An Appraisal of Warm Temperate Mangrove Estuaries as Food Patches using Zooplankton and RNA:DNA Ratios of *Gilchristella aestuaria* Larvae as Indicators

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2018

An Appraisal of Warm Temperate Mangrove Estuaries as Food Patches using Zooplankton and RNA:DNA Ratios of *Gilchristella aestuaria* Larvae as Indicators

Submitted in fulfilment of the requirements

for the degree of

Master of Science

at

Nelson Mandela University

by

Eugin Bornman

April 2018

Supervised by Prof. NA Strydom

Declaration

I, Eugin Bornman (student number: 217042082), hereby declare that, in accordance with Rule G5.6.3 of the Nelson Mandela University, the dissertation for the degree of Master of Science entitled, “An appraisal of warm temperate mangrove estuaries as food patches using zooplankton and RNA:DNA ratios of *Gilchristella aestuaria* larvae as indicators”, is my own work and that it has not previously been submitted for assessment to another university or for another qualification. At no point in this dissertation is another person’s text, data, graphs or figures presented, unless these have been suitably acknowledged and credited to the other person.

Eugin Bornman
4 December 2017

General Abstract

Mangrove habitats are considered as the ideal fish nursery as they are known to increase the growth and survival of juvenile fishes by providing enhanced food availability and protection. However, most studies have focused on tropical mangroves with a few recent warm temperate studies finding conflicting results. Furthermore, the nursery value of South African mangroves to fishes remain understudied in subtropical areas, while warm temperate mangroves are yet to be evaluated. This study aimed to assess whether mangrove presence leads to any advantage to the larvae of an important estuarine resident fish species, *Gilchristella aestuaria*, by comparing the food patch quality of South African warm temperate mangrove and non-mangrove estuaries. Results indicate that larvae fed primarily on the dominant prey species, *Pseudodiaptomus hessei*, *Paracrtia longipatella*, and *Acartiella natalensis*. However, postflexion larvae consumed more of the larger species, *P. hessei,* within the two mangrove estuaries (16.09 %V in Nahoon and 13.79 %V in Xhora) than the two non-mangrove estuaries (12.20 %V in Gonubie and 7.05 %V in Qora), despite other prey species occurring at similar densities. Results indicate that mangrove habitats acted as sediment sinks, slightly reducing the turbidity of these estuaries which resulted in postflexion larvae actively selecting larger, more nutritious prey, which in turn, significantly increased their individual instantaneous growth rates (0.11 ± 0.21 Gi) when compared to postflexion larvae in non-mangrove estuaries (0.09 ± 0.12 Gi). This study found that mangrove presence was significantly related to postflexion larval densities when coupled with abiotic (such as temperature and turbidity) and biotic factors (such as predator-prey interactions). Understanding the spatial and temporal dynamics, predator-prey interactions as well as the growth and survival of *G. aestuaria* is particularly important as they are key zooplanktivores that are prey to other species in estuarine food webs.

KEYWORDS: Nursery habitats, Estuarine roundherring, Fish larvae, Spatial and temporal dynamics, Fish feeding environments, Nutritional condition, Generalized Additive Models, Feeding ecology, Diet, Predator-prey interactions.

Table of Contents

List of Figures

List of Tables

Acknowledgements

This dissertation cannot be completed without mentioning all the individuals that have directly or indirectly helped in completing this long journey. I'd like to give thanks to a few people in particular, starting with my supervisor, Prof. Nadine Strydom, whose door was always open to me. Her gentle encouragement, relaxed demeanour and unsurpassed knowledge made for a good working relationship. A special thanks is extended to Dr. Catriona Clemmesen who kept me on the right lane (literally) during my stay in Germany. My gratitude is also extended to Prof. Tris Wooldridge who, despite being retired, spent months helping with the identifying and counting of zooplankton. His knowledge on intricate estuarine plankton dynamics were a valuable asset. Next I'd like to thank all the fellow students that were part of the fieldwork: Cuen Muller, Larize Nel, Taryn Smit, Kyle Hewett and Steven McGregor, their dedication as well as humour made short work of all the late nights we spent sampling on the various estuaries. I also need to mention the Zoology Department, Nelson Mandela University, which was my home (literally, during the final months). I also need to acknowledge the National Research Foundation for providing research funding under grant number 93570 as well as personal funding through the Innovation Masters Scholarship under grant number 107926. Lastly, no acknowledgments would be complete without giving thanks to my parents. Both have instilled a love of nature in me from a young age and have given me a good foundation on which I rely during everyday life. Thank you Mom for always being there for when I needed emotional support and for always saying how proud you are of me. Dad, thank you for teaching me about hard work, self-discipline and persistence which have kept me going.

Chapter 1: General Introduction

1.1 FISH NURSERIES

Estuaries are transitional zones that connect marine and freshwater systems. Each estuary is therefore a continuum of the two adjacent systems making estuaries unique, yet heterogeneous ecological networks that support diverse biological communities (Dahlgren et al., 2006; Elliott and Whitfield, 2011). Diversity of habitats, and therefore niche space, make estuaries ideal nursery areas for fishes, many of which are ecologically and economically important (Beck et al., 2001; Nagelkerken et al., 2015). These fish species undergo habitat shifts during their life-cycle and commonly use estuaries as nursery areas, often migrating during the late larval phase, where they may spend years before subsequently migrating to adult habitats (Boehlert and Mundy, 1988; Pattrick and Strydom, 2014; Potter et al., 2015). Estuaries provide juveniles with protection from predators by means of extensive habitat variety, much of which includes plant structures which create shelter as well as increased feeding opportunities due to a diverse array of primary and secondary producers (Orth et al., 1984; Laegdsgaard and Johnson, 2001; Cocheret De La Morinière et al., 2004; Nanjo et al., 2014).

A habitat is generally seen as a nursery if it supports elevated densities of juveniles per unit area or if the habitat contributes a greater proportion of recruits to the adult population (Orth et al., 1984; Beck et al., 2001; Heck et al., 2003; Dahlgren et al., 2006). The nursery quality of a particular habitat can thus be linked to (1) higher densities of larvae, (2) increased growth rate, (3) increased survival to adult stage, and (4) successful recruitment to adult habitats (Beck et al., 2001). Initial studies considered whole estuaries as nurseries to fishes. However, the need for specific conservation efforts resulted in the need to identify and evaluate key nursery habitats (Beck et al., 2001; Dahlgren et al., 2006). Plant structure was subsequently found to be important as higher abundances of juvenile fishes and invertebrates were found in structurally more complex habitats, such as marshes, mangroves and seagrasses (Heck and Wetstone, 1977; Orth et al., 1984; Nagelkerken et al., 2010; Edworthy and Strydom, 2016).

1.2 MANGROVES AS FISH NURSERIES

Mangroves are among the most productive habitats worldwide and are widely cited as the ideal fish nursery (Costanza et al., 1997; Beck et al., 2001). Mangroves are salt tolerant coastal trees that are tidally submerged and found mostly in tropical and subtropical regions with their distribution being

limited by the 20 °C seawater isotherm (Giri et al., 2014). The aerial roots, tree trunks and overhanging branches typical of mangrove habitats creates a complex submerged habitat which is thought to play a key role in determining the spatial distribution and abundance of a variety of fishes (MacArthur, 1965; Nagelkerken et al., 2010). The various physical structures provide shelter, which reduces the risk of predation (Rönnbäck et al., 1999; Laegdsgaard and Johnson, 2001). Leaf litter fall and sediment accretion of mangroves increases primary and secondary productivity, which enhances food availability to fishes (Emmerson, 1992; Laegdsgaard and Johnson, 2001; Sheaves, 2005; Rajkaran and Adams, 2010; Mazumder et al., 2011). The increase in food availability and protection from predators creates for an optimal feeding habitat for fishes, making them ideal nursery areas (Laegdsgaard and Johnson, 2001; Nagelkerken et al., 2010).

Mangroves are considered as ideal fish nurseries, however, there is still debate as to the importance of mangrove habitat to fishes relative to other habitats within estuaries (Faunce and Serafy, 2006; Blaber, 2007; Sheaves, 2017). Blaber (2007) maintains that most evidence is circumstantial as most studies have been conducted in the tropics, where mangroves form the dominant habitat, leaving few other habitats to accurately compare against. Recent studies conducted on fish in warm temperate mangrove systems in Australia and New Zealand have generally found that many of the species found in mangrove habitats were equally abundant in alternative habitats such as mudflats and saltmarshes (Clynick and Chapman, 2002; Smith and Hindell, 2005; Payne and Gillanders, 2009). Clynick and Chapman (2002) studied small mangrove patches in the Sydney Harbour by using seine and fyke nets and found that mean abundance, species richness and assemblages of fishes were similar in mangroves and adjacent mudflats. A study on the fish assemblages along a mangrove-mudflat gradient in three southern Australian estuaries found similar abundances and diversities of fishes between mangrove and adjacent mudflat habitats (Payne and Gillanders, 2009). These results suggest that structure is not the only or main attractant for fishes that utilise warm temperate mangrove systems, which contrasts most findings in tropical systems (Heck et al., 2003; Cocheret De La Morinière et al., 2004; Dahlgren et al., 2006; Nagelkerken et al., 2010). However, there has been limited research attention on the value of mangroves to fishes outside of tropical climates.

Similarly, little attention has been given to South African mangrove systems in terms of their nursery role to fishes. Mangroves readily fringe estuaries along the east coast of South Africa, extending down into upper warm temperate estuaries. Despite this, all research has been conducted in tropical systems. Cyrus and Forbes (1996) studied fishes in two KwaZulu-Natal estuaries on the north-east coast of South Africa which have been transformed into harbours. They used seine nets and found that fish abundance was strongly coupled with mangroves within both estuaries. Mangrove sites were found

to be the prime prawn habitat which are prey to certain fishes (Cyrus and Forbes, 1996). Another study also compared the fish assemblages of two estuaries, one with mangroves (Mngazana) and one without (Mngazi) and found that there was a higher abundance in the intermittently open Mngazi Estuary when compared to the permanently open Mngazana Estuary, however this pattern was not the same for diversity of fishes, given the different mouth conditions between these two estuaries (Mbande et al., 2005). They concluded that the intermittently open estuary, Mngazi Estuary, is more physically stable (when closed) which facilitates greater reproductive success for resident taxa (Mbande et al., 2005).

1.3 ASSESSING NURSERY VALUE

Most studies assessing the nursery value of mangrove habitats compare the abundance and diversity of fishes found within mangroves and the adjacent habitats (Beck et al., 2001; Dahlgren et al., 2006; Faunce and Serafy, 2006). However, the nursery value of a habitat also depends on the growth rate, survival and recruitment success of early stage fishes (Beck et al., 2001; Dahlgren et al., 2006). New research has shown that nutritional condition of fish larvae can be linked to ecosystem characteristics by using biochemical techniques such as the RNA:DNA ratio (Caldarone et al., 2001; Chícharo and Chícharo, 2008; Costalago et al., 2014). This is potentially very important in fodder fish species in estuaries that provide an important prey source for other fishes. The estuarine roundherring, *Gilchristella aestuaria* (Gilchrist, 1914) is an estuarine resident clupeid that is highly abundant in most South African estuaries (Wallace, 1975; Haigh and Whitfield, 1993; Strydom, 2015). This species is planktivorous, feeding predominantly on phytoplankton and zooplankton (Coetzee, 1982; White and Bruton, 1983) and plays a key ecological role in the transfer of energy between trophic levels (Whitfield and Harrison, 1996). This species have been found to spawn in the upper reaches throughout the year, with peak spawning occurring during summer (Strydom, 2015), and thus it is an ideal candidate species to assess the nursery value of estuaries.

1.4 RATIONALE

A school of thought exists where mangrove habitats are accepted as ideal fish nursery habitats (Beck et al., 2001). However, most studies have been conducted in the tropics with very few studies on the nursery value of mangroves in warm temperate and even sub-tropical regions (Faunce and Serafy, 2006; Blaber, 2007). Warm temperate mangroves in South Africa serve as the ideal *in situ* laboratory to explore the value of mangroves in the provisioning of good food patches for the important mid-trophic species, *Gilchristella aestuaria,* as similar estuaries with and without mangrove stands can be

compared which are in close geographical proximity to one another. Moreover, most studies focus on comparing fish abundance and diversity on an assemblage-level, with very few studies considering species-specific estimates of abundance, growth, and predator-prey interactions (Faunce and Serafy, 2006).

Thus, understanding the spatial and temporal dynamics, predator-prey interactions as well as the nutritional condition of larval *G. aestuaria* within estuaries would give an indirect indication of the food patch quality and in so doing, provide insight into the potential nursery role of estuarine mangrove habitats. Estuaries with mangroves present are expected to provide additional refuge and feeding opportunities to estuaries that are without mangroves. Therefore, *G. aestuaria* larvae should be more abundant, have better feeding opportunities and thus be in a better nutritional condition in estuaries with mangroves present.

1.5 AIMS AND OBJECTIVES

The main aim of this study was to compare the food patch quality of four similar warm temperate estuaries with and without mangroves.

The objectives of the present study were to:

1. Compare the spatial and temporal dynamics of the larval stages of *Gilchristella aestuaria* in mangrove and non-mangrove estuaries in warm temperate South Africa
2. Compare the diet of larval *Gilchristella aestuaria* in relation to plankton dynamics in mangrove and non-mangrove estuaries in warm temperate South Africa
3. Compare the body condition of larval *Gilchristella aestuaria* in relation to food patch quality in mangrove and non-mangrove estuaries in warm temperate South Africa

1.6 THESIS STRUCTURE

The thesis is written to facilitate the publication of the work and as such there is an unavoidable degree of repetition in the data chapters. The manuscript has been formatted and referenced according to the guidelines set by the journal Estuarine, Coastal and Shelf Science.

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Chapter 2: Spatial and Temporal Dynamics of *Gilchristella aestuaria* (Family Clupeidae) Larvae in Mangrove and Non-Mangrove Estuaries in Warm Temperate South Africa

2.1 ABSTRACT

Estuaries are important nursery areas to fishes as they provide a wide range of habitats that act as refugia to the early life stages of many economically and ecologically important fishes. Mangroves are among the most productive habitats worldwide and are widely cited as the ideal fish nursery habitat, however most studies focus on tropical mangroves. This study compared the larval density and distribution of a common mid-trophic fish species, *Gilchristella aestuaria* (Family Clupeidae), between similar warm temperate estuaries with and without mangrove habitats. Larval density were highest in the Qora Estuary with a mean ± range of 39.47 ± 230.59 (100 m^{-3}) and lowest in the Nahoon Estuary 6.54 ± 75.25 (100 m^{-3}). Thus, larval density was similar between mangrove and non-mangrove estuaries. However, densities differed spatially and temporally as there were significantly higher densities observed during the summer sampling season and within the upper reaches of the Nahoon and Gonubie Estuaries. Generalized Additive Models found that larval densities and distribution was best (68.10 %) explained by an interaction of temperature, conductivity, turbidity and pH. This study found no supporting evidence that increased larval densities of this species is as a result of the presence of mangrove habitats. This study adds some much-needed information to the nursery value of mangrove habitats by comparing the nursery value of warm temperate mangroves to fishes on an estuary wide scale.

2.2 INTRODUCTION

Estuaries are important nursery areas to fishes as they provide a wide range of habitats that act as refugia to the early life stages of many economically and ecologically important fishes (Beck et al., 2001; Dahlgren et al., 2006; Elliott and Whitfield, 2011; Vasconcelos et al., 2011). A nursery habitat is one that supports a high density, while increasing the growth and survival, of early stage fishes which then results in a higher number of individuals that can be recruited into the adult population (Beck et al., 2001). Therefore, estuaries are deemed as nursery areas that fishes use in multiple ways with differing dependency. Many marine fishes are actively recruited into estuaries during the postflexion stage, where they benefit from feeding on the abundant primary and secondary producers (Elliott et al., 2007). The abundance of these food items, however, varies between estuaries as they

are highly dynamic systems and the food patch quality can thus differ between estuaries, but also between the many habitats found within estuaries (Dahlgren et al., 2006). Mangroves are among the most productive habitats worldwide and are widely cited as the ideal fish nursery habitat (Costanza et al., 1997; Beck et al., 2001). The importance of estuaries as nurseries to fishes are well established (Beck et al., 2001; Able, 2005; Potter et al., 2015). However, the identification and evaluation of critical fish nursery habitats found within estuaries are still not fully understood, which restricts the development of appropriate conservation and management strategies (Able, 2005; Faunce and Serafy, 2006; Nagelkerken et al., 2015).

