the host intestinal tract and thus assimilate nutrients from food that has already been digested and processed by the host, hence because these nutrients are similar to those that the host assimilates both parasite and host show similar $\delta^{15}N$ values (Iken *et al.,* 2001). The nematodes, however, inhabit the stomachs of their respective hosts and thus feed off unprocessed food therefore the depletion pattern observed is as a result of the parasite selectively feeding on certain macromolecules or nutrients in the available food, which may differ to those assimilated by the host (Iken *et al.,* 2001). A similar result was obtained by Boag *et al.* (1998) who investigated the trophic relationships between the wild rabbit *Oryctolagus cuniculus* and its associated parasites. They found that the nematodes *Graphidium strigosum* and *Passalurus ambiguus*, as well as the flea *Spilopsyllus cuniculi*, were enriched in ^{15}N compared to their hosts, indicating that the organisms were feeding directly off the host tissue (Boag *et al.,* 1998). The cestodes *Cittataenia denticulata* and *Mosgovoyia pectinata*, however, showed depletion in ^{15}N compared to the host and the authors suggested that this may be due to the cestodes feeding off of significantly depleted fractions of the available food sources (Boag *et al.*, 1998).

These studies have indicated that there are confounding results regarding the enrichment or depletion of parasites compared to their hosts and that this is partly due to the high feeding selectivity of many parasites. Consequently, ecologists are still unsure of the trophic relationship exhibited between parasites and their hosts. Hence, the main objective of this study is to further investigate trophic interactions between parasites and their hosts in the marine ecosystem to attempt to bring some clarity to these perplexing results.

3.1.3- Aims and Hypotheses

The aim of this chapter is to use stable isotope analysis to investigate the trophic interactions between parasites and their hosts in the marine environment. This is achieved by analyzing gill copepod and monogenean parasites found parasitizing a broad trophic range of South African marine fish host species ranging from zooplanktivorous, small pelagic fish to piscivorous, apex fish predators. As the trophic positioning of the parasite compared to its host is of primary interest here, the major focus will be on the change of the $\delta^{15}N$ signature of the parasite compared to the host as this will provide insight as to whether or not the parasites are appearing at a higher trophic level than their associated hosts.

The specific research aims are shown below in bold, followed by their associated hypotheses:

a. **To assess whether the host gill tissue on which the parasite feeds differs in its isotopic signature ($\delta^{13}C$ and $\delta^{15}N$) compared to host muscle tissue, typically sampled in stable isotope studies, in order to select the most appropriate host tissue proxy with which to compare the parasite isotope ratios**:

H_0= There is no significant difference between the isotopic signatures of host gill and muscle tissues.

H_A= There is a significant difference between the isotopic signatures of host gill and muscle tissues.

Should they differ then host gill tissue will serve as a better proxy compared to host muscle tissue as this is what the parasites actually are feeding on.

b. To assess if gill parasites in general are enriched (in both ^{13}C and ^{15}N) compared to their hosts:

H_0= There are no significant differences between the isotopic signatures of the parasites and their associated host tissue proxy.

H_A= There is a significant difference between the isotopic signatures of the parasites and their associated host tissue proxy.

c. To assess if copepod and monogenean gill parasites show different levels of trophic fractionation (for both $\delta^{13}C$ and $\delta^{15}N$) compared to their associated hosts:

H_0= The trophic fractionation is not significantly different between copepod gill parasites and monogenean gill parasites.

H_A= The trophic fractionation is significantly different between copepod gill parasites and monogenean gill parasites.

d. To assess if trophic fractionation (for both $\delta^{13}C$ and $\delta^{15}N$) of monogenean gill parasites is affected by their feeding mode and different food sources:

H_0= The trophic fractionation of monopisthocotyleans and polyopisthocotyleans is not significantly different.

H_A= The trophic fractionation of monopisthocotyleans and polyopisthocotyleans is significantly different.

e. **To assess whether individual parasite taxa or species are enriched (in both ^{13}C and ^{15}N) compared to their hosts:**

$H_{0=}$ There is no significant differences in isotopic signatures between individual parasite species and their associated hosts.

H_A= There is a significant difference in isotopic signatures between individual parasite species and their associated hosts

3.2-Methods

3.2.1-Stable Isotope Sampling Protocol

In order to maximise the number of host-parasite observations and to cover the widest range of host-parasite interactions possible, individual fish samples were chosen by selecting fish with the highest abundance and diversity of parasites as indicated in Chapter 2 (Table 3.1). Stable isotope data were always collected from individual fish and from individual parasites when these were sufficiently large to provide a sufficient mass (0.2-0.5mg dry mass) for the mass spectrometer to combust; when parasites were too small to provide sufficient material, stable isotope data were collected from grouped samples (Table 3.1). On occasion, more than one individual parasite from a single host was analysed. Host gill tissue, host dorsal white muscle tissue and whole individual (or grouped) parasite samples were initially dried at 60°C for 24 hours and then individually ground and homogenised with a mortar and pestle. In the case of *Cardiodectes medusaeus* found infecting the heart *Lampanyctodes hectoris*, host heart tissue was sampled, dried and homogenized. Samples were then weighed into tin cups on a Sartorius M2P (Sartorius AG, Goettingen, Germany) microbalance to an accuracy of 1 microgram and then crushed to contain the sample within the cups. The samples were weighed to fall within the range of 0.4mg - 0.5mg, however the minimum sample mass from which the mass spectrometer could make an accurate measurement is 0.2mg (J. Sealy, UCT, pers. comm.), and all samples were above this lower threshold of the mass spectrometer's capabilities. After weighing, samples were then combusted in a Flash 2000 (Thermo Scientific, Bremen, Germany) organic elemental analyser and the gases directed to a Delta V Plus isotope ratio mass spectrometer via a Conflo IV (Thermo Scientific, Bremen, Germany) gas control unit. In-house standards used for calibrations were valine, Merck gel and seal bone. The valine is DL-Valine produced by SIGMA which has a $\delta^{15}N$ of 12.140‰ ± 0.149 and a $\delta^{13}C$ of -26.80 ‰ ± 0.139; Merck gel is a proteinaceous gel produced by Merck with a $\delta^{15}N$ of 7.50 ‰ ± 0.155 and a $\delta^{13}C$ of -20.05‰ ± 0.139; and seal bone is crushed bone which was demineralized and dissolved in acid and then reconstituted into a gel standard and has a $\delta^{15}N$ of 15.84‰ ± 0.127 and a $\delta^{13}C$ of -11.97‰ ± 0.101. All of these in-house standards have been calibrated with the International Atomic Agency standards, with nitrogen being expressed in terms of its value compared to atmospheric nitrogen, and carbon expressed in terms of its value compared to Pee-Dee Belemnite. The standardized ratios were then expressed as "δ" in the units "per mille" (‰) using equation 3.1, where δX is the standardized ratio, R_{Sample} is the uncorrected heavy to light isotope ratio of the sample and $R_{Standard}$ is the heavy to light isotope ratio of the standard.

(3.1)

Table 3.1: Fish host and gill parasites species analysed for δ^{13C} and $\delta^{15}N$. The values in parentheses indicate the number of samples analysed for each host and parasite species; C indicates copepod and M monogenean gill parasites; and (*x* x *y*)* indicates samples where parasites were grouped provide sufficient material for analysis with *x* showing the number of pooled samples analysed and *y* indicates the number of individual parasites pooled per sample. *Cardiodectes medusaeus* (grey) found infecting the heart of *Lampanyctodes hectoris,* is also included for later comparison.

Host Species (and common name)	Host Tissue	Parasite Taxa
Large Pelagics		
Thunnus albacares (Yellowfin tuna)	Muscle (4) Gill (5)	*Euryphorus brachypterus* (8) ^{C} *Pseudocycnus appendiculatus* (6) ^{C} Monogenean sp. 1 (1) ^{M}
Thunnus maccoyii (Southern bluefin tuna)	Muscle (2) Gill (2)	*Euryphorus brachypterus* (6) ^{C}
Thunnus alalunga (Albacore tuna)	Muscle (4) Gill (5)	*Euryphorus brachypterus* (10) ^{C} *Pseudocycnus appendiculatus* (11) ^{C}
Thunnus obesus (Bigeye tuna)	Muscle (8) Gill (8)	*Euryphorus brachypterus* (23) ^{C} *Hexostoma* sp. (15) ^{M} *Pseudocycnus appendiculatus* (3) ^{C}
Isurus oxyrinchus (Shortfin mako shark)	Muscle (2) Gill (2)	*Nemesis lamna lamna* (7) ^{C}
Prionace glauca (Blue shark)	Muscle (2) Gill (2)	*Kroyeria carchariaeglauci* (4x50)^{*C}
Xiphias gladius (Swordfish)	Muscle (2) Gill (2)	*Tristoma adcoccineum* (4) ^{M}

Table 3.1: (Continued)

Host Species (and common name)	Host Tissue	Parasite Taxa
Middle-Sized Pelagics		
Brama brama	Muscle (3)	*Hatschekia conifera* (6x50)^{*C}
(Atlantic pomfret)	Gill (3)	
Thyrsites atun	Muscle (5)	*Caligus dakari* (5) ^{C}
(Snoek)	Gill (5)	*Mazocraes* sp. (1x4)^{*C}
		Hatschekia conifera (2x20)^{*C}
Seriola lalandi	Muscle (5)	*Parabrachiella* sp. 1 (8) ^{C}
(Yellowtail amberjack)	Gill (4)	
Sarda sarda	Muscle (5)	*Caligus bonito* (14) ^{C}
(Atlantic bonito)	Gill (5)	
Demersal		
Genypterus capensis	Muscle (1)	*Chondracanthus colligens* (1) ^{C}
(Kingklip)	Gill (1)	
Merluccius capensis	Muscle (2)	*Anthocotyle merlucci* (2x3)^{*M}
(Shallow-water hake)	Gill (2)	
Small Pelagics		
Sardinops sagax	Muscle (2)	*Clavellisa ilishae* (1x10)^{*C}
(Sardine)	Gill (1)	
Trachurus capensis	Muscle (5)	*Gastrocotyle trachuri* (5x47)^{*M}
(Cape horse mackerel)	Gill (5)	
Lampanyctodes hectoris	Muscle (5)	*Cardiodectes medusaeus* (11)
Hector's lanternfish	Heart (5)	

3.2.2- Carbon Lipid Correction

To account for high lipid content in the tissues which affects the δ^{13}C values, samples which had a C:N ratio greater than 3.5 were corrected using the equation (Equation 3.2) for aquatic animals as proposed by Post *et al.* (2007).

(3.2)

In the cases whereby the correction was applied, the corrected values were used in further analyses whilst in the cases whereby the C:N was less than 3.5, the uncorrected values were used in further analyses. For simplicity and as the majority of samples required lipid correction, δ'^{13}C will be used from here onward to denote the $^{13}C/^{12}C$ isotopic ratios of both corrected and uncorrected samples.

3.2.3- Data Analysis

Data were checked for normality prior to each statistical test utilizing histograms and the Kolmogorov-Smirnov and Lilliefors tests. If the all variables within each analysis met the assumptions for normality then parametric tests were used, if not then non-parametric tests were employed. All statistics were analysed using the Statistica data analysis software package (Dell Inc. (2016) version 13. software.dell.com).

Because the main aim of this study was to assess whether or not gill parasites show a similar enrichment pattern as is typically observed in predator-prey studies, nitrogen isotope ratios were the primary focus of the present study as this provides an indication of an organism's trophic level (Minagawa and Wada, 1984). *Cardiodectes medusaeus* found infecting the heart of *Lampanyctodes hectoris* is not a gill parasite and thus neither the parasites nor the hosts were included in further analyses but will be referred to later.

Do host gill and muscle tissues have significantly different isotopic signatures?

The $\delta'^{13}C$ and $\delta^{15}N$ values of host white muscle tissue and host gill tissue for all samples combined were compared using a Wilcoxon matched pairs non-parametric test with significance set at $p<0.05$ to distinguish if there was a significant difference between their isotopic signatures. The relationship between $\delta^{15}N$ values of host gill and muscle tissue was further examined using regression analysis on individual data.

Are gill parasites enriched in general compared to their hosts?

In order to assess if gill parasites in general are enriched compared to their associated hosts, $\delta'^{13}C$ and $\delta^{15}N$ values of all parasites combined, regardless of type or species, were compared to the $\delta'^{13}C$ and $\delta^{15}N$ values of the selected tissue proxy of their associated hosts in a pair-wise comparison utilizing Wilcoxon matched pairs non-parametric tests with significance set at $p<0.05$.

Is trophic fractionation between parasites and their hosts different for copepod and monogenean gill parasites?

In order to investigate whether or not the trophic fractionation between parasites and their hosts is affected by the type of parasite (copepod or monogenean), the changes in isotopic signature for each parasite were calculated using Equation 3.3 for carbon and Equation 3.4 for nitrogen:

(3.3)

(3.4)

Parasites were then grouped according to their type (copepod or monogenean; see Table 3.1) and their changes in isotopic signatures (obtained using Equations 3.3 & 3.4) were compared using Mann-Whitney U non-parametric tests with significance set at $p<0.05$.

Are changes in trophic fractionation of monogeneans affected by feeding mode and food source?

The feeding mechanisms of copepod species analysed in this study could not be definitively determined due to a lack of literature on this topic. The monogeneans, however, are separated into two subclasses based on the way in which they attach to their hosts, feed and on their diets, thus enabling an assessment of whether trophic fractionation in these parasites is affected by feeding mode and food source. Hence monopisthocotyleans and polyopisthocotyleans were independently grouped (Table 3.2) and their mean trophic fractionation values for both carbon and nitrogen (obtained using Equations 3.3 & 3.4) were compared using Mann-Whitney U non-parametric tests with significance set at $p<0.05$.

Table 3.2: Individual species in each of the two feeding subclasses of monogeneans analysed in the present study

Monopisthocotyleans	**Polyopisthocotyleans**
Monogenean sp. 1 *Tristoma adcoccineum*	*Anthocotyle merlucci* *Gastrocotyle trachuri* *Hexostoma* sp. *Mazocraes* sp.

Are individual parasite species enriched compared to their associated hosts?

In order to assess whether individual parasite species are enriched compared to their associated hosts, the isotopic signatures ($\delta^{15}N$ and $\delta'^{13}C$) of each parasite were compared to the isotopic signatures ($\delta^{15}N$ and $\delta'^{13}C$) of the selected tissue proxy of their associated host in a pair-wise comparison utilizing Wilcoxon matched pairs non-parametric tests with significance at $p<0.05$. Host-parasite pairs that did not have a sufficient sample size ($n<3$) for statistical analysis were excluded.

The trophic fractionation values of individual parasite species which were found infecting multiple hosts were then further analysed to investigate if the trophic fractionation is consistent across multiple host species. The fractionation values of *Euryphorus brachypterus* in relation to its respective hosts (*Thunnus albacares, T. alalunga, T. obesus* and *T. maccoyii*), were compared using a Kruskal-Wallis non-parametric test with *post-hoc* comparison. Similarly, the trophic fractionation values of *Pseudocycnus appendiculatus,* found parasitizing *T. albacares, T. alalunga* and *T. obesus,* were also compared using a Kruskal-Wallis non-parametric test with *post-hoc* comparison. Trophic fractionation values of *Hatschekia conifera* found parasitizing *Thyrsites atun* and *Brama brama* were compared using a Mann-Whitney U non-parametric test. Significance for all tests was set at $p<0.05$.

Additionally, regression analysis was conducted whereby the δ^{15}N of the parasite was compared to the δ^{15}N of the selected host tissue proxy to further analyse if the trophic fractionation of these parasites is independent of host species.

Are individual parasite species enriched compared to their associated hosts' blood tissue?

The isotopic signatures of blood tissue from the hosts was not analysed in this study, however this tissue may constitute a large proportion of the diet for many of the examined parasites. Therefore, in order to ascertain whether the parasites are enriched compared to host blood, the isotopic ratio of their associated hosts' blood tissue was calculated using ratios obtained from Deudero *et al.* (2002). This was achieved by estimating the nitrogen and carbon signatures for blood and muscle tissue of *Clupea harengus, Melanogrammus aeglefinnus* and *Eutrigla gurnardus* from Deudero *et al.* (2002; their Figure 1) and utilizing the values to calculate a mean muscle to blood ratio (Table 3.3). The means of these three ratios were then utilized to convert the δ^{15}N and δ'^{13}C isotopic signatures of the hosts' muscle tissue analysed in the present study, to their estimated blood δ^{15}N and δ'^{13}C isotopic signatures. This is, however, based on a major assumption that muscle and blood tissue is consistently different across host species.

Table 3.3: The δ^{15}N and δ'^{13}C blood and muscle isotopic signatures of the three species analysed in Deudero *et al.* (2002), their associated muscle to blood ratios and the overall mean muscle to blood ratios.

Host Species	**Tissue**	**δ^{15}N**	**δ'^{13}C**	**Muscle:Blood δ^{15}N**	**Muscle:Blood δ'^{13}C**
M. aeglefinus	Blood	13.27	-17.43	1:0.848465	1:1.053172
	Muscle	15.64	-16.55		
C. harengus	Blood	11	-19.4	1:0.882117	1:1.02159
	Muscle	12.47	-18.99		
E. gurnadus	Blood	14.08	-19.14	1:0.931217	1:0.982546
	Muscle	15.12	-19.48		
Overall Mean Ratio				1:0,887266	1:1,019103

In order to assess whether individual parasite species are enriched compared to their associated hosts' blood tissue, the isotopic signatures (δ^{15}N and δ'^{13}C) of each parasite were compared to the calculated isotopic signatures ($\delta^{15}N$ and $\delta'^{13}C$) of the blood tissue of their associated host in a pair-wise comparison utilizing Wilcoxon matched pairs non-parametric tests with significance at $p<0.05$. Host-parasite pairs that did not have a sufficient sample size ($n<3$) for statistical analysis were excluded.

The trophic fractionation values of individual parasite species which were found infecting multiple hosts were then further analysed to investigate if the trophic fractionation is consistent across multiple host species. The fractionation values of *Euryphorus brachypterus* in relation to its respective hosts (*Thunnus albacares, T. alalunga, T. obesus* and *T. maccoyii*), were compared using a Kruskal-Wallis non-parametric test with *post-hoc* comparison. Similarly, the trophic fractionation values of *Pseudocycnus appendiculatus,* found parasitizing *T. albacares, T. alalunga* and *T. obesus,* were also compared using a Kruskal-Wallis non-parametric test with *post-hoc* comparison. Trophic fractionation values of *Hatschekia conifera* found parasitizing *Thyrsites atun* and *Brama brama* were compared using a Mann-Whitney U non-parametric test. Significance for all tests was set at $p<0.05$.

3.3- Results

A total of 290 samples comprising 15 species of fish and their associated gill parasites were analysed for their carbon and nitrogen isotopic ratios (Table 3.4).

Table 3.4: Mean (±SD) δ^{15}N, δ'^{13}N and C:N of all samples analysed in this study. *x* x *y** indicates samples where parasites were grouped in order to provide sufficient material for analysis, with *x* showing the number of pooled samples analysed and *y* indicating the number of individual parasites pooled per sample.

Host species	Sample Analysed	n	Mean δ^{15}N (±SD)	Mean δ^{13}C (±SD)	Mean C:N (±SD)
Thunnus albacares	Muscle	4	12,26 ± 0,44	-16,19 ± 0,95	3,04 ± 0,29
Yellowfin tuna	Gill	5	12,40 ± 0,18	-15,99 ± 0,35	3,48 ± 0,06
	Euryphorus brachypterus	8	13,06 ± 0,80	-16,15 ± 0,44	4,52 ± 0,54
	Monogenean sp. 1	1	13,98	-16,29	4,73
	Pseudocycnus appendiculatus	6	13,26 ± 0,37	-16,66 ± 0,23	4,34 ± 0,77
Thunnus maccoyii	Muscle	2	12,39 ± 1,42	-17,97 ± 0,16	3,29 ± 0,32
Bluefin tuna	Gill	2	11,83 ± 0,98	-17,44 ± 0,65	3,54 ± 0,01
	Euryphorus brachypterus	6	12,27 ± 1,33	-16,78 ± 0,29	4,86 ± 0,65
Thunnus alalunga	Muscle	4	12,12 ± 0,45	-17,81 ± 0,15	3,13 ± 0,01
Longfin tuna	Gill	6	11,16 ± 0,48	-17,37 ± 0,46	3,58 ± 0,05
	Euryphorus brachypterus	10	11,76 ± 0,67	-16,96 ± 0,56	4,54 ± 0,39
	Pseudocycnus appendiculatus	13	11,47 ± 2,42	-17,92 ± 0,38	3,09 ± 6,31
Thunnus obesus	Muscle	8	13,34 ± 0,67	-17,15 ± 0,73	3,52 ± 0,44
Bigeye tuna	Gill	8	12,54 ± 0,64	-16,58 ± 0,41	3,61 ± 0,03
	Euryphorus brachypterus	23	13,43 ± 0,60	-16,04 ± 0,45	4,65 ± 0,69
	Hexostoma sp.	15	12,81 ± 0,89	-15,82 ± 0,79	5,34 ± 0,50
	Pseudocycnus appendiculatus	3	13,28 ± 0,95	-17,03 ± 0,78	4,28 ± 0,31
Isurus oxyrinchus	Muscle	2	12,98 ± 0,13	-16,52 ± 0,32	2,74 ± 0,05
Mako shark	Gill	2	13,29 ± 0,38	-16,32 ± 0,43	2,90 ± 0,10
	Nemesis lamna lamna	7	13,67 ± 0,54	-14,62 ± 0,40	4,78 ± 0,41
Prionace glauca	Muscle	2	13,35 ± 0,81	-16,50 ± 0,07	2,72 ± 0,22
Blue Shark	Gill	2	13,06 ± 0,10	-16,58 ± 0,12	2,71 ± 0,38
	Kroyeria carchariaeglauci	4x50*	14,48 ± 0,20	-12,59 ± 0,77	7,57 ± 0,30
Xiphias gladius	Muscle	2	13,70 ± 0,64	-16,95 ± 1,37	6,50 ± 2,26
Swordfish	Gill	2	12,75 ± 0,32	-15,90 ± 0,01	3,69 ± 0,05
	Tristoma adcoccineum	4	15,03 ± 0,27	-16,02 ± 0,21	4,42 ± 0,18
Brama brama	Muscle	3	15,63 ± 0,67	-16,07 ± 1,19	5,94 ± 3,03
Atlantic pomfret	Gill	3	15,01 ± 0,18	-16,04 ± 0,06	3,83 ± 0,19
	Hatschekia conifera	6x50*	17,40 ± 0,35	-10,09 ± 1,50	13,20 ± 1,81
Thyrsites atun	Muscle	5	15,78 ± 0,28	-15,79 ± 0,29	3,29 ± 0,05
Snoek	Gill	5	15,58 ± 0,56	-15,80 ± 0,37	3,88 ± 0,16
	Caligus dakari	5	16,34 ± 0,62	-13,96 ± 0,12	6,76 ± 0,21
	Hatschekia conifera	2x20*	17,74 ± 0,15	-13,89 ± 1,09	7,58 ± 1,05
	Mazocraes sp.	1	15,60	-15,77	4,67

Table 3.4: (Continued)

Host species	Sample Analysed	n	Mean δ^{15}N (±SD)	Mean δ^{13}C (±SD)	Mean C:N (±SD)
Seriola lalandi	Muscle	5	14,43 ± 0,23	-15,61 ± 0,35	3,24 ± 0,07
Yellowtail amberjack	Gill	4	13,02 ± 0,34	-14,31 ± 0,45	3,35 ± 0,20
	Parabrachiella sp. 1	8	15,47 ± 0,41	-14,63 ± 0,11	4,53 ± 0,51
Sarda sarda	Muscle	5	15,06 ± 0,13	-15,89 ± 0,37	3,66 ± 0,43
Atlantic bonito	Gill	4	14,03 ± 0,09	-15,54 ± 0,30	3,79 ± 0,18
	Caligus bonito	14	16,88 ± 0,66	-15,07 ± 0,34	4,68 ± 0,48
Genypterus capensis	Muscle	1	15,83	-16,21	3,18
Kingklip	Gill	1	16,06	-15,77	3,84
	Chondracanthus colligens	1	15,82	-16,06	4,38
Merluccius capensis	Muscle	2	14,26 ± 0,08	-15,99 ± 0,10	3,19 ± 0,05
Shallow-water hake	Gill	2	14,80 ± 1,35	-15,81 ± 0,85	3,99 ± 0,13
	Anthocotyle merlucci	2x3*	14,64 ± 0,16	-15,56 ± 0,38	3,83 ± 0,65
Sardinops sagax	Muscle	2	11,34 ± 0,15	-16,50 ± 0,09	3,19 ± 0,02
Sardine	Gill	1	9,22	-17,51	3,78
	Clavellisa ilishae	1x10*	12,63 ± 0,21	-17,61 ± 0,54	6,93 ± 0,81
Trachurus capensis	Muscle	15	12,56 ± 0,50	-16,65 ± 0,47	3,24 ± 0,02
Cape horse mackerel	Gill	15	11,78 ± 0,51	-16,87 ± 0,60	3,76 ± 0,13
	Gastrocotyle trachuri	5x47*	12,39 ± 0,59	-17,55 ± 0,26	4,51 ± 0,10
Lampanyctodes hectoris	Muscle	5	13.01 ± 0.27	-17,15 ± 0.40	3.86 ± 0,36
Hector’s lanternfish	Heart	5	11.46 ± 0.93	-18.09 ± 0.40	4,09 ± 0,21
	Cardiodectes medusaeus	11	9.22 ± 0.37	-18.07 ± 0.51	5.93 ± 1.13

The bi-plot of host muscle δ^{15}N and δ'^{13}C values conforms the to the typical pattern seen in food-webs between predator and prey species whereby the lower trophic level species, such as sardine, *Sardinops sagax*, have lower δ^{15}N values whilst which the predatory species such as *Thyrsites atun* and the *Thunnus* species have higher δ^{15}N values (Figure 3.1). The δ'^{13}C values have very wide range, which is expected as the majority of the examined species are migratory with circumglobal dispersions and cover a wide range of habitats, from coastal to oceanic. There is also a very clear separation of species in iso-space with the middle-sized pelagic species (*Seriola lalandi*, *Brama brama*, *Thyrsites atun* and *Sarda sarda*) and demersal species (*Genypterus capensis* and *Merluccius capensis*) forming a separate group compared to the other species examined (Figure 3.1).

