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Ectoparasites of the Southern mullet *Liza richardsonii* in the Berg Estuary, South Africa

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Science in Applied Marine Science

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Declaration

I hereby declare that all of the work presented in this thesis is my own, except where otherwise stated in the text. This thesis has not been submitted in whole or in part for a degree at any other university.

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Date

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Abstract

The aims of this study were to observe changes in ectoparasite abundance and diversity on fish in relation to salinity gradients in the Berg Estuary. The host species studied was the commercially important, euryhaline southern mullet *Liza richardsonii* that is superabundant in estuaries of the Western Cape. A total of 210 fishes were sampled by using a single haul with a beach seine net at each of seven localities along a salinity gradient from the mouth to the upper reaches. Seven species of parasite were found. These included five species of Copepoda; a member of the family Caligidae, an *Ergasilus* species, a *Dermoergasilus* species, a *Brachiella* species and an unidentified caudal fin parasite. Two representatives of the Monogenea were found, one from the family Microcotylidae and another that was not identified. Infestation prevalence, mean intensity and site specificity were determined for the different parasite species. The mean infestation rate was highest at the stable saline extremes, freshwater (12 individuals) and marine (14 individuals). The lowest rate (four individuals) was observed at a highly variable saline environment situated 5 km from the mouth. Statistical analysis revealed that site selection for the gill parasites was a random process not affected by the behaviour or mobility of the parasite. The statistical analysis also revealed that there was no preference for infestation sites on the gills. It was concluded that salinity is not the single most important factor affecting parasite distribution and composition on the Southern mullet.

Key words: Ectoparasite, *Liza richardsonii*, salinity effects, Berg Estuary

Introduction

Utilisation of estuaries by fishes

It is well known how important estuaries are to marine fishes. Biologists have long maintained that the most important role of the estuarine environment, in respect of fish populations, is the provision of nursery grounds for juveniles of marine species (Wallace *et al.* 1984). This is because the environment in an estuary differs markedly from that of the sea. Marine organisms have to continually cope with wave action and a lack of shelter. Those found within estuaries experience an environment, which is smaller in area and is typically calm and shallow. It is also thought that because estuaries are turbid, they provide shelter from predation and, because they are very productive, they provide an abundance of food (Wallace *et al.* 1984).

However, not all marine fishes utilize the estuarine environment. Within South Africa, approximately 1500 species of continental shelf fishes have been recorded, but less than 100 species regularly occur in estuaries. The often abrupt changes in salinity, water temperature, dissolved oxygen and turbidity place considerable physiological demands on the fishes which utilize estuaries (Whitfield 1999). The majority of fishes that utilize estuaries enter the estuary as juveniles once egg and larval development has taken place in sea, close to the estuary mouth. This migratory instinct is very marked and during late winter, spring and early summer millions of fish fry enter estuaries, where the high temperatures and rich food supply favours rapid growth, and where they are protected from marine predators (Wallace *et al.* 1984). On the basis of the fact that the most important role the estuarine environment plays for marine fish populations is the provision of a nursery ground, the same authors developed a classification system for

South African associated fish fauna. This divides fish into six categories, according to their extent of dependence upon estuaries, as shown in Table 1.

Table 1. Levels of estuarine dependence of fishes found in South African estuaries, as defined by Wallace *et al.* (1984).

| Category | No. of species | Typical species | Relationship to South African Estuaries |
|----------|----------------|--|--|
| I | 8 | <i>Gilchristella aestuaris</i> | Dependence on estuaries during entire life cycle. |
| II | 22 | <i>Liza macrolepis</i> <i>Mugil cephalus</i> <i>Myxus capensis</i> <i>Valamugil cunnesius</i> <i>V. robustus</i> | Dependent on estuaries during juvenile phase of life cycle. |
| | 4 | | Dependent on estuaries during migration between rivers and the sea. |
| III | 19 | <i>Liza alata</i> <i>L. dumerili</i> <i>L. tricuspidens</i> | Largely, but not entirely, dependent on estuaries during juvenile phase of life cycle. |
| IV | 19 | <i>Crenimugil crenilabis</i> <i>Liza richardsonii</i> <i>Valamugil buechanani</i> | Benefit from, but only partially dependent on estuarine nursery areas. |
| V | 28 | | Stray into estuaries from the sea, not dependent on estuaries |
| VI | ± 100 | <i>Glossogobius giurus</i> | Miscellaneous species not dependent on estuaries. |

Table 1 gives a brief description of the categories, the total number of species in that category and the names of a few South African species important to the current study. The physical and biological structure of an estuary allow for numerous niches to be occupied by a range of species.

Whitfield (1996) outlines some of the factors determining fish distribution and abundance within estuaries which involve the following: salinity, river flow, turbidity, mouth condition, habitat variability, zoogeography and seasonality, catchment and estuary size, parasitism and productivity. Some of these factors also help to explain why fishes enter estuaries. For example, Whitfield *et al.* (1981) describes the salinity ranges of some Southern African fish species. Those, which are very good osmoregulators can,

penetrate further up an estuary and benefit from food resources not utilized by fishes, which are poor osmoregulators. An interesting aspect of their paper was that they found an inverse relationship between salinity and fish species diversity at St. Lucia. Gill-net catch rates during low salinities (< 20 ‰) increased by 100 % when compared to high salinity conditions (> 50 ‰). Thus, salinity can be regarded as an important factor determining fish distribution.

Another factor is turbidity. Blaber and Blaber (1980) have shown that juveniles that enter an estuary prefer highly turbid areas, compared to adults of the same species that were mostly recorded in low turbidity waters. They outlined that the main reason for this preference was due to the reduced light intensity, which minimized the effects of predation, and the possibility of increased surface zooplankton densities in very turbid areas providing a source of food.

Lastly, Whitfield (1996) briefly mentions that parasite infestation of fishes may also influence the successful utilization of southern African estuaries. Some fish carry high parasite loads, which may affect not only the fitness, but also the survival, of the host. However, he did not give a reason as to why or how fishes would benefit from utilizing an estuary in respect of their parasitic burden. Birkeland and Jacobsen (1997) have suggested that heavy infestation by salmon lice on sea trout may cause their premature return to estuaries and rivers. They suggest that physiological stress and high infection pressure in the sea result in this species returning to estuaries and freshwater. Heuch *et al.* (2002) indicated that species from the family Salmonidae, which are euryhaline fish, decrease their ectoparasites load once they have been exposed to low salinity for a short period. These findings give rise to the idea that fishes, which are

capable of tolerating low and high salinities, may benefit from entering estuarine environments as this is also a means by which they can reduce their parasite load and composition. The current study shall attempt to find similar evidence and observe if parasitism could be a possible factor for South African fishes entering estuaries.

Species selection for study

The ideal candidates for the current study are the fishes from the family Mugilidae, the reason being that the majority of these species are commonly found within estuaries, where they have to adapt to a constantly changing environment. They have a global distribution in temperate and tropical waters and can be found in marine, brackish and freshwater environments. One of the species, *Mugil cephalus*, is found in all the oceans from 42° N to 42° S and is the most widely known of the mullet species (De Silva 1980). This species has come into prominence among fisheries scientists due to its potential as a culture species in the tropics. A typical species such as *Mugil cephalus*, travels in schools and feeds on fine algae, diatoms, and detritus of bottom sediments (Thomson 1966).

Fifteen species of Mugilidae are found in South African waters and the most widely distributed of these is *Mugil cephalus*. Papers have been written on the biology (Thomson 1966; and De Silva 1980) and on the parasites and diseases (Paperna and Overstreet 1981) of this species. Less is known about other common species such as *Crenimugil crenilabis*, *Myxus capensis*, *Liza allata*, *Liza dumerilii*, *Liza richardsonii* and *Valamugil buchanani*. According to Whitfield *et al.* (1981) these mullet species can be

divided into four main groups, according to their origin and salinity tolerance. The species of interest in the current study are those that belong to the euryhaline marine fish group. This is the dominant group, where fishes penetrate estuaries for distances that vary according to their salinity tolerance. From their paper, it can be seen that the southern mullet *Liza richardsonii* is included in this group and can tolerate a range of conditions from freshwater to completely marine environment. Furthermore, this species is known to be extremely abundant within the Berg Estuary (De Villiers 1987).