The estuarine roundherring, *Gilchristella aestuaria* (Gilchrist, 1914), was selected as a candidate species as it is a small, mid-trophic fish that is highly abundant across most South African estuaries (Haigh and Whitfield, 1993; Strydom, 2015). This estuarine resident clupeid has a wide salinity tolerance, however elevated densities are usually found in the mesohaline zone (Strydom, 2015). Peak spawning occurs in the spring and summer months with sporadic events that extend throughout the year (Cyrus et al., 1993; Haigh and Whitfield, 1993). This species predominantly filter feeds on zooplankton and phytoplankton (White and Bruton, 1983; Strydom et al., 2014), but larger individuals have been reported to selectively forage on benthic invertebrates (Cyrus et al., 1993). It is also an important prey species to many ecologically and economically important fishes utilising estuaries. Therefore it plays a key ecological role in the transfer of energy between trophic levels (Blaber et al., 1981; White and Bruton, 1983; Whitfield and Harrison, 1996), which makes it an ideal candidate species to investigate the extent to which mangrove habitats contribute to the food patch quality and thus the nursery value to fishes.

Mangroves are salt tolerant coastal trees that are tidally submerged and found mostly in tropical and subtropical regions with their distribution being limited by the 20 °C seawater isotherm (Giri et al., 2014). Their complex root structures results in a complex submerged habitat which attracts a multitude of species by providing shelter as well as increased food availability (MacArthur, 1965; Nagelkerken et al., 2010). Mangroves enhance food availability by increasing nutrients either by sediment trapping or by leaf litter fall which increases primary and secondary productivity and thus enhances food items to fish (Emmerson, 1992; Laegdsgaard and Johnson, 2001; Sheaves, 2005; Rajkaran and Adams, 2010; Mazumder et al., 2011). The complex root structures reduces the risk of predation allowing smaller fishes to seek refuge from larger predators (Rönnbäck et al., 1999; Laegdsgaard and Johnson, 2001). These increases in food availability and protection from predators creates for an optimal feeding habitat for early stage fishes.

Although these factors allow mangrove habitats to support rich fish assemblages there is still debate as to the importance of mangrove habitat to fishes relative to other habitats within estuaries as well as other estuaries that do not have mangrove stands (Beck et al., 2001; Faunce and Serafy, 2006; Blaber, 2007; Sheaves, 2017). Very few studies have looked at their nursery value in other biogeographic regions where other habitat types are also present. Previous studies conducted on fish in warm temperate mangrove systems in Australia and New Zealand have generally found that many of the species found in mangrove habitats were equally abundant in alternative habitats such as mudflats and saltmarshes (Clynick and Chapman, 2002; Smith and Hindell, 2005; Payne and Gillanders, 2009). Mangrove habitats that are connected with seagrass habitats had greater species diversity (Nagelkerken et al., 2002) and broader isotopic niche widths (Muller and Strydom, 2017) than when habitats are considered on their own. Thus a thorough assessment of the value of mangroves to fish communities must incorporate the greater ecological area and not be restricted to single habitats (Nagelkerken et al., 2015). The warm temperate biogeographic region in South Africa is an ideal *in situ* location to compare the nursery value of mangrove habitats to fishes on an estuary wide scale, as mangroves reach the end of their southern latitudinal distribution here and estuaries with and without mangroves are situated in close proximity. This allows for comparative studies which have been lacking in the literature.

This study compared the spatial and temporal dynamics of a common, mid-trophic clupeid species in warm temperate estuaries with and without mangroves with the aim to assess the value of mangrove habitats to the nursery function on an estuary scale. Mangroves provide additional nutrients which would provide productivity increases thus in turn will increase feeding and therefore survival of *G. aestuaria* larvae. Therefore, estuaries with mangroves are expected to support a higher density of *G. aestuaria* larvae than estuaries that are devoid of mangroves.

2.3 MATERIALS AND METHODS

2.3.1 Study area

The Nahoon Estuary (27° 57’ 05” E, 32° 59’ 05” S) is situated near the city of East London, South Africa (numbered 1 in Figure 2.1). It is a permanently open estuary that is approximately 4.80 km long, has a mean depth of 2.32 m and an average temperature of 19.41 °C (Harrison, 2004; James and Harrison, 2016). The Nahoon river extends approximately 80 km inland and has a catchment area of about 580 km^2 (Talbot et al., 1985; Reddering and Esterhuysen, 1987). The Nahoon River has a reservoir, the Nahoon Dam, with a capacity of 5.9 x 10^6 m^3 and captures water from 87 % of the total catchment area (Reddering and Esterhuysen, 1987). The Nahoon Estuary is subject to contamination

from a variety of sources as well as pollutants resulting from occasional municipal waste water spills (Talbot et al., 1985; Newman and Watling, 2007). The estuary has a total area of 58.72 ha and comprises of the typical warm temperate vegetation types found in South African estuaries (Table 2.1). Currently the mangrove area at the Nahoon Estuary is non-natural and is relatively small <2 ha but is increasing at 0.06 ha y^{-1} since 1969 when *Avicennia marina* was planted and a few years later a few specimens of *Bruguiera gymnorrhiza* and *Rhizophora mucronata* were added among the larger *A. marina* trees (Steinke, 1972, 1986; Hoppe-Speer et al., 2015).

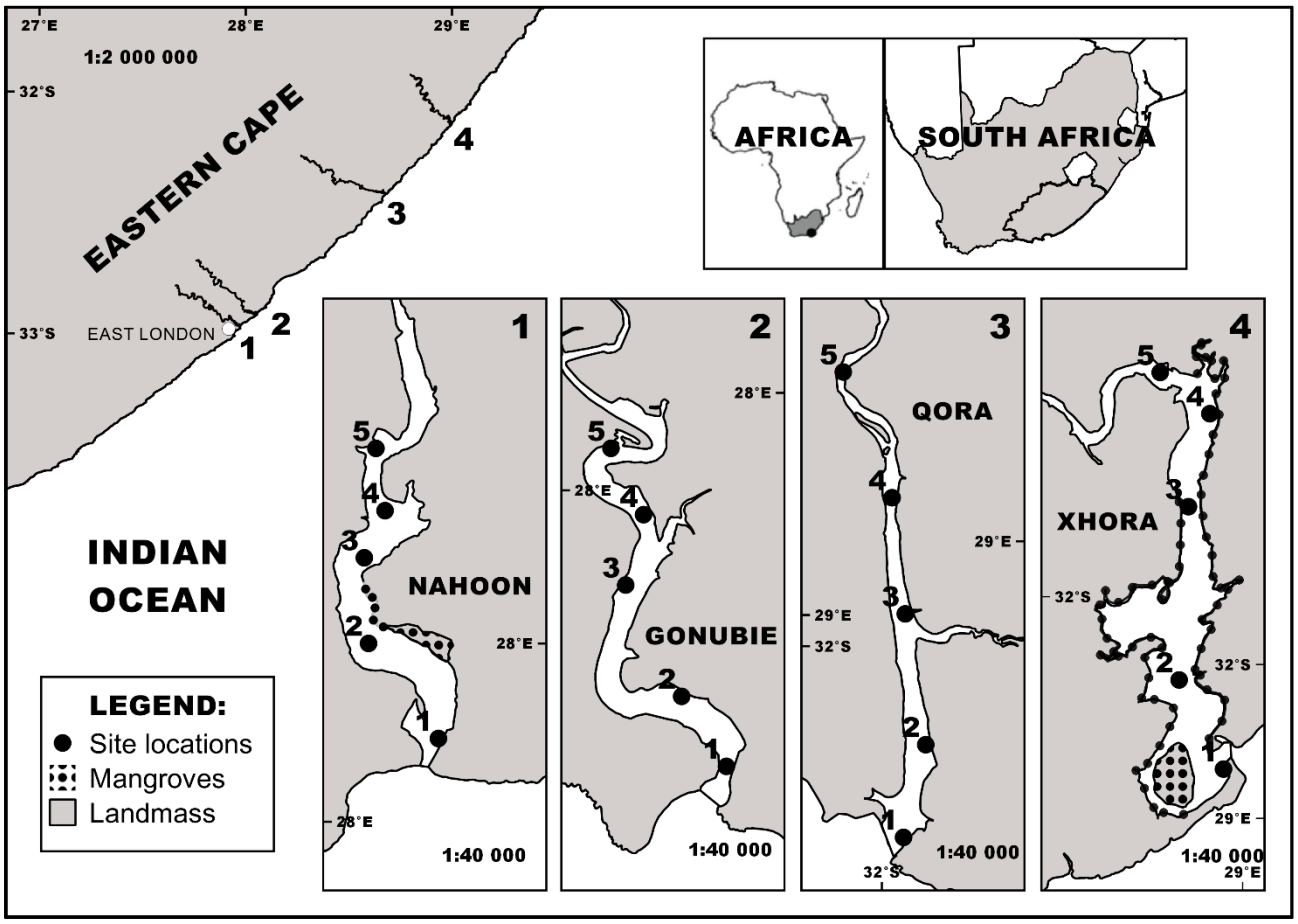

Figure 2.1: Geographic position of studied estuaries showing the location of sampling sites.

The Gonubie Estuary (28° 01' 59" E, 32° 55' 59" S) (numbered 2 in Figure 2.1) is situated approximately 10 km east of the neighbouring Nahoon Estuary. The Gonubie River extends approximately 80 km inland and has a catchment area of about 675 km^2 (Reddering and Esterhuysen, 1987). The Estuary is approximately 5 km long with a mean depth of 1.68 m and an average temperature of 19.98 °C which is similar to the other selected study estuaries (Harrison, 2004; James and Harrison, 2016). The Gonubie has no large reservoirs with only a small weir (personal observation of the authors) and limited impacts from anthropogenic pollutants (Adams et al., 2016). The Estuary has a total area of 53.4 ha and has no mangroves (Table 2.1).

The Qora Estuary (28° 40' 21" E, 32° 26' 50" S) is a permanently open estuary (numbered 3 in Figure 2.1). Qora has no reservoirs and limited impacts from anthropogenic pollutants (Adams et al., 2016). The estuary has a total area of 89.63 ha and has no mangroves (Table 2.1). Very little is known about this estuary due to its remote location, however it is of similar size and in relative close proximity to other study estuaries allowing for comparisons. This study forms part of a larger project that was the first to study the ichthyofauna of this estuary.

The Xhora Estuary (29° 05′ E, 32° 05′ S) (numbered 4 in Figure 2.1) is situated approximately 45 km north-east of the Qora Estuary and also falls within the warm temperate biogeographic zone. The mangroves at Xhora covers an area of 25.5 ha and consists of all three mangrove species present in South Africa (*A. marina*, *R. mucronata* and *B. gymnorrhiza*) (Hoppe-Speer et al., 2014). As with the Qora Estuary, very little is known about this estuary.

Table 2.1: Spatial extent of various habitat types within mangrove and non-mangrove estuaries on the warm temperate coast of South Africa. (Adams et al., 2016)

	Mangrove		**Non-mangrove**	
Habitat Type	**Nahoon**	**Xhora**	**Gonubie**	**Qora**
Intertidal salt marsh	2.80	0.00	3.70	0.00
Supratidal salt marsh	0.00	12.96	2.20	0.00
Submerged macrophytes	2.30	2.60	0.80	8.50
Reeds & Sedges	0.20	10.12	0.40	5.67
Mangroves	1.62	25.50	0.00	0.00
Sand/mud banks	4.50	17.13	6.30	10.23
Channel / water	47.30	91.45	40.00	65.23
Total Area (ha)	58.72	159.76	53.40	89.63

2.3.2 Field sampling

Samples were collected on a first quarter moon phase in summer 2015 and 2016 from five fixed sampling stations, at one kilometre intervals, along the main channel of four estuaries (Figure 2.1). This coincides with the known peak breeding period of the estuary-resident fish, *Gilchristella aestuaria* (Strydom 2015). Samples were collected isochronously after dark using two modified Working Party 2 (WP2) plankton nets (570 mm mouth diameter and 0.2 mm mesh aperture) fitted with calibrated Kahlsico 005 WA 130 flowmeters. The two nets were simultaneously lowered and towed horizontally alongside a 5 m boat for 3 min at a speed of 1-2 knots and sampled the upper 0.6 m of the water column and a mean volume of 189.76 ± 70.41 m^3 (Strydom and Whitfield, 2000). Two

replicate samples were collected at each of the five sites ranging from the upper to the lower reaches of each estuarine system (Fig. 1). Where possible an oblique course across the axis of the estuary was followed, thus enabling samples to be taken near the margins as well as in the mid-channel (Strydom et al., 2002). Sampling was conducted in complete darkness to limit any net avoidance by the fish. After each tow, flowmeter readings were recorded and the sample was immediately preserved in 10% buffered formaldehyde. Physico-chemical parameters were determined *in situ* at the time of sampling with a calibrated YSI sonde series 6600 multi-parameter probe with temperature, salinity, conductivity, total dissolved solids, turbidity, pH, and dissolved oxygen recorded every 0.5 m depths.

2.3.3 Laboratory analysis

All *G. aestuaria* were identified and removed from the samples using a Leica M80 stereomicroscope fitted with an eyepiece micrometer. Standard lengths of 50 randomly selected *G. aestuaria* were measured to the nearest 0.01 mm and staged into developmental stages according to Neira et al. (1998). The flowmeters on the nets allowed for the calculation of larval density. Flowmeters were calibrated in a controlled environment and it was determined that a value of 32.7 was the number of revolutions per m^3 of water filtered. Thus the following formula was used to calculate larval density:

$$\text{Density} = [\text{N} / (\text{r} / \text{c})] \times 100$$

where density is the number of *G. aestuaria* larvae per 100 m^3, *N* is the total number of larvae caught per haul, *r* the revolutions of the flowmeter and *c* the predetermined calibration value in m^3

2.3.4 Statistical analysis

Shapiro-Wilks and Levene's test was used to test data for normality and homogeneity of variance respectively. If these assumptions were not met, non-parametric tests were used. Kruskal-Wallis tests were used to test for differences in the four estuaries in terms of physico-chemical parameters. When significant ($P < 0.05$), the Mann-Whitney U test was used as a post-hoc on pairs of estuaries using a Bonferroni-corrected level of significance of $\alpha = 0.003$. Physico-chemical parameters violated the assumptions of normality and homogeneity of variances, and therefore, a Generalised Additive Models (GAMs) was used with a negative binomial distribution and log-link function, to quantify the relationship of larval density and length to the physico-chemical parameters. Mangrove presence was included in all models as it was the factor of primary concern. Best fit was determined via a forward stepwise approach using Akaike Information Criterion (AIC) and Chi-squared tests. All statistical analyses were performed using the statistical software R (v. 3.3.1) with mgcv and ggplot2 packages.

2.4 RESULTS

2.4.1 Environmental variability

Temperatures were similar in 2015 and 2016, however, temperatures were higher in summer than in winter ($P < 0.003$). Temperatures were similar in the Nahoon and Gonubie estuaries and in the Qora and Xhora estuaries, with the two northern estuaries, Qora and Xhora, being warmer than the two southern estuaries (Table 2.2). Salinity was similar between both sampling years, however estuaries were significantly less saline in summer ($P < 0.003$). The southern two estuaries were more saline than the two northern estuaries (Table 2.2). The Nahoon Estuary was the most saline than all the other estuaries (Table 2.2). Conductivity and total dissolved solids (TDS) differed seasonally with the two southern estuaries having significantly higher conductivity and higher TDS than the northern estuaries during summer (Table 2.2). Conductivity and TDS were significantly lower in the upper reaches than the other study sites ($P < 0.003$). During winter, the Gonubie Estuary had the highest TDS (Table 2.2). The estuaries were more turbid in 2016 than in 2015 and were more turbid during summer than during winter ($P < 0.003$). The Gonubie in summer 2016 was more turbid than all the other estuaries ($P < 0.003$). The Nahoon had a significantly lower turbidity during the summer of 2016, while it had the highest turbidity during winter 2015 (Table 2.2). There were no significant differences in turbidity across the five sites. The estuaries were more alkaline in 2015 than 2016 ($P < 0.003$). The pH was similar in all the estuaries with the only exception of winter 2015, where the pH in the Xhora Estuary was significantly lower than all the other estuaries ($P < 0.003$). The dissolved oxygen was higher in 2015 than in 2016 ($P < 0.003$). The Gonubie Estuary had the lowest dissolved oxygen concentration than all the other estuaries during summer (Table 2.2).