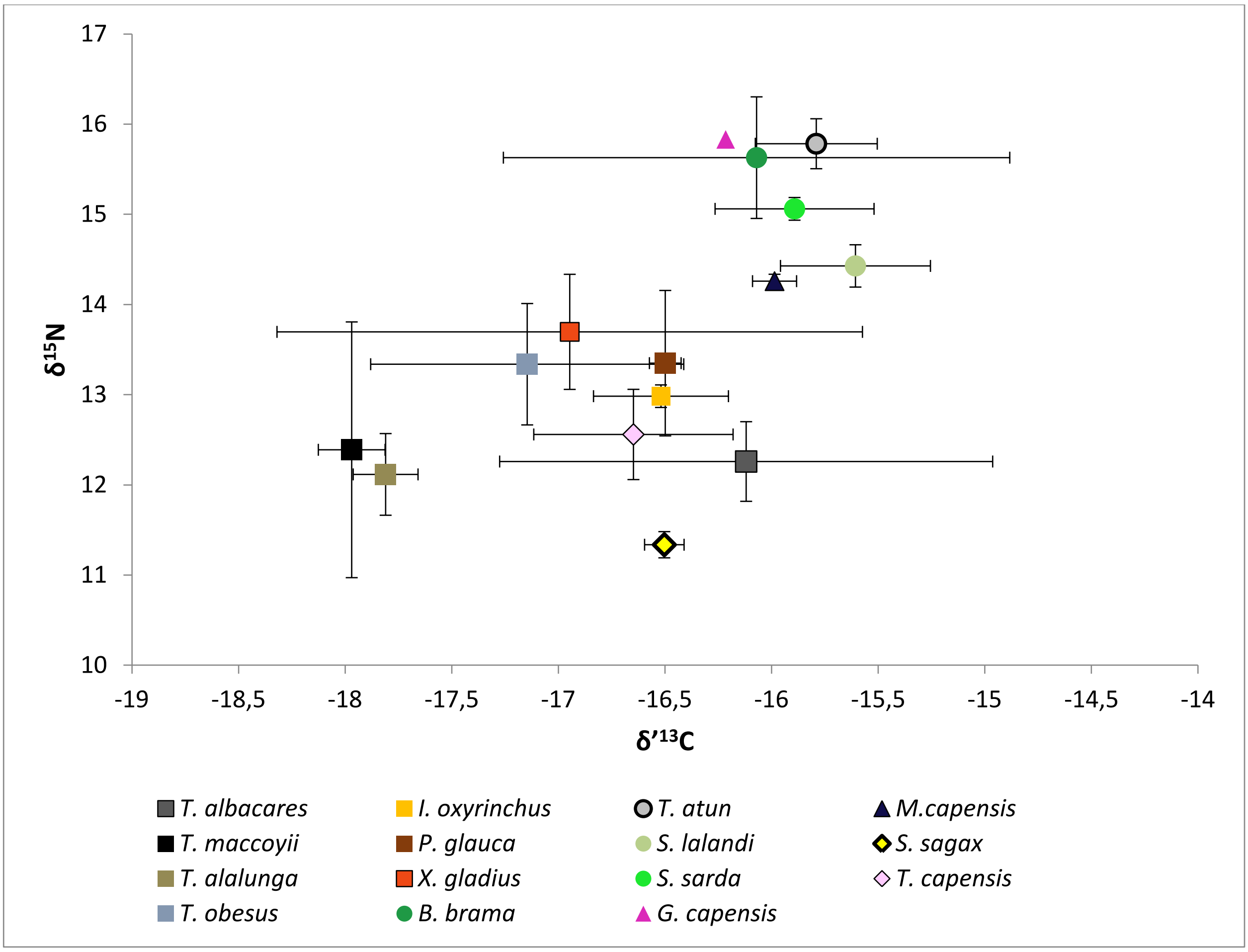

Figure 3.1: Bi-plot indicating the position of the examined fish host species' muscle tissue with respect to δ^{15}N (±SD) and δ'^{13}C (±SD). Squares (■) indicate the large pelagic species; circles (●) indicate the middle-sized pelagic species; triangles (▲) indicate the demersal species and diamonds (◆) indicate the small pelagic species**.**

The large pelagic fish host species, which include the *Thunnus* species, generally show a larger variance in their δ^{15}N and δ'^{13}C values compared to the middle-sized pelagic, demersal and small pelagic species examined (Figure 3.2). *Isurus oxyrinchus* has the lowest variance of these large pelagic species with variances in δ'^{13}C and δ^{15}N equalling 0.100‰^{2} and 0.016‰^{2}, respectively. *Brama brama*, although a middle-sized pelagic species, has a large variances in both δ'^{13}C (1.410‰^{2}) and δ^{15}N (0.454‰^{2}), which is similar to those of the larger pelagic species such as *X. gladius* with variance of 1.885‰^{2} for δ'^{13}C and 0.407‰^{2} for δ^{15}N. The remaining middle-sized pelagic, demersal and small pelagic species have relatively small variances with *Trachurus capensis* having the largest variance of 0.218‰^{2} for δ'^{13}C and 0.251‰^{2} for δ^{15}N.

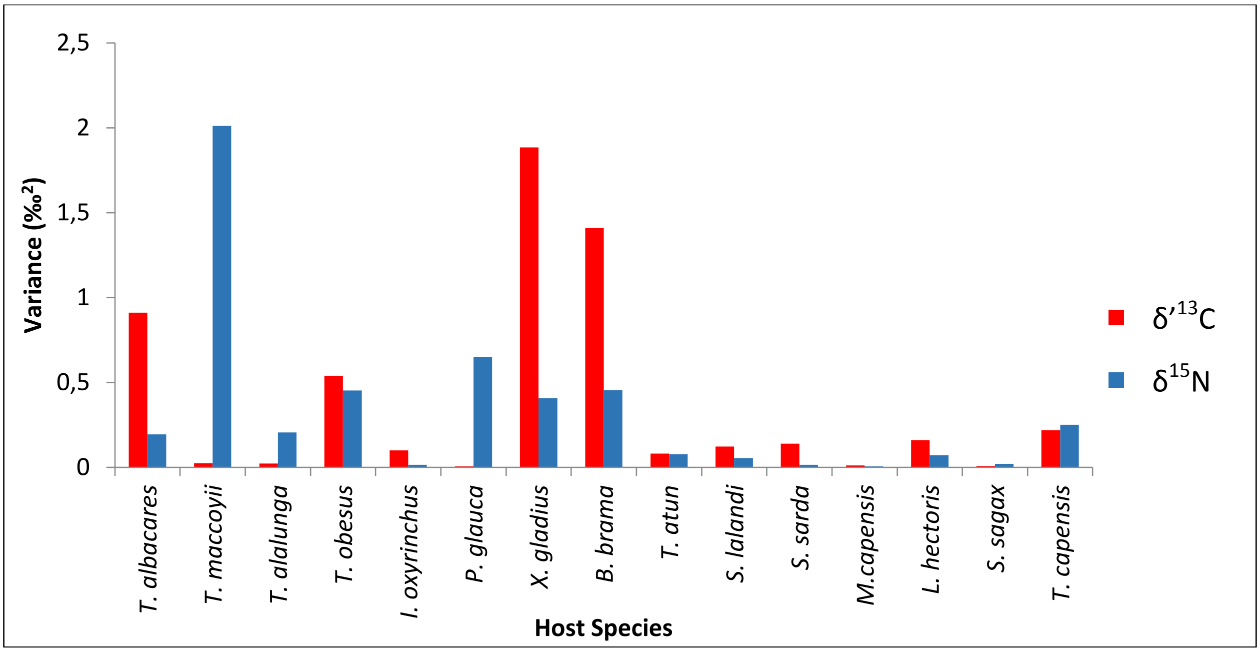

Figure 3.2: The variance (‰^{2}) of $\delta'^{13}C$ and $\delta^{15}N$ of the examined fish host species.

3.3.1- Comparing the isotopic signatures of host gill and white muscle tissue

The Wilcoxon matched pairs test comparing $\delta^{15}N$ of the host muscle to $\delta^{15}N$ of the host gill showed a highly significant difference (n=60; Z=5.66843; $p<0.00001$), with the muscle tissue showing a greater enrichment in ^{15}N (mean $\delta^{15}N$ of 13.53 ± 1.36‰), compared to gill tissue (mean $\delta^{15}N$ of 12.87 ± 1.53‰; Figure 3.3). The regression of host gill $\delta^{15}N$ against the host muscle $\delta^{15}N$ was significant ($p<0.001$), indicating that the $\delta^{15}N$ of the host gill tissue is directly related to the $\delta^{15}N$ of the host muscle tissue (Figure 3.4).

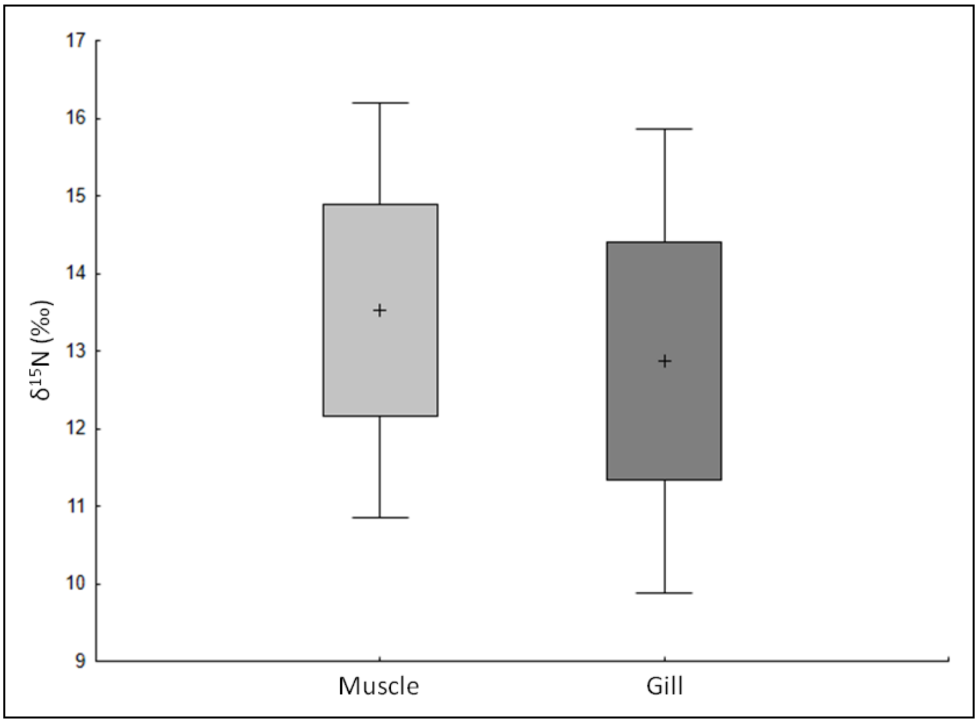

Figure 3.3: Mean δ^{15}N values of host muscle and host gill tissue from all fish hosts (excluding *Lampanyctodes hectoris*) analysed in this study (n= 60). (+) indicates the mean, the box indicates the standard deviation and the whiskers indicate the standard deviation x 1.96.

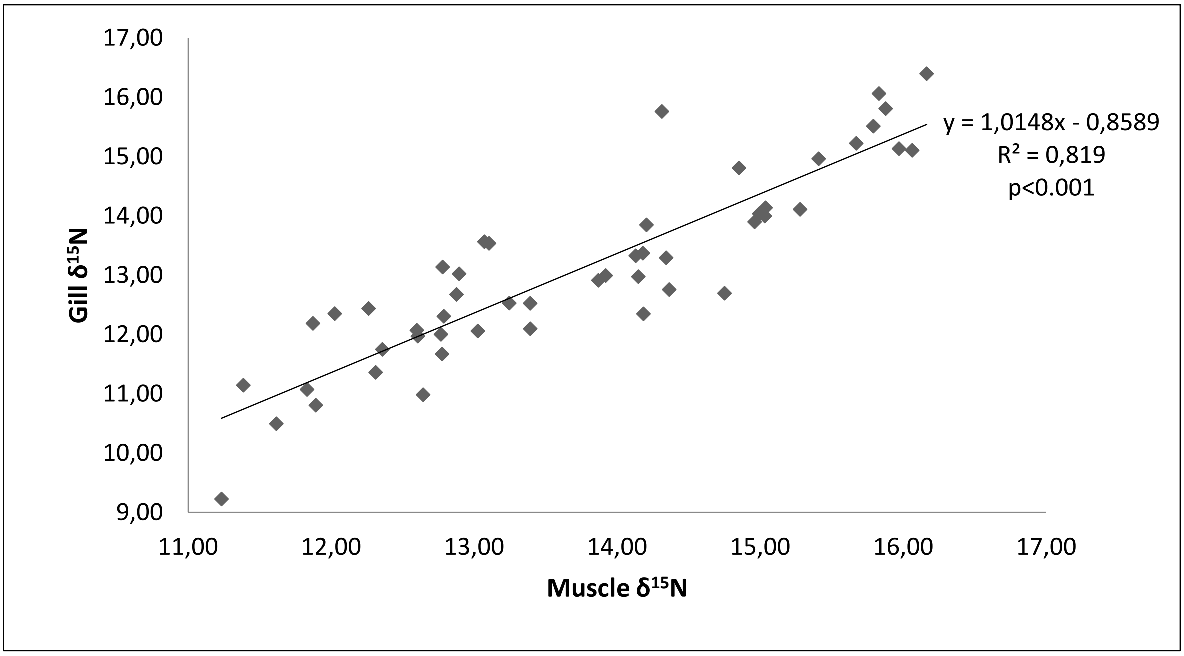

Figure 3.4: The relationship between δ^{15}N values of host gill and host muscle for all fish hosts examined (excluding *Lampanyctodes hectoris*).

Despite the substantial overlap in the standard deviations of the carbon signatures with respect to the different tissues (Figure 3.5), the Wilcoxon matched pairs test comparing the δ'^{13}C of host muscle to that of host gill also indicated a significant difference (n=60; Z=4.85813; p=0.03218), with gill tissue showing a higher enrichment in ^{13}C (mean $\delta'^{13}C$ of -16.33 ± 0.91‰) compared to white muscle tissue (mean $\delta'^{13}C$ of -16.53 ± 0.83‰; Figure 3.5).

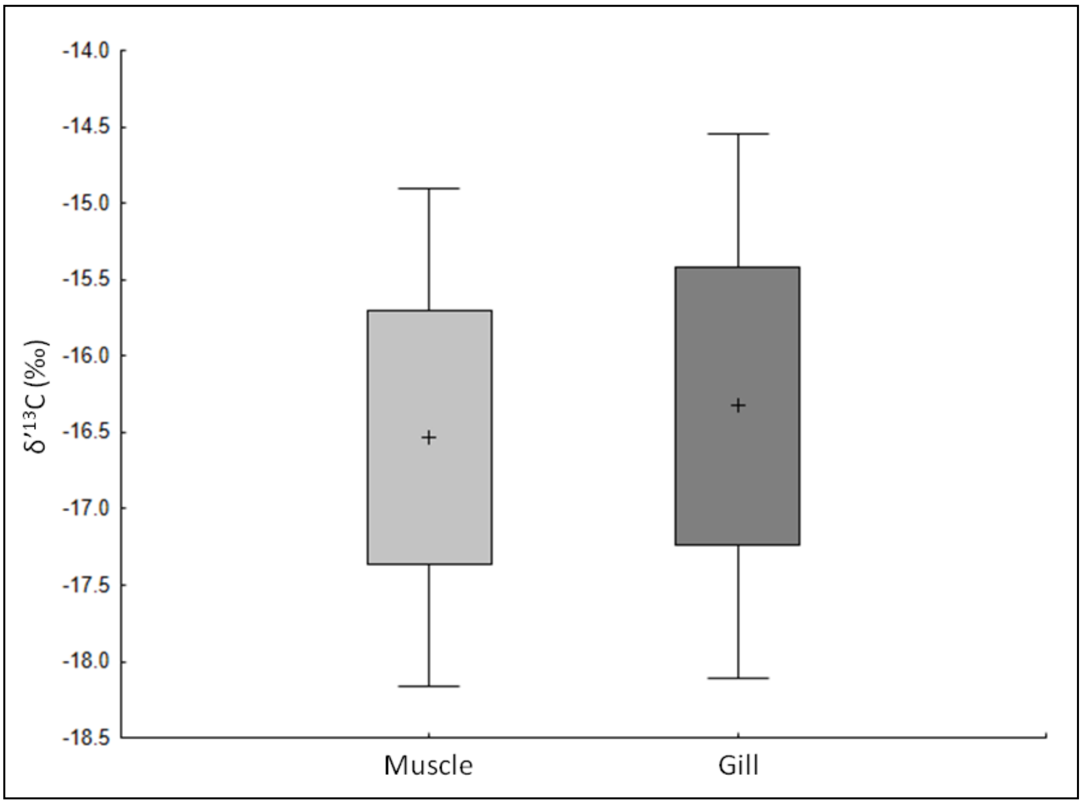

Figure 3.5: Mean $\delta'^{13}C$ values of host muscle and host gill tissue from all fish hosts (excluding *Lampanyctodes hectoris*) analysed in this study (n=60). (+) indicates the mean, the box indicates the standard deviation and the whiskers indicate the standard deviation x 1.96.

The different isotopic signatures of the host white muscle tissue and the host gill tissue are seen in the bi-plot (Figure 3.6). For the majority of examined species the gill tissue is more enriched in ^{13}C and the $\delta'^{13}C$ value lies to the right of the associated host muscle tissue, whilst the gill tissue is more depleted in ^{15}N and the $\delta^{15}N$ value lies below that of the associated muscle sample.

Given these difference the host gill tissue was selected as the appropriate host proxy to compare with isotopic signatures of the gill parasites.

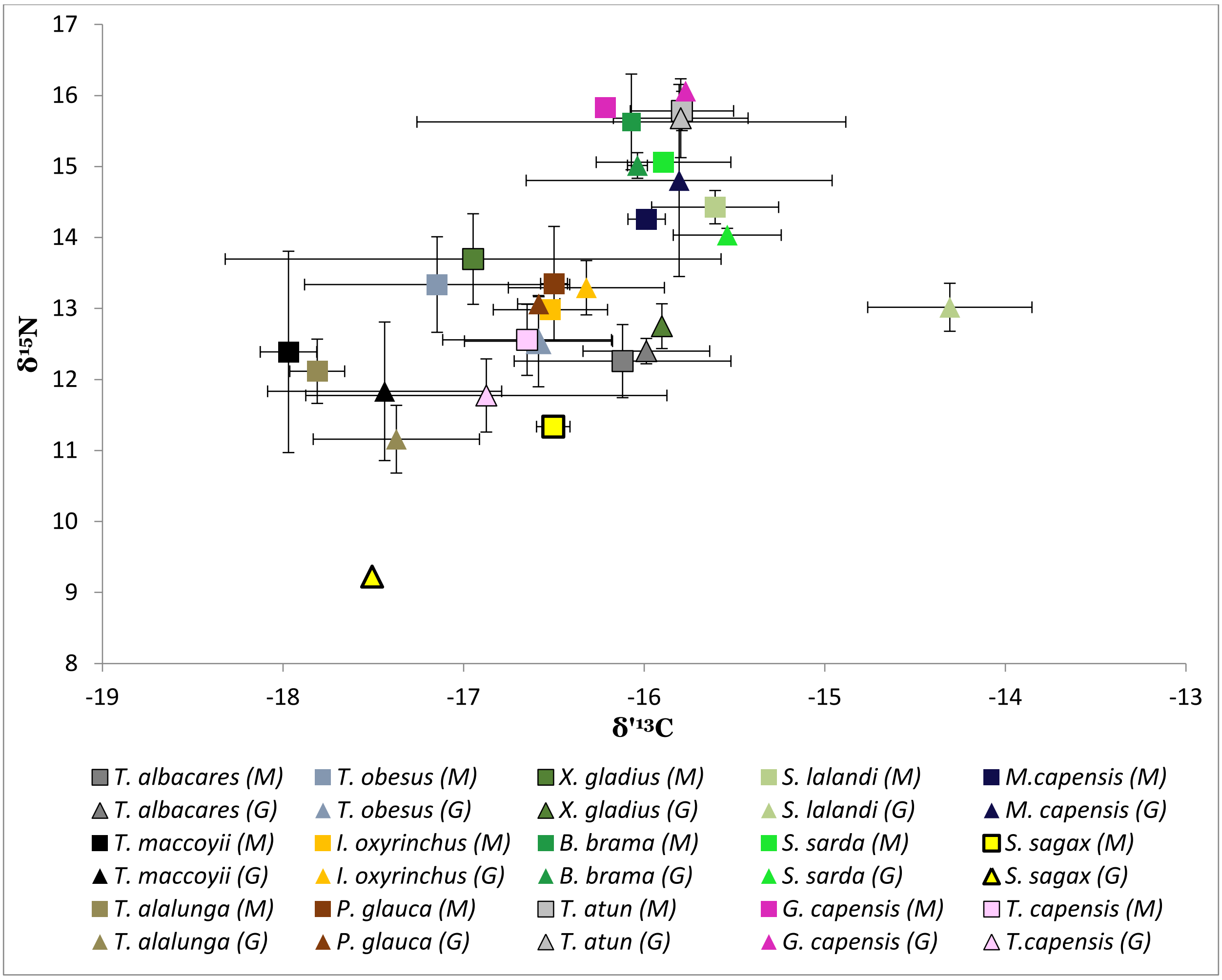

Figure 3.6: Bi-plot of the δ^{15}N (±SD) and δ'^{13}C (±SD) of the examined host species' gill and muscle tissues. In the legend, (M) indicates host white muscle and (G) indicates host gill tissue; on the plot, squares (■) indicate the host white muscle tissue and triangles (▲) indicate the host gill tissue.

3.3.2- Assessing if gill parasites in general are enriched in ^{13}C and ^{15}N compared to their hosts

The Wilcoxon matched-pairs test comparing the δ^{15}N values of the parasites to those of their associated host gill tissue showed a highly significant difference ($n=144$; $Z=9.767249$; $p<0.00001$; Figure 3.7), with the gill parasites in general showing a mean enrichment in ^{15}N of +1.15 ± 1.00‰ compared to their respective host δ^{15}N.

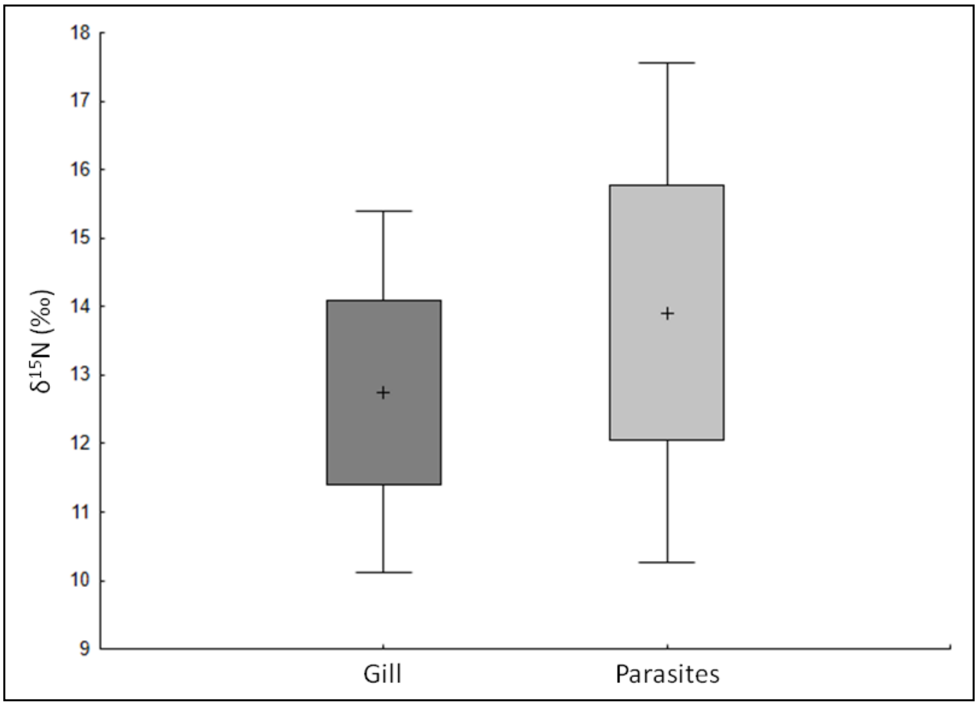

Figure 3.7: Comparing the $\delta^{15}N$ values of gill parasites and their associated host gill tissue. (+) indicates the mean, the box indicates the standard deviation and the whiskers indicate the standard deviation x 1.96.

The Wilcoxon matched-pairs test comparing the $\delta'^{13}C$ values of the parasites to those of their associated host gill tissue also showed a highly significant difference (n=144; Z=4.757796; $p<0.00001$; Figure 3.8), with the parasites in general showing a mean enrichment in ^{13}C (+0.65 ± 1.51‰) compared to their associated host gill tissue.

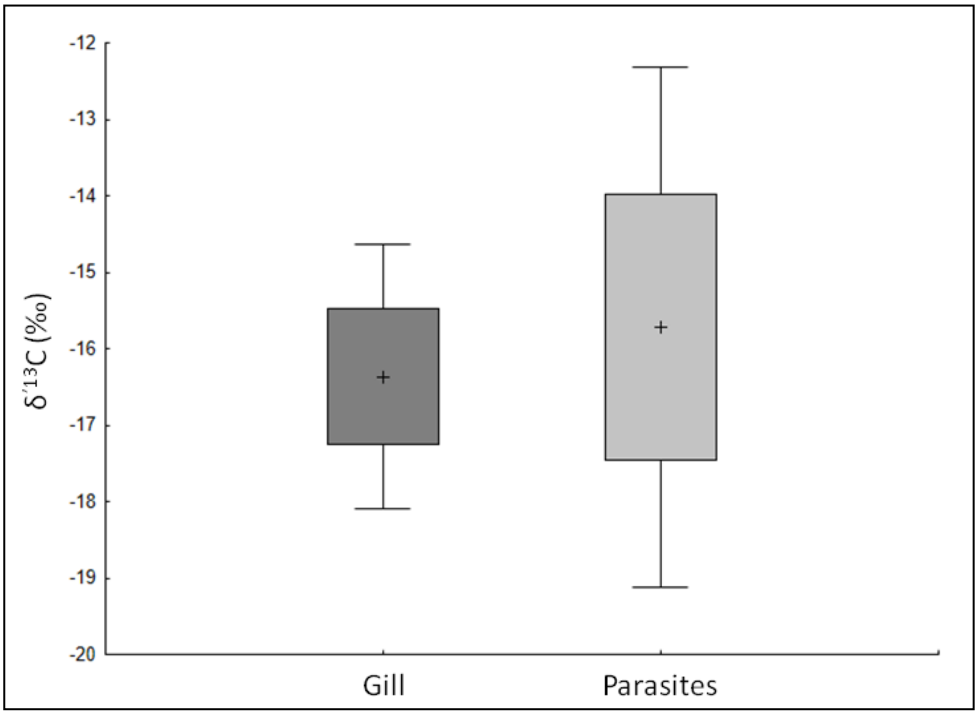

Figure 3.8: Comparing the δ'^{13}C values of gill parasites and their associated host gill tissue. (+) indicates the mean, the box indicates the standard deviation and the whiskers indicate the standard deviation x 1.96.

Figures 3.7 and 3.8 are however, coarse representations of the obtained data as both the host and parasite species are from multiple trophic levels with varying δ'^{13}C and δ^{15}N values.