According to Smith and Heemstra (1986) the endemic southern mullet *Liza richardsonii* (Smith, 1846) is regarded as one of the most abundant of the 15 species of Mugilidae found within South Africa and can be found from southern KwaZulu-Natal to northern Namibia. Most juveniles are commonly found in estuaries along the South African coastline, whereas adults (fishes longer than 200 mm or at an age of more than three years) are more commonly found in the near-shore marine environment. southern mullet found close to embayments and estuaries (Saldanha Bay, False Bay, Berg River) mostly had diatoms and detritus in their stomachs, whereas those caught in the open sea mostly contained zooplankton (De Villiers 1987). Southern mullet are also more abundant in estuaries during the summer season and temporarily decrease in numbers during the rainy winter period. However, they remain present along the coast between Port Nolloth and Still Bay throughout the year (De Villiers 1987). The period of greatest sexual development extends from September - March and spawning occurs throughout that period (Lasiak 1983).

This species can be identified by having an elongate shape with a pointed snout. The body has an overall silvery sheen, but is darker above and white below. A yellow

blotch can be found on the gill covers and adults can attain a length of 60 cm. The body is covered with 44-50 series of large distinct scales arranged along the flanks. The two dorsal fins are well developed. Thin lips surround the terminal mouth and while the jaws of adults are toothless, juveniles of less than 10 cm have distinctly pointed teeth. There are 90-100 rakers on the first gill arch and the eyes are covered by adipose tissue (Branch *et al.* 1994).

From Table 1 it can be seen that the southern mullet or Harder, *Liza richardsonii* is placed in category IV (Whitfield 1996). The juveniles occur mainly at sea, but are also abundant in estuaries. In particular, large populations occur in the Berg Estuary, where *Liza richardsonii* is subject to intensive gill net fishing by commercial fishermen (De Villiers 1987). Some 5-6 million fish are caught annually and 95 percent of these are landed between Cape Agulhas and False Bay. The firm white flesh provides excellent eating and is also good bait (Van Der Elst 1988). However, numerous parasites infecting this fish affect the fishing industry by causing mortalities within the fished populations.

Marine fish parasites have been given considerable attention over recent years due to certain species being harmful to man. Parasites not harmful to man, can also decrease the market value of commercially caught fish. To fishermen and mariculture operators, this type of information is becoming increasingly important, as fish diseases can lead to a reduction in fish yield and a reduction in commercial value of fish stock. Yashouv (1972) has shown that heavy infestations of copepod parasites on mullets within fishponds can decrease harvest by as much as 40 %. A 40 % decrease in harvest for the southern mullet fishery would cause considerable commercial harm to the local South African economy. As a result, information on the ecology and diversity of mullet parasites would be of great

benefit to those who manage the *Liza richardsonii* stock. It would allow for the proper implementation of preventative measures for the reduction of parasite load and composition.

Modes of infection by parasites

The difficulty with marine fish parasites, in particular those found around the South African coast, is that “one has to recognize and identify them, know their biology, life cycles, invasion pathways, relationships within food chains and many other biological phenomena pertaining to parasites” (Grabda, 1991).

In general there exist various modes in which pathogenic effects of parasites on hosts manifest themselves. Numerous parasites attach to their hosts through mechanical mechanisms, by using organs such as hooks, clamps and suckers, which enable them to stay on or in a host. This method of attachment generally damages the host’s body.

Another method is by depriving the host of food. As most parasites feed at the expense of their host they can do so either as intestinal parasites, digesting the host’s food or can feed on its blood and/or tissue fluid, thus taking away a considerable amount of nutritive substances from the host (Grabda 1991). As a result, the effected fishes lose weight and develop anaemia, particularly during a heavy invasion.

Lastly the parasites metabolites and glandular secretions may be toxic for the host. For example the branchiurian *Argulus* has a venom gland at the base of its stylet, which is used to pierce the skin and suck in the host’s blood and tissue juices. The toxins

irritate the fish skin and produce inflammation. They may be lethal to newly hatched fish and to the fry (Grabda 1991).

The host can respond to infections in an attempt to counteract the harmful effects of parasites. One defensive reaction to ectoparasites such as *Argulus*, leeches and copepods is to rub the body against sand, stones and submerged objects. This behaviour has been observed in the southern mullet (Gilchrist 1914). Other responses to skin and gill parasites include the intensified secretion of mucus (metaplasia), whereby the parasites are removed from the skin and gills with mucus. Parasites that bury themselves in the tissue can be removed through isolation. This is achieved as the host attempts to surround the parasite with connective tissue capsules, which may be impregnated with calcium salts. The capsules isolate the parasite and protect the host from its metabolites and toxins (Grabda 1991). Fishes may also produce antibodies and antitoxins, which can immobilize parasite toxins, making them harmless to the host. Lastly, the host may develop immunity to parasitic invasion. This immunity may be innate (host's reaction to the first contact with a parasite), or acquired, as immunity develops after a parasite or its metabolites have acted on the host and can protect the host for some time from recurrent invasion (Grabda 1991).

Ectoparasites within South Africa

A diverse array of parasites affects members of the Mugilidae. These include representatives Bacteria, Protozoa, Copepoda, Branchiuria, Isopoda, Monogenea, Nematoda Acanthocephala and Cestoda. A number of these have been studied in their

natural environment from the Northern Black Sea of Azov (Reshetnikova 1955), from Israel (Paperna 1964, 1975; Paperna and Lahav 1971, 1975) and from the south-eastern United States (Rawson 1973; Skinner 1974).

The most visible parasites on mullets are representatives of Copepoda, Branchiuria and Isopoda. These can be found externally between the fins, or on the gill filaments, gill rakers and the area around the mouth. Species, which attach to the gills, can become extremely detrimental to the host, in particular when the fish is confined to concentrated pools, or used in aquaculture. The gill parasites can cause severe damage by producing digestive secretions, which partially dissolve tissue. As the number of attached parasites increases, the destruction of the respiratory epithelium progresses (Paperna and Overstreet 1981), thus the ability of the fish to respire decreases and the fish becomes more stressed. In certain cases, when the intensity of infestation is high, damage to body tissue leads to asphyxiation, loss of blood, susceptibility to new diseases and ultimately death.

Most work conducted on fish parasites within South Africa has been taxonomic and little attention has been given to the ecology or biology of parasites of South African mullet species. Rawson (1977) mentions the development, seasonal abundance of parasite populations, and distribution of gill parasites on *Mugil cephalus*, Oldwage and van As (1988) described two new Ergasilidae species on *Mugil cephalus* and Schramm (1991) found that the southern mullet was parasitized by the cestode *Grillotia perelica*, but did not report the presence of other parasite species. Kruger *et al.* (1997) made a few ecological observations of the copepod, *Mugilicola smithae* on a number of species of Mugilidae from Transkei estuaries. Paperna (1996) documented haemogregarines and

trypanosomes infecting the blood and leeches from the inside of the mouth in mature fish (over 200 mm in length) of *Liza richardsonii* from the Kowie lagoon in the Southeastern Cape. Most effort has been directed at other fish species in the family Mugilidae, in particular *Mugil cephalus*. However, from all data produced, little has been reported as to how these fish remove their parasite burden. Furthermore, biologists are not sure how many species of copepods parasitize *Liza richardsonii*. According to Ho & Do (1982) and Oldewage & van As (1988) a relative of *Liza*, *Mugil cephalus*, is parasitized by more than 40 different species of copepods. This high number is likely due to its wide geographic range, which, in South Africa overlaps with that of *Liza richardsonii*. These closely related species could have similar parasite composition, but a comparison of their parasite fauna has never been attempted. Previous sampling efforts have shown that juvenile *Liza richardsonii* are parasitized by five different species of Copepoda, two species of Monogenea, one branchiurian and one isopod, as well as having myxosporian cysts. This suggests that the southern mullet can act as a host for a wide diversity of ectoparasites. Rawson (1977), who described the development and seasonal abundance of parasite populations and the distribution of gill parasites, studied the population biology of *Mugil cephalus* parasites. The majority of work describing ectoparasites comes from work done on *Mugil cephalus* (Ho and Do 1982; El-Rashidy and Boxshall 2001a, 2001b, 2001c, 2002).