2.4.2 Temporal and spatial trends in fish density

Larval density were highest in the Qora Estuary with a mean ± range of 39.47 ± 230.59 (100 m^{-3}) and lowest in the Nahoon Estuary 6.54 ± 75.25 (100 m^{-3}). Thus, larval density was similar between mangrove and non-mangrove estuaries, however densities differed spatially and temporally. Larval densities were significantly higher during the summer sampling season (Figure 2.2). Densities also differed among sites with the upper reaches (sites 4 and 5) of the Nahoon and Gonubie harbouring significantly higher *G. aestuaria* densities during summer than during winter (Figure 2.2). Larvae were distributed throughout the whole of the Qora Estuary during summer (Figure 2.2). Larval density peaked in the middle reaches in the Xhora Estuary during summer (Figure 2.2).

Table 2.2: Physico-chemical variation in the four studied estuaries where *Gilchristella aestuaria* were seasonally sampled in 2015 and 2016. Mean and range are given with significance codes that denote the following: *** $P < 0.0001$, ** $P < 0.001$; * $P < 0.003$, and ns is non-significant

	Mangrove		Non-mangrove	
Summer	**Nahoon**	**Xhora**	**Gonubie**	**Qora**
Temperature (°C)	20.17 (9.37)	23.41 (6.50) ***	20.44 (11.90)	24.12 (12.30) ***
Salinity	32.90 (6.81)	30.11 (19.71) ***	32.79 (5.01)	28.91 (30.95) ***
Conductivity ($mS.cm^{-1}$)	48.72 (18.98)	45.48 (33.01) *	48.57 (16.57)	44.72 (37.62) *
Total Dissolved Solids ($g.L^{-1}$)	34.59 (2.56)	32.17 (33.66) ***	34.21 (1.93)	28.71 (24.42) ***
Turbidity (NTU)	3.41 (13.50) *	4.89 (15.00) ns	5.77 (17.00) *	4.98 (13.20) ns
pH	8.30 (3.65) ns	8.62 (2.65) ns	8.71 (2.83) ns	8.29 (2.56) ns
Dissolved Oxygen ($mg.L^{-1}$)	8.13 (7.73)	7.42 (7.75)	7.32 (3.81) **	9.28 (8.96)
Dissolved Oxygen (%)	108.30 (102.80)	102.90 (99.90)	96.88 (42.30) ***	129.14 (101.70)
Total Rainfall (mm)	349.70 (103.80) ns	349.70 (103.80) ns	354.30 (1.20) ns	297.30 (73.80) ns
Winter				
Temperature (°C)	18.10 (3.80)	17.36 (4.20) ***	17.85 (4.90)	16.68 (3.90) ***
Salinity	34.33 (5.44)	33.90 (3.41)	34.28 (9.27)	33.59 (7.16)
Conductivity ($mS.cm^{-1}$)	45.17 (6.83)	44.01 (6.99)	44.93 (10.62)	42.99 (9.24)
Total Dissolved Solids ($g.L^{-1}$)	33.82 (5.54) ns	33.51 (2.97)	33.83 (7.48) *	33.21 (6.43)
Turbidity (NTU)	2.87 (9.20) ***	1.51 (5.90)	1.29 (8.09)	1.20 (6.50)
pH	8.07 (0.48)	8.02 (0.52) **	8.10 (1.08)	8.08 (0.14) ns
Dissolved Oxygen ($mg.L^{-1}$)	8.10 (6.04)	8.08 (3.05)	7.84 (5.54)	8.24 (2.59) *
Dissolved Oxygen (%)	105.32 (77.50) ns	103.16 (36.30) ns	101.72 (58.20) ns	103.62 (32.90) ns
Total Rainfall (mm)	441.05 (256.70) ns	441.05 (256.70) ns	354.00 (288.60) ns	179.25 (15.50) ns

2.4.3 Trends in larval density according to developmental stages

Larval density was similar between mangrove and non-mangrove estuaries, however, early stage *G. aestuaria* larvae occurred at significantly higher densities during the summer season (Figure 2.3). The Nahoon Estuary had significantly more larvae in the yolksac stage during summer while having less larvae in the postflexion stage (Figure 2.3). The Xhora Estuary had more larvae in the flexion and postflexion stages during summer than the Nahoon Estuary ($P < 0.003$). Preflexion larvae occurred in higher densities during summer in the Qora Estuary (Figure 3). Flexion larvae occurred in higher densities in the Xhora Estuary than the Qora Estuary ($P < 0.003$).

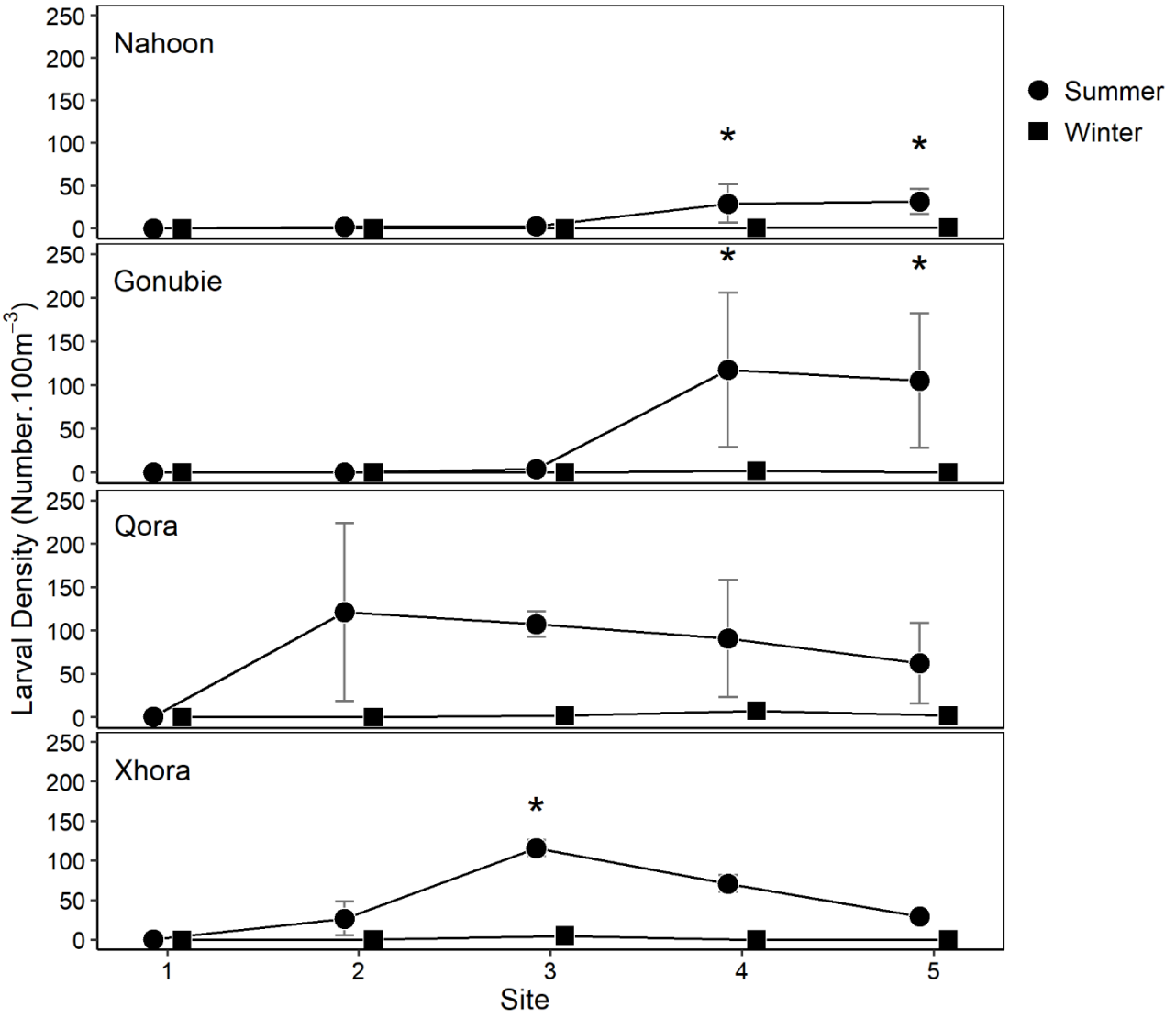

Figure 2.2: Mean larval density of *Gilchristella aestuaria* along the five sampled sites during summer and winter in mangrove and non-mangrove estuaries on the south east coast of South Africa. (Error bars denote range and * denote $P < 0.003$).

2.4.4 Distribution and environmental factors

Generalised Additive Models (GAMs) using a negative binomial distribution and the log link function revealed that *G. aestuaria* larval density could be best explained by an interaction of temperature, conductivity, turbidity and pH. The explained deviance was 68.1% with the estimated variance $\sigma^2 = 1$ and the AIC was 439.44. The smoothing term was significant at the 5% level, however the mangrove presence factor was not significant. The *P*-values of the individual levels indicate that the effects of temperature, conductivity, turbidity and pH are highly significant in explaining larval density (Figure 2.4). Larval density increased with temperature and turbidity, and peaked at conductivities of 39 – 44 S.m^{-1} and pH of around 8.07 (Figure 2.4).

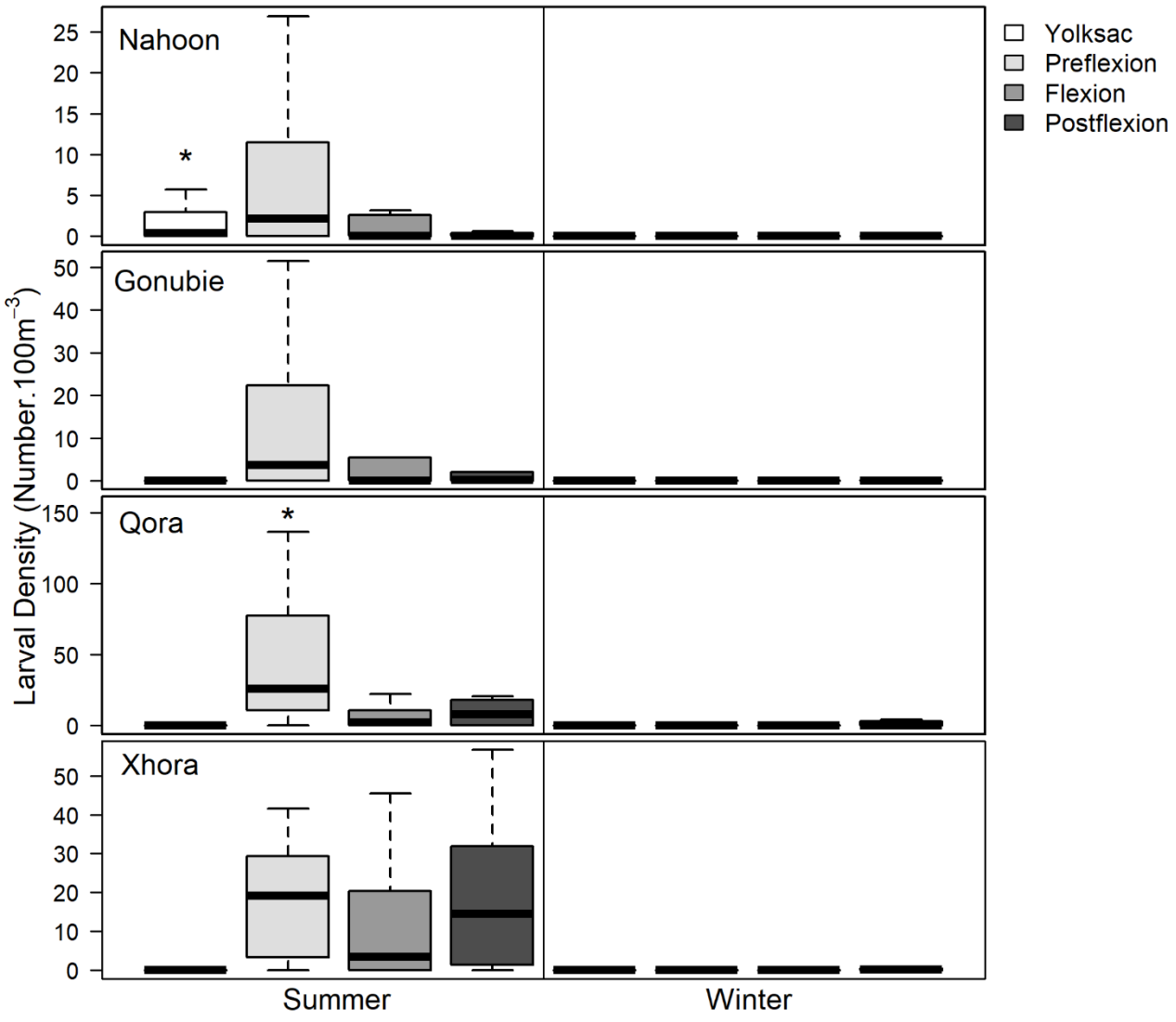

Figure 2.3: Larval *Gilchristella aestuaria* density by developmental stages in mangrove and non-mangrove estuaries on the south east coast of South Africa. (Median, interquartile, minimum and maximum are given, with * denotes $P < 0.003$).

2.5 DISCUSSION

The spatial and temporal variations in density of larval *Gilchristella aestuaria* was not related to the presence of mangrove habitats, however it can be explained by abiotic factors such as: temperature, conductivity, turbidity and pH. Temperatures were warmer during summer than in winter with the two northern estuaries, Qora and Xhora being the warmest as they are closest to the subtropics. Larval growth and thus survival are linked to temperature, where protein synthesis is more efficient in higher temperatures (Esteves et al., 2000; Buckley et al., 2008).

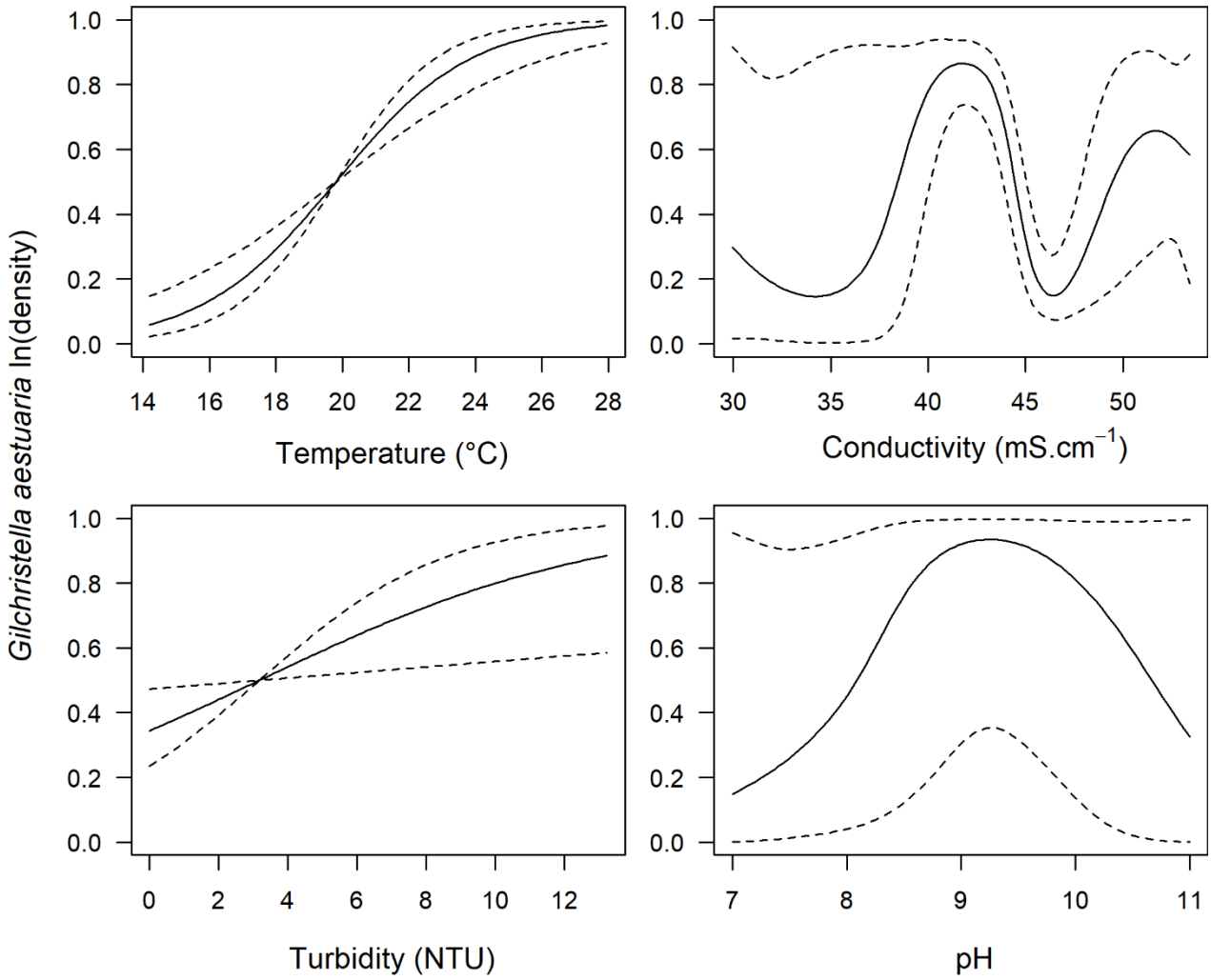

Figure 2.4: The relationship between environmental predictors and larval *Gilchristella aestuaria* density using a stepwise log-linked Generalized Additive Model. (Solid line denotes smooth terms and dashed lines denote the 95% confidence intervals)

This could explain the higher larval densities seen in the two northern estuaries, however, when it comes to explaining the nutritional condition and growth of *G. aestuaria* larvae, a recent paper found that coupled environmental factors (such as temperature, salinity, dissolved oxygen and turbidity) play a more determinant role as estuarine fish species have remarkably high tolerances to varying environmental factors (Costalago et al., 2015).