3.3.3- Assessing whether trophic fractionation differs between copepod and monogenean gill parasites

The Mann-Whitney U test comparing the enrichment in ^{15}N between copepod and monogenean gill parasites showed a significant difference (U=987; $n_{copepods}$=117; $n_{monogeneans}$=28; p=0.001121; Figure 3.9), with the copepods showing a greater enrichment in ^{15}N (mean trophic fractionation of +1.26 ± 0.98‰) compared to the monogeneans (mean trophic fractionation of +0.60 ± 0.92‰; Figure 3.13).

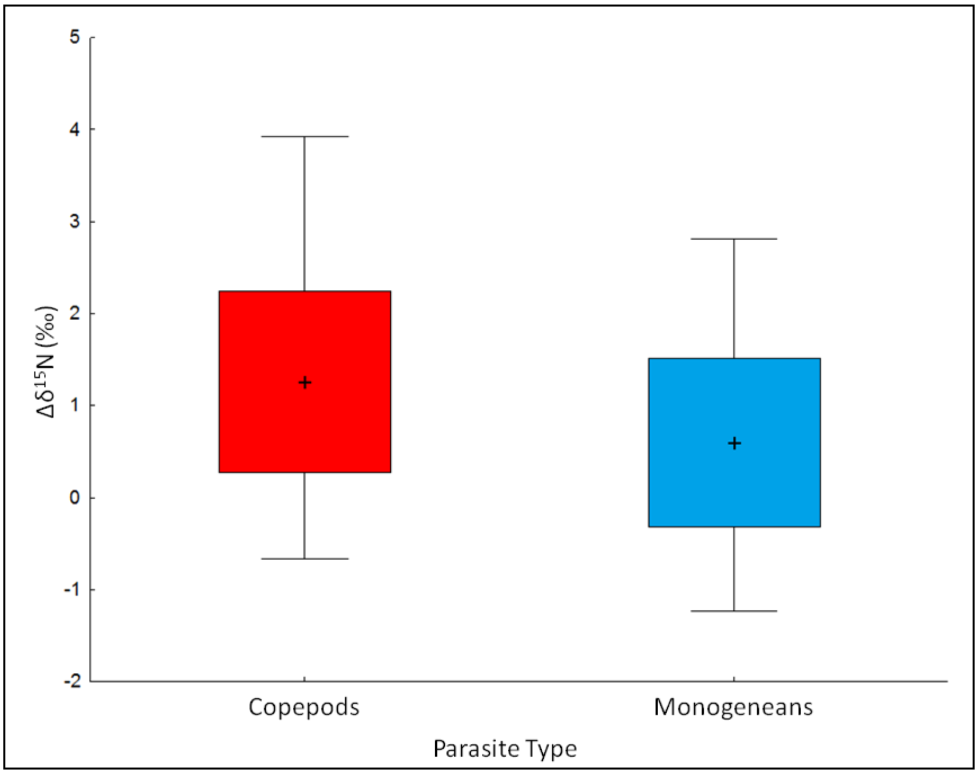

Figure 3.9: Mean trophic fractionation of nitrogen ($\Delta\delta^{15}N$) by parasite type, with copepods in red and monogeneans in blue. (+) indicates the mean, the box indicates the standard deviation and the whiskers indicate the minimum and maximum values.

The Mann-Whitney U Test comparing the enrichment in ^{13}C of copepod and monogenean gill parasites showed no significant difference ($U=1596$; $n_{copepods}=117$; $n_{monogeneans}=28$; $p=0.83533$; Figure 3.10), with copepods and monogeneans showing similar mean trophic fractionation values of +0.67 ± 1.58‰ and +0.43 ± 0.91‰, respectively.

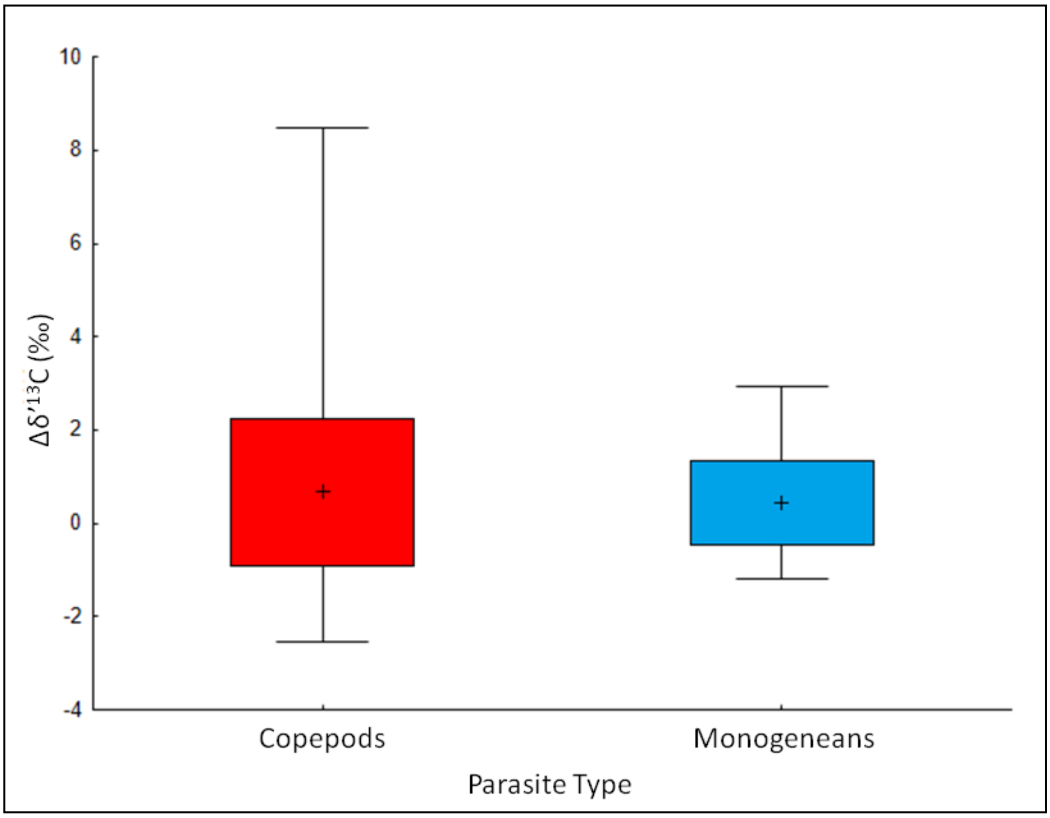

Figure 3.10: Mean trophic fractionation of carbon (Δδ’^{13}C) by parasite type, with copepods in red and monogeneans in blue. (+) indicates the mean, the box indicates the standard deviation and the whiskers indicate the minimum and maximum values.

3.3.4- Assessing if differences in isotopic signatures between monogeneans and their hosts are affected by parasite feeding mode

The Mann-Whitney U test comparing the nitrogen trophic fractionation between the two subclasses of monogenean gill parasites (monopisthocotyleans and polyopisthocotyleans) and their associated hosts showed a significant difference (U=22; $n_{monopisthocotyleans}$=5; $n_{polyopisthocotyleans}$=23; p=0.035776; Figure 3.11). The monopisthocotyleans had a greater mean enrichment in ^{15}N (mean trophic fractionation of +1.58 ± 1.32‰) compared to the polyopisthocotyleans (mean trophic fractionation of +0.38 ± 0.67‰).

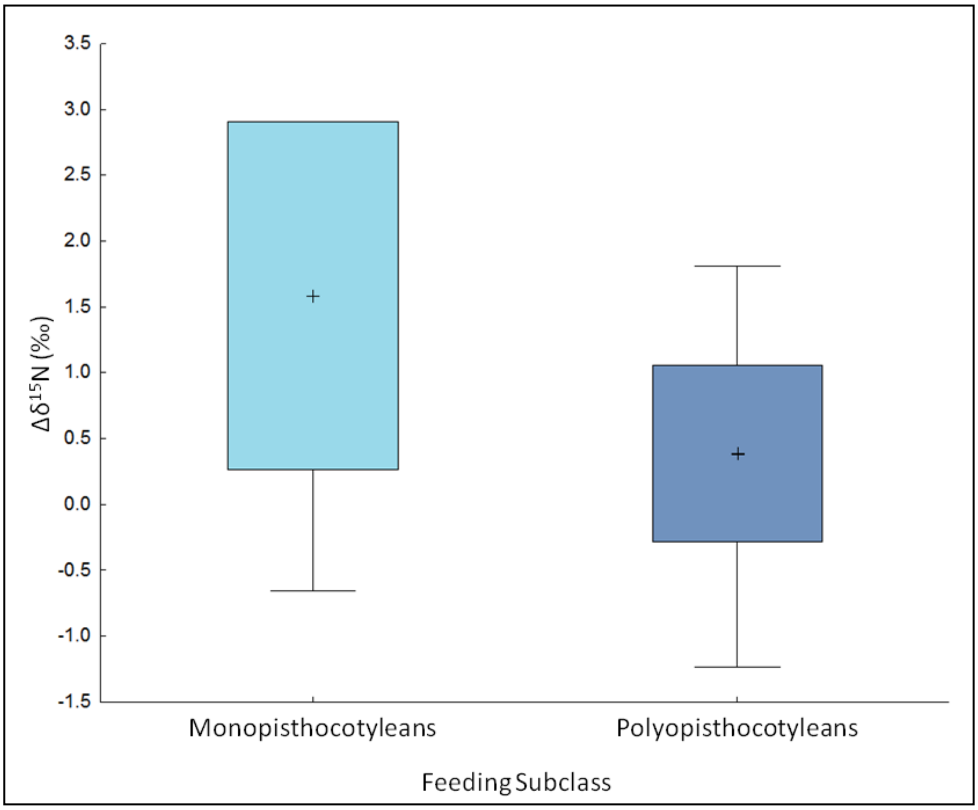

Figure 3.11: Mean trophic fractionation of nitrogen ($\Delta\delta^{15}N$) by monopisthocotyleans (tissue-scraping) and the polyopisthocotyleans (blood-feeding) monogeneans. (+) indicates the mean, the box indicates the standard deviation and the whiskers indicate the minimum and maximum values.

The Mann-Whitney U test comparing the carbon trophic fractionation values between the two subclasses of monogenean gill parasites (monopisthocotyleans and polyopisthocotyleans) and their associated hosts showed no significant difference (U=30; $n_{monopisthocotyleans}=5$; $n_{polyopisthocotyleans}=23$; p=0.105320; Figure 3.12) with the monopisthocotyleans having a mean trophic fractionation -0.116 ± 0.18‰ and the polyopisthocotyleans having a mean trophic fractionation +0.56 ± 1.0‰.

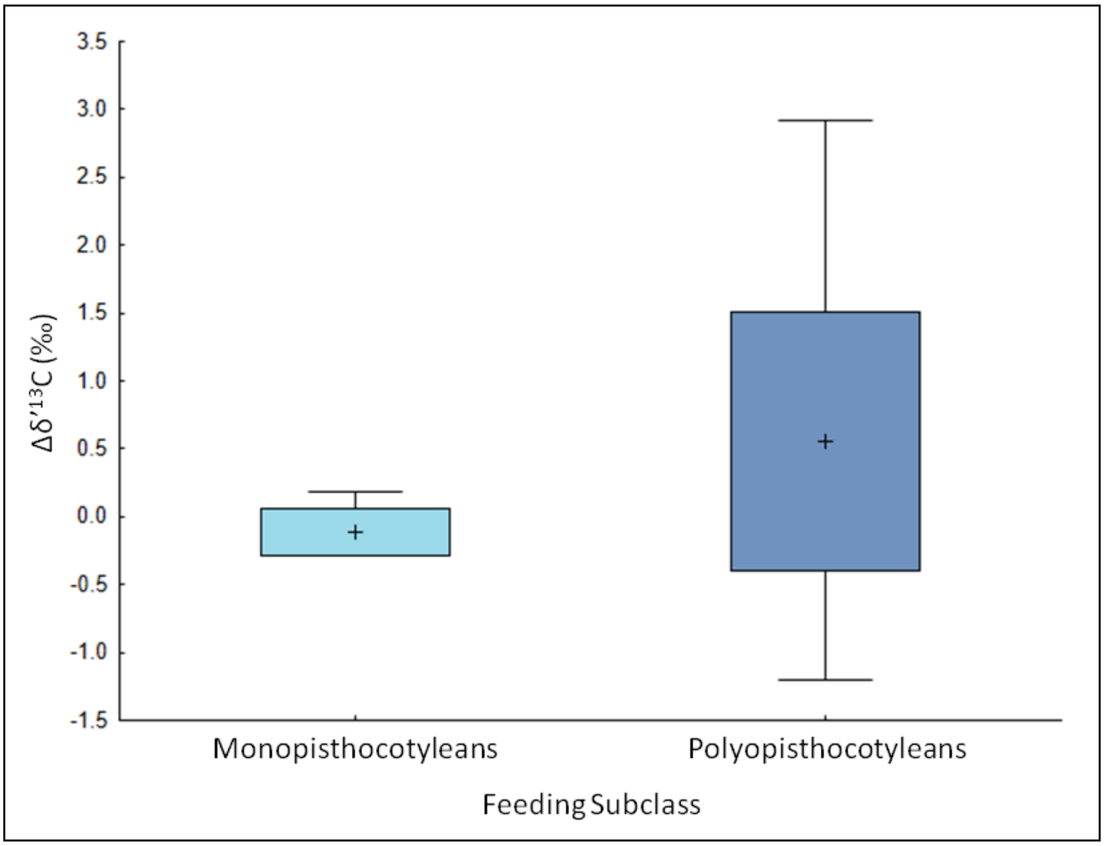

Figure 3.12: Mean trophic fractionation of carbon ($\Delta\delta'^{13}C$) by monopisthocotyleans (tissue-scraping) and the polyopisthocotyleans (blood-feeding) monogeneans. (+) indicates the mean, the box indicates the standard deviation and the whiskers indicate the minimum and maximum values.

3.3.5- Assessing whether individual parasite species are enriched compared to their associated hosts

Nine of a total of 18 comparisons between host gill $\delta^{15}N$ and parasite $\delta^{15}N$ values showed significant ($p<0.05$) enrichment by the parasite, with the 9 enriched parasites comprising one out of the four monogenean-host comparisons and eight out of the 14 copepod-host comparisons (Table 3.5; Figure 3.13). However, of the interactions which show no significant difference, three are close to the $P<0.05$ threshold ($p=0.06089$ for *Hexostoma* sp. parasitizing *Thunnus obesus*, $p=0.06789$ for *Kroyeria carchariaeglauci* parasitizing *Prionace glauca* and $p=0.06789$ for *Tristoma adcoccineum* parasitizing *Xiphias gladius*). In the cases of Monogenean sp. 1 parasitizing *Thunnus albacares*, *Mazocraes* sp. parasitizing *Thyrsites atun*, *Chondracanthus colligens* parasitizing *Genypterus capensis* and *Clavellisa ilishae* parasitizing *Sardinops sagax*, all the parasites except for *C. colligens*, were enriched compared to their associated host gill δ^{15}N, but this could not be statistically confirmed (Figure 3.13). In the case of *Kroyeria carchariaeglauci* parasitizing *Prionace glauca,* although appearing to show significant enrichment in ^{15}N compared to the host gill tissue (Figure 3.13), the statistical analysis returned a non-significant result. This can be attributed to a limited sample size. *Cardiodectes medusaeus* is not a gill parasite and thus was not included in the above results, however it showed significant ($p<0.05$) depletion in ^{15}N with respect to *Lampanyctodes hectoris* heart tissue (Table 3.5; Figure 3.13)

Table 3.5: The results of the Wilcoxon signed-rank matched paired tests comparing the δ^{15}N values of the parasites and the gill tissue of their associated hosts for which sufficient data were available for statistical analyses (*i.e.* excludes data for parasites of *G. capensis* and *S. sagax,* and for Monogenean sp. 1 parasitizing *T. albacares* and *Mazocraes* sp. parasitizing *T. atun*). In the case of *Cardiodectes medusaeus* infecting the heart of *Lampanyctodes hectoris* although not a gill parasite is also included here compared to host heart tissue for later comparison. The table indicates the n values, Z-values, p-values, the mean enrichment/depletion (±SD) and whether the parasites showed enrichment, depletion or no significant difference in δ^{15}N values. Tests were considered significant at $p<0.05$ and significant differences denoted by *.

Host Species	**Parasite Species**	**Wilcoxon Matched Pairs Test**				
		n	**Z**	**p-value**	**Mean Enrichment/ Depletion (‰)**	**Enriched/Depleted/No Difference**
T. albacares	*P. appendiculatus*	6	2.201398	0.02771*	0.868 ± 0.349	Enriched
	E. brachypterus	8	1.960392	0.04995*	0.709 ± 0.717	Enriched
T. maccoyii	*E. brachypterus*	6	1.572427	0.11585	0.435 ± 0.636	No Difference
T. obesus	*E. brachypterus*	23	4.136434	0.00004*	0.937 ± 0.636	Enriched
	P. appendiculatus	3	1.603567	0.10881	0.765 ± 0.090	No Difference
	Hexostoma sp.	15	1.874274	0.06089	0.362 ± 0.680	No Difference
T. alalunga	*E. brachypterus*	10	2.803060	0.00506*	0.564 ± 0.358	Enriched
	P. appendiculatus	13	3.179797	0.00147*	1.005 ± 0.418	Enriched
I. oxyrinchus	*N. lamna lamna*	7	1.352247	0.17630	0.342 ± 0.568	No Difference
P. glauca	*K. carchariaeglauci*	4	1.825742	0.06789	1.419 ± 0.253	No Difference
X. gladius	*T. adcoccineum*	4	1.825742	0.06789	2.275 ± 0.361	No Difference
B. brama	*H. conifera*	6	2.201398	0.02771*	2.381 ± 0.207	Enriched
T. atun	*C. dakari*	5	2.0226	0.04312*	0.645 ± 0.227	Enriched
	H. conifera	2	1.341641	0.17971	2.078 ± 0.355	No Difference
S. lalandi	*Parabrachiella* sp. 1	8	2.520504	0.01172*	2.315 ± 0.352	Enriched
S. sarda	*C. bonito*	14	3.295765	0.00098*	2.850 ± 0.651	Enriched
M. capensis	*A. merlucci*	2	0.447214	0.65472	-0.161 ± 1.515	No Difference
T. capensis	*G. trachuri*	5	2.0226	0.04312*	0.616 ± 0.198	Enriched
L. hectoris	*C. medusaeus*	11	2.934058	0.00335*	-2.401 ± 1.030	Depleted

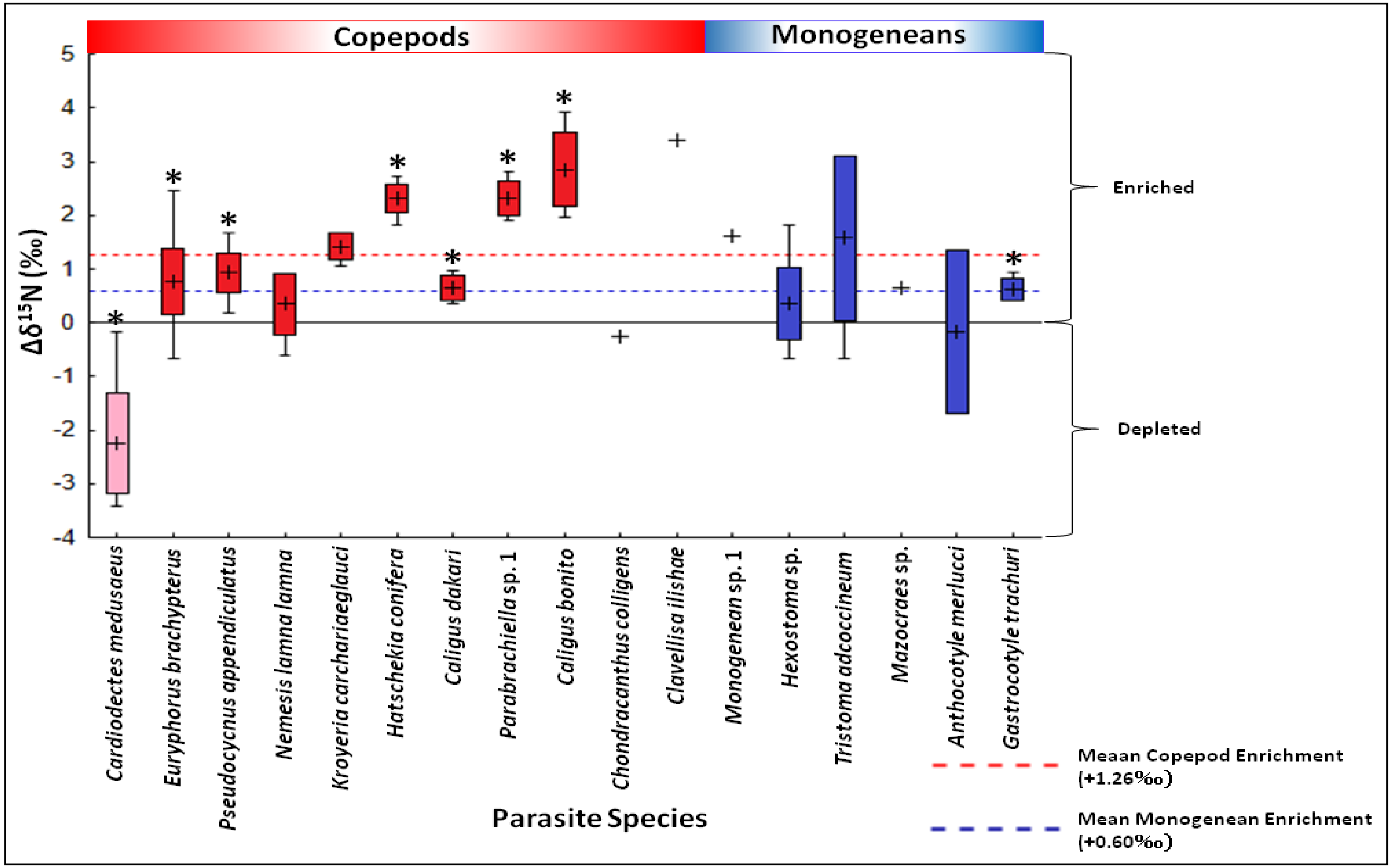

Figure 3.13: Mean trophic fractionation of nitrogen ($\Delta\delta^{15}N$) for each parasite species/taxa analysed in this study across all hosts on which that parasite was found. Copepod parasites are shown in red whilst monogenean parasites are shown in blue, (+) indicates the mean (or individual value if only one individual parasite or one group of pooled parasites was analysed), the box indicates the standard deviation, the whiskers indicate the minimum and maximum values and * indicates a significant difference between mean parasite and mean host gill $\delta^{15}N$ values. *Cardiodectes medusaeus* (pink) found infecting the heart of *Lampanyctodes hectoris*, although not a gill parasite is also included here compared to its host heart tissue for later comparison. ($\Delta\delta^{15}N$ of *Euryphorus brachypterus* parasitizing *Thunnus albacares*, *T. maccoyii*, *T. alalunga* and *T. obesus* did not differ significantly from one another, neither did *Pseudocycnus appendiculatus* parasitizing *T. albacares*, *T. alalunga* and *T. obesus*, nor did *H. conifera* parasitizing *B. brama* and *T. atun*, therefore parasites were grouped for simplification).

In order to analyse whether parasites infecting multiple host species showed a similar trophic fractionation of ^{15}N across their various hosts, the fractionation values of *Euryphorus brachypterus* found parasitizing *Thunnus albacares, T. alalunga, T. obesus* and *T. maccoyii* were compared using a Kruskal-Wallis test and indicated no significant difference (H (3, n=46) =4.1809; p=0.2426). The fractionation values of *Pseudocycnus appendiculatus* found parasitizing *T. albacares, T. alalunga* and *T.obesus* were also compared using a Kruskal-Wallis test and also showed no significant difference across host species (H (3, n=22) =2.4545; p=0.2931). The Mann-Whitney U test comparing the fractionation values of *Hatschekia conifera* found parasitizing *Thyrsites atun* and *Brama brama* also showed no significant difference (U=3.00, $n_{T.atun}$ =2; $n_{B.\ brama}$ = 6; p=0.4047).

The $\delta^{15}N$ values of these three parasite species that occur in multiple host species were then compared to the $\delta^{15}N$ of their associated hosts' gill via regressions (Figure 3.14). The regressions of *E. brachypterus* and *P. appendiculatus* were highly significant (p<0.001; Figures 3.14A & 3.14B), and neither of the regression slopes were statistically different to 1 (t=0.670, d.f.= 64, p=0.50524 for *E. brachypterus* and t=0.281, d.f.=39, p=0.7802 for *P. appendiculatus*). Both regressions followed the same pattern where an increase in host gill $\delta^{15}N$ value was associated with an increase parasite $\delta^{15}N$ value. The regression for *H. conifera* and its associated hosts, however, were not significant (p> 0.05; Figure 3.14C).