Whitfield *et al.* (1981) and Whitfield (1999) suggest that the prevailing salinity regime and estuarine type have a major influence on the ichthyofaunal community that develops. Thus the species richness of fish communities differs with changing salinity along an estuary. If these changes in species richness of fishes were observed, would

similar differences be observed in the parasite community associated with these fish? Rawson (1977) states that development of crustacean parasitism on mullets reflects rules for development of marine parasite fauna: (1) intensity and prevalence of infection increase with age, (2) changes in parasite composition reflect changes in host habitat or behaviour. Grabda (1991) stated that salinity is an important factor controlling development of fish parasites and that salinity effects are particularly pronounced in migratory fish. Those ascending the rivers or freshened coastal lakes from the sea lose their typically marine parasites and acquire freshwater ones, while the reverse takes place during descent. This transition has been observed for a long time in salmon and trout. However, the work done on these fishes only examined skin ectoparasites and did not include observations made about the effects of a changing saline environment on the gill parasites.

Gill parasites have been well documented for a number of fish species and for specific parasite species. Site preference of gill parasites was observed on the rabbit fish (Martens and Moens 1995) and the distribution and coexistence of gill ectoparasites was studied for *Cephalopholis argus* (Lo and Morand 2001). Less has been observed from gill parasites on representatives of the Mugilidae. Collins (1987) described the macrohabitat effects of copepods parasitizing the same species and El Hafidi *et al.* (1998) described the microhabitat distribution and coexistence of monogeneans parasitizing *Mugil cephalus*. No one has attempted a review of gill ectoparasites from the southern mullet *Liza richardsonii*. Fewer investigations are made on discovering how these fish rid themselves of their parasites, in particular their external parasites, which are greatly affected by the environment their host enters.

Aims of the study

One of the aims of the current study is to understand the relationship of gill parasites to their environment. Because so little is known about the gill parasites of *Liza richardsonii* it is not clear which species are host specific and if those are influenced by the absence or presence of competing species. Furthermore, it is not fully understood if a specific parasite prefers certain localities on the gill due to its morphology, mobility, and ability to access a specific site, competition, predation or ability to mate. Lo and Morand (2001) observed that the copepods under their investigation, showed a huge distribution over the gills of *Cephalopholis argus* and argued that this was due to their mobility allowing them to occupy different niches on the gills. Rawson (1977) on the other hand, indicated that a high degree of gill habitat subdivisions was encountered from parasites on *Mugil cephalus*, without any competitive exclusion. This still fails to indicate the role competition might play for gill parasites, in particular those on the southern mullet.

The hypothesis for this study is that juvenile southern mullet not only use estuaries as a nursery, but also to remove their parasite burden by moving from an ocean environment to that which is less saline. The aims are therefore: 1, to identify the parasites infecting juvenile southern mullet; 2, to observe parasite load and composition in relation to changing salinity; 3, to describe the spatial distribution of ectoparasites on the gills of *Liza richardsonii*, 4, to speculate on what factors are involved in site selection for the gill parasites.

Materials and Methods

Study area

The current study was conducted entirely in the Berg Estuary, which is situated about 32°46'S, 18°09'E in a winter rainfall area and is fed by the largest river in the Western Cape. The catchment area is about 4000 km² and has a mean annual runoff of 693 x 10⁶ m³. Flow rates are usually 0.2-2.0 m³s⁻¹ during winter, but they may attain 700 m³s⁻¹ during floods. The estuary meanders over an extensive floodplain and its bed falls only one meter over the last 50 km before reaching the sea, tidal effects are measurable up to 65 km from the sea (Bennett 1994). The natural mouth of the estuary has been closed and a new artificially-constructed mouth has been created to make a permanently open estuary. The channel has been dredged for the first four kilometres to a depth of four meters to allow the passage of purse-seine boats (Bennett 1994) (Fig.1).

Sampling

Sampling of southern mullet within the Berg Estuary was conducted from 4-6 November 2003. The surveyed area began at the mouth of the river and continued for about 30 km upstream, ending close to the farm Kliphoek (Fig. 1). Each sample (Bennett 1994) consisted of a single haul with a beach seine 20 m long, with a 12 mm stretched

mesh. Each sample covered an area of 200-600 m². The sites were chosen according to their salinity, which was measured on site using an optical-salinometer. Water samples were taken at each site and brought back to the laboratory where the salinity, conductivity and temperature were checked with the YSI Model 30 salinity meter. Seven sites were selected, at each of which 30 randomly-selected Southern Mullet were collected and kept in large containers aerated with oxygen pumps. On return to the camp, the live fishes had their spinal cord severed, after they were anaesthetized using clove-oil. Fishes were then measured (total length) and examined for external parasites. The left and right gills were excised, separated and placed in a Petri dish containing seawater. The gill arches were examined using a dissecting microscope, beginning with the first right gill. Left and right arches were numbered 1-4, with gill arch one nearest the operculum and arch four nearest the midline. Each gill arch was divided into three approximately equal regions dorsoventrally (sections I, II, III), according to the method of Lo and Morand (2001). The findings were recorded on data sheets, which indicated the parasite species, number found and locality on the fish.

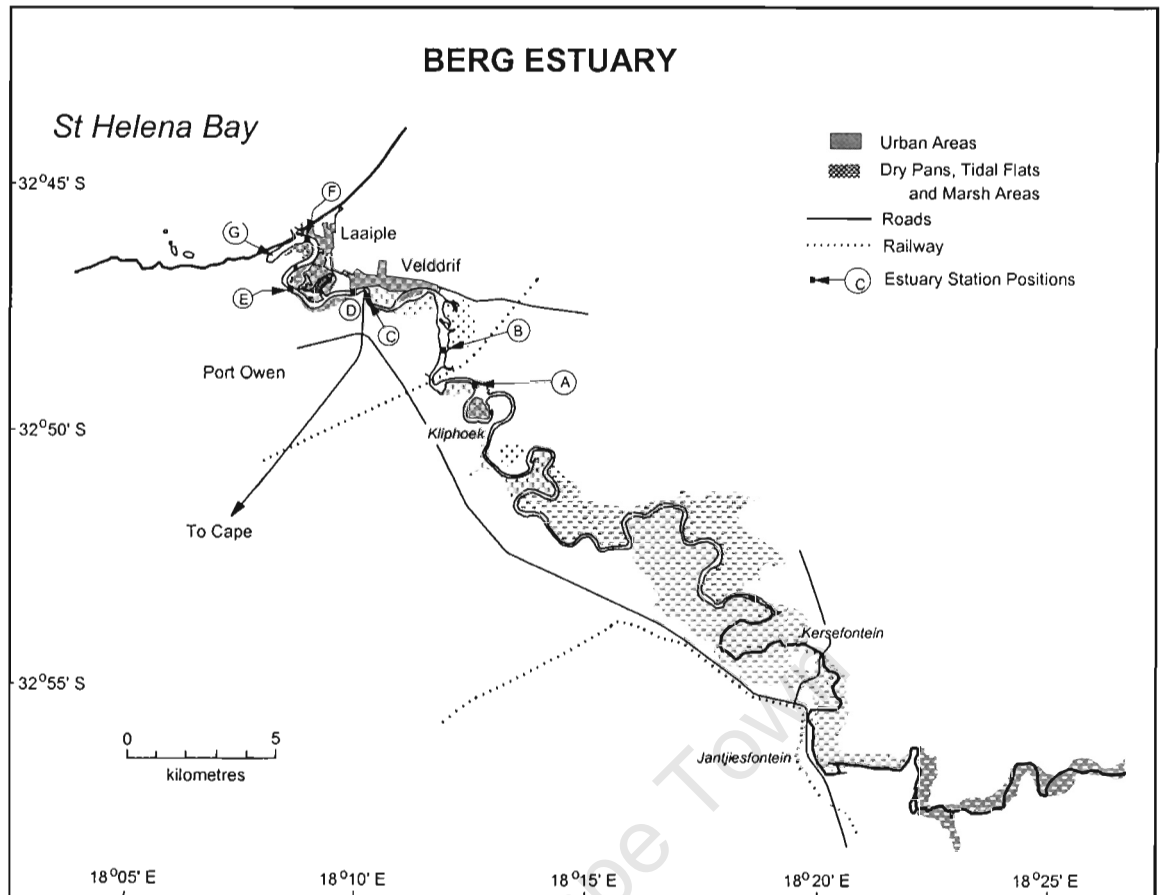


Fig. 1, The Berg Estuary, indicating the fishing stations used to catch *Liza richardsonii* on 4-6 November 2003.