Higher densities of *G. aestuaria* larvae have been repeatedly seen in the highly productive mesohaline zone (5-18) in most warm temperate estuaries (Strydom et al., 2014; Strydom, 2015). However, the studied estuaries were more saline when compared to most warm temperate estuaries, which might be due to their relatively small catchment sizes, yet permanently open mouth conditions. These high salinities may be the reason for the relatively low densities (< 100 larvae per 100 m^{-3}) found within

these estuaries when compared to other warm temperate estuaries. A study, which used a similar sampling technique, on the distribution and abundance of larval fishes in temperate South African estuaries, stated that a mean density of 543.60 (100 m^{-3}) was typical for warm temperate estuaries and a mean of 791.68 (100 m^{-3}) was typical for warm temperate/subtropical boundary estuaries (Strydom, 2015).

Despite the direct relationship of salinity and conductivity and the relatively similar salinities found within all the studied estuaries, conductivity played a significant role in explaining the density and distribution of *G. aestuaria* larvae. The Generalized Additive Models (GAMs) revealed that larval densities were highest at conductivities of 39 – 44 $mS.cm^{-1}$. Conductivity can impact mechanosensory-mediated behaviours such as predator avoidance as developing larval fish use neuromasts to sense vibrational cues and other forms of water displacement (Scott and Sloman, 2004; Linbo et al., 2006). Both the Nahoon and Gonubie, which are closest to human settlements, had higher mean salinities and conductivities during summer and winter. As the conductivity of an aqueous solution is not only related to salinity, but to electrolytes such as heavy metals and other industrial chemicals, it may be that the increased conductivities seen within the Nahoon and Gonubie may be as a result of anthropogenic contamination which has been found to negatively impact larval fish growth and survival in an experimental setting (Di-Toro et al., 2001; Linbo et al., 2006).

Mangrove habitats are sediment sinks which are known to reduce the turbidity of the surrounding habitats which may affect the density of larval fishes as it has implications for ease of feeding as well as differing predation pressures (Furukawa and Wolanski, 1996). Turbid waters decreases the contrast of larval fishes to the surrounding waters, which reduce the risk of predation (Utne-Palm, 2002). Thus, one might expect early stage fishes to be heavily preyed upon when waters are less turbid. However, the Nahoon had a high abundance of earlier staged larvae despite being the least turbid during summer. Early staged planktivorous larvae do not have well developed gillrakers and have been found to rely on their vision for particulate feeding once they become unaided by their yolksac (Costalago and Palomera, 2014). Gillrakers only start to develop from 8 – 9 mm for *G. aestuaria* which is before the fexion stage, therefore less turbid waters will be favourable to preflexion stages (Haigh and Whitfield, 1993). Despite this, the GAMs found that larval density increased with an increase in turbidity. The effect of turbidity on larval abundance and distribution is still not well understood within estuaries as turbidity is mostly as a consequence of freshwater inputs and is thus accompanied by a whole suite of environmental variables. However, the sharp decreases seen in subsequent flexion and postflexion larvae within the Nahoon during summer may indicate that larval survival was poor within this system when compared to the other estuaries in this study.

Extreme pH levels have been found to have a significant impact on the growth and survival of larval fishes (Baumann et al., 2012). The high surface-to-volume ratio of larvae, make them more vulnerable to diffusive processes across epithelia, which impacts mechanisms of acid–base regulation and are linked to gill function and muscle activity (Perry and Gilmour, 2006). Estuarine pH levels are linked to carbon dioxide and dissolved oxygen concentrations and are mostly due to fluctuations in biological activity, tides, freshwater inflow via leaching of soils, and anthropogenic impacts (Baumann et al., 2014). Despite the pH being relatively similar between estuaries, the pH was found to have a significant effect on larval density as GAMs, with pH as an explanatory variable, were significantly better at explaining larval density trends. A recent study on ocean acidification has found that fish eggs are more sensitive to increased pH levels than fish larvae (Baumann et al., 2012). Therefore, recruitment and survival of larvae may thus be affected by pH levels.

The two northern estuaries, the Qora and Xhora, had a higher number of smaller larvae than the southern estuaries, Nahoon and Gonubie. This might be as a consequence of larger abundances of resident spawning adults within these systems which could give rise to higher larval abundances within these systems. A greater catch per unite effort of adult *G. aestuaria* was found within the two northern estuaries during a parallel study (McGregor and Strydom, 2017). Larval abundances were higher during summer with more larvae being in earlier life stages than during winter where the population was dominated mainly by older postflexion larvae, indicating that larval peak breeding is during the summer season with sporadic breeding that occurs during the winter season. This low-scale breeding, seen throughout the winter season have been found in another study where *G. aestuaria* spawned as a result of freshwater pulses which act as a spawning cue (Strydom et al., 2002). The significantly higher abundance of yolksac stage larvae seen in the Nahoon and the subsequent low numbers of postflexion larvae may be as a consequence of delayed spawning which also indicates that the Nahoon Estuary is a poorer nursery than the other study estuaries (Scott and Sloman, 2004).

Larval density was not related to the presence of mangrove habitats, although the density and distribution of larvae within these warm temperate estuaries, were driven by an interaction of temperature, conductivity, turbidity and pH. Studies on the association of catch trends and environmental variables have found that *G. aestuaria* larval density is impacted by salinity, temperature, turbidity and river flow in two warm temperate estuaries further south of the current study estuaries (Strydom et al., 2002). It was concluded that river flow was the main driving factor impacting *G. aestuaria* densities. As these estuaries are nutrient limited, the nutrients from freshwater input may have a bigger contribution to the system than would mangrove habitats. Warm temperate mangroves have been found to

be less productive than tropical mangroves and thus their nursery value must not be considered the same across regions (Komiyama et al., 2008). This study is the first of its kind assessing the value of mangroves in driving production in fodder fish populations. The growth and survival of larvae are also dependent on food availability (Clemmesen, 1994). Previous studies have found *G. aestuaria* abundances to be positively correlated with Copepoda densities (Whitfield, 1999). Thus, it is recommended that future studies determining the nursery value of mangrove habitats incorporate growth and survival as well as biotic factors such as the match-mismatch with prey in order to further explore the possible intrinsic factors driving important fish nurseries.

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Chapter 3: Predator-prey Interactions Associated with Late Stage Larval Gilchristella aestuaria (Family Clupeidae) in Mangrove and Non-Mangrove Estuaries of Warm Temperate South Africa

3.1 ABSTRACT

Zooplankton and ichthyoplankton dynamics vary spatially and temporally in warm temperate estuaries. Therefore, the nursery value of these systems are likely to vary due to complex predator-prey interactions, as well as variations in freshwater supply, primary productivity as well as habitat availability for refuge. Mangrove habitats are among the most productive worldwide and are widely cited as the ideal fish nursery habitat, however most studies focus on tropical mangroves. This study compared the predator-prey interactions between the larvae of a common mid-trophic species, *Gilchristella aestuaria*, and dominant zooplankton between similar warm temperate estuaries with and without mangroves. Generalized Additive Models found that an interaction of mangrove presence, turbidity, copepod prey density and competition pressures by predatory Mysidacea were the most significant at explaining larval densities within warm temperate estuaries. Larvae fed primarily on the dominant prey species, *Pseudodiaptomus hessei*, *Paracrtia longipatella*, and *Acartiella natalensis*. However, postflexion larvae consumed more of the larger species, *P. hessei,* within the two mangrove estuaries (16.09 %V in Nahoon and 13.79 %V in Xhora) than the two non-mangrove estuaries (12.20 %V in Gonubie and 7.05 %V in Qora), despite other prey species occurring at high densities. This selective feeding may be as a consequence of decreased turbidities seen within mangrove estuaries. Therefore, mangrove presence was significantly related to postflexion larval densities when coupled with abiotic (such as temperature and turbidity) and biotic factors (such as predator-prey interactions). This study is the first of its kind assessing the value of mangroves in driving production in fodder fish populations. Thus, it is recommended that more studies are needed which incorporate predator-prey interactions when assessing the nursery value of mangrove habitats to fishes.

3.2 INTRODUCTION

Estuaries function as important nursery areas for many ecologically and economically important fish species. Estuaries link the freshwater and marine environments and thus are highly dynamic systems that allow for colonization by an array of species, many of which occur in high abundance and result in intricate food webs (Vinagre et al., 2011). Growth and survival of larval fishes in estuaries is

dependent on the interactive effects of both physico-chemical and biological variables which impact the availability and quality of prey items, as well as competition and predation impacts in the water column (Fortier and Harris, 1989; Cushing and Horwood, 1994; Welker et al., 1994). Estuarine larval fishes make extensive use of early copepodite stages of calanoid copepod species during early developmental stages (Strydom et al., 2014). The nutritional value of prey is likely to vary between prey species and thus, as suggested by the optimal foraging theory (Fortier and Harris, 1989), the ability to feed on the most nutritious prey item, with the least amount of energy spent capturing the prey, would result in fish that are more likely to survive to the adult stage (Esteves et al., 2000). However, predatory mysid shrimps have significant effects on Copepoda abundances and species composition which may affect prey availability and thus recruitment success of larval fishes (Wooldridge and Webb, 1988; Froneman, 2001). Therefore, the scale and effects of these predator-prey interactions will depend on the overlap in temporal and spatial distributions of possible prey and predator species in estuarine plankton. Within estuaries, nursery function is dependent on various factors, which include food availability (Beck et al., 2001; Heck et al., 2003) which is likely to vary due to complex predator-prey interactions (Heck et al., 2003), as well as variations in primary productivity which are associated with physical factors such as freshwater inflow (Vinagre et al., 2011) and the resultant productivity coupled with additional feeding opportunities derived from specific niche use, particularly plant communities within estuarine nurseries (Deegan and Garritt, 1997).

Mangroves are among the most productive habitats worldwide and are widely cited as the ideal fish nursery (Costanza et al., 1997; Beck et al., 2001). Their complex root structures creates a complex submerged habitat which attracts a multitude of species by providing shelter from predators as well as increased food availability (MacArthur, 1965; Nagelkerken et al., 2010). However, it is not known what the implications of these possible advantages of mangrove habitats are for plankton communities. This is particularly important for mid-trophic species such as the estuarine roundherring, *Gilchristella aestuaria* (Gilchrist, 1914) as they are key to estuarine food webs. This species was selected as a candidate species as it is highly abundant across most South African estuaries (Haigh and Whitfield, 1993; Strydom, 2015). This species is planktivorous, feeding predominantly on phytoplankton and zooplankton, and thus plays a key ecological role in the transfer of energy between trophic levels (Blaber et al., 1981; White and Bruton, 1983; Whitfield and Harrison, 1996).

A number of studies have focussed on *G. aestuaria* diet (Cyrus et al., 1993; Whitfield and Harrison, 1996), zooplankton dynamics (Wooldridge and Bailey, 1982) and links to environmental factors (Strydom et al., 2002, 2014). However very few have tried to link these to specific habitat types within estuaries. Moreover, the contribution to the nursery value of mangroves in the warm temperate

biogeographic region remains poorly assessed in relation to subtropical and tropical systems (Mbande et al., 2005; Blaber, 2007; Sheaves, 2017). The warm temperate region on the east coast of South Africa is an ideal *in situ* locale as mangroves reach the end of their latitudinal distribution. This allows similar estuaries, which are in close geographical proximity, with and without mangroves to be compared. The abundance and productivity of phytoplankton and zooplankton depends on nutrient inputs (mostly from riverine flow) into estuaries (Wooldridge and Bailey, 1982; Snow et al., 2000; Bate et al., 2002). However, mangroves have also been found to increase the nutrient levels and invertebrate communities within estuaries (Sheridan, 1997; Beck et al., 2001). It is not known to what extent mangrove-derived habitats facilitate the success of larval stages of resident fish species in estuaries.

This study aimed to assess whether mangrove presence provides ecological benefits to the larvae of an important estuarine fish species by comparing plankton communities in warm temperate mangrove and non-mangrove estuaries. More specifically the study aimed to assess the predator-prey interactions between zooplankton and *G. aestuaria* larvae in estuaries with and without mangroves. It is hypothesized that higher abundances of *G. aestuaria* larvae and their prey species will be found in estuaries with mangroves as these systems would provide an enhanced feeding environment for all species, compared to estuaries devoid of mangroves.

3.3 MATERIALS AND METHODS

3.3.1 Study area

Samples were collected seasonally from four warm temperate estuaries along the east coast of South Africa during 2015 and 2016 (Figure 3.1). The estuaries were selected based on shared similarities (under natural conditions) such as a permanently open mouth state, similar catchment, river and estuarine size, and similar vegetation type composition apart from mangrove presence. From the south, the Nahoon Estuary (27° 57’ 05” E, 32° 59’ 05” S) and the Gonubie Estuary (28° 01' 59" E, 32° 55' 59" S) are two similarly sized neighbouring estuaries situated near the city of East London. The Nahoon has a relatively small < 2 ha mangrove stand, but is increasing at 0.06 ha y^{-1} since 1969 when *Avicennia marina* was planted and a few years later a few specimens of *Bruguiera gymnorrhiza* and *Rhizophora mucronata* were added among the larger *A. marina* trees (Steinke, 1972, 1986; Hoppe-Speer et al., 2015). Despite the two estuaries being neighbouring, the Gonubie Estuary is devoid of mangroves (Hoppe-Speer et al., 2014). Anthropogenic impacts on the Nahoon Estuary include the Nahoon Dam as well as anthropogenic heavy metals and other pollutants from occasional municipal

waste water spills (Talbot et al., 1985; Newman and Watling, 2007). There are no known anthropogenic impacts on the Gonubie Estuary, however its close proximity to a populated area may result in impacts such as recreational activities. Both these rivers have farming activities in their catchment and may be subjected to agricultural runoff. The Qora (28° 40' 21" E, 32° 26' 50" S) and Xhora (29° 05′ E, 32° 05′ S) estuaries, are also similarly sized and in close proximity to each other. The mangroves at Xhora covers an area of 25.5 ha and consists of all three mangrove species present in South Africa (*A. marina*, *R. mucronata* and *B. gymnorrhiza*), while Qora is devoid of mangroves (Hoppe-Speer et al., 2014). This study forms part of a larger project that was the first to study the ichthyofauna of these two estuaries and thus very little is known about these two northern estuaries (Adams et al., 2016). Little to no anthropogenic impacts are likely in these estuaries due to their remote location. They are of similar size and in relative close proximity to the other study estuaries, which make them ideal for this comparative study.