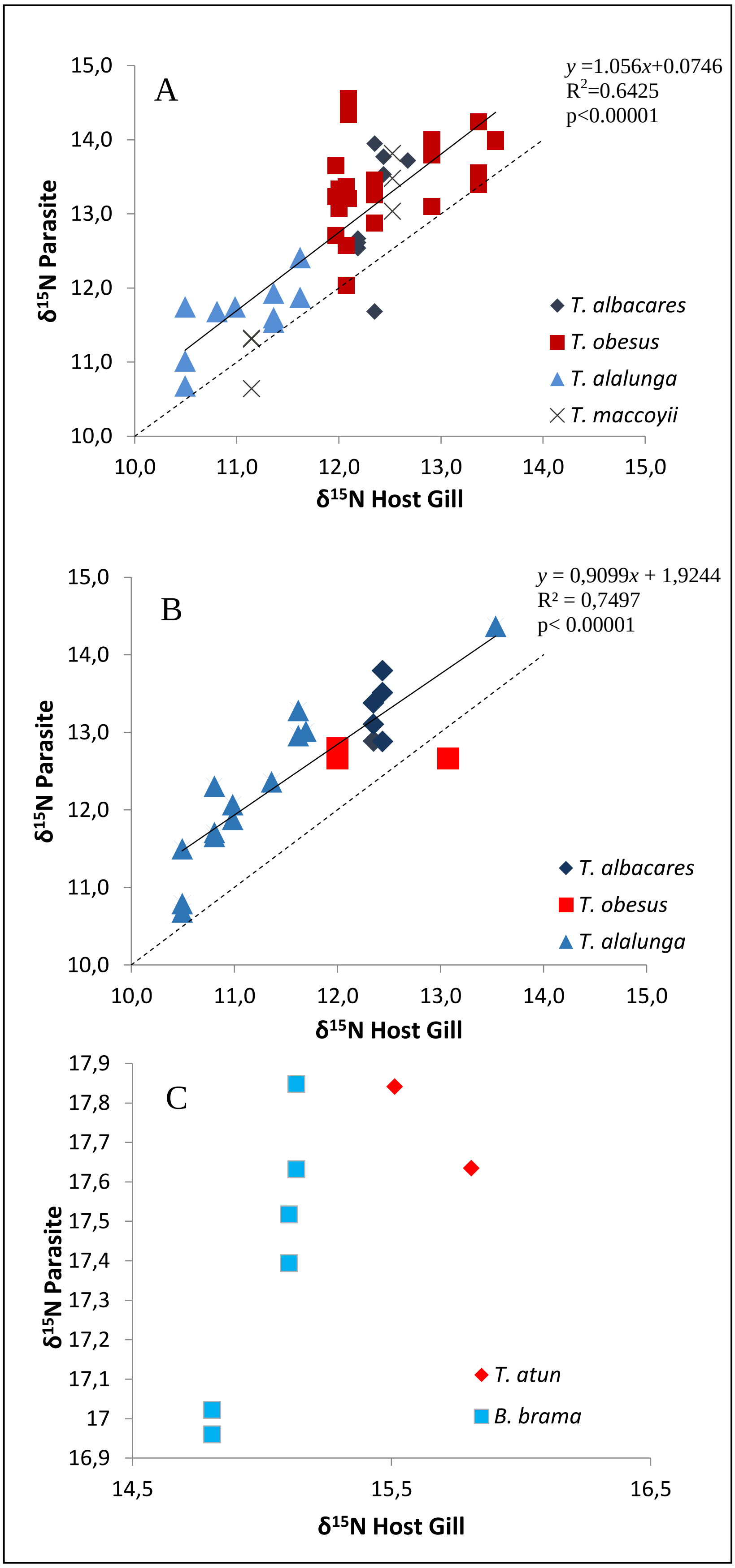

Figure 3.14: The relationship between δ^{15}N values of individual parasites and the gill tissue of their associated host for parasite species infecting multiple host species. (_ _ _) indicates the 1:1 line. A: *Euryphorus brachypterus* infecting *Thunnus albacares*, *T. obesus*, *T. alalunga* and *T. maccoyii;* B: *Pseudocycnus appendiculatus* parasitizing *T. albacares*, *T. obesus* and *T. alalunga;* and C: *Hatschekia conifera* parasitizing *Thyrsites atun* and *Brama brama.*

Of the 18 host gill δ’^{13}C versus parasite species δ’^{13}C comparisons in total, seven showed significant enrichment in ^{13}C ($p<0.05$), four showed significant depletion ($p<0.05$) and seven showed no significant difference (Table 3.6; Figure 3.15). However, of the interactions which show no significant difference, two are close to the $P<0.05$ threshold ($p=0.05934$ for *Euryphorous brachypterus* parasitizing *Thunnus alalunga* and $p=0.06789$ for *Kroyeria carchariaeglauci* parasitizing *Prionace glauca*). In the cases of Monogenean sp. 1 parasitizing *Thunnus albacares, Mazocraes* sp. parasitizing *Thyrsites atun*, *Chondracanthus colligens* parasitizing *Genypterus capensis* and *Clavellisa ilishae* parasitizing *Sardinops sagax,* all the parasites were depleted in ^{13}C compared to their associated host gill δ’^{13}C but this could not be statistically confirmed (Figure 3.15A). *Cardiodectes medusaeus* is not a gill parasite and thus was not included in the above results, however it showed no significant difference in δ’^{13}C compared to *Lampanyctodes hectoris* heart tissue (Table 3.6; Figure 3.15A)

The Mann-Whitney U test comparing the carbon fractionation of *Hatschekia conifera* from *Thyrsites atun* and *Brama brama* indicated no significant difference ($U=2.00$; $n_{T.atun}=2$; $n_{B.brama}=6$; $p=0.2433$). The Kruskal-Wallis analysis comparing the δ’^{13}C fractionation of *Euryphorus brachypterus* from *Thunnus albacares, T. maccoyii, T. obesus* and *T. alalunga* indicated a significant difference between one or more of the host species ($H\ (3, n=47) =14.6022$; $p=0.0022$). Further *post-hoc* tests indicated that the difference lies with the parasites infecting *T. albacares,* which were significantly less enriched in ^{13}C compared to the other three host species which were not significantly different from one another (Figure 3.15B). The Kruskal-Wallis comparing the δ’^{13}C fractionation of *Pseudocycnus appendiculatus* from *Thunnus albacares, T. obesus* and *T. alalunga* indicated a significant difference between one or more of the host species ($H\ (2, n=22) =6.1125$; $p=0.0471$). However, *post-hoc* comparisons did not indicate where the difference lies, which is as a result of the small sample sizes and the non-parametric tests utilizing ranks and not comparing the actual values (Figure 3.15C).

Table 3.6: The results of the Wilcoxon signed-rank matched paired tests comparing the $\delta'^{13}C$ values of the parasites and the gill tissue of their associated hosts for which sufficient data were available for statistical analyses (i.e. excludes data for parasites of *G. capensis* and *S. sagax,* and for Monogenean sp. 1 parasitizing *T. albacares* and *Mazocraes* sp. parasitizing *T. atun*). In the case of *Cardiodectes medusaeus* infecting the heart of *Lampanyctodes hectoris,* although not a gill parasite is also included here compared to host heart tissue for later comparison. The table indicates the n values, Z-values, p-values, the mean enrichment/depletion (±SD) and whether the parasites showed enrichment, depletion or no significant difference in $\delta'^{13}C$ values. Tests were considered significant at p<0.05 and significant differences denoted by *.

Host Species	**Parasite species**	**Wilcoxon Matched Pairs Test**				
		n	**Z**	**p-value**	**Mean Enrichment/ Depletion (‰)**	**Enriched/Depleted/No Difference**
T. albacares	*P. appendiculatus*	6	2.201398	0.02771*	-0.847 ± 0.169	Depleted
	E. brachypterus	8	0.420084	0.67442	-0.117 ± 0.477	No Difference
T. maccoyii	*E. brachypterus*	6	1.991741	0.04640*	0.651 ± 0.543	Enriched
T. obesus	*E. brachypterus*	23	4.197264	0.00003*	0.570 ± 0.229	Enriched
	P. appendiculatus	3	1.603567	0.10881	-0.420 ± 0.270	No Difference
	Hexostoma sp.	15	3.350975	0.00081*	0.895 ± 0.688	Enriched
T. alalunga	*E. brachypterus*	10	1.885695	0.05934	0.391 ± 0.544	No Difference
	P. appendiculatus	13	3.179797	0.00147*	-0.522 ± 0.617	Depleted
I. oxyrinchus	*N. lamna lamna*	7	2.366432	0.01796*	1.652 ± 0.215	Enriched
P. glauca	*K. carchariaeglauci*	4	1.825742	0.06789	3.991 ± 0.678	No Difference
X. gladius	*T. adcoccineum*	4	1.095445	0.27332	-0.114 ± 0.209	No Difference
B. brama	*H. conifera*	6	2.201398	0.02771*	5.945 ± 1.464	Enriched
T. atun	*C. dakari*	5	2.022600	0.04312*	1.612 ± 0.367	Enriched
	H. conifera	2	1.341641	0.17971	2.166 ± 0.658	No Difference
S. lalandi	*Parabrachiella sp. 1*	8	2.240448	0.02506*	-0.516 ± 0.350	Depleted
S. sarda	*C. bonito*	14	3.295765	0.00098*	0.501 ± 0.348	Enriched
M. capensis	*A. merlucci*	2	0.447214	0.65472	0.246 ± 0.463	No Difference
T. capensis	*G. trachuri*	5	2.022600	0.04312*	-0.676 ± 0.415	Depleted
L. hectoris	*C. medusaeus*	11	0.266733	0.78968	0.018 ± 0.248	No Difference

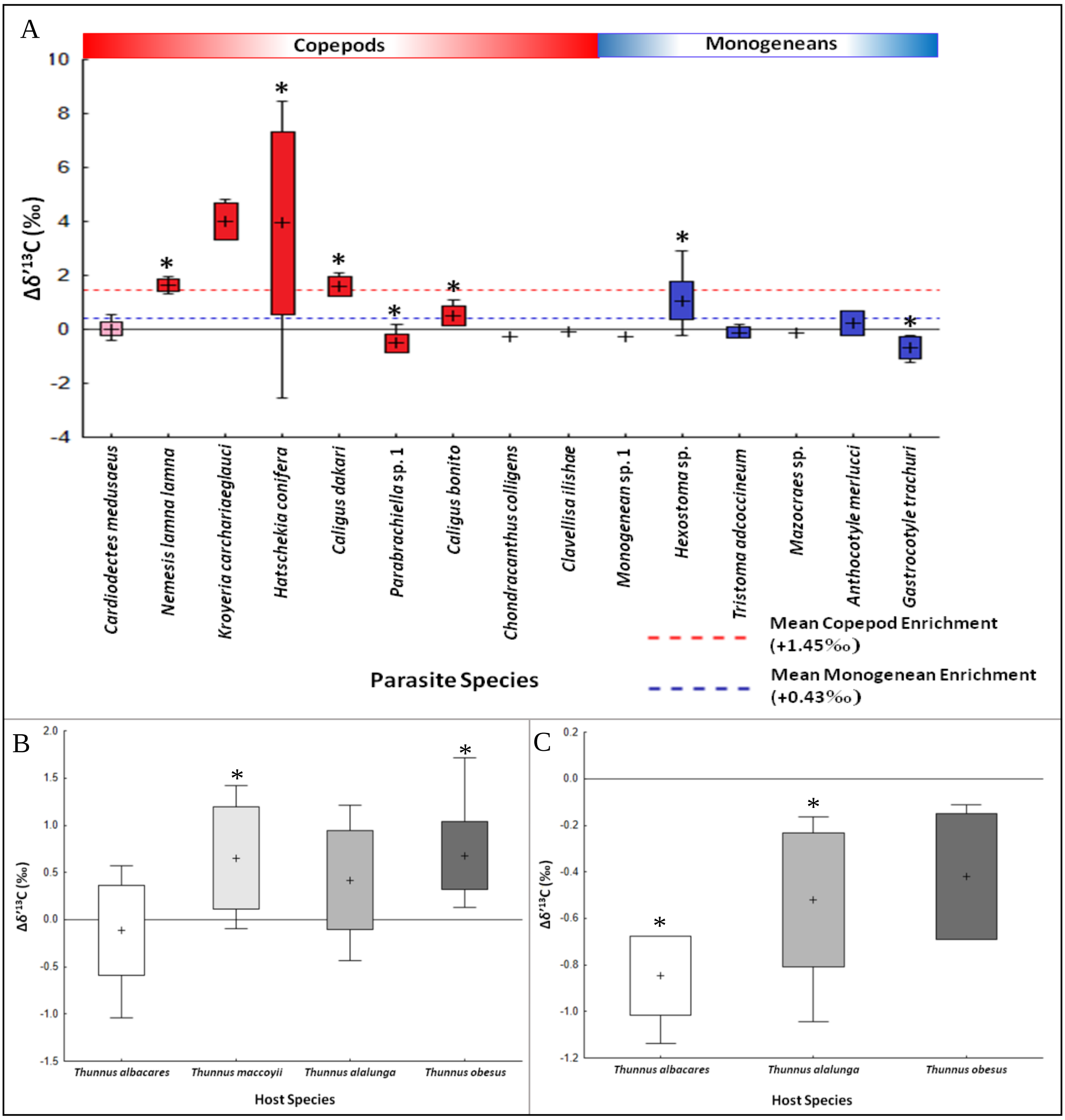

Figure 3.15: Mean trophic fractionation (Δδ’13C) for each parasite species/taxa analysed in this study. A: mean fractionation for each parasite species/taxa across all hosts on which that parasite was found. Copepod parasites are shown in red whilst monogenean parasites are shown in blue. Cardiodectes medusaeus (pink) found infecting the heart of *Lampanyctodes hectoris*, although not a gill parasite is also included here compared to its host heart tissue for later comparison. B: mean fractionation of *Euryphorus brachypterus* across its four host species. C: mean fractionation of *Pseudocycnus appendiculatus* across its three host species. (+) indicates the mean (or individual value if only one individual parasite or one group of pooled parasites was analysed), the box indicates the standard deviation, the whiskers indicate the minimum and maximum values and * indicates a significant difference between mean parasite and mean host gill $\delta'^{13}C$ values

3.3.6- Assessing whether individual parasite species are enriched compared to their associated hosts' blood

Eleven of a total of 19 comparisons between host blood $\delta^{15}N$, estimated from muscle $\delta^{15}N$ using data in Deudero *et al.* (2002), and parasite $\delta^{15}N$ values showed significant ($p<0.05$) enrichment of the parasite, with these comprising only of copepod-host interactions, one showed significant ($p<0.05$) depletion and seven showed no significant difference (Table 3.7; Figure 3.16). However, of the interactions which show no significant difference, three are close to the $P<0.05$ threshold ($p=0.06789$ for *Pseudocycnus appendiculatus* parasitizing *Thunnus albacares,* $p=0.06789$ for *Kroyeria carchariaeglauci* parasitizing *Prionace glauca* and $p=0.06789$ for *Tristoma adcoccineum* parasitizing *Xiphias gladius*). In the cases of *Mazocraes* sp. parasitizing *Thyrsites atun* and *Chondracanthus colligens* parasitizing *Genypterus capensis,* both parasites were enriched compared to their associated host blood $\delta^{15}N$, but this could not be statistically confirmed (Figure 3.16A). In the cases of *Kroyeria carchariaeglauci* parasitizing *Prionace glauca, Tristoma adcoccineum* parasitizing *Xiphias gladius, Anthocotyle merlucci* parasitizing *Merluccius capensis* and *Pseudocycnus appendiculatus* parasitizing *Thunnus albacares* and *T. obesus,* although appearing to show significant enrichment in ^{15}N compared to the host blood tissue (Figure 3.16), the statistical analysis returned a non-significant result. This can be attributed to a limited sample size. *Gastrocotyle trachuri* parasitizing *Trachurus capensis* showed a significant ($p<0.05$) depletion in ^{15}N compared to the host blood tissue and *Cardiodectes medusaeus,* although not a gill parasite and therefore was not included in the above results, also showed significant ($p<0.05$) depletion in ^{15}N with respect to *Lampanyctodes hectoris* blood tissue (Table 3.7; Figure 3.16).

The Mann-Whitney U test comparing the nitrogen fractionation of *Hatschekia conifera* from *Thyrsites atun* and *Brama brama* indicated no significant difference ($U=4.00$; $n_{T.atun}=2$; $n_{B.brama}$

=6; p=0.6171). The Kruskal-Wallis analysis comparing the $\delta^{15}N$ fractionation of *Euryphorus brachypterus* from *Thunnus albacares, T. maccoyii, T. obesus* and *T. alalunga* indicated a significant difference between one or more of the host species (H (3, n=43) =19.0224; p=0.0003). Further *post-hoc* tests indicated that the difference lies with the parasites infecting *T. albacares,* which were significantly more enriched in ^{15}N compared to *T. maccoyii* and *T. alalunga* (Figure 3.16B). *T. alalunga* was also significantly depleted in ^{15}N compared to *T. obesus* (Figure 3.16B). The Kruskal-Wallis comparing the $\delta^{15}N$ fractionation of *Pseudocycnus appendiculatus* from *Thunnus albacares, T. obesus* and *T. alalunga* indicated a significant difference between one or more of the host species (H (2, n=16) =9.6728; p=0.0079). Further *post-hoc* comparisons indicated that the difference lies with the parasites infecting *T. albacares* which were significantly more enriched in ^{15}N compared to the other two host species, which did not significantly differ from one another (Figure 3.16C).

Table 3.7: The results of the Wilcoxon signed-rank matched paired tests comparing the δ^{15}N values of the parasites and that estimated for the blood of their associated hosts for which sufficient data were available for statistical analyses (i.e. excludes data for parasites of *G. capensis* and *Mazocraes* sp. parasitizing *T. atun*). In the case of *Cardiodectes medusaeus* infecting the heart of *Lampanyctodes hectoris,* although not a gill parasite is also included here compared to host blood tissue for later comparison. The table indicates the n values, Z-values and p-values, the mean enrichment/depletion (±SD) and whether the parasites showed enrichment, depletion or no significant difference in δ^{15}N values. Tests were considered significant at $p<0.05$ and significant differences denoted by *.

Host Species	**Parasite Species**	**Wilcoxon Matched Pairs Test**				
		n	**Z**	**p-value**	**Mean Enrichment/ Depletion (‰)**	**Enriched/Depleted/No Difference**
T. albacares	*E. brachypterus*	7	2.366432	0.01796*	2.477 ± 0.480	Enriched
	P. appendiculatus	4	1.825742	0.06789	2.443 ± 0.410	No Difference
T. maccoyii	*E. brachypterus*	6	2.201398	0.02771*	1.276 ± 0.469	Enriched
T. obesus	*E. brachypterus*	23	4.197264	0.00003*	1.588 ± 0.675	Enriched
	P. appendiculatus	3	1.603567	0.10881	1.850 ± 0.776	No Difference
	Hexostoma sp.	15	2.839809	0.00451*	0.770 ± 0.804	Enriched
T. alalunga	*E. brachypterus*	7	2.366432	0.01796*	0.720 ± 0.265	Enriched
	P. appendiculatus	9	2.665570	0.00769*	1.004 ± 0.450	Enriched
I. oxyrinchus	*N. lamna lamna*	7	2.366432	0.01796*	2.142 ± 0.535	Enriched
P. glauca	*K. carchariaeglauci*	4	1.825742	0.06789	2.639 ± 0.503	No Difference
X. gladius	*T. adcoccineum*	4	1.825742	0.06789	2.874 ± 0.520	No Difference
B. brama	*H. conifera*	6	2.201398	0.02771*	3.529 ± 0.286	Enriched
T. atun	*C. dakari*	5	2.022600	0.04312*	2.258 ± 0.405	Enriched
	H. conifera	2	1.341641	0.17971	3.689 ± 0.200	No Difference
S. lalandi	*Parabrachiella* sp. 1	8	2.520504	0.01172*	2.698 ± 0.539	Enriched
S. sarda	*C. bonito*	14	3.295765	0.00098*	3.530 ± 0.668	Enriched
M. capensis	*A. merlucci*	2	1.341641	0.17971	1.991 ± 0.230	No Difference
S. sagax	*C. ilishae*	3	1.069045	0.28505	1.481 ± 2.080	No Difference
T. capensis	*G. trachuri*	5	2.022600	0.04312*	-1.489 ± 0.566	Depleted
L. hectoris	*C. medusaeus*	11	2.934058	0.00335*	-2.400 ± 0.299	Depleted

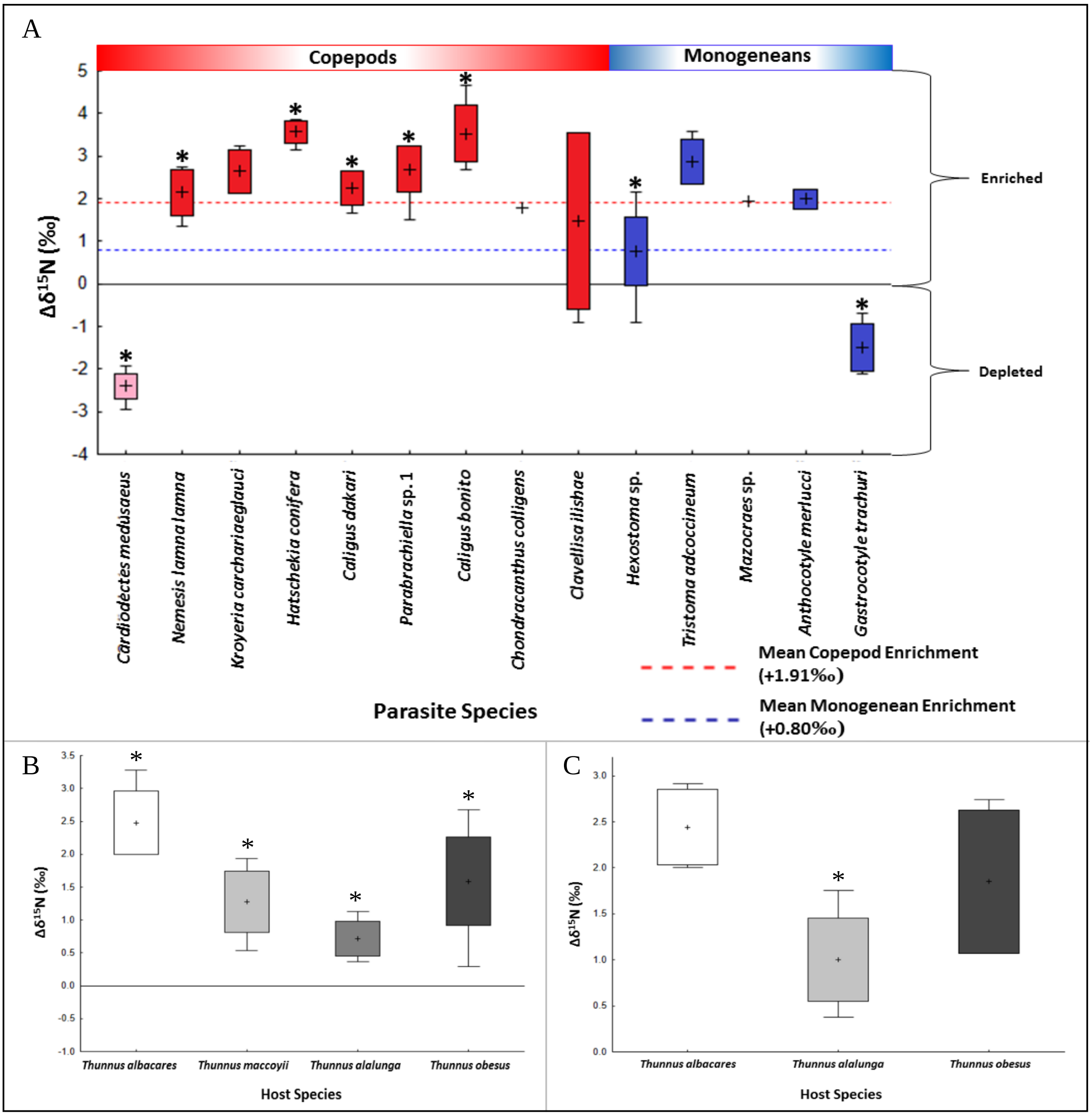

Figure 3.16: Mean trophic fractionation ($\Delta\delta^{15}N$) for each parasite species/taxa analysed in this study compared to their associated hosts' blood nitrogen isotopic signature. A: mean fractionation for each parasite species/taxa across all hosts on which that parasite was found. Copepod parasites are shown in red whilst monogenean parasites are shown in blue. Cardiodectes medusaeus (pink) found infecting the heart of *Lampanyctodes hectoris*, although not a gill parasite is also included here for later comparison. B: mean fractionation of *Euryphorus brachypterus* across its four host species. C: mean fractionation of *Pseudocycnus appendiculatus* across its three host species. (+) indicates the mean (or individual value if only one individual parasite or one group of pooled parasites was analysed), the box indicates the standard deviation, the whiskers indicate the minimum and maximum values and * indicates a significant difference between mean parasite and mean host blood $\delta^{15}N$ values

Of the 19 host blood $\delta'^{13}C$ versus parasite species $\delta'^{13}C$ comparisons in total, nine showed significant enrichment in ^{13}C ($p<0.05$), one showed significant depletion ($p<0.05$) and nine showed no significant difference (Table 3.8; Figure 3.17). However, of the interactions which show no significant difference, three are close to the $P<0.05$ threshold ($p=0.06789$ for *Pseudocycnus appendiculatus* parasitizing *Thunnus albacares,* $p=0.06789$ for *Kroyeria carchariaeglauci* parasitizing *Prionace glauca* and $p=0.06789$ for *Tristoma adcoccineum* parasitizing *Xiphias gladius*). In the cases of *Mazocraes* sp. parasitizing *Thyrsites atun* and *Chondracanthus colligens* parasitizing *Genypterus capensis,* both parasites were enriched in ^{13}C compared to their associated host blood $\delta'^{13}C$ but this could not be statistically confirmed (Figure 3.17A). *Cardiodectes medusaeus* is not a gill parasite and thus was not included in the above results, however it showed significant ($p<0.05$) depletion of ^{13}C compared to *Lampanyctodes hectoris* blood tissue (Table 3.8; Figure 3.17A)

The Mann-Whitney U test comparing the carbon fractionation of *Hatschekia conifera* from *Thyrsites atun* and *Brama brama* indicated no significant difference ($U=1.00$; $n_{T.atun}=2$; $n_{B.brama}=6$; $p=0.1336$). The Kruskal-Wallis analysis comparing the $\delta'^{13}C$ fractionation of *Euryphorus brachypterus* from *Thunnus albacares, T. maccoyii, T. obesus* and *T. alalunga* indicated a significant difference between one or more of the host species ($H\ (3, n=43) = 19.5787$; $p=0.0002$). Further *post-hoc* tests indicated that the difference lies with the parasites infecting *T. albacares,* which were significantly less enriched in ^{13}C compared to *T. maccoyii* and *T. obesus* but not significantly different from *T. alalunga,* whilst the other three host species which were not significantly different from one another (Figure 3.17B). The Kruskal-Wallis comparing the $\delta'^{13}C$ fractionation of *Pseudocycnus appendiculatus* from *Thunnus albacares, T. obesus* and *T. alalunga* indicated a significant difference between one or more of the host species ($H\ (2, n=16) = 9.2990$; $p=0.0096$). Further, *post-hoc* comparisons indicated that the difference lies with the parasites infecting *T. albacares* which generally showed greater depletion in ^{13}C compared to the other two hosts, which themselves were not significantly different from one another (Figure 3.17C).

Table 3.8: The results of the Wilcoxon signed-rank matched paired tests comparing the δ'^{13}C values of the parasites and that estimated for the blood of their associated hosts for which sufficient data were available for statistical analyses (i.e. excludes data for parasites of *G. capensis* and *Mazocraes* sp. parasitizing *T. atun*). In the case of *Cardiodectes medusaeus* infecting the heart of *Lampanyctodes hectoris*, although not a gill parasite is also included here compared to host blood tissue for later comparison. The table indicates the n values, Z-values and p-values, the mean enrichment/depletion (±SD) and whether the parasites showed enrichment, depletion or no significant difference in δ^{13}C values. Tests were considered significant at $p<0.05$ and significant differences denoted by *.