In the laboratory, gill copepods were randomly chosen according to their morphology and location on the gill and cleared in 88% lactic acid and stained in 1 g: 10 ml Lignin Pink in Lactic acid for one hour (pers comm, N. Smit). These were then examined and drawn using a *camera lucida*, and split into species. Body width and length were recorded for 20 specimens of each species. Monogeneans were stained using the Mayer Carmalum method for staining helminths. They were then photographed and identified.

Prevalence (percentage of host population infected) and mean intensity (average number of parasites per host in the infected population) was calculated for all the parasite

species and for each locality. Comparisons of community composition with regard to parasite abundance and changing salinity were completed using the PRIMER software package (Plymouth Marine Laboratory). ANOSIM (based on Bray-Curtis similarities) was used to detect significant differences between parasite communities from each site. All data were checked for homoscedasity and homogeneity of variances using the Kolmogrov-Smirnof and Levenes tests respectively. Differences in parasite intensity between left and right gill arches were tested using a Student's t-test. In order to test if the gill parasites preferred a specific gill, the Kruskal Wallace non-parametric ANOVA was used. Finally, ANOVA was used to compare infestation levels between the different sections on the gill. All univariate statistical tests were performed using the software STATISTICA.

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Results

Altogether 210 juvenile southern mullet (*Liza richardsonii*) were captured in the study period, ranging from 46-172 mm in fish length with an average of 90 mm. The total number of parasite species found was seven. All of the species observed were found at different localities on the fish (Fig. 2). Two species of copepods were encountered externally and parasitized the fins. The unidentified caudal fin parasite was mainly found on the caudal fin, but could also be found embedded in the dorsal fin. Only three individuals were found and could only be identified to order. The head of this parasite was found embedded within the soft tissue of the fins, whereas the rest of its body remained external. It has a short neck, which is attached to a small, flat shaped thorax. The egg sac is cylindrical and is longer and larger than the thorax. These individuals were among the larger species observed and measured 6-8 mm in length.

The other copepod species found externally belonged to the family Lernaepodidae. These individuals were identified as *Brachiella* species of which all five specimens were exclusively found embedded beneath the pectoral fin. This species is small, 2-4 mm in length, with a sac-like body; segmentation is obliterated so that only two parts of the body can be discerned: the cephalothorax with antennae and mouth parts, and the thorax formed by the fusion of the terminal thoracic segments, the genital segment, and the abdomen (Grabda 1991).

Five species of gill parasites were found, of which three belonged to the Copepoda and two to the Monogenea. One caligid species was found on the gill filament. It was nearly 3 mm in length and was light brown. The second antennae and maxillipeds

were used as additional attachment organs, accompanied by its cephalothorax, which functions as a sucker, holding fast to the gills (Fig. 2). Two gill parasites came from the family Ergasilidae, one from the genus *Dermoergasilus* (688 individuals) and the other from the genus *Ergasilus* (832 individuals). Twenty individuals were taken from each genus based on the parasite's locality on the gills and measured for width and length in order to provide average measurements for the entire population observed. Those from the genus *Dermoergasilus* averaged 0.72 mm in length and 0.29 mm in width, while the *Ergasilus* averaged 0.62 mm in length and 0.29 mm in width. These parasites were found on two separate locations on the gill. The *Ergasilus* species were only found on the gill rakers, where they were hard to find as their white texture blended with the texture of the gill rakers. The *Ergasilus* have strong claw-like antennae adapted for burrowing in the hard gill tissue. The *Dermoergasilus* were slightly darker in colour and were beige. They were only found at the base of the gill filament and used their second antenna with hooks to tightly clasp the soft filament tissue.

Two species from the class Monogenea were found on the gills (Fig. 2). Both species were mobile, but differed in respect of the localities in which they were found on the gills. Those from the genus *Microcotyle* (23 individuals) were mostly found in between the middle of the gill filaments. The other, unidentified, monogenean species (75 individuals) had a flattened disc-like shape, with large black dots covering its body. Its opisthaptor resembled clamps, which it used to attach to the host. The unidentified monogenean was tubular in shape and white. Its length (0.3 mm) and width (0.1 mm) was much smaller than the *Microcotyle* species that had a length of 1.5 mm and a width of 1 mm. It was mainly found at the roof and between the gill filaments. The opisthaptor on

this species contained suckers for attachment. Myxosporian cysts were also observed, but are not considered any further.

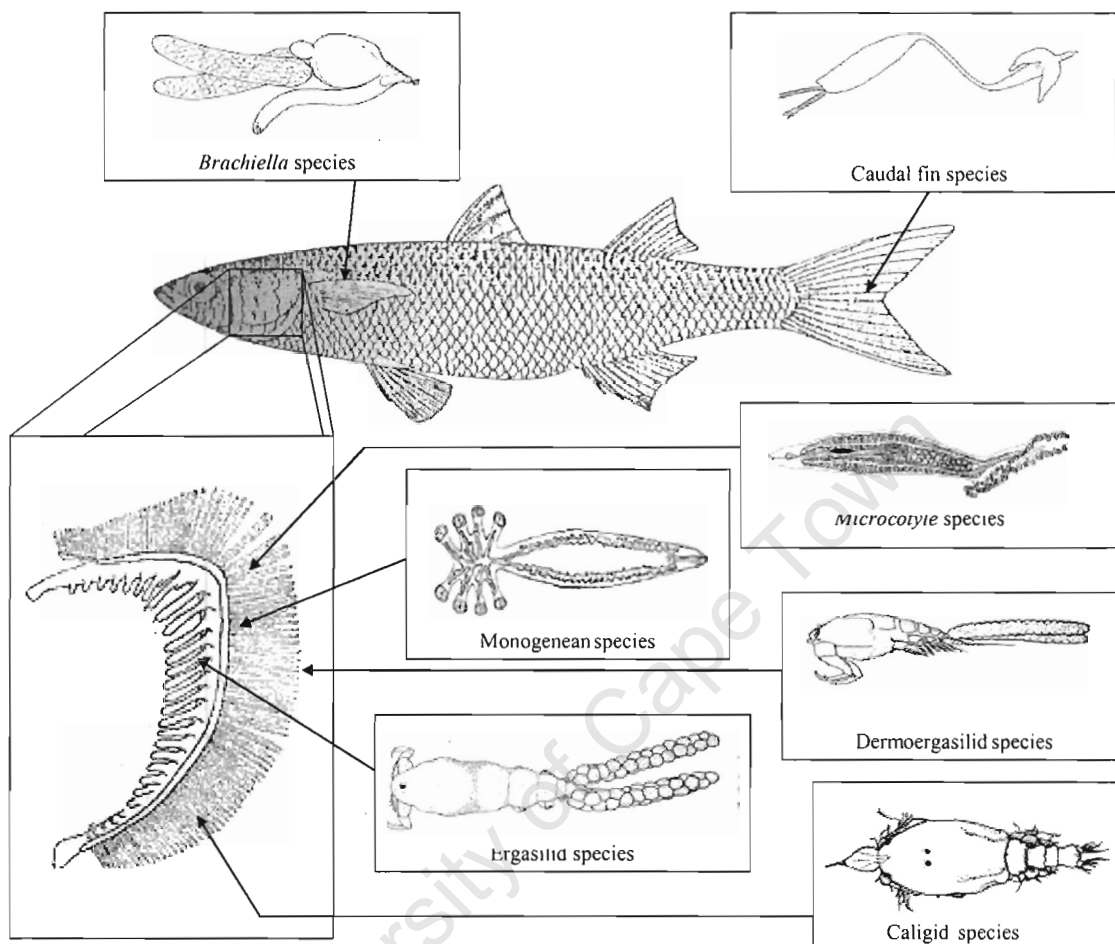


Fig. 2 Diagram of all the parasite species observed and where they were found on the Southern mullet.