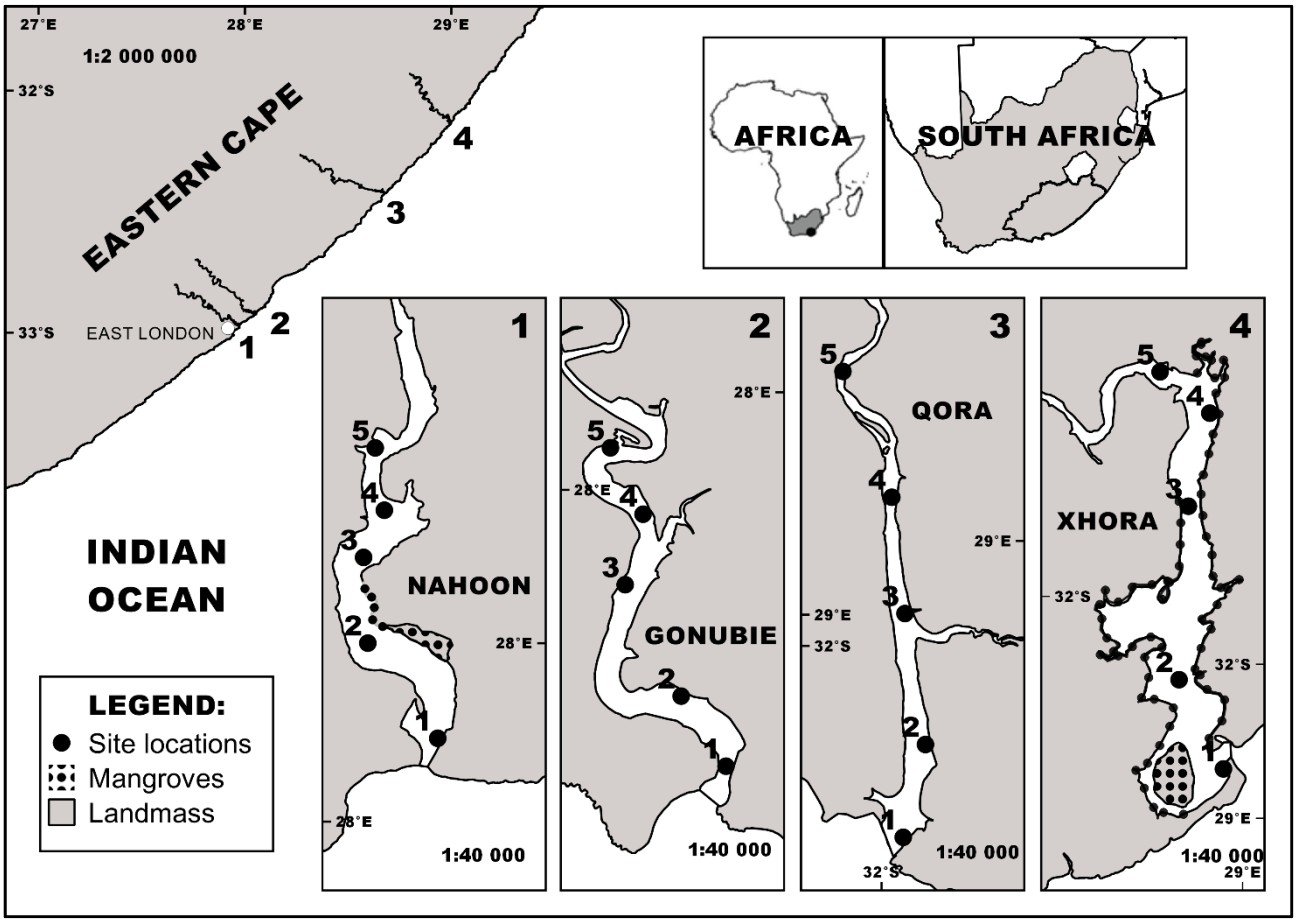

Figure 3.1: Geographic position of studied estuaries showing the location of sampling sites.

3.3.2 Field sampling

Plankton samples were collected isochronously after dark on a first quarter moon phase from five

fixed sampling stations (each are one kilometre apart) along the main channels of the four estuaries in order to keep any feeding periodicity or diel rhythms constant (Figure 3.1). Samples were collected using two modified Working Party 2 (WP2) plankton nets (570 mm mouth diameter and 0.2 mm mesh aperture) fitted with calibrated Kahlsico 005 WA 130 flowmeters. The two nets were simultaneously lowered and towed horizontally alongside a 5 m boat for 3 min at a speed of 1-2 knots and sampled the upper 0.6 m of the water column at a mean volume of 189.76 ± 70.41 m^3 (Strydom and Whitfield, 2000). Two replicate samples were collected at each of the five sites ranging from the upper to the lower reaches of each estuarine system (Figure 3.1). Where possible, an oblique course across the axis of the estuary was followed, thus enabling samples to be taken near the banks as well as in the mid-channel (Strydom et al., 2002). After each tow, flowmeter readings were recorded, and the sample was immediately preserved in 10% buffered formaldehyde. Physico-chemical parameters were determined *in situ* at the time of sampling with a calibrated YSI sonde series 6600 multi-parameter probe with temperature, salinity, conductivity, total dissolved solids, turbidity, pH, and dissolved oxygen recorded every 0.5 m.

3.3.3 Larval density

All *G. aestuaria* were identified according to Neira et al. (1998) and removed from the samples using a Leica M80 stereomicroscope fitted with an eyepiece micrometer. Standard lengths of 50 randomly selected *G. aestuaria* were measured to the nearest 0.01 mm and staged into developmental stages according to Neira et al. (1998). The flowmeters on the nets allowed for the calculation of larval density. Flowmeters were calibrated in a controlled environment and it was determined that a value of 32.7 was the number of revolutions per m^3 water filtered. Thus the following formula was used to calculate larval density:

$$\text{Density} = [\text{N} / (\text{r} / \text{c})] \times 100$$

where density is the number of *G. aestuaria* larvae per 100 m^3, *N* is the total number of larvae caught per haul, *r* the revolutions of the flowmeter and *c* the predetermined calibration value in m^3

3.3.4 Gut content analysis

Stomachs of postflexion *G. aestuaria* larvae were removed and opened using a Leica M80 stereo-microscope fitted with an eyepiece micrometer. Food items were then identified, counted and flattened in a 1 mm deep tray, marked with 1mm^2 grids to calculate the volume of each prey item. Identification was completed to the lowest possible taxon, sexed and staged using *inter alia* (Kasturirangan, 1963; Jerling and Wooldridge, 1989; Mattheus, 2012; Conway, 2013). This data was then used to calculate the frequency of occurrence (%F), numerical occurrence (%N) and volumetric

occurrence (%V) of each prey item expressed as a percentage of the total stomach contents (Hyslop, 1980).

3.3.5 Zooplankton density

Samples were diluted by adding freshwater to a predetermined volume (up to 2 litres on average) and three subsamples were drawn off by using a wide-mouthed pipette after agitation (Wooldridge and Melville-Smith, 1979). These samples were then placed on a tray and identified using Mattheus (2012). The dominant copepod *Pseudodiaptomus hessei* was divided into a number of classes: mature males, ovigerous females, nonovigerous females and juveniles according to Jerling and Wooldridge (1989). Mysids were examined under a stereo microscope fitted with an eyepiece micrometer, measured (anterior tip of carapace to posterior tip of telson, excluding spines) and separated into seven classes which relate to the degree of sexual maturity (Wooldridge and Bailey, 1982). These classes are based on those described by Mauchline (1973): (i) Juveniles - secondary sexual characteristics not developed (ii) Immature males (iii) Immature females (iv) Females with developing young in the brood pouch (v) Females with rounded embryos (vi) Females with empty marsupia; young released (vii) Mature males. The results were expressed as the number of individuals of each species per cubic meter of water (Wooldridge and Melville-Smith, 1979).

3.3.6 Statistical analysis

Shapiro-Wilks and Levene's test was used to test data for normality and homogeneity of variance respectively. If these assumptions were not met, non-parametric tests were used. All of the physico-chemical parameters as well as larval and zooplankton densities violated the assumptions of normality and homogeneity of variances. Therefore, Kruskal-Wallis tests were used to test for differences in the four estuaries. When significant ($P < 0.05$), the Mann-Whitney U test was used as a post-hoc on pairs of estuaries using a Bonferroni-corrected level of significance of $\alpha = 0.003$. Descriptive statistics were used to show trends in %N, %V and %F in the diet of each species of larval fish among estuaries. A Generalised Additive Models (GAMs) was used with a negative binomial distribution and log-link function, to quantify the relationship between larval density, zooplankton density and physico-chemical parameters. Mangrove presence was included in all models as it was the factor of primary concern. Best fit was determined via a forward stepwise approach using Akaike Information Criterion (AIC) and Chi-squared tests. All statistical analyses were performed using the statistical software R (v. 3.3.1) with mgcv and ggplot2 packages.

3.4 RESULTS

3.4.1 Environmental variability

Temperatures were similar in 2015 and 2016, however, temperatures were higher in summer than in winter ($P < 0.003$). Temperatures were similar in the Nahoon and Gonubie estuaries and in the Qora and Xhora estuaries, with the two northern estuaries, Qora and Xhora, being warmer than the two southern estuaries (Table 3.1). Despite that the overall seasonal rainfall being similar, the two southern estuaries were more saline than the two northern estuaries with the Nahoon Estuary being the most saline, with a mean of 32.90 (26.09 – 39.71) during summer and 34.33 (28.89 - 39.77) during winter (Table 3.1). Conductivity and total dissolved solids (TDS) differed seasonally with the two southern estuaries being significantly more conductive and had higher TDS than the northern estuaries during summer (Table 3.1). Conductivity and TDS were significantly lower in the upper reaches of all the estuaries ($P < 0.003$).

Table 3.1: Physico-chemical variation in the four studied estuaries where *Gilchristella aestuaria* were seasonally sampled in 2015 and 2016. Mean and range are given with significance codes that denote the following: *** $P < 0.0001$, ** $P < 0.001$; * $P < 0.003$, and ns is non-significant

	Mangrove		**Non-mangrove**	
Summer	**Nahoon**	**Xhora**	**Gonubie**	**Qora**
Temperature (°C)	20.17 (9.37)	23.41 (6.50) ***	20.44 (11.90)	24.12 (12.30) ***
Salinity	32.90 (6.81)	30.11 (19.71) ***	32.79 (5.01)	28.91 (30.95) ***
Conductivity (mS.cm^{-1})	48.72 (18.98)	45.48 (33.01) *	48.57 (16.57)	44.72 (37.62) *
Total Dissolved Solids (g.L^{-1})	34.59 (2.56)	32.17 (33.66) ***	34.21 (1.93)	28.71 (24.42) ***
Turbidity (NTU)	3.41 (13.50) *	4.89 (15.00) ns	5.77 (17.00) *	4.98 (13.20) ns
pH	8.30 (3.65) ns	8.62 (2.65) ns	8.71 (2.83) ns	8.29 (2.56) ns
Dissolved Oxygen (mg.L^{-1})	8.13 (7.73)	7.42 (7.75)	7.32 (3.81) **	9.28 (8.96)
Dissolved Oxygen (%)	108.30 (102.80)	102.90 (99.90)	96.88 (42.30) ***	129.14 (101.70)
Total Rainfall (mm)	349.70 (103.80) ns	349.70 (103.80) ns	354.30 (1.20) ns	297.30 (73.80) ns
Winter				
Temperature (°C)	18.10 (3.80)	17.36 (4.20) ***	17.85 (4.90)	16.68 (3.90) ***
Salinity	34.33 (5.44)	33.90 (3.41)	34.28 (9.27)	33.59 (7.16)
Conductivity (mS.cm^{-1})	45.17 (6.83)	44.01 (6.99)	44.93 (10.62)	42.99 (9.24)
Total Dissolved Solids (g.L^{-1})	33.82 (5.54) ns	33.51 (2.97)	33.83 (7.48) *	33.21 (6.43)
Turbidity (NTU)	2.87 (9.20) ***	1.51 (5.90)	1.29 (8.09)	1.20 (6.50)
pH	8.07 (0.48)	8.02 (0.52) **	8.10 (1.08)	8.08 (0.14) ns
Dissolved Oxygen (mg.L^{-1})	8.10 (6.04)	8.08 (3.05)	7.84 (5.54)	8.24 (2.59) *
Dissolved Oxygen (%)	105.32 (77.50) ns	103.16 (36.30) ns	101.72 (58.20) ns	103.62 (32.90) ns
Total Rainfall (mm)	441.05 (256.70) ns	441.05 (256.70) ns	354.00 (288.60) ns	179.25 (15.50) ns

The Gonubie Estuary had the highest mean TDS of 33.83 $g.L^{-1}$ during winter than all the other estuaries (Table 3.1). The dissolved oxygen concentration differed seasonally and between the estuaries with the Gonubie Estuary having the lowest dissolved oxygen concentration of 7.32 $mg.L^{-1}$ during summer and the Qora Estuary having the highest of 8.24 $mg.L^{-1}$ during winter (Table 3.1). Estuaries were more turbid during summer than during winter ($P < 0.003$). The Gonubie in summer 2016 was more turbid than all the other estuaries with a mean turbidity of 5.77 NTU ($P < 0.003$). The Nahoon had a significantly lower turbidity of 3.41 NTU during summer, while during winter, it had the highest turbidity of 2.87 NTU (Table 3.1). The pH was similar in all the estuaries with the only exception of the Xhora Estuary that had a significantly lower pH of 8.02 than all the other estuaries during winter (Table 3.1).

3.4.2 Larval density

Catches of larval *G. aestuaria* were similar between the studied estuaries with no difference between mangrove and non-mangrove estuaries, however, densities differed seasonally and spatially (see previous chapter). Densities also differed among sites with the upper reaches of the Nahoon and Gonubie harbouring significantly higher postflexion *G. aestuaria* densities (Figure 3.3). Larvae were distributed more uniformly throughout the Qora Estuary, with most being from sites in the mid to upper estuary and peaked in the middle reaches (site 3) in the Xhora Estuary (Figure 3.3).

3.4.3 Diet of *Gilchristella aestuaria*

In total, 593 larvae were analysed for stomach content with only 43.34 % having any food items in the stomach. The diet of postflexion *G. aestuaria* larvae were dominated by Copepoda species: *Pseudodiaptomus hessei*, *Paracartia longipatella* and *Acartiella natalensis*. Larvae consumed more *P. hessei* in the Nahoon (18.40 %V) and Xhora (17.25 %V) than in the Gonubie (16.26 %V) and Qora (9.72 %V) (Table 3.2). Larvae consumed more *P. longipatella* in the two southern estuaries than the two northern estuaries, while *A. natalensis* were consumed more in the two northern estuaries (Table 3.2). After Copepoda, unidentified algal matter formed the second largest proportion of the diet, while a few opportunistic Mysidacea prey items, that included eyes of an unidentified *Mesopodopsis spp*. and the eggs of *Rhopalophthalmus terranatalis*, were found (Table 3.2).

Table 3.2: Diet composition of postflexion *Gilchristella aestuaria* in the four studied estuaries during 2015 and 2016. The various dietary metrics being: the number of individuals of a particular food item out of the total number of food items (%N), the volume of food item out of the total volume of stomach contents (%V), and the number of stomachs in which each prey item occurred out of the total number of stomachs examined (%F).

Prey item	Mangrove						Non-mangrove					
	Nahoon			Xhora			Gonubie			Qora		
	%N	%V	%F	%N	%V	%F	%N	%V	%F	%N	%V	%F
Unidentified food items	23.58	9.20	21.43	0.39	0.49	1.30	10.19	7.32	23.81	6.75	5.86	10.36
Plant-like matter	58.49	41.38	14.29	55.77	21.04	23.04	62.04	40.65	19.05	46.31	27.59	24.09
Pseudodiaptomus hessei												
Adults	3.77	16.09	14.29	5.91	13.79	13.48	3.70	12.20	4.76	2.59	7.05	6.72
Juveniles				1.51	3.46	6.96	1.39	4.07	4.76	1.36	1.94	4.20
Eggs										0.63	0.15	0.56
Fragments	0.94	2.30	7.14							0.28	0.58	1.96
Total	4.72	18.39	21.43	7.42	17.25	20.43	5.09	16.26	9.52	4.86	9.72	13.45
Paracartia longipatella												
Adults	6.60	18.39	7.14	0.39	0.99	1.74	5.56	11.38	14.29	3.50	6.83	9.80
Juveniles	0.94	2.30	7.14							0.10	0.24	0.84
Females	0.94	2.30	7.14									
Fragments										0.45	0.44	1.12
Total	8.49	22.99	21.43	0.39	0.99	1.74	5.56	11.38	14.29	4.05	7.51	11.76
Acartiella natalensis												
Adults	1.89	4.60	7.14	31.56	54.84	36.09	9.72	13.82	9.52	27.65	37.58	19.61
Females										0.14	0.24	0.28
Fragments				0.23	0.27	1.74				0.59	0.66	1.40
Total	1.89	4.60	7.14	31.79	55.11	37.83	9.72	13.82	9.52	28.38	38.48	21.29
Copepoda fragments	2.83	3.45	14.29	4.09	4.51	14.35	7.41	10.57	23.81	9.54	10.55	18.21
Copepoda eggs										0.03	0.05	0.28
Total Copepoda	17.92	49.43	64.29	43.68	77.86	74.35	27.78	52.03	57.14	46.87	66.31	64.99
Mesopodopsis sp.												
Eyes				0.12	0.38	0.87						
Rhopalophthalmus terranatalis												
Eggs				0.04	0.22	0.43				0.07	0.24	0.56
Total Mysidacea	0.00	0.00	0.00	0.15	0.60	1.30	0.00	0.00	0.00	0.07	0.24	0.56

3.4.4 Predator-prey interaction

Dominant prey species density did not differ between mangrove and non-mangrove estuaries, however species relationships to environmental factors such as temperature and salinity were observed seasonally and spatially (Figure 3.3). Dominant prey species were *P. hessei*, *P. longipatella*, and *A. natalensis* which occurred in higher densities in the upper reaches of the studied estuaries. Densities of *P. hessei* were higher in the Gonubie with a mean ± range of 3401.00 ± 19357 m^{-3} and Xhora estuaries 3996.60 ± 10686 m^{-3} during summer ($P < 0.003$), while *P. longipatella* was similar in all the estuaries, while *A. natalensis* were at higher densities in the Qora with a mean ± range of 17203.60 ± 56300.00 m^{-3} and Xhora estuaries 17656.60 ± 70858.00 m^{-3} ($P < 0.003$) (Figure 3.3). Larval *G. aestuaria* densities positively correlated with the dominant prey items, with the strongest positive correlation being *P. longipatella* (Table 3.3). Postflexion *G. aestuaria* densities did not correlate with

P. hessei males, nor females, but significantly correlated with the juvenile forms, indicating that the larvae prefer to feed on juvenile *P. hessei* (Table 3.3).