Host Species	**Parasite Species**	**Wilcoxon Matched Pairs Test**				
		n	**Z**	**p-value**	**Mean Enrichment/Depletion (‰)**	**Enriched/Depleted/No Difference**
T. albacares	*E. brachypterus*	7	1.352247	0.17630	0.271 ± 0.515	No Difference
	P. appendiculatus	4	1.825742	0.06789	-0.639 ± 0.692	No Difference
T. maccoyii	*E. brachypterus*	6	2.201398	0.02771*	1.527 ± 0.298	Enriched
T. obesus	*E. brachypterus*	23	4.197264	0.00003*	1.501 ± 0.492	Enriched
	P. appendiculatus	3	1.603567	0.10881	-0.153 ± 0.040	No Difference
	Hexostoma sp.	15	3.407771	0.00066*	1.833 ± 0.661	Enriched
T. alalunga	*E. brachypterus*	7	2.366432	0.01796*	0.993 ± 0.448	Enriched
	P. appendiculatus	9	0.533114	0.59396	0.075 ± 0.267	No Difference
I. oxyrinchus	*N. lamna lamna*	7	2.366432	0.01796*	2.177 ± 0.233	Enriched
P. glauca	*K. carchariaeglauci*	4	1.825742	0.06789	4.221 ± 0.710	No Difference
X. gladius	*T. adcoccineum*	4	1.825742	0.06789	1.255 ± 1.022	No Difference
B. brama	*H. conifera*	6	2.201398	0.02771*	6.285 ± 2.244	Enriched
T. atun	*C. dakari*	5	2.022600	0.04312*	1.906 ± 0.340	Enriched
	H. conifera	2	1.341641	0.17971	2.346 ± 0.758	No Difference
S. lalandi	*Parabrachiella* sp. 1	8	2.520504	0.01172*	1.008 ± 0.312	Enriched
S. sarda	*C. bonito*	14	3.295765	0.00098*	1.152 ± 0.402	Enriched
M. capensis	*A. merlucci*	2	1.341641	0.17971	0.732 ± 0.278	No Difference
S. sagax	*C. ilishae*	3	1.603567	0.10881	-1.801 ± 0.539	No Difference
T. capensis	*G. trachuri*	5	2.022600	0.04312*	-1.383 ± 0.252	Depleted
L. hectoris	*C. medusaeus*	11	2.845147	0.00444*	-0.578 ± 0.355	Depleted

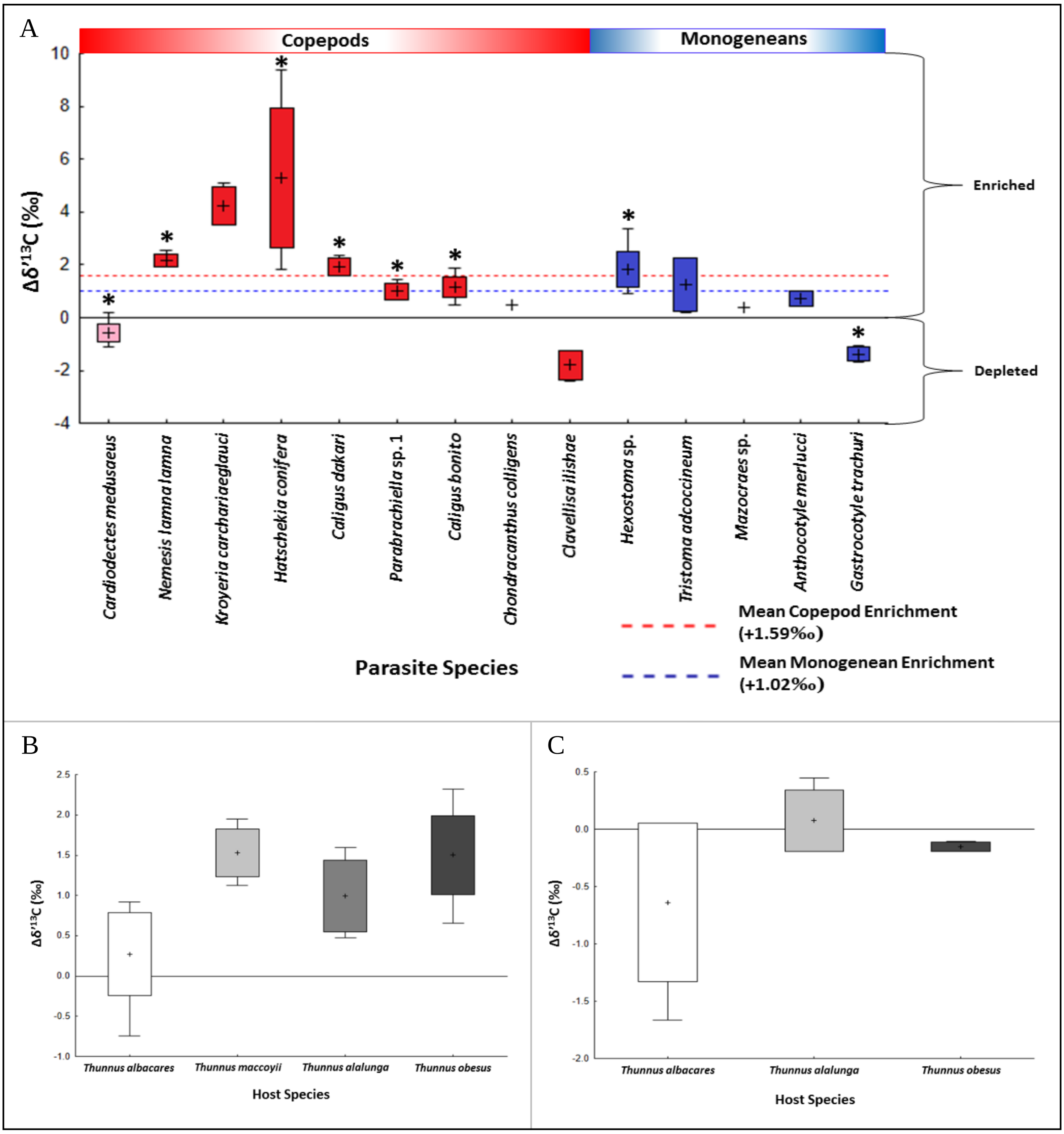

Figure 3.17: Mean trophic fractionation (Δδ'^{13}C) for each parasite species/taxa analysed in this study compared to their associated hosts' blood carbon isotopic signature. A: mean fractionation for each parasite species/taxa across all hosts on which that parasite was found. Copepod parasites are shown in red whilst monogenean parasites are shown in blue. Cardiodectes medusaeus (pink) found infecting the heart of *Lampanyctodes hectoris*, although not a gill parasite is also included here for later comparison. B: mean fractionation of *Euryphorus brachypterus* across its four host species. C: mean fractionation of *Pseudocycnus appendiculatus* across its three host species. (+) indicates the mean (or individual value if only one individual parasite or one group of pooled parasites was analysed), the box indicates the standard deviation, the whiskers indicate the minimum and maximum values and * indicates a significant difference between mean parasite and mean host blood δ^{13}C values

3.4- Discussion

This study is the first to investigate the trophic interactions between parasites and their South African marine fish hosts using stable isotope analysis, and compared the carbon and nitrogen stable isotope ratios of 17 species/taxa of gill parasites with the measured carbon and nitrogen stable isotope ratios of the gill and the estimated carbon and nitrogen stable isotope ratios of the blood tissues of their associated 15 fish hosts.

3.4.1- Fish species in iso-space

The bi-plot of host muscle δ^{15}N and δ'^{13}C (Figure 3.1) conforms to the typical pattern of interaction between predator and prey species whereby as one moves up the food-web the fish tend to become more enriched in ^{15}N, and the stable isotope ratios of the species examined in this study are mostly similar to values previously reported for some of these species. *Sardinops sagax* is a clupeoid, commonly eaten by pelagic predators in the Benguela Upwelling System (van der Lingen, 2002) and occupies a relatively low trophic level, as revealed by Figure 3.1. The isotopic signature of Benguela sardines obtained in this study are similar to those from other studies (van der Lingen and Miller, 2011; Moseley *et al.*, 2012), as is that for shallow-water hake *Merluccius capensis* (van der Lingen and Miller, 2014). Nitrogen stable isotope signatures of the two shark species (*Prionace glauca* and *Isurus oxyrinchus*) from this study are, however, somewhat lower than those previously reported (van der Lingen and Miller, 2011), but this may be attributed to the small sample sizes of both host species. As one moves up trophic levels, the larger predatory species tend to have higher values of both isotope ratios but also greater variation associated with their respective δ^{15}N and δ'^{13}C values. The middle-sized pelagic and demersal predatory species all appeared more enriched in ^{15}N as well as having different δ'^{13}C values compared to the larger pelagic predators (Figure 3.1). These differences likely reflect their different distributions, with the tuna and sharks being from the oceanic domain whereas the hakes, kingklip, snoek and Atlantic pomfret have a more coastal distribution (van der Elst, 1993;

Heemstra and Heemstra, 2014). This is supported by the variances of both $\delta'^{13}C$ and $\delta^{15}N$ for each host species whereby the larger pelagic species generally have a larger variances compared to the middle-sized pelagic, demersal and small pelagic species (Figure 3.2). The isotopic signature of an individual organism integrates a number of trophic interactions over time and space into a single value, and individuals within the various species that have a cosmopolitan distribution, undertake long migrations and have long life spans, such as the large pelagic species, will feed on many different food sources from different localities. Hence the wide dietary breadth of these migratory species may contribute to the variability observed in the carbon and nitrogen signatures. The middle-sized pelagic and demersal predatory species mostly do not undergo long-distance migrations (van der Elst, 1993; Heemstra and Heemstra, 2014), and likely remain within or near the Benguela Upwelling System for the majority of the year and thus feed off similar diet sources with similar isotopic ratios year round. Another plausible explanation for the variation in isotopic ratios of the fish species examined may be due to spatial influences, such as temperature and depth, on the basal food sources as these effects are known to influence stable isotope signatures (Polunin *et al.,* 2001; Barnes *et al.,* 2007; Bergmann *et al.,* 2009; Olson *et al.,* 2010). However, this is beyond the scope of this thesis as the variability in host stable isotope signatures is not of primary focus.

3.4.2- Comparing the $\delta^{15}N$ isotopic signatures of host gill and white muscle tissue

The analysis comparing the $\delta^{15}N$ values of host white muscle and gill tissue showed a significant difference, with host white muscle showing significantly higher enrichment in ^{15}N compared to host gill tissue (Figure 3.3). This difference in $\delta^{15}N$ of the white muscle tissue and gill tissue is a trend commonly observed in fish stable isotope studies (e.g. Serrano *et al.,* 2007; Banas *et al.,* 2009; Svensson *et al.,* 2014). Svensson *et al.* (2014) investigated the difference in isotopic ratios between gill and white muscle tissue on nine different species of fish of the coast of the

Netherlands and found that in majority of their samples the white muscle was generally more enriched in ^{15}N compared to the gill tissue. A similar result was obtained by Serrano *et al.* (2007) investigating the isotopic composition of farmed and wild gilthead sea bream tissues.

There are a number of proposed reasons as to why white muscle tissue is more enriched in ^{15}N compared to gill tissue. One such reason is that the white muscle tissue has a higher metabolic activity, and as a result, there is a higher turnover rate of nitrogen isotopes within the muscle compared to the gill. This was observed in a study by Xia *et al.* (2013) who investigated the turnover rates of nitrogen in various tissues of the grass carp, *Ctenopharyngodon idellus,* and found that the long-term ^{15}N enrichment of the gill tissue was primarily associated with the growth of the fish, with a very slow turnover rate of nitrogen associated with low metabolic activity. The white muscle tissue however, had a far higher turnover rate of nitrogen compared to the gill tissue, indicating that the white muscle tissue had a higher metabolic activity (Xia *et al.,* 2013). As the lighter ^{14}N isotope is more readily utilized and metabolized than the heavier ^{15}N, tissues having a higher turnover rate will have a higher sequestration of the ^{15}N, which explains the higher δ^{15}N signature in muscle than gill tissue (Fry, 2006).

Another reason why the δ^{15}N ratios of the gill and white muscle tissues differ can be attributed to varying amino acid compositions of the tissues, as various tissues are synthesized by different groups of proteins with varying amino acid combinations (Ostrowski and Divakaran, 1989; Ng and Hung, 1994; Wolf *et al.,* 2009). The δ^{15}N varies between amino acids, and thus varying proportions of individual amino acids pertaining to the different proteins that make up the various tissues will affect the δ^{15}N of the tissue as a whole (Wolf *et al.*, 2009).

The majority of predator-prey studies utilizing stable isotopes to investigate trophic interactions have leant toward using white muscle tissue as a proxy in order to determine the isotopic composition of large vertebrates such as fish (e.g. Vander Zanden *et al.*, 1999; Yoshii *et al.*, 1999; Thorburn *et al.,* 2014). This is understandable as white muscle tissue constitutes the majority of a fish’s total body mass (dos Santos *et al.,* 2004; Sumich and Morrissey, 2004), therefore when a predator consumes a prey item the largest proportion of the diet consists of the prey muscle tissue and thus the isotopic composition of the prey muscle tissue serves as a good proxy for the entire organism. Parasites, however, are highly selective of which host tissue they feed on and, unlike in typical predator-prey interactions, parasites do not consume their entire host (Lafferty *et al.,* 2008). With this in mind as well as the significantly different $\delta^{15}N$ signatures of the white muscle tissue compared to the gill tissue, it is clear that host gill tissue serves as a better proxy to test host-gill parasite trophic interactions than host muscle tissue. Hence, comparing the parasite’s $\delta^{15}N$ signature to the host white muscle tissue’s $\delta^{15}N$ in order to discern any enrichment pattern would be illogical, as the parasites are not feeding off the white muscle tissue and with the tissues’ higher $\delta^{15}N$ any enrichment of the parasite compared to the host may be obscured.

3.4.3- Comparing the $\delta^{15}N$ of parasites to their associated host gill and blood tissue $\delta^{15}N$

If the trophic interactions between parasites and their hosts conform to those typically observed between predators and their prey, one would expect an enrichment of ^{15}N in the parasite compared to the host (Minagawa and Wada, 1984). This however has not always been the case for previous parasite-fish host stable isotope studies. The majority of the few studies conducted to date have shown either no significant difference in $\delta^{15}N$ between the parasite taxa and their associated host tissues (Iken *et al.,* 2001; Demopoulos and Sikkel, 2015) or a depletion in ^{15}N of the parasite (Iken *et al.,* 2001; Pinnegar *et al.,* 2001; Deudero *et al.,* 2002; Nachev *et al.,* 2017), with significant enrichment in ^{15}N of the parasite seldom reported (Demopoulos and Sikkel, 2015; Nachev *et al.,* 2017).

The results of the present study have however shown that the majority of the gill parasites are enriched in ^{15}N compared to the host gill and blood tissues, with an overall mean enrichment in ^{15}N of +1.15 ± 1.00‰ and 1.66 ± 1.69‰, respectively. Therefore, the null hypothesis of no significant difference in $\delta^{15}N$ between parasites and their associated hosts is rejected. This enrichment pattern however, is not consistent across the two main parasite types analysed here: copepod parasites show a significantly higher mean enrichment compared to monogenean parasites with respect to host gill $\delta^{15}N$, with mean trophic fractionation values of +1.26 ± 0.98‰ and +0.60 ± 0.92‰, respectively (Figure 3.9 & 3.13). Therefore the null hypothesis that the trophic fractionation will not differ between gill copepod parasites and gill monogenean parasites with respect to their associated hosts, is also rejected.

The limited literature on the feeding mechanisms of the copepods at a species level precluded definitive identification of the feeding modes of the copepod parasites analysed in this study, hence only the two feeding subclasses of monogeneans could be compared. This comparison indicated that the monopisthocotyleans were more enriched in ^{15}N compared to the polyopisthocotyleans with respect to host gill $\delta^{15}N$ (mean trophic fractionations of +1.58 ± 1.32‰ and +0.38 ± 0.67‰, respectively; Figure 3.11) therefore the null hypothesis of no significant difference between enrichment values of the monopisthocotyleans and polyopisthocotyleans is rejected. A plausible explanation for the differences in enrichment values may be the differences in the isotopic signatures of their diets. Monopisthocotylean monogeneans feed by scraping and feeding off the host epithelial tissues (Buchmann and Lindenstrøm, 2002; Buchmann *et al.,* 2004; Hayward, 2005; Whittington, 2005; Rubio-Godoy, 2007; Goater *et al.,* 2014), whereas polyopisthocotylean monogeneans feed off the host blood by gently lacerating the gill epithelium to expose the blood in the capillary network beneath (Wootten, 2012). These different host tissues can have differing isotopic signatures, as was shown in a study by Deudero *et al.* (2002) where the blood tissue of *Melanogrammus* *aeglefinnus* was depleted in ^{15}N compared to the gill tissue. By using the muscle tissue to blood ratios for three species of fish (*Clupea harengus, Melanogrammus aeglefinnus* and *Eutrigla gurnardus*) analysed by Deudero *et al.* (2002) and assuming that muscle and blood tissue is consistently different across host species, an estimate of the blood nitrogen isotopic ratio was calculated for each host species examined in the present study (Table 3.3) and compared to their associated parasites. *Gastrocotyle trachuri,* a known polyopisthocotylean found parasitizing *Trachurus capensis,* is significantly depleted in ^{15}N compared to the estimated host blood nitrogen isotopic ratio (Table 3.7; Figure 3.16), whilst *Tristoma adcoccineum,* a known monopisthocotylean found parasitizing *Xiphias gladius,* is enriched in ^{15}N compared to the host gill tissue (Table 3.4; Figure 3.12). The blood-feeding polyopisthocotyleans generally showed less enrichment in ^{15}N with respect to host blood compared to the epithelial-feeding monopisthocotyleans, which generally showed a greater enrichment in ^{15}N compared to host gill (Tables 3.5 & 3.7; Figures 3.13 & 3.16A). Any deviations from this pattern may be as consequence of the major assumption that the muscle to blood isotopic ratios hold across all fish species, thus affecting the results.

As the present study has documented this difference in ^{15}N trophic fractionation values between blood-feeding polyopisthocotylean monogeneans and tissue-scraping monopisthocotylean monogeneans, fractionation patterns could possibly be used to infer the diet of a parasite if this pattern of fractionation holds across other parasite types. The trophic fractionation values of the Caligid copepods (*Euryphorus brachypterus, Caligus dakari* and *C. bonito*) analysed in the present study, which are known to feed by scraping off and consuming the epithelial tissues of the hosts' gill (Kabata, 1974), can be compared to those of a known copepod blood-feeder, *Cardiodectes medusaeus* which feeds by protruding a section of its cephalothorax directly into the host's heart and consuming its blood (Perkins, 1983; 1985). All three tissue-scraping caligid copepods are significantly enriched in ^{15}N compared to their associated hosts' gill tissues (Table

3.5; Figure 3.13), whilst the blood-feeding *C. medusaeus* is significantly depleted in ^{15}N compared to the hosts' blood tissue (Table 3.7; Figure 3.16A). Therefore, the application of stable isotope fractionation values has the potential to be used to infer parasite diets, however further analyses of a broader range of parasite types as well as the empirical analyses of host tissues, such as blood, will be required to confirm this.

At a species-specific level, the null hypothesis that there would be no significant differences in $\delta^{15}N$ values of the parasite compared to $\delta^{15}N$ values of the host gill tissues was rejected. Of the 22 host-parasite interactions examined, 13 showed enrichment in ^{15}N compared to their associated host gill tissue (Table 3.5; Figure 3.13), and 11 showed enrichment and one showed depletion to their associated hosts' estimated blood tissue (Table 3.7; Figure 3.16). Only three studies have previously investigated the trophic interactions of gill parasites and their hosts (Table 3.9). These studies have found confounding results with the majority of host-parasite interactions showing no significant difference between the isotope signatures of the hosts compared to those of their associated gill parasites (Pinnegar *et al.*, 2001; Deudero *et al.*, 2002). Iken *et al.* (2001) did however observe an enrichment pattern from host to parasite when comparing their $\delta^{15}N$ isotopic signatures, with an observed mean enrichment in ^{15}N of +2.8‰.

Table 3.9: Summary of past studies which have investigated host-parasite interactions using stable isotope analysis focusing on teleost gill parasites. * indicates cases whereby the sample size limited statistical analyses between host $\delta^{15}N$ and parasite $\delta^{15}N$.

Host	**Host Proxy Tissue**	**Parasite**	**n**	**Change of $\delta^{15}N$ isotopic ratio compared to host**	**Reference**
Coryphaenoides armatus	Muscle	Copepod sp.	10x2	Enriched	Iken *et al.*, 2001
Gadus morhua	Muscle	*Clavella adunca*	1	Depleted*	Deudero *et al.*, 2002
Gadus morhua	Muscle	*Laernaeocera branchialis*	2	No Difference*	Deudero *et al.*, 2002
Melanogrammus aeglefinus	Muscle	*Laernaeocera branchialis*	3	No Difference	Deudero *et al.*, 2002
Merlangius merlangus	Muscle	*Clavella adunca*	5	No Difference	Deudero *et al.*, 2002
Merlangius merlangus	Muscle	*Laernaeocera branchialis*	6	No Difference	Deudero *et al.*, 2002
Merlangius merlangus	Muscle	Copepod sp.	2	Depleted*	Deudero *et al.*, 2002
Platichthys flesus	Muscle	*Laernaeocera branchialis*	3	No Difference	Pinnegar *et al.*, 2001

Deudero *et al.* (2002) and Pinnegar *et al.* (2001) attributed their results to the high selectivity shown by many parasites in what they feed on and assimilate. Many parasites have a limited ability to manufacture amino acids (Power and Klein, 2004), and thus rely on the direct assimilation of amino acids from the host tissue on which they feed (Deudero *et al.,* 2002). However, many of these essential amino acids are depleted in ^{15}N compared to the δ^{15}N of the host tissue (Hare, 1991). Therefore, when comparing the isotopic signatures of the parasites to their host tissues, there would be a lack of enrichment due to the depleted amino acid source.

However, the variation in these results may also be attributed to the sample sizes of the studies as many lacked a sufficient sample size to statistically analyse the differences in isotopic signatures between the parasites and their hosts (Table 3.9). Of the four interactions that did have a large enough sample size, two had the bottom limit of n=3 (Table 3.9). Whereas the present study had small sample sizes for a few of the host-parasite interactions, the majority had a large enough sample size to allow statistical comparisons of the δ^{15}N of the parasites to the host gill tissue and blood tissue, and these provide convincing results of enrichment (Table 3.5 & 3.7).

Another likely reason for the differences in the results of the previous studies can be attributed to the host tissue used as a proxy to obtain a δ^{15}N value from the host organism. All of the studies cited above used host muscle tissue, which is a common proxy to use in stable isotope studies of trophic interactions. However, the gill parasites parasitize and obtain nutrients from the gill tissue, which shows a significant depletion in ^{15}N compared to the host muscle tissue in both this (Figure 3.3) and previous studies (Deudero *et al.,* 2002; Serrano *et al.,* 2007; Banas *et al.,* 2009; Svensson *et al.,* 2014). In addition, many gill parasites, such as the polyopisthocotylean monogeneans, feed off their associated hosts' blood tissue, which can also be depleted in ^{15}N compared to host white muscle tissue (Deudero *et al.,* 2002). Therefore, as white muscle is generally more enriched in ^{15}N compared to both the gill and blood tissue, the difference between the white muscle $\delta^{15}N$ and parasite $\delta^{15}N$ will be less than the difference between the gill and blood $\delta^{15}N$ and the parasite $\delta^{15}N$, leading to the contrasting results between the present study and past studies.

The results of this study corroborate Doucett *et al.* (1999), who highlighted that parasites should be enriched compared to their diets, and thus conform to the typical enrichment patterns observed between predators and their prey. However, the magnitudes of enrichment expressed by the enriched parasites in this study were all less than that obtained by Doucett *et al.* (1999), who obtained enrichment values of +3.5‰ when comparing the isotopic values of *Nanocladius* chironomid larvae parasitizing nymphs of the knobbed salmonfly *Pteronarcys biloba*. This difference may be due to the relative trophic position of the hosts examined in the two studies, with the nymphs analysed by Doucett *et al.* (1999) occupying the first trophic level as primary consumers and feeding off particulate organic matter. Hussey *et al.* (2014) investigated the trophic positioning of organisms relative to their $\delta^{15}N$ by modelling various food chains (see Chapter 1; Section 1.4.2), and reported that the enrichment of ^{15}N does not increase by 3.4‰ per trophic level in a linear fashion but rather is scaled relative to the trophic position of the animal, with enrichment between the lower trophic levels being greater than 3.4‰ and that between organisms at higher trophic levels being less than 3.4‰ (Figure 1.6). A similar decreasing trend in enrichment with increasing trophic level was observed in the present study when examining the enriched copepods, which showed higher enrichment when parasitizing lower trophic level hosts compared to those parasitizing higher trophic level hosts (Figure 3.18). For example, although the data for *Clavellisa ilishae* only consist of a single value due to its small sample size and low prevalence on *Sardinops sagax* which resulted in the need to combine individual parasites and precluded statistical analyses, it had a high enrichment in ^{15}N of +3.41‰. In contrast, *Pseudocycnus appendiculatus* and *Euryphorus brachypterus*, both of which are commonly found parasitizing the predatory *Thunnus* species, had much lower mean enrichment values of +0.93‰ and +0.74‰, respectively. Three of the four parasites infecting the middle-sized pelagic fish had mean enrichment values of between +2.31 and +2.85‰, although one (*Caligus dakari* found in *Thyrsites atun*) had the lowest mean enrichment value of any of the significantly enriched copepod gill parasites (Figure 3.18). These results provide a direct corroboration of the observation of Hussey *et al.* (2014) that enrichment in ^{15}N between successive trophic levels decreases from lower to higher levels.

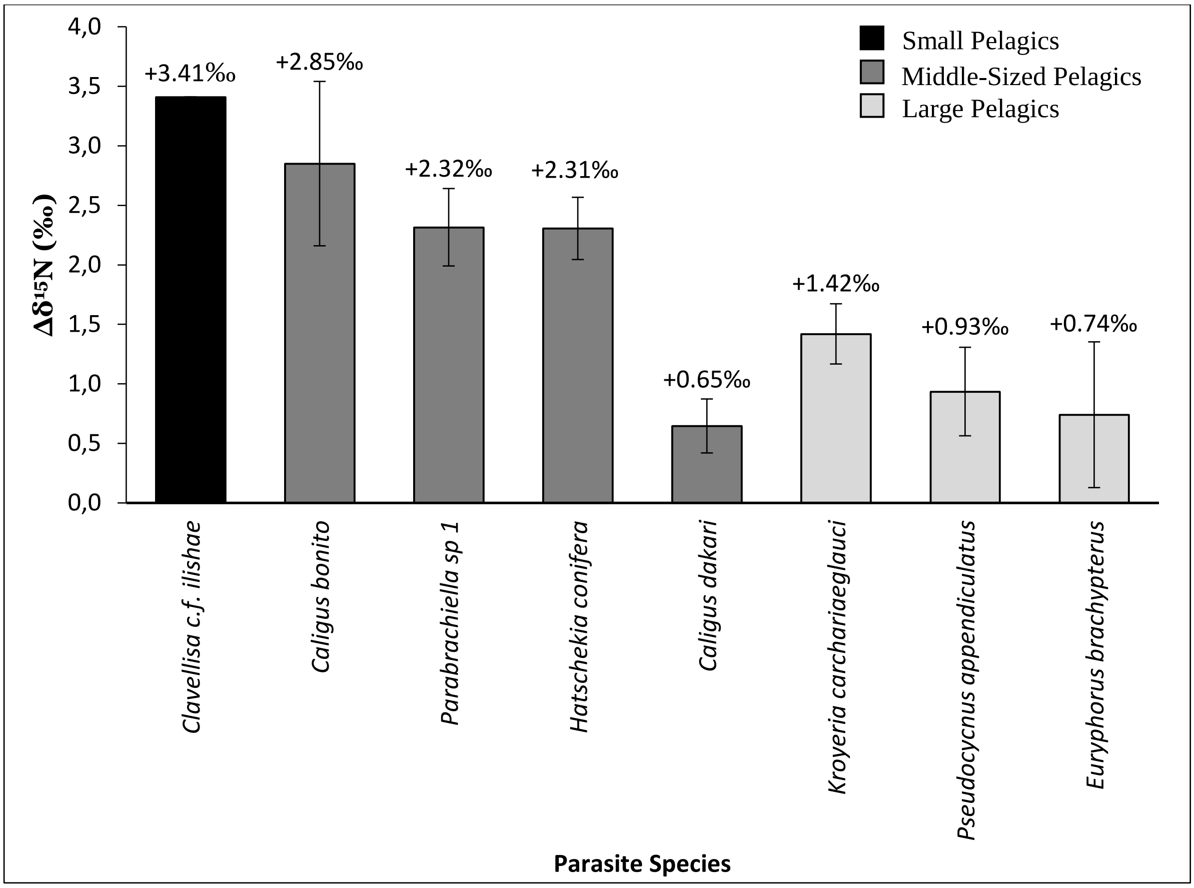

Figure 3.18: The mean change in nitrogen trophic fractionation ($\Delta\delta^{15}$N) of the significantly enriched copepod parasites compared to the host gill (δ^{15}Nparasite - δ^{15}N host gill). The parasites are ordered in terms of the relative trophic position of their hosts.