The distribution of each species in relation to salinity is shown on Fig. 3. The greatest number of species (seven) was found at the highest salinity (32.7 ppt). The gill parasites were common throughout the range of salinities investigated. The second highest number of parasite species found was at a salinity of 14.5 ppt, where five species

were found. Parasites found externally, such as those from the genus *Brachiella*, were only found at salinities >14.5 ppt.

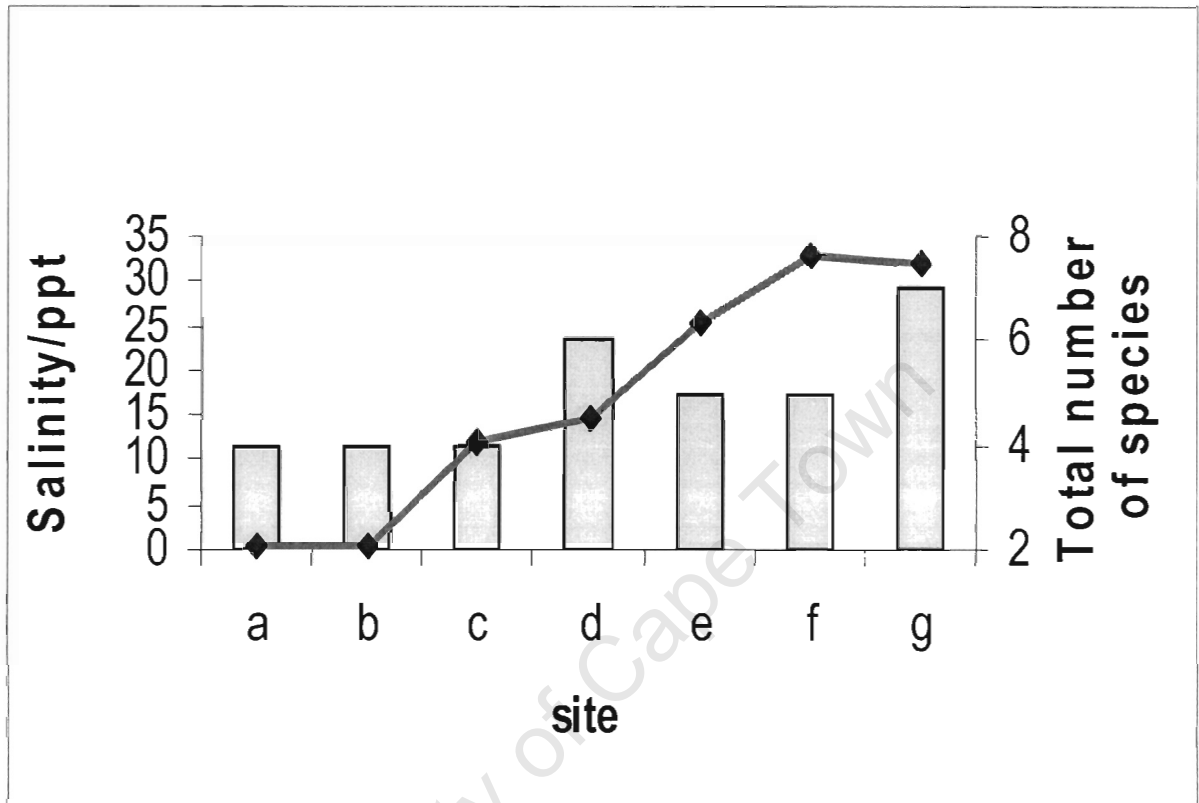


Fig. 3 Bar graph showing species richness for all the seven sites. The line graph indicates the salinity recorded at each site.

The parasite community found on juvenile mullet at each locality investigated can be seen on Fig. 4. The gill parasites were observed from low salinities 0.4 ppt up to the highest salinity 32.7 ppt. except for the caligid species that was only found at salinities similar to the marine environment. Both external copepods were found in high salinities <30 ppt (Fig. 4), however the unidentified caudal fin parasite was not present in the highest salinity found at Site F (32.7 ppt).

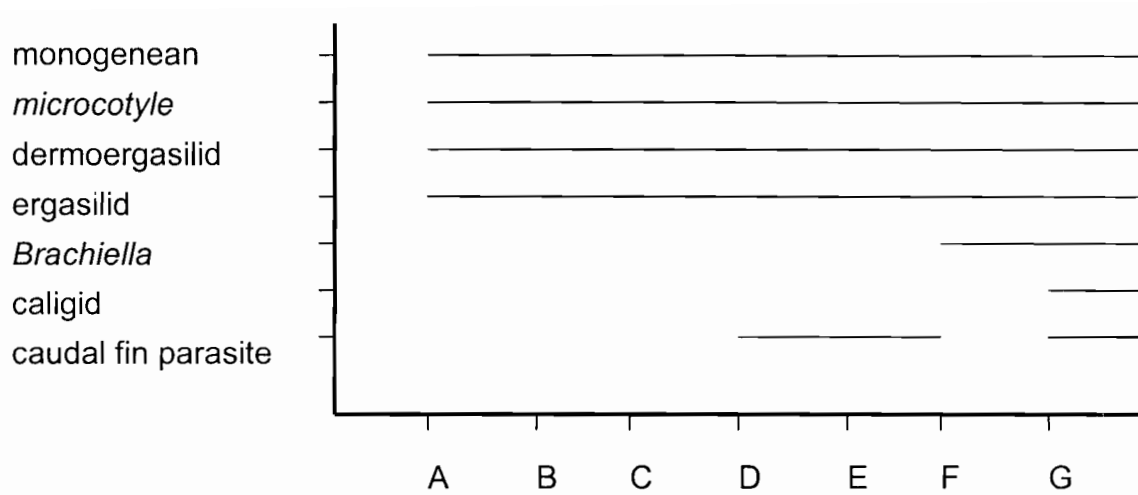


Fig. 4 The presence of each parasite species found at the various locations observed.

Figure 5 shows the total parasite load per 30 fish in relation to salinity. The highest parasite burden of 429 individuals was found at the highest salinity of 32.7 ppt. This gradually decreased to 146 individuals at a salinity of 14.5 ppt at Site D, after which it increased again to 376 parasites at a salinity of 0.4 ppt at Site A. Thus there are huge abundances of parasites at the extremes of the salinity range and lower numbers in the middle range. For individual parasites the distributional trends are mostly similar (Fig. 7). Abundance for the unidentified monogenean peaked at 86 individuals at Site E, and was lowest at seven individuals at Site B. Specimens from the Microcotylidae peaked at five individuals at Sites F and G, and was lowest at two individuals at Sites A, B and D. The most abundant species was from the genus *Ergasilus* with 242 individuals at Site F, and a minimum of 23 individuals at Site E. Abundance of the *Dermoergasilus* peaked at 179 individuals at Site A, and was lowest at Site B with 49 individuals. Individuals from the *Brachiella* species peaked at Site G with three individuals. Only single specimens of the Caligidae and the unidentified caudal fin parasite were observed at Site G.