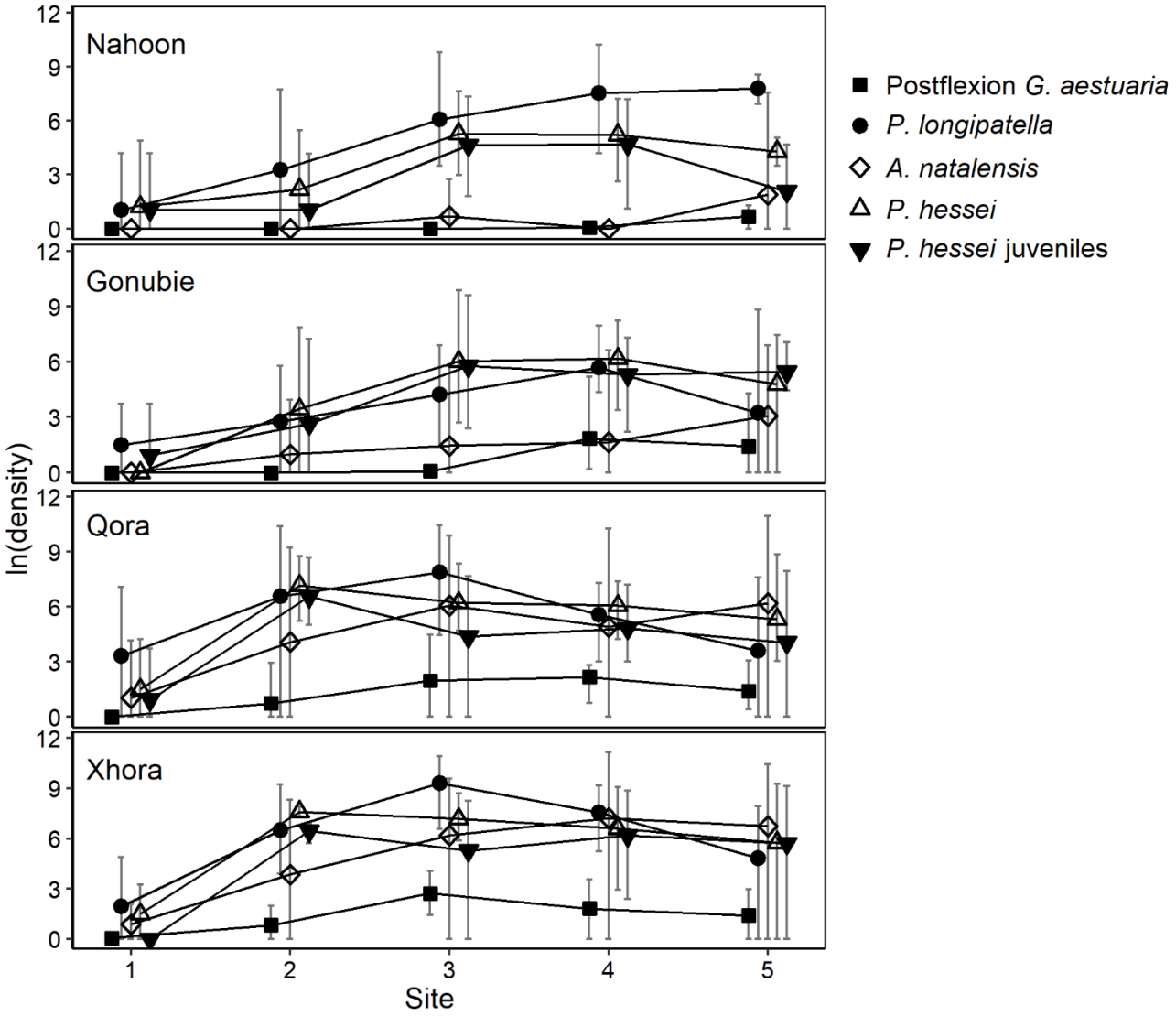

Figure 3.2: Spatial changes in mean postflexion *Gilchristella aestuaria* and dominant zooplankton prey density at all sites in mangrove and non-mangrove estuaries on the south east coast of South Africa. (Error bars denote the range)

Predatory Mysidacea (*Mesopodopsis wooldridgei* and *Rhopalophthalmus terranatalis*) and larvae of Brachyura species were found to co-occur in the upper reaches where *G. aestuaria* larvae were most abundant (Figure 3.4). The densities of these species correlated with *G. aestuaria* densities (Figure 3.4). Similar densities of *M. wooldridgei* were found in mangrove and non-mangrove estuaries, however, *R. terranatalis* occurred in higher densities in mangrove estuaries ($P < 0.003$).

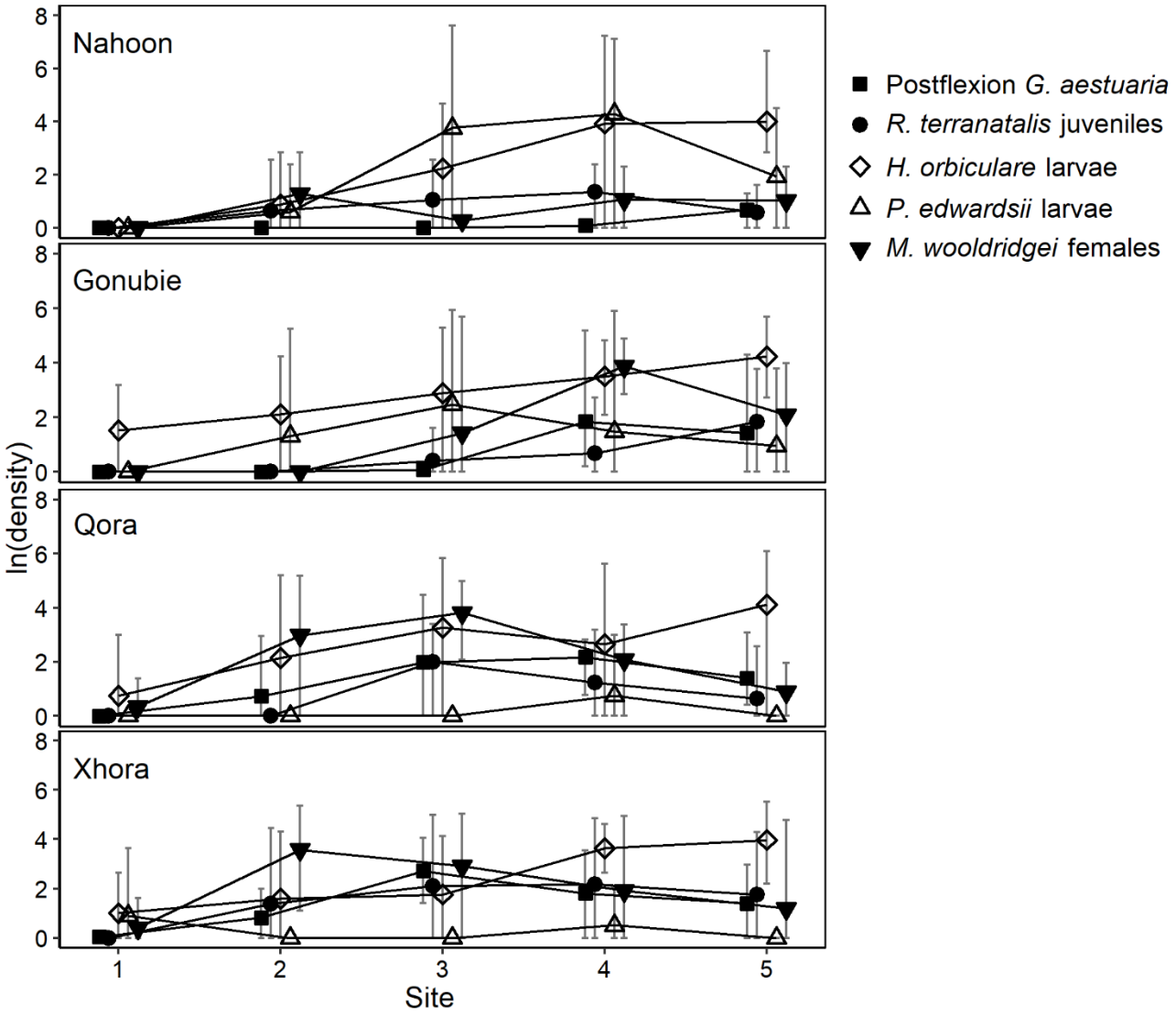

Figure 3.3: Spatial changes in mean postflexion *Gilchristella aestuaria* and co-occurring predatory zooplankton density at all sites in mangrove and non-mangrove estuaries on the south east coast of South Africa. (Error bars denote the range)

The densities of both these species correlated with the densities of *G. aestuaria* larvae, the female *M. wooldridgei*, that had young in their brooding pouches, negatively correlated with pre- and post-flexion stage *G. aestuaria*, while juvenile *R. terranatalis* negatively correlated with the density of larvae in the flexion stage (Table 3.3). The larvae of co-occurring Brachyura species, *Hymenosoma orbiculare* and *Paratylodiplax edwardsii*, also negatively correlated with *G. aestuaria* densities (Figure 3.4), with strongest correlation between *H. orbiculare* larvae and preflexion larval densities (Table 3.3).

Table 3.3: Output of a Generalized Additive Model with mangrove presence as a fixed factor and *Gilchristella aestuaria* densities per developmental stages as response variables and explanatory variables selected by means of a forward stepwise approach.

Response variable	Explanatory variable	Z-value (factor)	edf	Chi squared (Smoother)	Deviance explained (%)
All Larval stages	Mangrove presence/absence ns	0.22			
	Temperature***		3.54	93.18	
	Conductivity***		5.41	45.18	
	*P. longipatella***		1.06	16.74	
	M. wooldridgei brooding females***		4.16	37.92	84.30
Preflexion	Mangrove presence/absence ns	2.19			
	Temperature***		3.84	68.21	
	M. wooldridgei brooding females***		4.81	31.36	
	H. orbiculare larvae***		1.74	37.19	88.20
Flexion	Mangrove presence/absence ns	1.82			
	Temperature***		5.14	27.40	
	R. terranatalis juveniles**		1.00	9.66	
	P. hessei males**		2.70	12.02	77.60
Postflexion	Mangrove presence/absence*	2.34			
	Turbidity*		3.15	12.73	
	*P. longipatella***		1.00	6.92	
	P. hessei juveniles**		1.52	10.62	
	M. wooldridgei brooding females***		3.46	34.97	63.40

Models were fitted with negative binomial distribution and log-linked
edf = estimated degrees of freedom
(significance codes *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns = non-significant)

3.4.5 Factors influencing the spatial trends of larval density

The Generalised Additive Models (GAMs) revealed that *G. aestuaria* larval density could best be explained by an interaction of temperature, conductivity, *P. longipatella* density and *M. wooldridgei* brooding female density. These explanatory variables were all significant at the 5% level, apart from the fixed factor, mangrove presence, that was not significant (Table 3.3). Larval density correlated positively with temperature and the important prey species *P. longipatella*, while negatively correlated with *M. wooldridgei* brooding female density and peaking at conductivities of 39 – 44 $S.m^{-1}$. The GAMs also found that preflexion and flexion stage density were positively correlated with temperature, however, preflexion stage larvae were negatively correlated with *M. wooldridgei* brooding females and *H. orbiculare* larvae and flexion stage larvae showed a negative correlation with juveniles of *R. terranatalis* and *P. hessei* males (Table 3.3).

Postflexion stage larval densities were strongly related to turbidity, *P. longipatella*, juvenile *P. hesseii* and *M. wooldridgeii* brooding female densities (Figure 3.5). Mangrove presence, as a fixed factor, was only significant in the postflexion density model (Table 3.3).

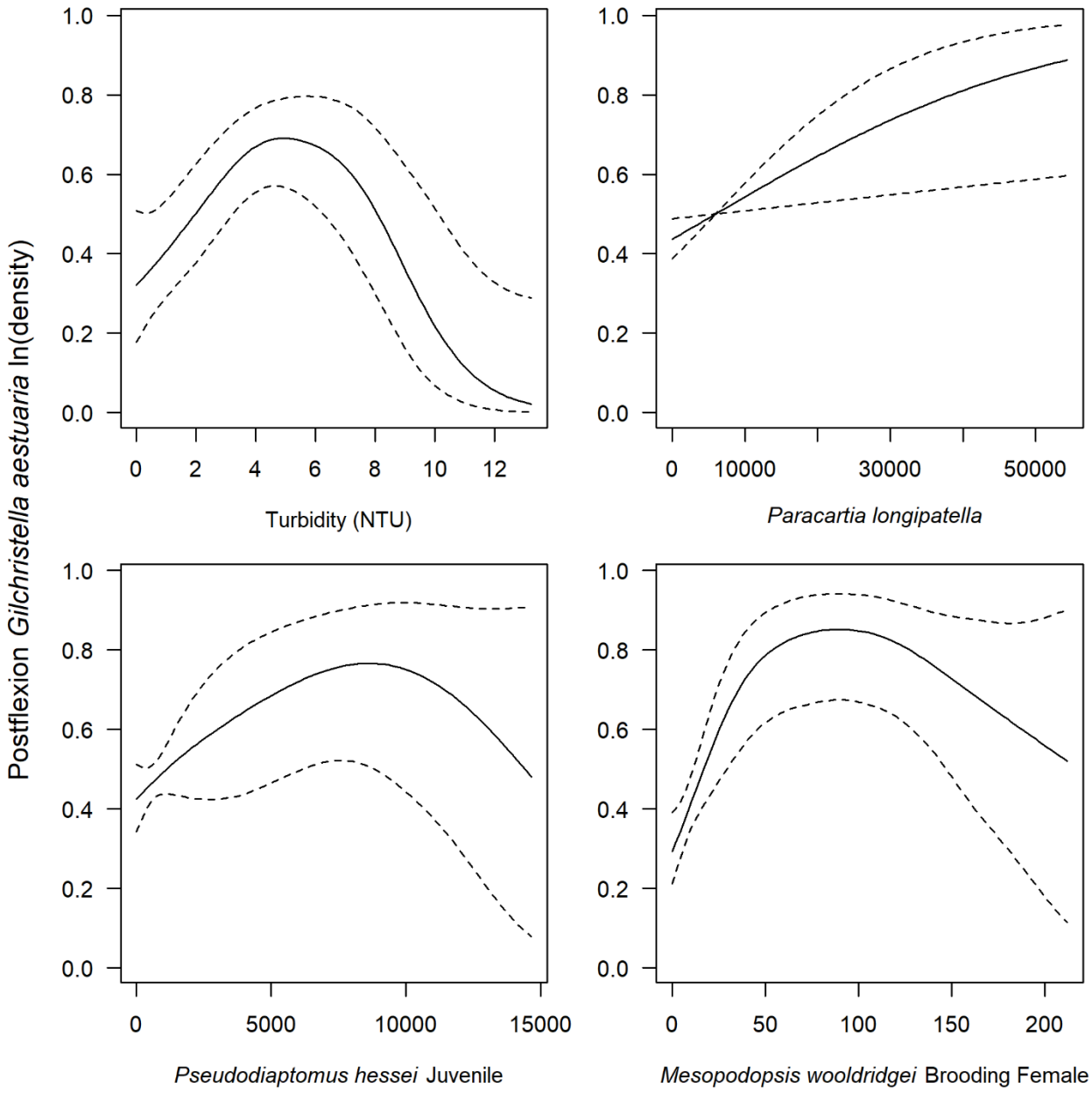

Figure 3.4: The relationship between explanatory variables and postflexion *Gilchristella aestuaria* density using a forward stepwise, log-linked Generalized Additive Model. (Zooplankton densities given in (Number.m^{-3}), Solid line denotes smooth terms and dashed lines denote the 95% confidence intervals)

3.5 DISCUSSION

Larval densities of *G. aestuaria* were considerably lower in the studied estuaries when compared to findings of other studies (Strydom, 2015). A study, which used a similar sampling technique, on the distribution and abundance of larval fishes in temperate South African estuaries, stated that a mean density of 543.60 (100 m^{-3}) was typical for warm temperate estuaries and a mean of 791.68 (100 m^{-3}) was typical for warm temperate/subtropical boundary estuaries (Strydom, 2015). Estuaries in the latter study mostly had good freshwater supply, giving rise to mesohaline conditions which were not observed in the present study where lower densities of < 100 (100 m^{-3}) were observed. Zooplankton density were also relatively low when compared to other warm temperate estuaries (Wooldridge and Bailey, 1982; Wooldridge, 2010). The low densities observed in the studied estuaries might be due to the relatively high salinities seen when compared to most warm temperate estuaries (James and Harrison, 2016). This might be due to their relatively small catchment sizes, yet permanently open mouth conditions.