Lafferty *et al.* (2008) stated that due to the confounding results of many previous studies investigating host-parasite interactions using stable isotopes, with some showing enrichment in ^{15}N of the parasite compared to the associated host, some showing depletion and some showing no significant difference, the use of δ^{15}N to determine a parasite's trophic level may be challenging. Hence, the authors suggested that in order to determine the trophic level of a parasite, the assessment of how the parasite affects the food-web topology as a whole by incorporating parasite-host interactions into simple food-web matrices will be the best approach. This study however, has convincingly shown that the analysis of stable isotopes can be applied to host-parasite interactions as a plausible method to discern parasite trophic levels with the majority of the parasites in the present study showing enrichment in ^{15}N compared to their associated hosts. Hence, these parasites are appearing at higher trophic levels and can therefore be considered as the hosts' "predators". However, all parasites draw resources from their hosts and therefore, whether enriched in ^{15}N or not, are theoretically predators. Since the present study utilizes the trophic fractionation of ^{15}N as a sole indicator of trophic level position, the results of this study also indicate that the use stable isotopes to distinguish trophic levels does not necessarily work in all cases. Therefore in cases whereby the parasite is not distinctly enriched in ^{15}N compared to the host, other methods should be employed in conjunction with stable isotopes to investigate these host-parasite trophic interactions. These methods need to take into account the site and feeding specificity of parasites as the present study also highlights the importance of analysing the host tissue that the parasite actually is feeding on order for accurate comparisons to be made.

3.4.4- Comparing the δ'^{13}C isotopic signatures of host gill and white muscle tissue

The analysis comparing host white muscle δ'^{13}C and host gill δ'^{13}C showed a significant difference, with the host gill showing significantly higher enrichment in ^{13}C compared to the white muscle tissue (Figure 3.5), thus rejecting the null hypothesis and accepting the alternate hypothesis. A similar result was found by Svensson *et al.* (2014) who compared the isotopic signatures of gill and white muscle tissue of fish. Those authors found that the majority of gill-white muscle tissue comparisons showed no significant difference in their δ^{13}C values, however there were a number of species which showed a higher enrichment of ^{13}C in the gill tissue compared to that of the white muscle tissue with a differences in carbon isotopic signatures ranging from 0.01‰ to 2.2‰. This was attributed to high content of ^{13}C depleted lipids in the gill tissues compared to the muscle tissues (Svensson *et al.,* 2014).

3.4.5- Comparing the δ'^{13}C of parasites to their associated host gill tissue δ'^{13}C

Trophic fractionation of carbon between predators and their prey typically ranges from -3 to +4‰ with an overall mean value of 0.4 ± 1.3‰ (Post, 2002). This very small fractionation in δ^{13}C enables ecologists to identify source production as producers can have varying δ^{13}C signatures which can be traced through food-webs (DeNiro and Epstein, 1978; Michener and Schell, 1994).

The results of the present study showed a significant overall enrichment in ^{13}C of the parasites compared to their associated host gill and blood tissues (Figures 3.15 & 3.17) with an overall mean trophic fractionations of +0.63 ± 1.47‰ and +1.39 ± 1.93‰, respectively. This rejects the null hypothesis that there would be no significant differences in the δ'^{13}C signatures of parasites compared to their associated host tissue proxy and accepts the alternate hypothesis. This fractionation pattern conforms to that of typical predator-prey interactions whereby there is only a slight enrichment in ^{13}C when comparing the resource to the consumer (Post, 2002).

Parasite type did not seem to affect the fractionation mean carbon when comparing the trophic fractionation of copepods and monogeneans in general with respect to host gill δ'^{13}C, as there was no significant difference between the two, thus accepting the null hypothesis (Figure 3.10). Nor did the feeding mode of the monogeneans seem to affect the fractionation, as monopisthocotylean and polyopisthocotylean monogeneans showed no significant differences in their fractionation values with respect to host gill $\delta'^{13}C$, thus accepting the null hypothesis (Figure 3.12).

At a species-specific level, the null hypothesis that there would be no significant differences in $\delta'^{13}C$ when comparing the $\delta'^{13}C$ of the parasite to the $\delta'^{13}C$ of the host tissue proxy was rejected since of the 18 host gill-parasite interactions analysed, 4 showed significant depletion of ^{13}C in the parasite, seven showed no significant differences in $\delta'^{13}C$ between the parasites and their hosts and seven showed significant enrichment in ^{13}C (Table 3.6; Figure 3.15) whilst of the 19 host blood-parasite interactions analysed, one showed significant depletion, nine showed no significant differences and nine showed significant enrichment (Table 3.8; Figure 3.17). Although *Kroyeria carchariaeglauci* appears to be highly enriched in ^{13}C compared to its host gill and blood tissues (Figure 3.15A & 3.17A), these were not significant due to the small sample size. Similarly, *Pseudocycnus appendiculatus* infecting *Thunnus obesus* shows depletion in ^{13}C, but these too were not significant due to a limited sample size (Figures 3.15C & 3.17C).

Over 77% (77.12%; n=271) of all the samples analysed had a C:N greater than 3.5, and of the 147 parasite samples analysed 146 required the mathematical lipid correction equation to be applied due to high lipid content (Table 3.4). The present study applied the lipid correction equation proposed by Post *et al.* (2007) to account for the high lipid content in the majority of samples. Post *et al.* (2007) compared the $\Delta\delta^{13}C$ against the C:N ratio of 16 different species of aquatic organisms with varying lipid content and produced a highly significant ($p<0.001$) linear regression with an R^2 value of 0.907, which should be relatively accurate in correcting the $\delta^{13}C$ values (Post *et al.*, 2007). As the trophic fractionation of carbon ranges between -3‰ to +4‰ (Post, 2002), the mathematical lipid correction equation appears to have worked for the majority of the analysed samples as their associated fractionation values fall within this range (Figure 3.15). However, the only possible way in which to confirm whether the mathematical corrections have accurately accounted for the lipids would be to compare the mathematically corrected carbon signatures to samples which have had the lipids manually extracted. It is unlikely, however, that the mathematical correction was successful in correcting for high lipid content in all of the samples analysed, especially those of parasites for which the C:N was greater than 6.9. This value was considered by Post *et al.* (2007) to be the upper limit of applicability for that equation and that extrapolating the regression equation beyond a C:N of 6.9 may result in inaccuracies as the relationship between C:N and $\delta^{13}C$ may become non-linear. Samples for which the equation likely unsuccessfully corrected for lipids included *Hatschekia conifera* found infecting *Brama brama* and *Thyrsites atun*, as well as *Kroyeria carchariaeglauci* found infecting *Prionace glauca*. The mean trophic fractionation of these parasites, even after they had been corrected for lipids, fell outside of the -3‰ to +4‰ expected range and this may be as a result of their very high lipid contents (mean C:N of 13.20 for *H. conifera* parasitizing *B. brama,* 7.58 for *H. conifera* parasitizing *T. atun* and 7.57 for *K. carchariaeglauci* parasitizing *P. glauca*) and hence inaccurate mathematical correction. The C:N ratios of the other parasite samples fell below this 6.9 threshold (Table 3.4) and thus the equation could be successfully applied. In addition, the species for which the C:N exceeds 6.9 have a very high lipid content but lipids are depleted in ^{13}C (Post *et al.,* 2007), hence these parasites should be depleted in ^{13}C compared to their respective hosts. This however is not the case as both *H. conifera* and *K. carchariaeglauci* are highly enriched in ^{13}C compared to their host gill tissue (Figures 3.15A & 3.17A).

There have been multiple mathematical correction equations proposed to remove the effect of lipids and correct the $\delta^{13}C$ signature (McConnaughey and McRoy, 1979; Kiljunen *et al.*, 2006; Sweeting *et al.,* 2006; Post *et al.,* 2007), but some of these mathematical corrections have been heavily criticized due to multiple assumptions having to be made across species and the majority of these proposed equations having focused on aquatic species (Sweeting *et al.,* 2006). de Lecea and de Charmoy (2015) investigated whether it is better to chemically extract lipids from samples or if mathematical corrections will suffice in order to provide an accurate $\delta^{13}C$

signature. They analysed various tissues of eight marine organisms ranging from teleost and elasmobranch fish to marine invertebrates, corrected the $\delta^{13}C$ value using three chemical extraction methods, and compared the results to five proposed mathematical correction equations (de Lecea and de Charmoy, 2015). It was concluded that the utilization of mathematical models cannot be accurately applied to correct for lipids and the best method is to chemically extract the lipids and then analyse a treated and untreated sample to attain accurate $\delta^{13}C$ and $\delta^{15}N$, respectively (de Lecea and de Charmoy, 2015). This being said, the mathematical correction equation produced by Post *et al.* (2007) appears to have successfully accounted for lipids in the majority of samples analysed in the present study, but mathematical corrections in general should be approached with caution especially when the samples in question have very high lipid content. Therefore, if it is logistically and economically viable, manual lipid extraction and the analysis of a treated sample will provide a more accurate $\delta^{13}C$ signature.

Of the samples that fell below the 6.9 C:N threshold and for which the mathematical correction appeared to have worked, there was still variability in the fractionation patterns between species (Figures 3.15 & 3.17). This variability may be attributed to the high selectivity of parasites on what they feed, and those that have a lipid-rich diet are often depleted in ^{13}C as lipids themselves are depleted in ^{13}C compared to the whole tissue (Deudero *et al.,* 2002, Post *et al.,* 2007; Demopoulos and Sikkel, 2015). However, due to the high lipid content of many of the parasites and the lack of lipid extracted samples with which to confirm the accuracy of the mathematical corrections, further biological inferences could not be drawn to avoid misinterpretation.

Conclusion

The results of the present study have provided strong evidence for a pattern of general enrichment in the ^{15}N of gill parasites compared to their associated host gill and blood tissues. However, this enrichment is not consistent across all gill parasite taxa examined, with parasite type, parasite diet and relative trophic level of the host having an effect on the trophic fractionation of ^{15}N. High lipid content affected the carbon signatures of the majority of the samples analysed thus limiting the inferences that could be drawn in this study, highlighting that the prior manual extraction of lipids is important when measuring $\delta^{13}C$.

These results lend themselves toward a better understanding of host-parasite trophic interactions which, once a comprehensive understanding is achieved, will enable ecologists to incorporate parasites into food-web models and ecological studies, providing a far better representation of species within a community and a better understanding of how organisms within a community interact on a trophic basis.

3.5- References

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Chapter 4: Summary and recommendations

The current study has documented the head ectoparasite assemblage of 17 species of marine fish ranging from large apex predators to small pelagic species caught within South African waters. Thirty-two parasite taxa were found comprising two new host records and 15 new locality records, thereby further contributing to the known parasite biodiversity within the South African marine environment.

The host-parasite trophic interactions of 15 species of fish and their copepod and monogenean gill parasites were assessed by means of stable isotope analysis employing carbon and nitrogen isotopes. Analyses indicated that the host fish nitrogen isotopic signature was tissue specific with white muscle tissue showing a higher enrichment in ^{15}N compared to the gill tissue, as was also reported by Serrano *et al.*(2007), Banas *et al.* (2009) and Svensson *et al.* (2014). Because of this difference and because gill parasites feed off the gills or associated tissue, host gill tissue was selected as the host proxy tissue for stable isotope measurement, and the isotopic signature of host blood was estimated from white muscle signatures, for trophic comparisons. Most of the parasites showed enrichment in ^{15}N compared to host gill and blood tissues. Although a general pattern of enrichment was observed, there was a significant difference in the nitrogen trophic fractionation values between copepods and monogeneans and their respective host gill tissue. There was also a significant difference between the nitrogen trophic fractionation values of the two feeding modes of monogenean parasites examined, with the tissue-scraping monopisthocotyleans showing significantly greater enrichment compared to the blood-feeding polyopisthocotyleans with respect to their host gill tissue. Hence, parasite type and parasite diet can have an influence on their respective nitrogen isotopic signatures, as was previously reported for cestodes and trematodes by Boag *et al.* (1998), and trematodes and nematodes by Iken *et al.* (2001). This study indicated that the relative trophic level of the hosts also appears to play a role in trophic fractionation, with enrichment values decreasing as the trophic level of the host increases. This finding provides a direct corroboration that trophic fractionation is not linear across food-webs but is scaled relative to trophic level as suggested by Hussey *et al.* (2014).

The majority of both fish host and parasite samples required mathematical correction of the $\delta^{13}C$ values due to high lipid content. The correction appeared to have successfully accounted for lipids in the majority of these, which fell below the upper threshold of a C:N ratio of 6.9 for the correction equation to be applicable (Post *et al.*, 2007). However, whether the corrections sufficiently accounted for high lipid content could not be verified due to a lack of lipid-extracted samples with which to compare the mathematical corrections, thus prohibiting any further biological inferences from being drawn regarding the enrichment of ^{13}C between parasites and their hosts and emphasizing the necessity for manual lipid extraction.

By using stable isotope analysis and other such methods, such as fatty acid analysis, to investigate host-parasite trophic interactions we can begin to better incorporate parasites into food-web models, enabling a more comprehensive understanding of community interactions and ecosystems as a whole.

Limitations

Although every attempt was made to be as thorough as possible during the present study, there were a few limitations.

Firstly, a number of monogenean parasites could not be identified using morphological characteristics because these become indistinct if the sample has been frozen. As the second chapter focuses on the copepod and monogenean gill parasite assemblage of several marine fish hosts from South Africa, comparisons and the identification of new geographic or host records was not possible for these unidentified species. Identification of monogeneans from morphological characteristics can be difficult even in fresh specimens, therefore in order to correctly identify the monogeneans down to the lowest possible level of classification one could employ molecular characterizations as demonstrated by Christison and Baker (2007) who compared genetic sequences of monogenean parasite samples to those uploaded on the National Center for Biotechnology Information BLAST database.

Every attempt was made to have sufficiently large sample sizes to statistically compare the isotopic signatures, but this could not be achieved in all cases. Increasing the sample sizes, especially regarding the small pelagic species and their associated parasites, will provide more definitive results. This however, would require the sampling of a greater number of individual host specimens as the prevalence and infection intensities of those parasites were generally very low.

In order to obtain accurate $\delta^{13}C$ signatures of both parasite and host tissue, the manual extraction of lipids is required. The mathematical corrections appeared to have worked for the majority of the samples, but as multiple other studies have indicated, mathematical corrections may not always provide accurate results, especially regarding samples that have very high lipid content.

The Way Forward

Very little literature was available on the feeding modes of many of the copepods, but trophic fractionation from host to parasite varied among the monogeneans and differed between tissue-scraping and blood-feeding subclasses. Future studies should investigate how the copepod parasites are feeding and on what tissues they are feeding, as well as assess the host pathology. This will aid in comprehensively understanding the parasite- host trophic interactions as well as gain valuable insight into the potential effects that the parasite infections may have on their hosts. These studies could then be incorporated into stable isotope studies to identify the most appropriate host tissue proxy with which to compare the isotopic signatures of the parasite. Another refinement for future studies would be to analyse the carbon and nitrogen isotopic signatures of multiple host tissues including host gill tissue, blood and mucus. These could then utilized to hypothesize the dietary composition of the parasites, adding further insight into their feeding mechanisms and biology.

The present study is highly focused, comparing the parasite isotopic ratios of copepod and mongenean gill parasites to that of their respective host gill tissue. Therefore, further studies should investigate parasite-host trophic interactions with other parasites including endoparasites, ensuring that isotopic analysis is conducted on the tissue on which the parasite feeds (or is presumed to feed) in order to discern possible enrichment patterns.

The application of stable isotope analysis to determine isotopic signatures and the fractionation of nitrogen to infer the diet of a parasite appears to be highly plausible. Therefore future studies could investigate this further by looking at a broader range of parasites and host tissues. A possible future study would be to investigate these host-parasite interactions using experimentally-held or farmed fish hosts and analyzing their isotopic ratios. There was variation in the isotopic signatures of individuals within single host species in which can be attributed to the individual hosts in the present study having been wild caught and thus each having consumed varying food sources. By analysing hosts that have been fed a constant food source with a relatively constant isotopic signature, the variation in the host isotope ratios would be reduced. This lends itself toward a better comparison between host and parasite as external influences such as varying diet which have an effect on the host’s isotopic signature would be limited.

The present study has provided additional steps toward improving the incorporation of parasites into food-web models, which when achieved, will provide biologists and scientists alike a far more realistic idea of how communities are really structured based on their trophic interactions.

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Appendices

Appendix 1: Parasite Identification Literature

Appendix 1A: Literature used for copepod parasite identification

Species	**Reference**
Euryphorus brachypterus	Pillai, 1985
Caligus bonito	Pillai, 1985
Caligus dakari	Boxshall and El-Rashidy, 2009
Caligus aesopus	Jones, 1988
Chondracanthus colligens	Barnard, 1955b
Eobrachiella elegans	Ho and Do, 1984
Parabrachiella sp. 1	Barnard, 1955a
Parabrachiella sp. 2	Barnard, 1955a
Pseudocycnus appendiculatus	Pillai, 1985
Nemesis lamna lamna	Pillai, 1985
Hatschekia conifera	Kabata, 1988
Kroyeria carchariaeglauci	Izawa, 2008
Cardiodectes medusaeus	Perkins, 1983; Barnard 1955b
Clavellisa ilishae	El-Rashidy and Boxshall, 2010
Nothobomolochus fradei	El-Rashidy and Boxshall, 2010

Appendix 1B: Literature used for monogenean parasite identification

Species	**Reference**
Anthocotyle merlucci	Akmirza, 2013
Gastrocotyle trachuri	Jones, 1933
Hexostoma sp.	Price, 1961
Tristoma adcoccineum	Chisholm and Whittington, 2007
Nasicola klawei	Kohn *et al.*, 2004
Mazocraes sp.	Chauhan, 1950

Appendix 2: Parasite Assemblage Raw Data

Appendix 2A: The gill parasite assemblage of *Thunnus albacares*

ID	**Head Length (cm)**	**Fork Length (cm)**	***Euryphorus brachypterus***	***Pseudocycnus appendiculatus***	***Caligus* sp. 1**	***Hexostoma* sp.**	**Monogeneaen sp. 1**	***Nasicola klawei***	**Monogenean sp. 2**
Y-11-36			1	3	0	0	4	0	0
04,03,001	26,3		1	1	0	0	0	5	0
140,01,22	31		11	0	0	0	0	0	0
140,5,63	33		6	6	0	0	0	2	0
140,5,68	25,9		1	0	0	0	0	3	0
140,5,46	26,5	99	0	0	0	0	0	4	0
140,5,6	26		0	0	0	0	0	0	0
140,05,021		107	0	1	0	0	0	0	1
140,5,46			0	3	0	0	0	0	2
140,5,80	26,2		0	2	0	0	0	0	0
140,5,098		97	0	4	0	0	0	0	0
140,5,18		125	3	11	0	0	0	0	1
140,05,87		136	1	6	0	0	0	0	0
140,04,20	31	126	1	2	0	0	0	0	0
140,4,25	35,3	141	0	1	0	0	0	0	0
140,5,67	26,5	98	1	0	0	0	0	0	0
140,5,069	26		0	0	0	0	0	1	0
140,5,26	25,3		0	1	0	0	0	2	0
140,5,32	33,9		11	4	0	4	0	0	0
140,5,49	26,6		6	0	0	0	1	4	0
140,5,78	25,5	97	0	0	0	0	0	0	0
140,5,89	25,9	103	3	0	0	0	0	0	0

ID	**Head Length (cm)**	**Fork Length (cm)**	***Euryphorus brachypterus***	***Pseudocycnus appendiculatus***	***Caligus* sp. 1**	***Hexostoma* sp.**	**Monogeneaen sp. 1**	***Nasicola klawei***	**Monogenean sp. 2**
140,5,93	25,4	100	4	3	0	0	0	1	0
140,5,94	27,5	106	1	0	0	0	0	0	0
140,5,97	26,8		0	1	0	0	0	2	0
140,04,08	33	131	0	1	0	0	0	0	0
140,05,61	33,4	134	0	3	0	0	0	0	0
140,5,66	26,5	98	3	4	0	0	0	0	0
140,4,22	36,2	139	1	1	0	0	0	1	0
140,4,29	34,6	136	2	1	0	1	0	0	0
140,5,58	29,8		19	0	0	0	1	0	0
140,5,56	26,3		0	0	0	0	1	0	0
140,5,65	30,7	131	6	6	0	0	0	1	0
140,3,36	32,5		2	2	0	0	0	2	0
140,5,86	26,3	98	0	0	0	0	0	2	0
140,04,30	31,8		8	0	0	0	0	0	0
140,5,11	33	122	0	0	0	0	0	1	0
140,5,53	33	139	0	0	0	0	0	0	0
140,4,21	33,5	131	0	0	0	0	0	0	0
140,5,14	31	136	0	0	0	0	0	0	0
140,4,27	31,5	111	0	0	0	0	0	0	0
Y-13-26			13	0	0	0	0	0	0
Y-13-7			0	0	1	0	0	0	0

Appendix 2B: The gill parasite assemblage of *Thunnus maccoyii*

ID	**Head Length (cm)**	**Fork Length (cm)**	***Euryphorus brachypterus***
140,2,49	47,9		24
140,1,14	45		1
140,1,19	53		68
140,3,048	35,5	113	4

Appendix 2C: The gill parasite assemblage of *Thunnus alalunga*

ID	**Head Length (cm)**	**Fork Length (cm)**	***Euryphorus brachypterus***	***Pseudocycnus appendiculatus***	**Copepod sp. 1**
A-11-31			2	0	0
A-11-33			0	3	0
A-11-36			0	1	0
140,02,66,79,92		89	3	1	0
140,02,092		64	1	2	0
140,02a		80	0	4	0
140,02b		68	10	1	0
140,02,79		82,5	0	6	0
140,02,66	20,8		2	4	0
140,3,17	25		0	0	0
D400		87	0	0	0
D401		85	1	0	0
D402		89	0	3	0
D403		91	0	2	0
D404		83	0	0	0
D405		88	0	5	0
D406		90	0	1	0

ID	**Head Length (cm)**	**Fork Length (cm)**	***Euryphorus brachypterus***	***Pseudocycnus appendiculatus***	**Copepod sp. 1**
D407		80	1	5	0
D408		78	0	7	0
D409		89	0	0	0
D410		92	0	8	0
D411		82	0	0	0
D412		87	0	0	0
D413		89	0	4	0
D414		87	0	3	0
D415		82	0	4	0
D416		87	0	4	0
D417		87	2	1	0
D418		84	0	6	0
D419		89	1	14	0
A-11-38			0	1	0
A-11-42			4	0	1
A-11-43			5	2	0
140,3,43	24,8		6	6	0
A-11-001			7	0	0
A-11-39			0	0	0
A-11-41			18	0	0
A-11-44			2	0	0
A-11-31			3	4	0
A-11-34			1	2	0
A-11-35			4	15	0
A-13-8			1	0	0
140,6,7	26,8		3	0	0

Appendix 2D: The gill parasite assemblage of *Thunnus obesus*

ID	Head Length (cm)	Fork Length (cm)	*Euryphorus brachypterus*	*Pseudocycnus appendiculatus*	*Hexostoma* sp.	Monogeneaen sp. 1	*Nasicola klawei*
140,4,17	39,5		34	0	0	0	0
140,5,37	38,6		11	0	1	0	0
140,4,28	33	114	1	0	2	0	0
06,10,01	45,5		4	0	2	0	0
140,04,13	42,9		61	0	6	0	0
140,4,7	32		17	0	6	0	0
140,05,64		105	27	0	1	0	0
140,05,60			5	0	5	0	0
140,04,23		129	13	0	4	0	0
140,5,34	36	129	5	0	11	0	0
140,4,12	31	107	23	2	0	3	0
140,04,009	31,9	102	13	0	0	0	0
140,04,26	32	128	2	1	0	0	2
140,5,67	38	137	0	0	0	0	0
140,5,55	30	102	0	0	0	0	0

Appendix 2E: The parasite assemblage of *Isurus oxyrinchus*

ID	Fork Length (cm)	*Nemesis lamna lamna*
155,1,2	123	35
155,2,8	135,2	59

Appendix 2F: The gill parasite assemblage of *Prionace glauca*

ID	Fork Length (cm)	*Kroyeria carchariaeglauci*	Copepod sp. 2	Copepod sp. 3	Copepod sp. 4
155,7,6	116	185	1	1	0
06,09,01	152,5	121	0	0	2

Appendix 2G: The gill parasite assemblage of *Xiphias gladius*

ID	Eye Diameter (cm)	Lower Jaw (cm)	*Tristoma adcoccineum*	Monogeneaen sp. 3	Monogeneaen sp. 4	Monogeneaen sp. 5
140,04,14	5	36,5	7	0	0	0
140,04,82	4,5	36,3	7	1	0	0
140,04,004	3,8	28	0	0	0	0
140,04,31	6	53	1	0	0	0
140,05,016	5,7	45	4	0	1	0
140,05,70	4,7	38,2	0	0	0	0
140,5,39	5	36,5	5	0	0	1
140,4,5	5,2		3	0	0	0
140,3,37	5,9	30,8	1	0	0	0

Appendix 2H: The gill parasite assemblage of *Brama brama*

ID	Fork Length (cm)	*Hatschekia conifera*	Monogeneaen sp. 6
am001	42,1	153	1
am002	39,4	143	8
am003	39,9	2	0
am004	47,2	210	8
am005	43,5	30	4