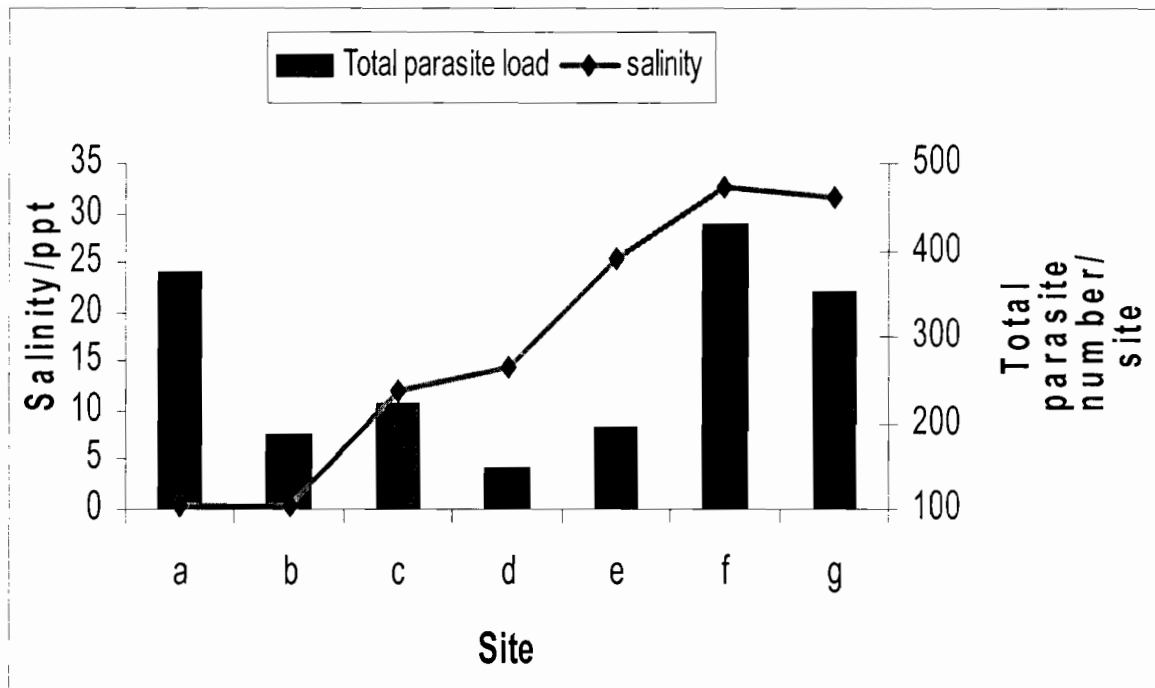


Fig. 5 Parasite load found on 30 fish from each site. The line graph shows the salinity at each site.

Figure 6 shows the mean parasite load for thirty fishes collected at each site. This graph indicates that at salinities of > 30 ppt and < 12 ppt the mean parasite infestation was high and was greatest at Site F (14 parasites per fish). The lowest value observed, was at Site D where the mean parasite number was four. Maximum and minimum bars indicated that at each locality there were fishes that were not parasitized. However, some fishes carried high parasite loads (64 parasites at Site E) and these were mainly observed at the extremes of the saline range.

These findings are also reflected in Tables 2 and 3, which show the prevalence (% of hosts parasitized) and mean intensity of infection (average number of parasites per host). The gill copepod (*Ergasilus* and *Dermoergasilus*) were the most prevalent parasites, infecting 147 of the 210 fishes analyzed. At the different localities, their prevalence fluctuated considerably but no pattern can be seen. In contrast, the

microcotilid had a stable prevalence, infesting at least two fish at each locality, and slightly increasing at higher salinities. Only one individual Caligidae was found at a salinity of 31.7 ppt. Very low prevalences can also be seen for the *Brachiella* species and the unidentified caudal fin parasites, which infected one fish at the sites they were found.

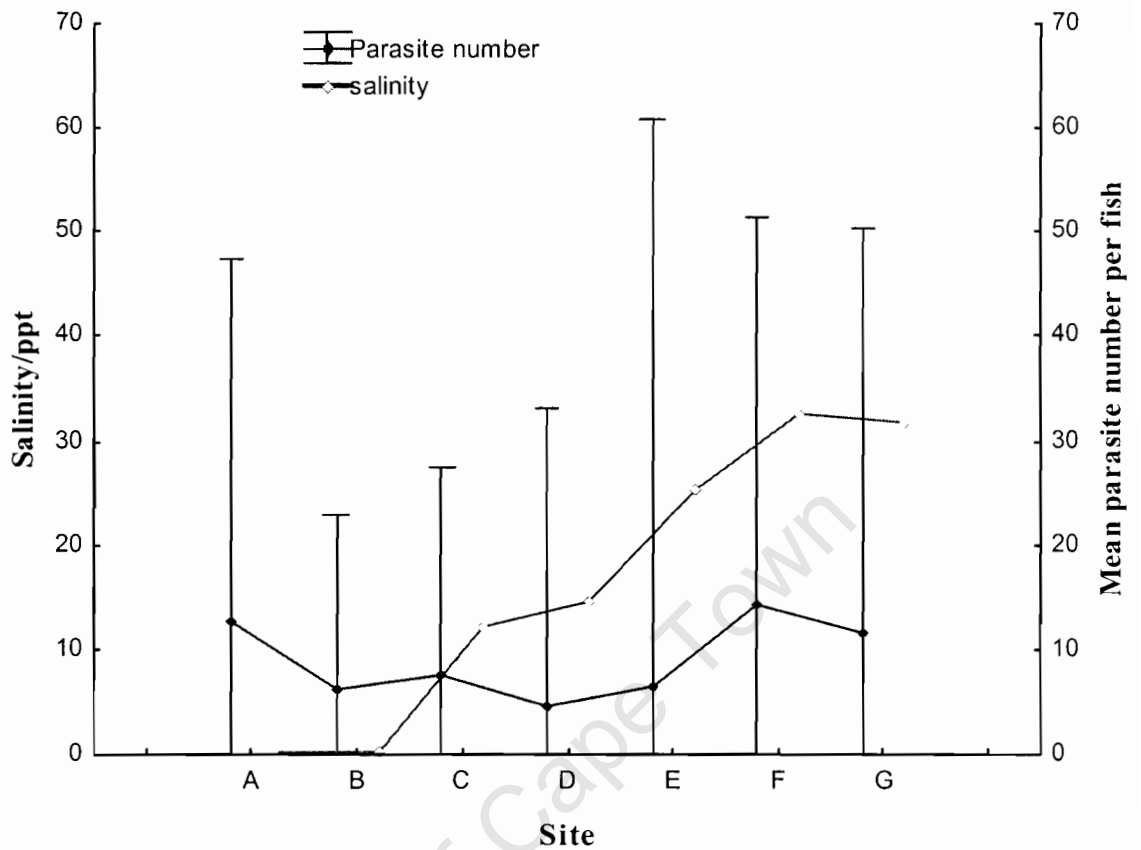


Fig. 6 Mean parasite load found on 30 fish from each site max. and min. bars are included. Line graph shows the salinity at each site.

The mean intensity of infestation for all the parasites species was low at five individuals per infected fish, whereby the *Ergasilus* had the highest mean intensity of infection of nine per fish at the highest salinity. This intensity decreased in the brackish waters to around two per fish and rose slightly again to six per fish in fresher water (>1

ppt). For the other gill parasites, this trend was not clearly repeated as their intensity of infection did not fluctuate significantly with changes in salinity.