The environmental variables that most influenced the spatial variation of larval *G. aestuaria* density were temperature, conductivity and turbidity. The studied estuaries were warmer during summer, with the Qora and Xhora estuaries being warmer than Nahoon and Gonubie estuaries. The Qora and Xhora estuaries are the two northern estuaries and thus are closer to the tropics which would explain their warmer temperatures. The Generalized Additive Models (GAMs) revealed that larval densities were highest at conductivities of 39 – 44 $mS.cm^{-1}$. Both the Nahoon and Gonubie, which are closest to human settlements and are thus more prone to damming and water abstraction, had higher mean salinities and conductivities during summer and winter. As the conductivity of an aqueous solution is not only related to salinity, but to electrolytes such as dissolved minerals leached from soils as well as anthropogenic heavy metal and other industrial chemicals, it may be that the increased conductivities seen within the Nahoon and Gonubie may be as a result of anthropogenic contamination. These environmental pollutants have been experimentally found to negatively affect larval fish growth, survival and behaviour (Di-Toro et al., 2001; Scott and Sloman, 2004; Linbo et al., 2006). The GAMs found that postflexion larval density peaked at a narrow range of 5-7 NTU. The effect of turbidity on larval abundance and distribution is still not well understood within estuaries as turbidity is mostly a consequence of freshwater inputs as well as wind driven disturbances, and is thus accompanied by a whole suite of abiotic and biotic variables. However, turbidity have been found to impact predation risk and feeding success of fish larvae (Utne-Palm, 2002). More turbid waters decrease the contrast of early stage fishes to their surrounding waters, lowering the risk of predation, while later stage fishes

are negatively affected by turbidity as it impedes feeding success (Utne-Palm, 2002). The spatial and temporal variation of larval densities were best explained by coupling multiple environmental factors, however, the growth and survival of larvae are also affected by biotic factors such as prey density and predator-prey interactions.

The diet of postflexion *G. aestuaria* larvae mainly consisted of the dominant Copepoda species found in the studied estuaries. These included: *P. hessei*, *P. longipatella*, and *A. natalensis* which co-occurred with high *G. aestuaria* densities. Previous studies found that at least 50% of the dietary requirements of *G. aestuaria* consisted of *P. hessei* in the permanently open warm temperate Sundays Estuary (Whitfield and Harrison, 1996; Strydom et al., 2014). In this study, however, *P. hessei* only contributed to 15.41% of the stomach volume. The larvae in this study showed some selective feeding behaviour. In the two northern estuaries *A. natalensis* was found in higher densities than the two southern estuaries and replaced *P. longipatella* in the diet, despite *P. longipatella* still being present at high densities within these estuaries. Thus, postflexion *G. aestuaria* preferred *A. natalensis* over *P. longipatella*. The density of *A. natalensis* was negatively correlated with salinity which supports previous findings that *A. natalensis* prefers lower salinities than *P. longipatella* (Wooldridge and Melville-Smith, 1979). It was also found that *A. natalensis* was more prevalent during summer, while *P. longipatella* was present during both seasons. Thus, *G. aestuaria* larval densities were better explained by *P. longipatella* rather than *A. natalenesis* densities. The number of *P. hessei* found within the stomach of *G. aestuaria* larvae were similar in mangrove and non-mangrove estuaries, however, the volume of *P. hessei* consumed was larger in mangrove estuaries. The larger size of *P. hessei* compared to the other Copepoda prey species may offer more nutritional value which may be the reason for larvae actively selecting these larger prey species.

The density and spatial distribution of larval *G. aestuaria* not only depends on prey density and environmental variables, but were also influenced by competition with predatory mysid species. Juvenile *R. terranatalis* and adult *M. wooldridgei* readily prey on the copepod *P. hessei* (Wooldridge and Webb, 1988), which is a dominant prey item for *G. aestuaria* (Strydom et al., 2014). However, the scale and impact of these predator-prey interactions will rely on the overlap in spatial distributions in estuaries. Larval *G. aestuaria* and *M. wooldridgei* co-occurred in the upper reaches of the studied estuaries. The densities of all the larval stages, with the exception of the flexion stage, were negatively correlated with *M. wooldridgei*, with the strongest negative correlation with brooding females. Densities of more than a 100 m^3 brooding female *M. wooldridgei* caused a marked decrease in postflexion *G. aestuaria* density. Brooding female *M. wooldridgei* will need to replenish energy that was spent on producing offspring. These two species are of similar size and thus are likely competing

for the same prey source. Predatory crab larvae, in particular *H. orbiculare*, were also negatively correlated with preflexion *G. aestuaria* densities which may be due to predation pressure.

Mangrove presence had no significant effect on preflexion and flexion stage larval densities. However, postflexion larvae showed some relationship with the presence of mangroves. The slight decrease in turbidity within mangrove estuaries and the consumption of larger *P. hessei* individuals may be a possible reason. Mangrove habitats are sediment sinks which are known to reduce the turbidity of the surrounding habitats which may affect the density of larval fishes as it affects feeding success as well as predation pressure (Furukawa and Wolanski, 1996). Less turbid waters will favour later stage fishes with better developed fins to evade predators and aid in active feeding on selected prey items (Utne-Palm, 2002). This study thus supports the optimal foraging theory where larvae maximised their energy gain while expending the least amount of energy in the cost of foraging (Fortier and Harris, 1989). Postflexion *G. aestuaria* larvae maximised their energy gain by actively feeding on larger *P. hessei* individuals in less turbid mangrove estuaries.

The spatial and temporal estuarine zooplankton and subsequent ichthyoplankton dynamics in warm temperate estuaries are highly variable and have been found to relate to freshwater inflow, as it is the main source of nutrients of these systems driving productivity (Wooldridge and Bailey, 1982; Deegan and Garritt, 1997; Vinagre et al., 2011; Strydom et al., 2014). Adult *G. aestuaria* have been found to rely on freshwater flow as a spawning cue (Strydom et al., 2002) and both the larvae and adults are dependent upon Copepoda densities as a prey source (Whitfield and Harrison, 1996; Strydom et al., 2014). However, this study found that the match between larvae and prey were not the only driver of larval *G. aestuaria* density. The better feeding opportunities for postflexion larvae in less turbid mangrove systems may result in increased growth and survival of these larvae. However, predator-prey dynamics of estuarine plankton communities remain understudied. Thus, it is recommended that future studies assessing fish nursery habitats should not only focus on fish abundance and diversity but should include a suite of factors, which include predator-prey interactions of early stage fishes.

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Chapter 4: Appraisal of Warm Temperate South African Mangrove Estuaries as Habitats to Enhance Larval Nutritional Condition and Growth of Gilchristella aestuaria (Family Clupeidae) using RNA:DNA Ratios

In press in the journal, Estuaries and Coasts

4.1 ABSTRACT

Estuaries are highly dynamic systems that serve as nursery areas to fishes and are likely to vary in nursery function, mostly due to habitat quality and food availability. Mangroves are thought to be good nurseries as they enhance food availability and protection, improving growth and survival of juvenile fishes. Food quantity and quality may be reflected in nutritional condition, which may in turn be a useful proxy for growth and survival of larval fishes. This study compared the nutritional condition and growth rate of 793 late stage larvae of estuarine roundherring, *Gilchristella aestuaria*, by using RNA:DNA indices to indirectly compare the feeding environment among similar warm temperate mangrove and non-mangrove estuaries in South Africa during the summer of, 2015 and 2016. Results indicated that *G. aestuaria* larvae had differing nutritional conditions within the sampling years and within the estuaries. The standardised RNA:DNA (*sRD*) as well as the RNA residual index values were higher within mangrove estuaries only in 2016. The instantaneous growth rate (*Gi*) of larvae in mangrove and non-mangrove estuaries were similar, however, post-flexion larvae were found to have a higher *Gi* and *sRD* in mangrove estuaries. Turbidity was the major factor influencing the nutritional condition of *G. aestuaria* larvae. Mangroves have been found to act as sediment sinks and thus may provide advantages that increase feeding success for post-flexion larvae, however more is yet to be understood in terms of feeding environment dynamics and how habitat quality influences the survival of larval fishes.

4.2 INTRODUCTION

Estuaries are highly dynamic and productive systems that serve as nursery areas for economically and ecologically important species (Beck et al., 2001; Heck et al., 2003; Able, 2005; Dahlgren et al., 2006; Elliott and Whitfield, 2011; Vasconcelos et al., 2011). Estuaries often encompass a large diversity and abundance of primary and secondary producers, and thus provide fishes with a range of habitat choices (Rönnbäck et al., 1999; Laegdsgaard and Johnson, 2001). Due to heterogeneity of habitats and food resources among estuaries, both the nursery value and species assemblage vary

among estuarine systems. Within estuaries, nursery function is highly dependent on various factors, which include food availability, predation, competition pressures and abiotic factors such as temperature, salinity, oxygen and turbidity (Beck et al., 2001; Able, 2005; Strydom, 2015).

Mangrove root structures are ideal nursery habitats for fishes and are known to enhance food availability while reducing predation (Laegdsgaard and Johnson, 1995; Rönnbäck et al., 1999). The aerial roots, tree trunks and overhanging branches, typical of mangrove habitats, create a complex intertidal habitat that is thought to play a key role in determining the spatial distribution and abundance of a variety of fishes (MacArthur, 1965; Cocheret De La Morinière et al., 2004; Nagelkerken et al., 2010). Mangrove forests also provide increased dissolved organic carbon through the decomposition of leaf litter (Emmerson, 1992; Sheaves, 2005; Rajkaran and Adams, 2010; Mazumder et al., 2011), which may enhance food quantity and quality to fishes (Laegdsgaard and Johnson, 2001). However, there is still some uncertainty as to the extent of the nursery value of mangroves to fishes (Mbande et al., 2005; Blaber, 2007; Sheaves, 2017). Blaber (2007) maintains that most evidence is circumstantial as most studies have been conducted in the tropics, where mangroves form the dominant habitat, leaving few other habitats to accurately compare against. Moreover, warm temperate and subtropical mangroves remain poorly evaluated in relation to tropical systems. Mangroves in warm temperate areas provide an ideal opportunity to evaluate the role of these habitats in enhancing the feeding environment for fishes. On the south-eastern coast of South Africa, mangroves reach the end of their latitudinal distribution and estuaries with and without mangroves are situated in close proximity, allowing for comparative studies.

The nutritional condition of early life stages is a good predictor of survival as larvae in poor condition are more likely to be affected by predation, disease, unfavourable environmental conditions and are less efficient at feeding due to impaired swimming ability (Amara and Galois, 2004; Silva et al., 2014). Biochemical tools based on nucleic acid indices such as the RNA:DNA ratio have been successfully used on fish larvae to reveal changes in feeding conditions and growth after periods of only three to four days (Clemmesen, 1994, 1996; Caldarone et al., 2001; Chícharo and Chícharo, 2008). The RNA:DNA ratio is a measure of nutritional condition reflecting a larva's potential to make proteins, and can be related to individual growth rates. However, this method depends on larval age and size (Clemmesen, 1994; Esteves et al., 2000; Teodósio et al., 2017). Thus, by using a residual RNA index with an independently determined variable such as dry weight or standard length, one can remove the allometric effect of larval size (Suthers et al., 1996; Chícharo et al., 1998). Temperature is also related to the rate at which translation occurs (protein synthesis per unit RNA), thus the estimation of growth must be done by applying laboratory-derived RNA:DNA-growth models that contain

a temperature term (Esteves et al., 2000; Buckley et al., 2008). Buckley et al. (2008) used a meta-analysis of published data with eight species, including herring (*Clupea harengus*), to develop a multi-species growth model that is independent of temperature. Consequently, the nutritional condition of larvae can be used to assess feeding environments among different habitat types at different temperatures (Buckley et al., 2008; Chícharo et al., 2012; Costalago et al., 2015).

The estuarine roundherring, *Gilchristella aestuaria* (Gilchrist, 1914) is an estuarine resident clupeid that is highly abundant in most South African estuaries (Haigh and Whitfield, 1993; Strydom, 2015). This species is planktivorous, feeding predominantly on phytoplankton and zooplankton and plays a key ecological role as a mid-trophic species (Blaber et al., 1981; White and Bruton, 1983; Whitfield and Harrison, 1996). While the abundance and productivity of phytoplankton and zooplankton depends on nutrient inputs (mostly from riverine flow) into estuaries (Paterson and Whitfield, 1997; Strydom et al., 2002), mangroves have been found to increase nutrient levels and invertebrate abundance (Sheridan, 1997; Beck et al., 2001). It is not known to what extent the mangrove-derived estuarine feeding environments in warm temperate estuaries relate to the nutritional condition of resident larvae and therefore species success (Costalago et al., 2014). Thus, nutritional condition indices are potentially useful tools to understand feeding environments, especially for mid-trophic planktivorous forage fishes such as *Gilchristella aestuaria*. Estuaries with mangroves present, are expected to provide additional refuge and better feeding environments than estuaries where mangroves are absent. Therefore, fish larvae are expected to be more abundant and in a better nutritional condition than larvae occurring in estuaries without mangroves present.

This study compared the nutritional condition and growth rate of *G. aestuaria* larvae in four warm temperate South African estuaries, with and without mangrove habitats, using the RNA:DNA ratio method to ascertain whether estuaries with mangrove habitats provide a better feeding environment for the larvae of this ecologically important species. Understanding the nutritional condition of larval *G. aestuaria* relative to the physico-chemical conditions within estuaries could give an indirect indication of the feeding environment and in so doing, provide insight into the potential nursery role of estuarine mangrove habitats.

4.3 MATERIALS AND METHODS

4.3.1 Study area

The Nahoon Estuary (27° 57’ 05” E, 32° 59’ 05” S) is situated near the city of East London, South Africa (numbered 1 in Figure 4.1). It is a permanently open estuary that is approximately 4.80 km

long, has a mean depth of 2.32 m and an average temperature of 19.41 °C which is similar to the other selected study estuaries (Harrison, 2004; James and Harrison, 2016). The Nahoon river extends approximately 80 km inland and has a catchment area of about 580 km^2 (Talbot et al., 1985; Reddering and Esterhuysen, 1987). The Nahoon River has a reservoir, the Nahoon Dam, with a capacity of 5.90 x 10^6 m^3 and captures water from 87 % of the total catchment area (Reddering and Esterhuysen, 1987). The Nahoon Estuary is subject to anthropogenic metals from a variety of sources as well as pollutants resulting from occasional municipal waste water spills (Talbot et al., 1985; Newman and Watling, 2007). The estuary has a total area of 58.72 ha and comprises of the typical warm temperate vegetation types found in South African estuaries which includes saltmarshes (2.80 ha), reeds and sedges (0.20 ha), submerged macrophyte beds (2.30 ha) and mangroves (van Niekerk and Turpie, 2012; Adams et al., 2016). Currently, the mangrove area at the Nahoon Estuary is non-natural and relatively small <2 ha. However, the area has been increasing at a rate of 0.06 ha y^{-1} since 1969, when *Avicennia marina*, *Bruguiera gymnorrhiza* and *Rhizophora mucronata* were planted (Steinke, 1972, 1986; Hoppe-Speer et al., 2015).