ID	Fork Length (cm)	*Hatschekia conifera*	Monogeneaen sp. 6
am006	45,5	198	3
am007	46	587	1
am008	37	112	4
am009	39,1	179	1
am011	36,9	80	0
am010	41,2	131	3

Appendix 2I: The gill parasite assemblage of *Thyrsites atun*

ID	Head Length (cm)	Fork Length (cm)	*Hatschekia conifera*	*Caligus dakari*	*Mazocraes* sp.
250		89,6	18	1	0
MW001	21		0	0	0
MW002	22,5		0	0	0
MW003	15,4		1	0	1
MW004	20,5		19	0	1
MW005	20,9		2	0	0
MW006	20,1		3	0	1
MW007	17,7		0	0	0
MW008	19,5		7	0	0
MW009	18,4		3	0	0
MW010	17,4		0	3	0
MW011	18,8		1	0	0
MW012	23		0	0	4
MW013	21,5		0	0	0
MW014	20,4		0	0	0
MW015	18,4		3	0	1
MW013,1	11,6		0	0	0

ID	Head Length (cm)	Fork Length (cm)	*Hatschekia conifera*	*Caligus dakari*	*Mazocraes* sp.
MW014,1	21,9		1	3	0
MW015,1	18,5		8	7	0
MW016	15,7		2	0	0
MW017	22		1	3	0
MW018	19,5		1	4	0
MW019	19,3		3	0	0
MW020	19,4		3	0	0
MW021	18		0	7	0
MW022	20		24	0	0
MW023	20,1		0	3	0
MW024	20		0	3	0
MW025	16,6		0	0	0
MW026	21,7		4	0	0
MW027	19,4		0	3	0
MW028	19,6		0	0	1
MW029	19,8		6	1	1
MW030	18,8		0	1	0
MW031	19,7		0	0	0

Appendix 2J: The gill parasite assemblage of *Seriola lalandi*

ID	Head Length (cm)	*Caligus aesopus*	*Eobrachiella elegans*	*Parabrachiella* sp. 1	*Parabrachiella* sp. 2	Copepod sp. 5
FH 22/001	17,5	0	0	1	0	1
FH 21/002	19,5	0	0	1	0	0
FH 21/003	17,5	0	0	0	0	0
FH 21/004	17	0	0	0	0	0
FH 21/005	16,5	0	0	0	0	0

ID	**Head Length (cm)**	***Caligus aesopus***	***Eobrachiella elegans***	***Parabrachiella* sp. 1**	***Parabrachiella* sp. 2**	**Copepod sp. 5**
FH 21/006	17	0	0	2	0	0
FH 21/007	21	0	0	0	1	0
FH 21/008	20,2	0	0	7	0	0
FH 24/001	19,5	0	0	0	0	0
FH 24/002	15,6	0	0	0	0	0
FH 24/003	17,4	0	0	0	0	0
FH 24/004	17,6	0	0	0	0	0
FH 24/005	18,1	0	0	0	0	0
FH 24/006	19,2	0	0	0	0	0
FH 24/007	18,2	0	0	0	0	0
FH 24/008	18,5	0	0	0	0	0
FH 24/009	19,6	0	0	0	0	0
FH 24/010	20	0	0	0	0	0
FH 24/011	19,5	0	0	0	0	0
FH 24/012	17,1	0	0	7	0	0
FH 21/009	17,3	0	0	0	0	0
FH 21/010	17,9	0	0	1	0	0
FH 21/011	18,6	0	0	0	0	0
FH 21/012	17,6	0	0	0	0	0
FH 24/013	16,9	0	0	0	0	0
FH 24/014	18,5	0	0	0	1	0
FH 24/015	18,2	0	0	0	0	0
FH 24/016	18,2	0	3	0	0	3
FH 21/013	16,9	0	0	0	1	0
FH 21/014	18,5	0	0	0	0	0
FH 24/018	14,1	0	0	0	0	0
FH 24/019	18,3	0	0	0	1	0
FH 24/020	12,2	0	0	0	0	0

ID	**Head Length (cm)**	***Caligus aesopus***	***Eobrachiella elegans***	***Parabrachiella* sp. 1**	***Parabrachiella* sp. 2**	**Copepod sp. 5**
FH 24/021	20,5	0	0	0	0	0
FH 24/022	17,7	0	0	2	0	0
FH 24/023	18,6	3	0	0	0	0
FH 24/024	17,5	0	0	2	0	0
FH 24/025	19,2	0	1	0	0	1
FH 24/026	18,9	0	2	6	0	2
FH 24/027	18,8	0	0	0	0	0
FH 24/028	17,8	0	0	0	0	0
FH 24/017	17,8	0	0	2	0	0
FH 24/018	14,1	0	0	0	0	0
FH 24/019	18,3	0	0	0	1	0
FH 24/020	12,2	0	0	0	0	0

Appendix 2K: The gill parasite assemblage of *Sarda sarda*

ID	**Fork Length (cm)**	***Caligus bonito***
AB004	35,6	2
AB005	37,3	3
AB006	48,8	9
AB007	52,4	6
AB008	50,9	1
AB009	51,8	3
AB010	50,6	2
AB011	53,5	0
AB012	49,8	1
AB013	60,5	0
AB014	53,1	1

ID	Fork Length (cm)	*Caligus bonito*
AB015	52,3	1
AB016	56,3	3
AB017	52,7	0
AB018	51,3	0
AB019	51,7	4

Appendix 2L: The gill parasite assemblage of *Genypterus capensis*

ID	Fork Length (cm)	*Chondracanthus colligens*
AM0163		1
13,9,1	72,4	0
T16	89,6	0
T11,1	80,3	0
T11,2	67	0
T11,3	62,5	0
T11,4	62,6	0
T3,1	67	0
T3,2	70,6	0
T3,3	64	0
T3,4	71,3	0
T20,1	67,8	0
T20,2	89	0
T20,3	94	0
T20,4	72,8	0
D00495	92,6	1
D00641	83,4	0
AM177	74,7	0

ID	Fork Length (cm)	*Chondracanthus colligens*
AM178	68,5	3
AM179	55,2	0
AM180	54,4	3
AM181	53,9	0

Appendix 2M: The gill parasite assemblage of *Merluccius paradoxus*

ID	Fork Length (cm)	*Anthocotyle merlucci*
D00827,96,1	34	0
D00827,96,2	32	0
D00827,96,3	33	0
D00827,96,4	32	0
D00827,96,5	30	0
D00827,96,6	57	1
D00827,96,7	58	0
D00827,96,8	57	0
D00827,96,9	56	0
D00827,96,10	71	0
01,09,01	50,5	2
01,09,02	58,3	0
01,09,03	56	1
01,09,04	54,7	0
01,09,05	65,6	2
01,09,06	50,8	0

Appendix 2N: The gill parasite assemblage of *Merluccius capensis*

ID	**Fork Length (cm)**	***Anthocotyle merlucci***
D00783,54,1	38	3
D00783,54,2	46	4
D00783,54,3	46	0
D00783,54,4	49	1
D00783,54,5	52	0
D00829,98,1	30	2
D00829,98,2	36	0
D00829,98,3	35	2
D00829,98,4	35	1
D00829,98,5	37	1

.

Appendix 2O: The gill parasite assemblage of *Sardinops sagax*

ID	**Standard Length (cm)**	***Clavelisa ilishae***	***Nothobomolochus fradei***
SSM1	19,6	0	0
SSM2	17,8	0	0
SSM3	15,9	0	0
SSM4	16,1	0	0
SSM5	17,7	0	0
SSM6	17,5	0	0
SSM7	16,7	0	0
SSM8	17,6	0	0
SSM9	16,2	0	0
SSM10	16,8	0	0
SSM11	16,1	0	0

ID	Standard Length (cm)	*Clavellisa ilishae*	*Nothobomolochus fradei*
SSM12	18,6	0	0
SSM13	16,8	0	0
SSM14	18,6	0	0
SSM15	18,4	0	0
SSM16	17,5	0	0
SSM17	16	0	0
SSM18	17,3	0	0
SSM19	17,6	0	0
SSM20	16,1	0	0
SSM21	17,8	0	0
SSM22	17	0	0
SSM23	16,9	0	0
SSM24	17,6	0	0
SSM25	18,2	0	0
SSM26	18,5	0	0
SSM27	17,7	0	0
SSM28	17,8	0	0
SSM29	17,1	0	0
SSM30	18,1	0	0
SSM31	17,2	0	0
SSM32	19,2	0	0
SSM33	18,5	0	0
SSM34	18,2	0	0
SSM35	20,2	0	0
SSM36	17	0	0
SSM37	17,5	0	0
SSM38	19,5	0	0
SSM39	18,7	0	0

ID	**Standard Length (cm)**	***Clavellisa ilishae***	***Nothobomolochus fradei***
SSM40	18,2	0	0
SSM41	18,6	0	0
SSM42	18,3	0	0
SSM43	17,8	0	0
SSM44	16,7	0	0
SSM45	18	0	0
SSM46	18,5	2	0
SSM47	18,9	0	0
SSM48	17,7	0	0
SSM49	17,5	0	0
SSM50	18,4	0	0
SSM51	18,6	0	0
SSM52	19,4	0	0
SSM53	18,7	0	0
SSM54	18,7	0	0
SSM55	18,4	0	0
SSM56	18,5	0	0
SSM57	18,4	1	0
SSM58	15	0	0
SSM59	18,4	0	0
SSM60	19,6	0	0
SSM61	17,3	0	0
SSM62	17,9	0	0
SSM63	13	0	0
SSM64	12,9	0	0
SSM65	12,5	0	0
SSM66	12,4	0	0
SSM67	11,4	0	0

ID	**Standard Length (cm)**	***Clavelisa ilishae***	***Nothobomolochus fradei***
SSM68	11,5	0	0
SSM69	12,7	0	0
SSM70	11,8	1	0
SSM71	11,5	0	0
SSM72	14,1	0	0
SSM73	12,1	0	0
SSM74	12	0	0
SSM75	18,3	0	0
SSM76	16	0	1
SSM77	17,7	0	0
SSM78	15,6	0	0
SSM79	18,6	0	0
SSM80	18	0	0
SSM81	17,3	0	0
SSM82	18,2	0	0
SSM83	17,4	0	0
SSM84	19,6	0	0
SSM85	17,4	0	0
SSM86	18,6	0	0
SSM87	18,2	0	0
SSM88	17,2	0	0
SSM89	17	0	0
SSM90	18,1	0	0
SSM91	17,4	0	0
SSM92	17,6	0	0
SSM93	16	0	0
SSM94	16,9	0	0
SSM95	17,6	0	0

ID	**Standard Length (cm)**	***Clavelisa ilishae***	***Nothobomolochus fradei***
SSM96	18,2	0	0
SSM97	18,6	0	0
SSM98	16,6	8	0
SSM99	18,4	0	0
SSM100	18,7	0	0
SSM101	18	0	0
SSM102	18	0	1
SSM103	16,7	0	0
SSM104	17,8	0	0
SSM105	16,6	0	0
SSM106	17,2	0	0
SSM107	17,3	0	0
SSM108	18,1	0	0
SSM109	18,2	0	0
SSM110	19,6	0	0
SSM111	18,5	0	0
SSM112	18,2	0	0
SSM113	17,4	0	0
SSM114	18,6	0	0
SSM115	16,6	0	0
SSM116	18,7	0	0
SSM117	19,6	0	0
SSM118	18,5	0	0
SSM119	17,9	0	0
SSM120	17,9	0	0
SSM121	16,8	0	0
SSM122	17,6	0	0
SSM123	17,5	0	0

ID	**Standard Length (cm)**	***Clavelisa ilishae***	***Nothobomolochus fradei***
SSM124	15,5	0	0
SSM125	16,7	0	0
SSM126	18,2	0	0
SSM127	18,8	0	0
SSM128	20	0	0
SSM129	17,8	0	0
SSM130	15,7	0	0
SSM131	17,2	0	0
SSM132	19	0	0
SSM133	18,5	0	0
SSM134	17,4	0	0
SSM135	17	0	0
SSM136	18,2	0	0
SSM137	17,8	0	0
SSM138	18,1	0	0
SSM139	17,3	0	0
SSM140	17,5	0	0
SSM141	16,4	0	0
SSM142	17	0	0
SSM143	17,5	0	0
SSM144	16,4	0	0
SSM145	19	0	0
SSM146	18,1	0	0
SSM147	17,8	0	0
SSM148	17,5	0	0
SSM149	17,7	0	0
SSM150	19,5	0	0
SSM151	16,8	0	0

ID	Standard Length (cm)	*Clavelisa ilishae*	*Nothobomolochus fradei*
SSM152	17,9	0	0
SSM153	17,9	0	0
SSM154	17,6	0	0
SSM155	16,1	0	0
SSM156	17,8	0	0

Appendix 2P: The gill parasite assemblage of *Trachurus capensis*

ID	Fork Length (cm)	*Gastrocotyle trachuri*	*Caligus dakari*
9,11,1	34	0	0
9,11,2	36,8	0	0
9,11,3	35,8	3	0
9,11,4	32,4	0	0
9,11,5	30	0	0
9,11,6	33	0	0
9,11,7	36	1	0
10,11,1	32,5	0	0
10,11,2	33,7	3	0
10,11,3	35,5	5	0
10,11,4	35	3	0
10,11,5	32,9	0	0
10,11,6	32,3	7	0
10,11,7	32,8	3	0
10,11,8	32	4	0
10,11,9	31,5	1	0
10,11,10	33,4	0	0
288,1	22,5	3	0

ID	**Fork Length (cm)**	***Gastrocotyle trachuri***	***Caligus dakari***
288,2	27,2	0	0
288,3	23	0	0
288,4	24,2	2	0
288,5	23,6	2	0
288,6	25,8	0	0
288,7	22,6	3	0
288,8	23,5	1	0
288,9	23,7	0	0
288,10	22	2	0
288,11	19,5	0	0
288,12	21,8	2	0
288,13	23,5	0	0
288,14	22,6	3	0
288,15	19,7	2	0
288,16	20,8	1	0
288,17	19,2	1	0
288,18	21,6	0	0
288,19	21,2	1	0
288,20	19,8	2	0
288,21	20,6	2	0
288,22	21,4	6	0
HMM1	17,3	1	0
HMM2	17,2	4	0
HMM3	16,8	6	0
HMM4	17,7	0	0
HMM5	17,8	0	0
HMM6	16,6	1	0
HMM7	16,7	1	0

ID	**Fork Length (cm)**	***Gastrocotyle trachuri***	***Caligus dakari***
HMM8	17,2	4	0
HMM9	17,2	2	0
HMM10	17,4	0	0
HMM11	25,5	3	0
HMM12	22,4	5	0
HMM13	23,2	1	0
HMM14	24,7	1	0
HMM15	20,3	0	0
HMM16	19,3	0	0
HMM17	21,5	0	0
HMM18	20,6	0	0
HMM19	21,2	0	0
HMM20	21,4	1	0
HMM21	19,5	1	0
HMM22	18,7	1	0
HMM23	20,2	0	0
HMM24	21,5	8	0
HMM25	21	3	0
HMM26	22,5	5	0
HMM27	19,4	3	0
HMM28	21,8	2	0
HMM29	21,4	3	0
HMM30	20,5	0	0
HMM31	22,3	3	0
HMM32	22,4	2	0
HMM33	18,8	1	0
HMM34	18,6	4	0
HMM35	21	1	0

ID	Fork Length (cm)	*Gastrocotyle trachuri*	*Caligus dakari*
HMM36	24	14	0
HMM37	21,2	9	0
HMM38	24,6	2	1
HMM39	21,5	4	0
HMM40	22,4	4	0
HMM41	23,4	2	0
HMM42	24,4	1	0
HMM43	24,5	7	0
HMM44	23,3	9	1
HMM45	23	5	0
HMM46	22,7	6	0
HMM47	16	1	0
HMM48	13,7	3	0
HMM49	14,7	0	0
HMM50	15	2	0
HMM51	14	3	0
HMM52	14,9	1	0
HMM53	13,8	1	0
HMM54	16,1	2	0
HMM55	15,7	2	0
HMM56	15,1	1	0
HMM57	14,9	1	0
HMM58	14,2	0	0
HMM59	15	0	0
HMM60	14,8	1	0
HMM61	14,9	0	0
HMM62	15,9	2	0
HMM63	12,7	1	0

ID	**Fork Length (cm)**	***Gastrocotyle trachuri***	***Caligus dakari***
HMM64	12,7	0	0
HMM65	15,5	1	0
HMM66	14	1	0
HMM67	20,8	5	0
HMM68	21,4	2	0
HMM69	21,4	3	0
HMM70	22	2	0
HMM71	19,9	4	0
HMM72	17,3	5	0
HMM73	22,9	5	0
HMM74	20,4	2	0
HMM75	19,9	5	0
HMM76	21,1	4	0
HMM77	21,3	0	0
HMM78	21,4	11	0
HMM79	21,1	3	0
HMM80	20,4	5	0
HMM81	20,1	9	0
HMM82	20,5	1	0
HMM83	19,6	3	0
HMM84	20,5	2	0
HMM85	22,3	0	0
HMM86	12,4	1	0
HMM87	13,6	4	0
HMM88	13,6	1	0
HMM89	13,8	2	0
HMM90	14,1	0	0
HMM91	14,5	1	0

ID	**Fork Length (cm)**	***Gastrocotyle trachuri***	***Caligus dakari***
HMM92	14,7	1	0
HMM93	14,8	1	0
HMM94	16	6	0
HMM95	16,7	6	0
HMM96	17	8	0
HMM97	17	3	0
HMM98	17,1	3	0
HMM99	17,9	6	0
HMM100	19,6	5	0
HMM101	19,6	0	0
HMM102	20,3	4	0
HMM103	21,2	0	0
HMM104	21,5	5	0
HMM105	21,7	3	0
HMM106	20,9	1	0
HMM107	21,5	0	0
HMM108	20,7	6	0
HMM109	21,2	0	0
HMM110	22,1	3	0
HMM111	22	9	0
HMM112	16,6	2	0
HMM113	20,1	2	0
HMM114	16,7	0	0
HMM115	18,5	0	0
HMM116	15,7	0	0
HMM117	18,4	0	0
HMM118	16,5	3	0
HMM119	17,3	1	0

ID	**Fork Length (cm)**	***Gastrocotyle trachuri***	***Caligus dakari***
HMM120	16,6	0	0
HMM121	15,5	0	0
HMM122	15,1	1	0
HMM123	16,1	1	0
HMM124	16,9	0	0
HMM125	16,4	0	0
HMM126	18,2	1	0
HMM127	14,7	1	0
HMM128	18	4	0
HMM129	19,6	4	0
HMM130	18,4	0	0
HMM131	14,5	0	0
HMM132	18,7	0	0
HMM133	19,8	2	0
HMM134	15,1	1	0
HMM135	18,4	0	0
HMM136	18,8	4	0
HMM137	22,4	4	0
HMM138	19,9	2	0
HMM139	21	3	0
HMM140	20	6	0
HMM141	19,2	7	0
HMM142	18	7	0
HMM143	18,5	2	0
HMM144	18,4	1	0
HMM145	23,4	0	0
HMM146	23	6	0
HMM147	20	6	0

ID	**Fork Length (cm)**	***Gastrocotyle trachuri***	***Caligus dakari***
HMM148	21,7	1	0
HMM149	23,3	2	0
HMM150	23,1	0	0
HMM151	22,2	0	0
HMM152	21,6	2	0
HMM153	20,7	1	0

Appendix 3: Stable Isotope Analysis Raw Data

Appendix 3: Raw data obtained from the stable isotope analyses. Table indicates the individual sample ID; host species; the type of sample analysed; the uncorrected δ^{13}C; the corrected δ'^{13}C; the δ^{15}N and the C:N.

ID	Host Species	Sample Analysed	δ^{13}C	δ'^{13}C	δ^{15}N	C:N
04,03,001,1	*Thunnus albacares*	Muscle	-15,09	-15,09	12,03	2,81
140,5,49,1	*Thunnus albacares*	Muscle	-16,4	-16,4	11,87	2,95
140,5,63,1	*Thunnus albacares*	Muscle	-15,9	-15,9	12,26	2,94
140,5,68,1	*Thunnus albacares*	Muscle	-17,49	-17,37	12,88	3,47
04,03,001,2	*Thunnus albacares*	Gill	-16,43	-16,23	12,35	3,55
140,5,49,2	*Thunnus albacares*	Gill	-16,56	-16,39	12,19	3,52
140,5,63,2	*Thunnus albacares*	Gill	-15,58	-15,53	12,44	3,41
140,5,68,2	*Thunnus albacares*	Gill	-15,89	-15,75	12,67	3,5
Y-11-36	*Thunnus albacares*	Gill	-16,09	-16,03	12,35	3,42
04,03,001,3	*Thunnus albacares*	*Euryphorus brachypterus*	-17,19	-16,12	13,95	4,44
140,5,49,5	*Thunnus albacares*	*Euryphorus brachypterus*	-17,76	-16,35	12,54	4,78
140,5,49,6	*Thunnus albacares*	*Euryphorus brachypterus*	-17,23	-16,49	12,67	4,1
140,5,49,7	*Thunnus albacares*	*Euryphorus brachypterus*	-16,88	-16,33	12,61	3,9
140,5,63,6	*Thunnus albacares*	*Euryphorus brachypterus*	-17,15	-15,6	13,77	4,91
140,5,63,7	*Thunnus albacares*	*Euryphorus brachypterus*	-16,78	-16,05	13,53	4,09
140,5,68,3	*Thunnus albacares*	*Euryphorus brachypterus*	-17,82	-16,78	13,72	4,4
Y-11-36	*Thunnus albacares*	*Euryphorus brachypterus*	-17,62	-15,46	11,68	5,53
04,03,001,7	*Thunnus albacares*	*Pseudocycnus appendiculatus*	-17,79	-17,04	12,89	4,12
140,5,63,3	*Thunnus albacares*	*Pseudocycnus appendiculatus*	-17	-16,4	13,8	3,96
140,5,63,4	*Thunnus albacares*	*Pseudocycnus appendiculatus*	-17,64	-16,67	13,51	4,33
140,5,63,5	*Thunnus albacares*	*Pseudocycnus appendiculatus*	-18,94	-16,44	12,88	5,88
Y-11-36	*Thunnus albacares*	*Pseudocycnus appendiculatus*	-17,29	-16,71	13,38	3,93
Y-11-36	*Thunnus albacares*	*Pseudocycnus appendiculatus*	-17,2	-16,71	13,11	3,84
Y-11-36	*Thunnus albacares*	Monogenean sp. 1	-17,65	-16,29	13,98	4,73
04,03,001,5	*Thunnus albacares*	*Nasicola klawei*	-18,25	-15,62	14,1	6,01
04,03,001,6	*Thunnus albacares*	*Nasicola klawei*	-17,65	-15,38	14	5,65
140,5,49,3	*Thunnus albacares*	*Nasicola klawei*	-18,11	-16,65	13,35	4,82
140,5,49,4	*Thunnus albacares*	*Nasicola klawei*	-17,86	-16,28	13,16	4,95
140,5,63,10	*Thunnus albacares*	*Nasicola klawei*	-17,78	-15,12	14,11	6,04
140,5,63,9	*Thunnus albacares*	*Nasicola klawei*	-19,23	-15,65	14,46	6,97
140,5,68,4	*Thunnus albacares*	*Nasicola klawei*	-18,93	-14,9	13,64	7,42
140,5,68,5	*Thunnus albacares*	*Nasicola klawei*	-17,47	-15,35	13,68	5,5
140,1,19,1	*Thunnus maccoyii*	Muscle	-18,08	-18,08	11,39	3,06
140,2,49,1	*Thunnus maccoyii*	Muscle	-18,02	-17,86	13,39	3,52
140,1,19,2	*Thunnus maccoyii*	Gill	-18,08	-17,89	11,14	3,54
140,2,49,2	*Thunnus maccoyii*	Gill	-17,15	-16,98	12,52	3,53
140,1,19,3	*Thunnus maccoyii*	*Euryphorus brachypterus*	-18,37	-17,12	11,32	4,62
140,1,19,4	*Thunnus maccoyii*	*Euryphorus brachypterus*	-18,83	-16,47	11,31	5,73

ID	Host Species	Sample Analysed	$\delta^{13}C$	$\delta'^{13}C$	$\delta^{15}N$	C:N
140,1,19,5	*Thunnus maccoyii*	*Euryphorus brachypterus*	-18,41	-16,87	10,64	4,9
140,2,49,3	*Thunnus maccoyii*	*Euryphorus brachypterus*	-18,56	-16,44	13,48	5,49
140,2,49,4	*Thunnus maccoyii*	*Euryphorus brachypterus*	-17,8	-17,07	13,82	4,09
140,2,49,5	*Thunnus maccoyii*	*Euryphorus brachypterus*	-17,67	-16,73	13,04	4,29
140,02,66,2	*Thunnus alalunga*	Muscle	-17,83	-17,83	11,62	3,14
140,02,66,79,92,2	*Thunnus alalunga*	Muscle	-17,59	-17,59	12,31	3,14
140,02,92,2	*Thunnus alalunga*	Muscle	-17,86	-17,86	12,64	3,12
140,2, b,2	*Thunnus alalunga*	Muscle	-17,95	-17,95	11,89	3,12
140,02,66,1	*Thunnus alalunga*	Gill	-17,79	-17,56	10,49	3,59
140,02,66,79,92,1	*Thunnus alalunga*	Gill	-17,79	-17,55	11,36	3,59
140,02,92,1	*Thunnus alalunga*	Gill	-18,04	-17,84	10,98	3,56
140,2, b,1	*Thunnus alalunga*	Gill	-17,96	-17,68	10,81	3,63
A-11-31	*Thunnus alalunga*	Gill	-17,2	-16,93	11,62	3,63
A-11-34,1	*Thunnus alalunga*	Gill	-16,82	-16,68	11,69	3,49
140,02,66,6	*Thunnus alalunga*	*Euryphorus brachypterus*	-19,17	-17,7	11,02	4,84
140,02,66,7	*Thunnus alalunga*	*Euryphorus brachypterus*	-18,54	-17,09	10,68	4,82
140,02,66,79,92,3	*Thunnus alalunga*	*Euryphorus brachypterus*	-17,95	-16,34	11,93	4,98
140,02,66,79,92,4	*Thunnus alalunga*	*Euryphorus brachypterus*	-18,08	-16,81	11,6	4,63
140,02,66,79,92,5	*Thunnus alalunga*	*Euryphorus brachypterus*	-18,17	-16,45	11,54	5,09
140,02,92,3	*Thunnus alalunga*	*Euryphorus brachypterus*	-18,79	-17,67	11,75	4,48
140,2, b,3	*Thunnus alalunga*	*Euryphorus brachypterus*	-18,48	-17,62	11,68	4,21
A-11-31	*Thunnus alalunga*	*Euryphorus brachypterus*	-17,16	-16,44	12,41	4,08
A-11-31	*Thunnus alalunga*	*Euryphorus brachypterus*	-17,28	-16,39	11,87	4,25
A-11-34,5	*Thunnus alalunga*	*Euryphorus brachypterus*	-17,76	-17,11	13,1	4,01
140,02,66,3	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-19,8	-18,37	10,79	4,8
140,02,66,4	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-20,49	-18,32	10,69	5,54
140,02,66,5	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-19,21	-18,24	11,5	4,33
140,02,66,79,92,6	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-19,62	-17,9	12,36	5,08
140,02,92,4	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-19,32	-18,19	11,88	4,49
140,02,92,5	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-19,87	-18,4	12,07	4,84
140,2, b,4	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-18,84	-17,87	12,31	4,33
140,2, b,5	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-18,83	-17,92	11,66	4,28
140,2, b,6	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-18,37	-17,84	11,71	3,88
A-11-31	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-18,29	-17,27	13,28	4,38
A-11-31	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-19,39	-17,34	12,96	5,43
A-11-34,2	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-19,81	-17,72	12,68	5,47
A-11-34,3	*Thunnus alalunga*	*Pseudocycnus appendiculatus*	-19,21	-17,56	13,02	5,02
04,03,002,1	*Thunnus obesus*	Muscle	-17,18	-17,18	12,61	3,24
06-10-1,1	*Thunnus obesus*	Muscle	-17,26	-17,05	14,18	3,56
140,04,26	*Thunnus obesus*	Muscle	-15,66	-15,66	13,1	3,14
140,05,34,1	*Thunnus obesus*	Muscle	-19,33	-18,22	14,18	4,47
140,4,12,1	*Thunnus obesus*	Muscle	-17,01	-17,01	12,77	3,17
140,4,13,1	*Thunnus obesus*	Muscle	-17,34	-16,99	13,87	3,7
140,4,17,1	*Thunnus obesus*	Muscle	-18	-17,73	12,6	3,62
140,4,7,1	*Thunnus obesus*	Muscle	-17,31	-17,31	13,39	3,29