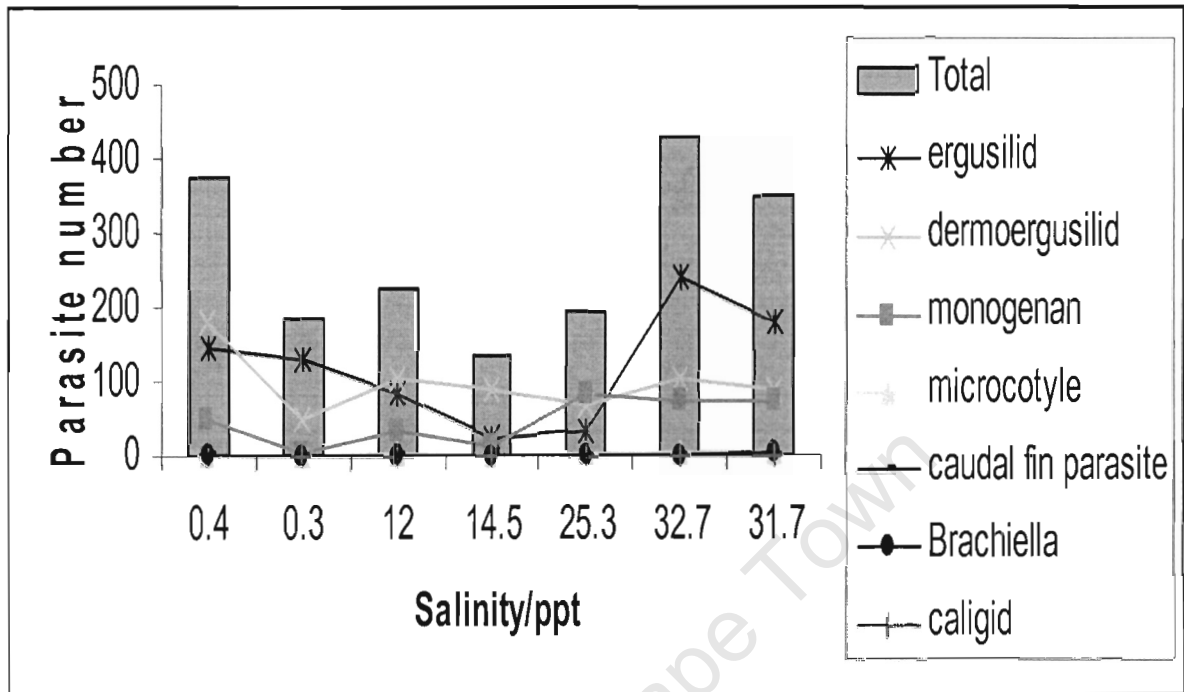


Fig. 7 Trends in individual parasite load for every 30 fish investigated in relation to salinity.

Of the 210 specimens of *Liza richardsonii* sampled in the current study, 90 % were infected by at least one parasite species and 66 % were parasitized by at least two species. Double infestations were most common, occurring in 41 % of the fish investigated. This was followed by single (23 %), triple (21 %) and quadruple (2 %) infections respectively. No specimen was found to be infested by all parasite species observed.

Table 2. Percentage of the *Liza richardsonii* infected by each of the parasite species (N=210, average fish length =90.5mm). The total prevalence of each parasite species for 210 fishes investigated is summarised.

| Site | Salinity/ppt | Prevalence | | | | | | |
|---------|--------------|------------|----------------|-----------------------|------------------|------------------|-------------------|-----------|
| | | Monogenea | Microcotylidae | <i>Dermoergasilus</i> | <i>Ergasilus</i> | Caudal fin para. | <i>Brachiella</i> | Caligidae |
| A | 0.4 | 40 | 6 | 83 | 76 | 0 | 0 | 0 |
| B | 0.3 | 10 | 6 | 33 | 83 | 0 | 0 | 0 |
| C | 12 | 33 | 6 | 70 | 66 | 0 | 0 | 0 |
| D | 14.5 | 30 | 6 | 66 | 53 | 3 | 0 | 0 |
| E | 25.3 | 53 | 6 | 80 | 30 | 3 | 0 | 0 |
| F | 32.7 | 66 | 10 | 66 | 93 | 0 | 3 | 0 |
| G | 31.7 | 60 | 13 | 43 | 86 | 3 | 10 | 3 |
| Total % | | 37 | 6 | 63 | 70 | 1 | 2 | 0.4 |

Table 3. Mean number of parasites for 30 *Liza richardsonii* at each site (N=210, average fish length =90.5mm).

| Site | salinity/ppt | Mean Intensity of infection | | | | | | |
|------|--------------|-----------------------------|----------------|-----------------------|------------------|------------------|-------------------|-----------|
| | | Monogenea | Microcotylidae | <i>Dermoergasilus</i> | <i>Ergasilus</i> | Caudal fin para. | <i>Brachiella</i> | Caligidae |
| A | 0.4 | 4 | 1 | 7 | 6 | 0 | 0 | 0 |
| B | 0.3 | 2 | 1 | 5 | 5 | 0 | 0 | 0 |
| C | 12 | 3 | 2 | 5 | 4 | 0 | 0 | 0 |
| D | 14.5 | 2 | 1 | 5 | 2 | 1 | 0 | 0 |
| E | 25.3 | 5 | 2 | 3 | 4 | 1 | 0 | 0 |
| F | 32.7 | 4 | 2 | 5 | 9 | 0 | 1 | 0 |
| G | 31.7 | 4 | 1 | 7 | 7 | 1 | 1 | 1 |
| Mean | | 3.4 | 1.4 | 5.3 | 5.3 | 0.4 | 0.3 | 0.1 |

Significant differences of community composition with regard to parasite abundance and changing salinity were not detected (ANOSIM $R= 0.176$, $p>0.05$). The Student t-test indicates that the infestation intensity of the gill parasites between the left and right gills was not significantly different ($T= 1.40$, $p>0.05$). Therefore, the data for left and right gills were pooled for further analyses. A significant difference was also not found for preference between the gills (Kruskal Wallace Test $H= 0.0912$, $p>0.05$). Lastly, ANOVA ($F=0.83$, $p<0.05$) also showed that there was no significant preference for a specific segment of the gill.

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Discussion

The results show that a variety of ectoparasites, dominated by the copepods, infested the southern mullet *Liza richardsonii*. Comparisons of the parasites found in the southern mullet from the Berg Estuary with reports from Mugilidae from other regions, such as Sapelo Island, Georgia (Rawson 1975), Japan (Ho and Do 1982), Southern Africa (Oldewage and van As 1988) and the Philippines (El-Rashidy and Boxshall 2001) show that similar parasite faunas were found. Further taxonomic investigations of the copepods may indicate if some of the species found are identical to the ones found in the other reports or are previously unreported species.

Significant differences of ectoparasite load and composition on juvenile southern mullet with regards to salinity were not observed. However, certain species such as the individual from the family Caligidae, the *Brachiella* species and the unidentified caudal fin species were only found at salinities of 16 ppt or higher. It is, therefore, concluded that these species are marine organisms, which are incapable of tolerating low salinities. Due to the few specimens observed at each site and the location on which they were found on the fish, other factors such as predators, which feed on these parasites, or abrasive habits of the host, could also be reasons why these parasites were not observed in low salinities. Gilchrist (1914) has shown that southern mullet uses benthic structures, such as stones and submerged objects, to rid themselves of external parasites. Paperna and Overstreet (1981) stated that occasionally the incidence of a parasite would fluctuate because predators not involved in the parasitic life cycle eat the majority of the intermediate host population. However, one must understand that under natural

conditions high parasite loads are not commonly found unless the hosts are confined to crowded environments where parasites can easily find new hosts.

The only species that were found throughout the range of salinities under investigation were the gill parasites, excluding the *Caligus* species (Fig. 4). These were the unidentified monogenean, the *Microcotyle* species, the *Dermoergasilus* and the *Ergasilus*. These species are capable of adjusting to a changing environment that ranges from marine to freshwater. This factor and that only a small number of parasite species were found, allowed for no significant differences to be seen between parasite load and composition with regard to changing salinity.

A pilot study during the late summer season has shown that other parasites also occur on juvenile southern mullet; these included an isopod (*Anilocra*), a branchiurian (*Argulus*) and an unidentified species of copepod. It is thus apparent that *Liza richardsonii* can accommodate a greater diversity of ectoparasites than recorded during the present survey and that parasite load and species composition varies seasonally. Rawson (1977) has indicated that the prevalence of *Argulus* species declined significantly during the summer and fall period. Future studies should thus incorporate samples taken from different seasons, thus giving insight as to what is the maximum number of species the juvenile mullet can accommodate. It will also indicate whether the mullet swimming upriver affects the other species of parasites under natural conditions.

Variation of parasite infection upstream

It was expected that species composition would either follow the salinity curve, or have high species richness at the saline extremes (Fig. 3). It was assumed that in low salinities freshwater parasites would become the common burden. However, different species of parasites were not observed in the fresher reaches of the Berg Estuary. Curiously, at Site D a large number of species were recorded. An explanation for this could be that at salinities of 15 ppt those species, which do not tolerate lower salinities, begin to die off. Those, which remain attached, are capable of tolerating low salinities. This area within the river is therefore the zone at which weaker osmoregulators are removed from their fish host.