ID	**Host Species**	**Sample Analysed**	**$\delta^{13}C$**	**$\delta'^{13}C$**	**$\delta^{15}N$**	**C:N**
04,03,002,2	*Thunnus obesus*	Gill	-16,97	-16,73	11,97	3,6
06-10-1,2	*Thunnus obesus*	Gill	-16,6	-16,29	13,37	3,67
140,04,26	*Thunnus obesus*	Gill	-16,29	-16,02	13,54	3,62
140,05,34,2	*Thunnus obesus*	Gill	-17,11	-16,85	12,35	3,62
140,4,12,2	*Thunnus obesus*	Gill	-17,15	-16,91	12	3,6
140,4,13,2	*Thunnus obesus*	Gill	-16,38	-16,18	12,91	3,55
140,4,17,2	*Thunnus obesus*	Gill	-16,73	-16,47	12,07	3,61
140,4,7,2	*Thunnus obesus*	Gill	-17,46	-17,22	12,1	3,6
04,03,002	*Thunnus obesus*	*Euryphorus brachypterus*	-17,11	-16,45	12,71	4,02
04,03,002,3	*Thunnus obesus*	*Euryphorus brachypterus*	-17,26	-16,25	13,23	4,38
04,03,002,4	*Thunnus obesus*	*Euryphorus brachypterus*	-19,01	-15,93	13,65	6,46
06-10-1,3	*Thunnus obesus*	*Euryphorus brachypterus*	-17,32	-15,65	14,24	5,04
06-10-1,4	*Thunnus obesus*	*Euryphorus brachypterus*	-18,03	-15,63	13,4	5,78
06-10-1,5	*Thunnus obesus*	*Euryphorus brachypterus*	-16,93	-15,5	13,56	4,8
140,04,26	*Thunnus obesus*	*Euryphorus brachypterus*	-16,06	-15,31	13,98	4,11
140,04,26	*Thunnus obesus*	*Euryphorus brachypterus*	-16,1	-15,29	14	4,17
140,05,34,3	*Thunnus obesus*	*Euryphorus brachypterus*	-17,28	-16,25	13,26	4,4
140,05,34,4	*Thunnus obesus*	*Euryphorus brachypterus*	-17,71	-16,66	13,46	4,42
140,05,34,5	*Thunnus obesus*	*Euryphorus brachypterus*	-17,68	-16,32	12,88	4,73
140,4,12,10	*Thunnus obesus*	*Euryphorus brachypterus*	-16,88	-16,13	13,17	4,11
140,4,12,8	*Thunnus obesus*	*Euryphorus brachypterus*	-16,87	-16,22	13,08	4,01
140,4,12,9	*Thunnus obesus*	*Euryphorus brachypterus*	-17,05	-16,07	13,33	4,35
140,4,13,6	*Thunnus obesus*	*Euryphorus brachypterus*	-17,96	-15,28	13,8	6,06
140,4,13,7	*Thunnus obesus*	*Euryphorus brachypterus*	-17,25	-16	14	4,62
140,4,13,8	*Thunnus obesus*	*Euryphorus brachypterus*	-16,61	-15,58	13,1	4,39
140,4,17,3	*Thunnus obesus*	*Euryphorus brachypterus*	-16,92	-16,27	13,37	4,01
140,4,17,4	*Thunnus obesus*	*Euryphorus brachypterus*	-17,23	-16,34	12,04	4,25
140,4,17,5	*Thunnus obesus*	*Euryphorus brachypterus*	-18,21	-15,9	12,57	5,69
140,4,7,3	*Thunnus obesus*	*Euryphorus brachypterus*	-17,59	-16,47	13,21	4,49
140,4,7,4	*Thunnus obesus*	*Euryphorus brachypterus*	-17,68	-16,61	14,34	4,43
140,4,7,5	*Thunnus obesus*	*Euryphorus brachypterus*	-17,62	-16,76	14,56	4,21
140,04,26	*Thunnus obesus*	*Pseudocycnus appendiculatus*	-16,7	-16,13	14,37	3,92
140,4,12,3	*Thunnus obesus*	*Pseudocycnus appendiculatus*	-18,51	-17,45	12,66	4,43
140,4,12,4	*Thunnus obesus*	*Pseudocycnus appendiculatus*	-18,63	-17,52	12,8	4,47
04,03,002	*Thunnus obesus*	*Hexostoma* sp.	-17,93	-15,56	12,31	5,75
06-10-1,6	*Thunnus obesus*	*Hexostoma* sp.	-17,29	-15,22	13,59	5,44
06-10-1,7	*Thunnus obesus*	*Hexostoma* sp.	-17,16	-15,06	13,26	5,47
140,05,34,6	*Thunnus obesus*	*Hexostoma* sp.	-17,5	-15,89	11,68	4,98
140,05,34,7	*Thunnus obesus*	*Hexostoma* sp.	-18,79	-17,07	12,37	5,1
140,05,34,8	*Thunnus obesus*	*Hexostoma* sp.	-17,63	-16,46	14,16	4,53
140,4,12,5	*Thunnus obesus*	*Hexostoma* sp.	-18,29	-16,35	11,7	5,31
140,4,12,6	*Thunnus obesus*	*Hexostoma* sp.	-17,33	-13,98	13,03	6,74
140,4,12,7	*Thunnus obesus*	*Hexostoma* sp.	-17,89	-15,61	12,43	5,66
140,4,13,3	*Thunnus obesus*	*Hexostoma* sp.	-17,38	-15,26	14,47	5,5

ID	Host Species	Sample Analysed	δ^{13}C	δ'^{13}C	δ^{15}N	C:N
140,4,13,4	*Thunnus obesus*	*Hexostoma* sp.	-17,15	-15,44	13,35	5,08
140,4,13,5	*Thunnus obesus*	*Hexostoma* sp.	-17,43	-15,86	13,53	4,94
140,4,7,6	*Thunnus obesus*	*Hexostoma* sp.	-18,46	-16,5	11,76	5,33
140,4,7,7	*Thunnus obesus*	*Hexostoma* sp.	-18,27	-16,38	12,16	5,26
140,4,7,8	*Thunnus obesus*	*Hexostoma* sp.	-18,37	-16,7	12,41	5,04
140,04,26	*Thunnus obesus*	*Nasicola klawei*	-16,91	-14,64	14,87	5,65
140,04,26	*Thunnus obesus*	*Nasicola klawei*	-17,07	-14,74	14,69	5,7
155,1,2	*Isurus oxyrinchus*	Muscle	-16,29	-16,29	13,07	2,7
155,2,8,1	*Isurus oxyrinchus*	Muscle	-16,74	-16,74	12,89	2,77
155,1,2	*Isurus oxyrinchus*	Gill	-16,01	-16,01	13,56	2,83
155,2,8,2	*Isurus oxyrinchus*	Gill	-16,62	-16,62	13,02	2,98
155,2,8,3	*Isurus oxyrinchus*	*Nemesis lamna lamna*	-16,46	-15,05	13,79	4,78
155,2,8,4	*Isurus oxyrinchus*	*Nemesis lamna lamna*	-17,15	-15,03	13,9	5,49
155,2,8,5	*Isurus oxyrinchus*	*Nemesis lamna lamna*	-16,4	-14,86	13,01	4,91
155,1,2	*Isurus oxyrinchus*	*Nemesis lamna lamna*	-15,26	-14,18	14,33	4,44
155,1,2	*Isurus oxyrinchus*	*Nemesis lamna lamna*	-15,68	-14,5	13,5	4,55
155,1,2	*Isurus oxyrinchus*	*Nemesis lamna lamna*	-15,59	-14,69	14,22	4,26
155,1,2	*Isurus oxyrinchus*	*Nemesis lamna lamna*	-15,72	-14,05	12,96	5,04
06-09-01,1	*Prionace glauca*	Muscle	-16,45	-16,45	12,78	2,56
155,7,6	*Prionace glauca*	Muscle	-16,55	-16,55	13,92	2,87
06-09-01,2	*Prionace glauca*	Gill	-16,5	-16,5	13,14	2,98
155,7,6	*Prionace glauca*	Gill	-16,67	-16,67	12,99	2,45
06-09-01,3	*Prionace glauca*	*Kroyeria carchariaeglauci*	-16,25	-11,68	14,2	7,97
06-09-01,4	*Prionace glauca*	*Kroyeria carchariaeglauci*	-16,42	-12,23	14,58	7,59
155,7,6	*Prionace glauca*	*Kroyeria carchariaeglauci*	-17,12	-13,25	14,5	7,26
155,7,6	*Prionace glauca*	*Kroyeria carchariaeglauci*	-17,27	-13,21	14,66	7,46
140,4,5,1	*Xiphias gladius*	Muscle	-20,68	-15,98	13,25	8,1
140,5,16,1	*Xiphias gladius*	Muscle	-19,45	-17,92	14,15	4,9
140,4,5,2	*Xiphias gladius*	Gill	-16,27	-15,89	12,53	3,73
140,5,16,2	*Xiphias gladius*	Gill	-16,21	-15,91	12,97	3,66
140,4,5,3	*Xiphias gladius*	*Tristoma adcoccineum*	-16,87	-15,71	15,34	4,53
140,4,5,4	*Xiphias gladius*	*Tristoma adcoccineum*	-17,27	-16,09	14,69	4,55
140,5,16,3	*Xiphias gladius*	*Tristoma adcoccineum*	-17,27	-16,2	15,09	4,44
140,5,16,4	*Xiphias gladius*	*Tristoma adcoccineum*	-16,87	-16,06	14,99	4,16
AM 004	*Brama brama*	Muscle	-20,62	-14,71	14,85	9,32
AM 006	*Brama brama*	Muscle	-16,68	-16,57	16,06	3,47
AM 007	*Brama brama*	Muscle	-18,59	-16,93	15,97	5,03
AM 004	*Brama brama*	Gill	-16,76	-16,07	14,81	4,05
AM 006	*Brama brama*	Gill	-16,38	-15,97	15,1	3,77
AM 007	*Brama brama*	Gill	-16,38	-16,06	15,13	3,67
AM 004	*Brama brama*	*Hatschekia conifera*	-19,79	-10,27	16,96	12,97
AM 004	*Brama brama*	*Hatschekia conifera*	-19,2	-12,14	17,02	10,49
AM 006	*Brama brama*	*Hatschekia conifera*	-20,19	-7,5	17,39	16,17
AM 006	*Brama brama*	*Hatschekia conifera*	-19,87	-10,03	17,52	13,29
AM 007	*Brama brama*	*Hatschekia conifera*	-20,06	-10,6	17,85	12,91

ID	Host Species	Sample Analysed	$\delta^{13}C$	$\delta'^{13}C$	$\delta^{15}N$	C:N
AM 007	*Brama brama*	*Hatschekia conifera*	-19,95	-10,01	17,63	13,4
mw004,1	*Thyrsites atun*	Muscle	-16,16	-16,16	15,79	3,27
mw012,1	*Thyrsites atun*	Muscle	-15,86	-15,86	15,41	3,36
mw015,1,1	*Thyrsites atun*	Muscle	-15,85	-15,85	16,16	3,31
mw018,1	*Thyrsites atun*	Muscle	-15,38	-15,38	15,67	3,3
mw022,1	*Thyrsites atun*	Muscle	-15,7	-15,7	15,88	3,21
mw004,2	*Thyrsites atun*	Gill	-16,84	-16,36	15,51	3,84
mw012,2	*Thyrsites atun*	Gill	-15,97	-15,62	14,96	3,7
mw015,1,2	*Thyrsites atun*	Gill	-16,67	-15,89	16,4	4,14
mw018,2	*Thyrsites atun*	Gill	-15,9	-15,36	15,22	3,9
mw022,2	*Thyrsites atun*	Gill	-16,21	-15,75	15,81	3,82
mw015,1,3	*Thyrsites atun*	*Caligus dakari*	-17,21	-14	16,95	6,59
mw015,1,4	*Thyrsites atun*	*Caligus dakari*	-17,23	-13,78	16,99	6,85
mw018,3	*Thyrsites atun*	*Caligus dakari*	-17,3	-14,08	15,98	6,61
mw018,4	*Thyrsites atun*	*Caligus dakari*	-17,6	-13,9	15,58	7,09
mw018,5	*Thyrsites atun*	*Caligus dakari*	-17,31	-14,02	16,18	6,68
mw004,3	*Thyrsites atun*	*Hatschekia conifera*	-18,11	-14,66	17,84	6,84
mw022,3	*Thyrsites atun*	*Hatschekia conifera*	-18,04	-13,12	17,63	8,32
mw012,3	*Thyrsites atun*	*Mazocraes* sp.	-17,07	-15,77	15,6	4,67
FH 21/008	*Seriola lalandi*	Muscle	-15,08	-15,08	14,34	3,16
FH 24/14	*Seriola lalandi*	Muscle	-15,68	-15,68	14,36	3,27
FH 24/19	*Seriola lalandi*	Muscle	-16,04	-16,04	14,55	3,3
FH 24/22	*Seriola lalandi*	Muscle	-15,51	-15,51	14,13	3,19
FH 24/26	*Seriola lalandi*	Muscle	-15,72	-15,72	14,75	3,3
FH 21/008	*Seriola lalandi*	Gill	-13,9	-13,9	13,29	3,24
FH 24/14	*Seriola lalandi*	Gill	-14,65	-14,65	12,76	3,39
FH 24/22	*Seriola lalandi*	Gill	-15	-14,75	13,33	3,61
FH 24/26	*Seriola lalandi*	Gill	-13,93	-13,93	12,69	3,15
FH 24/26	*Seriola lalandi*	*Eobrachiella elegans*	-15	-14,46	16,11	3,9
FH 24/26	*Seriola lalandi*	*Eobrachiella elegans*	-16,6	-14,82	14,25	5,16
FH 24/26	*Seriola lalandi*	*Eobrachiella elegans*	-14,96	-14,39	16,08	3,93
FH 24/26	*Seriola lalandi*	*Eobrachiella elegans*	-14,41	-14	14,02	3,77
FH 21/008	*Seriola lalandi*	*Parabrachiella* sp. 1	-15,11	-14,52	15,87	3,95
FH 21/008	*Seriola lalandi*	*Parabrachiella* sp. 1	-15,46	-14,67	15,88	4,15
FH 21/008	*Seriola lalandi*	*Parabrachiella* sp. 1	-15,62	-14,58	15,36	4,4
FH 21/008	*Seriola lalandi*	*Parabrachiella* sp. 1	-16,01	-14,67	15,71	4,71
FH 24/22	*Seriola lalandi*	*Parabrachiella* sp. 1	-15,81	-14,87	15,39	4,3
FH 24/22	*Seriola lalandi*	*Parabrachiella* sp. 1	-15,57	-14,56	15,42	4,37
FH 24/26	*Seriola lalandi*	*Parabrachiella* sp. 1	-16,87	-14,62	14,6	5,63
FH 24/26	*Seriola lalandi*	*Parabrachiella* sp. 1	-15,91	-14,58	15,51	4,7
FH 24/14	*Seriola lalandi*	*Parabrachiella* sp. 2	-16,85	-14,38	14,73	5,85
FH 24/19	*Seriola lalandi*	*Parabrachiella* sp. 2	-15,52	-14,63	15,33	4,26
AB006,1	*Sarda sarda*	Muscle	-15,59	-15,54	15,28	3,4
AB007,1	*Sarda sarda*	Muscle	-16,83	-16,26	14,99	3,93
AB009,1	*Sarda sarda*	Muscle	-15,96	-15,85	14,96	3,47

ID	Host Species	Sample Analysed	$\delta^{13}C$	$\delta'^{13}C$	$\delta^{15}N$	C:N
AB016,1	*Sarda sarda*	Muscle	-17,2	-16,29	15,03	4,28
AB019,1	*Sarda sarda*	Muscle	-15,52	-15,52	15,04	3,24
AB006,2	*Sarda sarda*	Gill	-15,32	-15,1	14,11	3,57
AB007,2	*Sarda sarda*	Gill	-16,09	-15,66	14,04	3,79
AB009,2	*Sarda sarda*	Gill	-16,1	-15,77	13,9	3,68
AB016,2	*Sarda sarda*	Gill	-15,91	-15,37	14	3,91
AB019,2	*Sarda sarda*	Gill	-16,46	-15,8	14,14	4,02
AB006,3	*Sarda sarda*	*Caligus bonito*	-16,27	-14,94	17,46	4,69
AB006,4	*Sarda sarda*	*Caligus bonito*	-16,18	-14,83	16,24	4,72
AB007,3	*Sarda sarda*	*Caligus bonito*	-17,29	-15,47	15,99	5,2
AB007,4	*Sarda sarda*	*Caligus bonito*	-17,13	-15,26	16,38	5,23
AB007,5	*Sarda sarda*	*Caligus bonito*	-16,73	-15,31	17,96	4,79
AB009,3	*Sarda sarda*	*Caligus bonito*	-16,08	-15,58	17,33	3,86
AB009,4	*Sarda sarda*	*Caligus bonito*	-16,35	-15,65	17,82	4,06
AB009,5	*Sarda sarda*	*Caligus bonito*	-16,96	-15,18	16,16	5,15
AB016,3	*Sarda sarda*	*Caligus bonito*	-15,61	-15,03	17,36	3,95
AB016,4	*Sarda sarda*	*Caligus bonito*	-16,02	-14,8	16,93	4,59
AB016,5	*Sarda sarda*	*Caligus bonito*	-16,09	-14,7	17,26	4,76
AB019,3	*Sarda sarda*	*Caligus bonito*	-15,87	-14,82	16,69	4,41
AB019,4	*Sarda sarda*	*Caligus bonito*	-16,72	-14,69	16,16	5,41
AB019,5	*Sarda sarda*	*Caligus bonito*	-16,05	-14,69	16,56	4,73
D00495.1	*Genypterus capensis*	Muscle	-16,21	-16,21	15,83	3,18
D00495.2	*Genypterus capensis*	Gill	-16,26	-15,77	16,06	3,84
D00495.3	*Genypterus capensis*	*Chondracanthus colligens*	-17,07	-16,06	15,82	4,38
D00783,54,1,1	*Merluccius capensis*	Muscle	-16,06	-16,06	14,2	3,22
D00783,54,2,1	*Merluccius capensis*	Muscle	-15,91	-15,91	14,31	3,16
D00783,54,1,1	*Merluccius capensis*	Gill	-16,94	-16,4	13,85	3,89
D00783,54,2,2	*Merluccius capensis*	Gill	-15,92	-15,21	15,76	4,08
D00783,54,1,1	*Merluccius capensis*	*Anthocotyle merlucci*	-16,76	-15,83	14,76	4,29
D00783,54,2,3	*Merluccius capensis*	*Anthocotyle merlucci*	-15,29	-15,29	14,53	3,37
SSM 70	*Sardinops sagax*	Muscle	-16,44	-16,44	11,44	3,21
SSM 98.2	*Sardinops sagax*	Muscle	-16,57	-16,57	11,23	3,18
SSM 98.1	*Sardinops sagax*	Gill	-17,93	-17,51	9,22	3,78
SSMp1	*Sardinops sagax*	*Clavelisa ilishae*	-21,44	-17,15	12,42	7,69
SSMp2	*Sardinops sagax*	*Clavelisa ilishae*	-21,13	-17,49	12,63	7,03
SSMp3	*Sardinops sagax*	*Clavelisa ilishae*	-20,9	-18,2	12,85	6,07
HMM 2.1	*Trachurus capensis* (1)	Muscle	-17,16	-17,16	12,79	3,23
HMM 6.1	*Trachurus capensis* (1)	Muscle	-16,84	-16,84	12,72	3,21
HMM 9.1	*Trachurus capensis* (1)	Muscle	-17,5	-17,5	13,11	3,27
HMM 2.2	*Trachurus capensis* (1)	Gill	-17,83	-17,34	12,31	3,85
HMM 6.2	*Trachurus capensis* (1)	Gill	-17,08	-16,81	12,24	3,63
HMM 9.2	*Trachurus capensis* (1)	Gill	-17,95	-17,5	12,32	3,81
HMM 26.1	*Trachurus capensis* (3)	Muscle	-16,15	-16,15	11,9	3,27
HMM 27.1	*Trachurus capensis* (3)	Muscle	-16,56	-16,56	12,78	3,25
HMM 28.1	*Trachurus capensis* (3)	Muscle	-16,23	-16,23	12,03	3,24

ID	Host Species	Sample Analysed	δ^{13}C	δ'^{13}C	δ^{15}N	C:N
HMM 26.2	*Trachurus capensis* (3)	Gill	-16,2	-15,87	11,57	3,69
HMM 27.2	*Trachurus capensis* (3)	Gill	-17,76	-17,14	11,67	3,98
HMM 28.1	*Trachurus capensis* (3)	Gill	-16,88	-16,47	11,05	3,77
HMM 40.1	*Trachurus capensis* (5)	Muscle	-16,29	-16,29	12,07	3,24
HMM 43.1	*Trachurus capensis* (5)	Muscle	-16,33	-16,33	11,83	3,19
HMM 44.1	*Trachurus capensis* (5)	Muscle	-16,14	-16,14	12,02	3,21
HMM 40.2	*Trachurus capensis* (5)	Gill	-16,94	-16,63	10,88	3,67
HMM 43.2	*Trachurus capensis* (5)	Gill	-16,32	-16,21	11,07	3,47
HMM 44.2	*Trachurus capensis* (5)	Gill	-16,28	-16,01	11,16	3,63
HMM 69.1	*Trachurus capensis* (7)	Muscle	-17	-17	13,03	3,25
HMM 72.1	*Trachurus capensis* (7)	Muscle	-16,37	-16,37	12,86	3,22
HMM 75.2	*Trachurus capensis* (7)	Muscle	-17,11	-17,11	13,12	3,27
HMM 69.2	*Trachurus capensis* (7)	Gill	-17,94	-17,53	12,06	3,76
HMM 72.2	*Trachurus capensis* (7)	Gill	-17,18	-16,74	12,24	3,79
HMM 75.2	*Trachurus capensis* (7)	Gill	-18,09	-17,52	12,24	3,93
HMM 102.1	*Trachurus capensis* (8)	Muscle	-16,19	-16,19	13,35	3,23
HMM 91.1	*Trachurus capensis* (8)	Muscle	-16,52	-16,52	12,44	3,23
HMM 93.1	*Trachurus capensis* (8)	Muscle	-17,33	-17,33	12,36	3,25
HMM 102.2	*Trachurus capensis* (8)	Gill	-17,05	-16,59	12,14	3,82
HMM 91.2	*Trachurus capensis* (8)	Gill	-17,3	-16,89	11,93	3,77
HMM 93.2	*Trachurus capensis* (8)	Gill	-18,3	-17,87	11,75	3,79
HMMp1	*Trachurus capensis* (1)	*Gastrocotyle trachuri* (1)	-19,04	-17,91	13,22	4,49
HMMp3	*Trachurus capensis* (3)	*Gastrocotyle trachuri* (3)	-18,88	-17,69	11,95	4,55
HMMp5	*Trachurus capensis* (5)	*Gastrocotyle trachuri* (5)	-18,52	-17,23	11,71	4,65
HMMp7	*Trachurus capensis* (7)	*Gastrocotyle trachuri* (7)	-18,5	-17,49	12,61	4,38
HMMp8	*Trachurus capensis* (8)	*Gastrocotyle trachuri* (8)	-18,54	-17,42	12,46	4,48
Lan 6.1	*Lampanyctodes hectoris*	Muscle	-16,88	-16,48	13,52	3,76
Lan 8.1	*Lampanyctodes hectoris*	Muscle	-17,92	-17,44	12,85	3,84
Lan 25.1	*Lampanyctodes hectoris*	Muscle	-17,50	-17,33	12,99	3,52
Lan 18.1	*Lampanyctodes hectoris*	Muscle	-18,19	-17,09	13,23	4,47
Lan 17.1	*Lampanyctodes hectoris*	Muscle	-17,78	-17,42	12,95	3,72
Lan 6.2	*Lampanyctodes hectoris*	Heart	-18,26	-17,47	11,98	4,16
Lan 8.2	*Lampanyctodes hectoris*	Heart	-19,17	-18,11	9,62	4,43
Lan 25.2	*Lampanyctodes hectoris*	Heart	-19,04	-18,44	11,55	3,95
Lan 18.2	*Lampanyctodes hectoris*	Heart	-18,33	-17,79	11,94	3,90
Lan 17.2	*Lampanyctodes hectoris*	Heart	-19,11	-18,48	12,15	4,00
Lan 6.3	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-20,92	-17,49	9,67	6,81
Lan 6.4	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-20,33	-17,32	9,50	6,40
Lan 8.3	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-21,75	-17,58	9,47	7,57
Lan 8.4	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-20,59	-18,25	8,76	5,71
Lan 25.3	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-19,89	-18,59	8,57	4,67
Lan 25.4	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-20,66	-18,50	9,28	5,54
Lan 25.5	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-19,16	-18,38	9,13	4,14
Lan 18.3	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-21,05	-17,97	9,43	6,47

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Lan 18.4	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-21,78	-17,62	9,44	7,55
Lan 17.3	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-19,81	-18,24	9,44	4,94
Lan 17.4	*Lampanyctodes hectoris*	*Cardiodectes medusaeus*	-20,88	-18,86	8,73	5,39