It would also be of interest to observe if adult *Liza richardsonii* from the open sea in St. Helena Bay are infested by the same diversity of ectoparasites as observed on juveniles in the current study. A higher diversity observed in the adult fishes could indicate that the juveniles benefit by remaining in fresher waters. On the other hand Martens and Moens (1995) have suggested that this trend could be a likely result of changes in the diet of the adult and juvenile fish.

The highest parasite load per locality was observed at the higher range of salinity of 32.7 ppt at Site F. The majority of the individuals were gill parasites, in particular from the genus *Ergasilus*. Of the total of 1882 parasites collected, 832 were *Ergasilus*, 688 *Dermoergasilus*, 342 unidentified monogeneans, 23 *Microcotyle*, five *Brachiella*, two unidentified caudal fin parasites and one caligid. This hierarchy in species domination was observed at most sites, in particular Site F. A slight trend can be seen whereby there is a larger parasite load in the high salinities compared to the lower salinities (Fig. 5). The mean parasite load per fish, whereby the highest values were shown at the saline

extremes, supported these findings. There is a 30 % reduction of parasite infection when comparing total load between Sites F and G, and A and B. This provides an indication that *Liza richardsonii* could benefit from swimming up the Berg Estuary.

The lowest parasite load was found at Site D at a salinity of 14.5 ppt. This site is likely to experience the greatest variation in salinity and would thus be most stressful for the parasites. It is clear that at this location of the river the majority of individuals do not survive, giving rise to the low number of individuals observed. Furthermore, the mean number of infestation at this locality was also low at four parasites per fish (Fig. 6). Only the gill parasites were capable of tolerating the low salinities, and were able to increase in numbers, which was shown by the peak at Site A.

One difficulty in interpreting the current results is that one does not know the ectoparasites' duration of exposure to their new environment, the variation in salinity at this particular site and if this affects their abundance and diversity. This is because there was no control over the mobility of the juvenile mullet. Therefore, it was not known if fishes caught at a particular site, were resident at that site or recently came from a different locality. In order to control southern mullet mobility, it is advised that for future experiments that caught fishes be kept in pens with varying salinities. This would ensure that observations made about parasite load were due to the exact environment the fishes came from. It would also allow for an accurate insight as to the type of environment the ectoparasites prefer and allow for experimental manipulation. This study has assumed that salinity is the single most important environmental variable for parasites. However, Paperna and Overstreet (1981) state that the relationship between salinity and temperature often outweighs the importance of salinity alone. Future research should also

incorporate the question if it is possible that ectoparasites are removed from their host due to changes in temperature. This is because the juvenile southern mullet migrate from a cool, deep marine environment to a shallow warm estuarine environment once egg and larval development has taken place. Thus, sudden temperature changes could influence the ectoparasite load and composition between sites. In the current study such temperature measurements were not recorded, and could be an additional factor if, for example, temperature between the sampling sites did not vary drastically.

From the data collected it is concluded that *Liza richardsonii* enters the estuary and finds a locality where salinity fluctuates regularly and constantly. These changes affect the ectoparasites considerably as shown in figures 5, 6 and 7, as the ectoparasites are not abundant at Site D. The impact of the tides and freshwater input create a highly variable saline environment around 5 km from the mouth where the majority of southern mullet are found (per comm. B. Clark). In environments where the salinity is not as variable the survival rate of the ectoparasite is improved. This is supported by the results, as the ectoparasites are more abundant in either freshwater or marine (Fig. 7). However, when the parasites find themselves in a saline environment that fluctuates constantly, their chances of survival are drastically reduced as shown by abundance levels at Site D. Furthermore, the highest abundance of ectoparasites (429 individuals) was observed close to the mouth where according to Bennet (1994) the southern mullet is most abundant (5 km from the mouth of the Berg Estuary).

The most prevalent species of parasites were those found within the gills of *Liza richardsonii*, as can be seen on Table 2. Trends could not be seen from Table 2 between parasite prevalencies at each site and salinity. A slight trend could be seen for the

monogenean species, with a larger percentage of fish infested at high, compared to low, salinities. Curiously the *Microcotyle* species maintained a constant rate of percentage fish infested at almost every site investigated. The unidentified caudal fin species, the *Brachiella* species and the caligid all had extremely low prevalencies. These four species only infested a total of 22 fish from the 210 examined. This could possibly also indicate that *Liza richardsonii* is not their preferred host and examinations could be done to observe if there are higher prevalencies of these parasites on the closely related species *Mugil cephalus*.

It is known that fishes kept in confined pools or aquariums usually have a mean intensity of infection, which is higher than that seen in the natural environment (Paperna and Overstreet 1981). The values given in Table 3 indicate that fishes from the Berg River are part of a healthy population. It should be understood that not all of the fish investigated were parasitized. At each locality 1-3 fish were uninfected by any species of ectoparasite. However, mullet would still increase their health by removing a percentage of the parasite load they carry, as each individual parasite damages tissue or removes fluids. Table 3 also shows that the most common parasites also have the highest intensity of infestation. These were the *Ergasilid* species and the *Dermoergasilus* species. These gill parasites had a large surface area to attach to without affecting attachment sites for conspecifics. This does not seem the case for *Bracheilla* species and the unidentified caudal fin species, as they attach to specific localities on the fish, in such a manner that it may prevent conspecifics attaching to the host.

Variation of infection site on fish

It was expected that no preferences would be seen as to which side of the fishes' gills the gill parasites would attach to. It was believed that attachment to the gills would be a random process, which is not influenced by parasite behaviour. This view was supported by the Student's t-test, which revealed that no significant difference was found between the two.

Furthermore, it was expected that there would be a difference observed between parasite loads attached to the various gills. Numbers of attached individuals on the first gill could be high, because it is the first gill encountered. The second or third gill could also show high number, as they have the largest surface area to attach to compared with gills one and four. Rawson (1977) and Martens and Moens (1995) have shown that parasite infestations were not seen on the first gill, but preferred the third. The Kruskal Wallance test showed that there was no significant difference of parasite loads between all the gills observed. This indicates that the selection of a specific gill by parasites of the Southern Mullet is a random process.

Lastly, the ANOVA showed that there was no preference observed as to which segment of the gill most parasites attached too. This suggests that competition does play a significant role in segment selection as the parasites were distributed evenly across the gills. However, it is expected that there were far too few parasites observed, on each segment for habitat restrictions to occur. Another reason as to why no preference was observed could be, the manner in which the gills were divided into segments. In this study the gills were divided into three segments, however Lo and Morand (2001) chose to partition their gills into 12 sections, which were defined as the smallest identifiable

territorial units that can contain members of the parasite community. Thus by increasing the number of segments, it is more likely to find specific parasites occupying a particular habitat.

Added to this, careful analysis of the gill copepods showed that each was morphologically different to such an extent that they could not co-exist in the same microhabitat. The *Dermoergasilus* had its interlocking antennae adapted to grasp the soft gill filaments. The *Ergasilus* had its antennae adapted for grasping on to the much harder gill rakers. The manner of which these antennae were designed allows for each species to occupy a particular niche of the gill. Furthermore, these antenna limited the mobility of the species, thus restricting them to the first attachment site they encountered during their larval stages. It is likely that due to the small number of monogeneans collected and the reduced partitioning of the gill size that no significant difference in attachment sites was observed. Both species of monogeneans preferred the gill filaments. The *Microcotyle* was only found in the middle part of gill at the base of the gill filaments. The unidentified monogenean species was also only found in the gill filaments, usually at the base, but was located close to the exterior of the gill filaments. It is therefore believed that competitive exclusion did not play a significant role in this study.

Conclusion

The current investigation does not statistically prove that the effects of a wide-ranging saline environment determine the distribution, composition and abundance of parasites found infecting the southern mullet. However, no causal effect could be established as there could be additional confounding factors affecting parasite abundance and diversity. These other factors could not be analysed within the scope and time of this current study. Factors such as temperature, fish shoal size, turbidity of the water and the chemical composition of the water could also affect the distribution, composition and abundance of the ectoparasites. Therefore, it is suggested that a more elaborate investigation is developed, required to clearly define the range and contributing power of a variety of potential factors using multivariate analysis techniques.

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