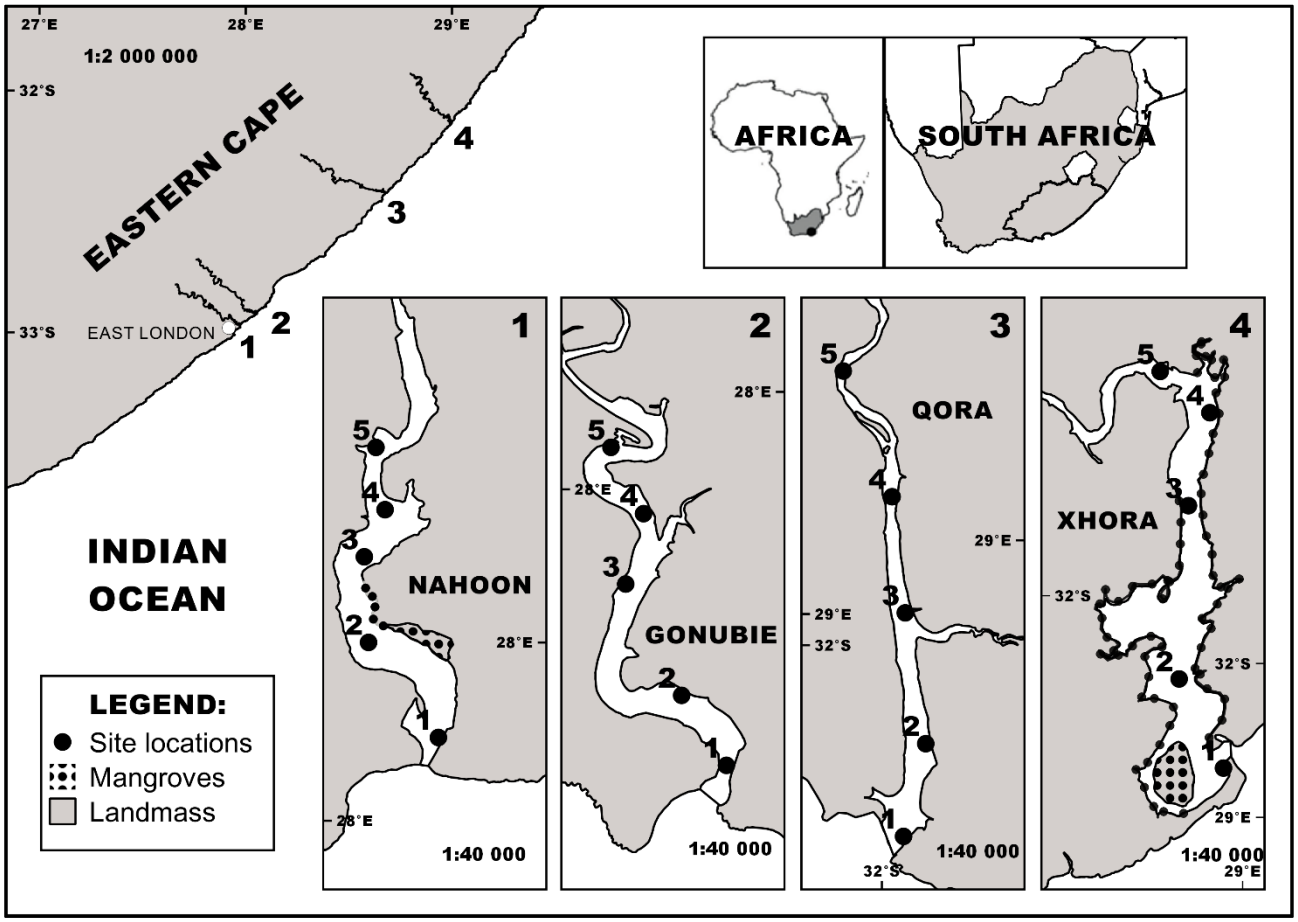

Figure 4.1: Geographic position of studied estuaries showing the location of sampling sites.

The Gonubie Estuary (28° 01' 59" E, 32° 55' 59" S) (numbered 2 in Figure 4.1) is situated approximately 10 km north of the neighbouring Nahoon Estuary. The Gonubie River extends approximately 80 km inland and has a catchment area of about 675 km^2 (Reddering and Esterhuysen, 1987). The estuary is approximately 5 km long with a mean depth of 1.68 m and an average temperature of 19.98 °C which is similar to the other selected study estuaries (Harrison, 2004; James and Harrison, 2016). The Gonubie has no large reservoirs with only a small weir (personal observation of the authors) and limited impacts from anthropogenic pollutants (Adams et al., 2016). The estuary has a total area of 53.4 ha and comprises of 5.90 ha of saltmarshes, 0.40 ha of reeds and sedges, 0.80 ha of submerged macrophyte beds, 6.30 ha of sand/mud banks, and no mangroves (van Niekerk and Turpie, 2012).

The Qora Estuary (28° 40' 21" E, 32° 26' 50" S) is a permanently open estuary (numbered 3 in Figure 2.1). Qora has no reservoirs and limited impacts from anthropogenic pollutants (Adams et al., 2016). The estuary has a total area of 89.63 ha and comprises of no saltmarsh, 5.67 ha of reeds and sedges, 8.50 ha of submerged macrophytes, 10.23 ha of sand/mud banks, and no mangroves (van Niekerk and Turpie, 2012). Very little is known about this estuary due to its remote location, however it is of similar size and in relative close proximity to other study estuaries allowing for comparisons. This study forms part of a larger project that was the first to study the ichthyofauna of this estuary.

The Xhora Estuary (29° 05′ E, 32° 05′ S) (numbered 4 in Figure 4.1) is situated approximately 45 km North of the Qora Estuary and also falls within the warm temperate biogeographic zone. The estuary has a total area of 159.76 ha and comprises of 12.96 ha of saltmarsh, 10.12 ha of reeds and sedges, 2.60 ha of submerged macrophytes, and 17.13 ha of sand/mud banks (van Niekerk and Turpie, 2012). The mangroves at Xhora covers an area of 25.5 ha and consists of all three mangrove species present in South Africa (*A. marina*, *R. mucronata* and *B. gymnorrhiza*) (Hoppe-Speer et al., 2014). As with the Qora Estuary, very little is known about this estuary.

4.3.2 Field sampling

Samples were collected on a first quarter moon phase in summer 2015 and 2016 from five stations (each are one kilometre apart) along the main channel of four estuaries (Figure 4.1). This coincides with the known peak breeding period of the estuary-resident fish *Gilchristella aestuaria* (Strydom 2015). Samples were collected isochronously after dark using two modified Working Party 2 (WP2) plankton nets (570 mm mouth diameter and 0.2 mm mesh aperture) fitted with calibrated Kahlsico 005 WA 130 flowmeters. The two nets were simultaneously lowered and towed horizontally alongside a 5 m boat for 3 min at a speed of 1-2 knots and sampled a mean ± SD volume of 189.8 ± 70.4 m^3

(Strydom and Whitfield, 2000). Two replicate samples were collected at each of the five sites ranging from the upper to the lower reaches of each estuarine system (Figure.4.1). Where possible an oblique course across the axis of the estuary was followed, thus enabling samples to be taken near the banks as well as in the mid-channel (Strydom et al., 2002). Sampling was conducted in complete darkness to limit any net avoidance by the fish. Physico-chemical parameters (temperature, salinity, turbidity, pH and dissolved oxygen) were taken vertically at intervals of 0.5 m depth with an YSI sonde series 6600 multi-parameter probe at each site. An additional plankton tow was performed at the site in each estuary where *G. aestuaria* larvae were most abundant, and 100 randomly selected *G. aestuaria* larvae were sorted directly after sampling and preserved in individual vials containing RNAlater (Sigma-Aldrich) for subsequent nucleic acid analysis.

4.3.3 Larval density

All *G. aestuaria* were identified and removed from the plankton samples using a Leica M80 stereomicroscope fitted with an eyepiece micrometer. Standard lengths of 50 randomly selected *G. aestuaria* were measured to the nearest 0.1 mm and staged into developmental stages according to Neira et al. (1998). The flowmeters on the nets allowed for the calculation of larval density. Flowmeters were calibrated in a controlled environment and it was determined that a value of 32.7 was the number of revolutions per m^3 water filtered. Thus, the following formula was used to calculate larval density:

$$\text{Density} = [N / (r / c)] \times 100$$

where Density is the number of *G. aestuaria* larvae per 100 m^3, *N* is the total number of larvae caught per haul, *r* the revolutions of the flowmeter and *c* the predetermined calibration value in m^3

4.3.4 Morphological measurements

Photographs were taken prior to nucleic acid extraction of each individual *G. aestuaria* larva. The standard length, body depth, myomere height, and eye diameter to the nearest 0.01 mm were measured using ImageJ v1.47 software.

4.3.5 Nucleic acid extraction

Individual samples were rinsed in deionised water and frozen for 5 min at -80 °C before freeze drying for at least 18 hours at -50 °C and 0.100 mbar using a Christ alpha 1-4 freeze dryer. Once dried, each sample was weighed to the nearest 0.001 mg using a Sartorius SC2 micro balance to obtain an accurate dry weight. Individual samples were homogenised mechanically by adding differently sized

glass beads (2 mm and 0.17-0.50 mm), and then chemically using a defined volume (800 µl, but 400 µl for larvae <150 µg dry weight) of Tris-SDS buffer (Tris 0.05 M; NaCl 0.1 M; SDS 0.01 %; EDTA 0.01 M; pH 8) that was added to each sample and incubated for 30 min on ice. Samples were then shaken for 15 min in a RETSCH type MM2 shaker at room temperature. Once homogenised, the samples were transferred to a Sigma 3-18 K centrifuge running for 8 min at a speed of 6803 RPM (RCF: 3829 g, temperature: 1 °C). The supernatant of each sample was then transferred into a new vial for further dilution steps or directly into a black 96-well-cliniplate. Preliminary tests had indicated that larger larvae (>450 µg) had to be diluted in order for their nucleic acid content to stay in the range of the defined calibration curves of RNA ($y = 39.21(\pm 2.40)x$; $R^2 = 0.998 \pm 0.002$; 16S-23S-ribosomal, Roche) to avoid a loss in quality. The DNA calibration curve was calculated by multiplying the slope value of the RNA calibration curve with the factor of 2.2, which adjusts for the relative fluorescence intensity difference of RNA and DNA (LePecq and Paoletti, 1966). A control homogenate (prepared from a large group of larvae) was also measured on each cliniplate.

4.3.6 Nucleic acid quantification

Two dispensers of an Ascent Fluoroscan (Thermo Fisher) were prepared with Ethidium bromide (EB, 2.5 mg mL^{-1} dilution, Roth 2218.2) and TE buffer (Tris 0.05 M; NaCl 0.1 M; EDTA 0.01 M; pH 8). Measurements were conducted at an excitation wavelength of 355 nm and an emission wavelength of 590 nm at a temperature of 25 °C. For determination of the RNA:DNA ratio, fluorescence was measured in three steps: 1) the pure samples (self-fluorescence). 2) after addition of EB (total fluorescence), and 3) the remaining DNA fluorescence after incubation in RNase (Serva Ribonuclease A, from bovine pancreas) for 30 min at 37 °C. Subtracting the total fluorescence from the DNA fluorescence provided the RNA fluorescence. With the aid of calibration curves and dilution factors, the relative fluorescence values could then be converted into weight (µg) values of RNA and DNA for each individual *G. aestuaria* larva. The RNA:DNA ratios derived from a slope ratio of 2.2 were then standardized (*sRD*) using the reference slope ratio of 2.4 according to the method outlined in Caldarone et al. (2006).

4.3.7 Growth rate calculation

The *sRD* values were used to determine the growth rate of larvae. As temperature is related to the rate at which translation occurs (protein synthesis per unit RNA), the multi-species growth model developed by Buckley et al. (2008) was used to calculate larval instantaneous growth rates (*Gi*) in order to eliminate the possible bias due to the differences in temperatures between estuaries:

$$Gi = 0.0145 \times sRD + 0.0044 \times (sRD \times T) - 0.078$$

where *Gi* is the instantaneous growth rate, *sRD* the standardized RNA:DNA ratio and *T* the temperature the *G. aestuaria* larvae experienced. Results were interpreted such that a value of 0 would mean no growth at all and a value of 1 would be a doubling of the weight of the larva per day (Buckley et al., 2008).

4.3.8 Statistical analysis

Shapiro-Wilks and Levene's test was used to test data for normality and homogeneity of variance respectively. If these assumptions were not met, non-parametric tests followed. Kruskal-Wallis tests were used to test for differences in terms of physico-chemical, morphological and growth rates, which were tested among the four estuaries within each sampling year. When significant ($P < 0.05$), Mann-Whitney U post-hoc test was used on pairs of estuaries using a Bonferroni-corrected level of significance of $\alpha = 0.003$. Spearman's rank-order correlation analysis was used to determine any correlations between *sRD* and the studied variables (larva length, body depth, myomere height, eye diameter, dry weight, temperature, salinity, turbidity, pH, and dissolved oxygen). All statistical analyses were performed using the statistical software R (v. 3.3.1).

4.4 RESULTS

3.4.1 Environmental variability

Physico-chemical measurements revealed that the surface temperatures were higher in 2016 than in 2015, with the Qora Estuary being significantly warmer in 2016 than all the other estuaries (Table 4.1). In 2015, the Nahoon and Gonubie estuaries were significantly more saline than the other estuaries (Table 4.1). In terms of turbidity, the Gonubie Estuary, was significantly more turbid in 2016 than the other estuaries (Table 4.1). The pH values were significantly higher in 2015 than in 2016 (Table 4.1). The dissolved oxygen concentrations were higher in 2015 than in 2016 (Table 4.1). The Qora Estuary in 2015 had a significantly higher dissolved oxygen concentration than all the other estuaries. Data obtained from the nearest weather station revealed that the total monthly rainfall was higher in 2016 than in 2015 with most rainfall in the Qora Estuary in 2016 and the least in the Qora Estuary in 2015 (Table 4.1). However, the total yearly rainfall was higher in 2015 than in 2016 (Table 4.1).

Table 4.1: Physico-chemical variables of the surface waters (< 1 m) where *Gilchristella aestuaria* larvae were sampled in the four studied estuaries during summer, 2015 and 2016. Average and (range) of physico-chemical variables and average monthly rainfall with total rainfall for each year are given. Significance codes that denote the following: *** $P < 0.0001$, ** $P < 0.001$; * $P < 0.003$, and ns is non-significant.

	Mangrove		**Non-mangrove**	
2015	**Nahoon**	**Xhora**	**Gonubie**	**Qora**
Temperature (°C)	20.38 (8.40)	23.23 (2.78) *	19.57 (6.29)	22.24 (7.34) *
Salinity	34.82 (2.28)	30.00 (19.03) **	34.41 (2.49)	27.25 (25.98) **
Conductivity (mS.cm^{-1})	52.87 (3.14)	46.12 (33.01) **	52.42 (3.46)	42.47 (37.62) ***
Turbidity (NTU)	0.04 (0.60) ***	2.56 (2.40)	0.65 (3.80) **	2.95 (13.20)
pH	13.40 (63.06) ns	9.70 (0.95)	9.63 (2.14)	9.02 (2.32)
Dissolved Oxygen (mg.L^{-1})	8.95 (3.36)	8.92 (4.52)	7.08 (1.72) **	11.73 (2.91) **
Dissolved Oxygen (%)	120.59 (37.80)	122.25 (50.50)	93.70 (24.20) ***	158.38 (31.00) ***
Total Rainfall (mm)	14.40 / 867.20	89.80 / 505.70	14.40 / 867.20	10.00 / 852.00
2016				
Temperature (°C)	21.94 (9.10)	24.91 (4.90) *	22.09 (11.90)	26.41 (6.20) *
Salinity	30.23 (1.97)	28.10 (10.28) ***	30.67 (0.46)	28.75 (6.12) *
Conductivity (mS.cm^{-1})	43.74 (6.84)	42.74 (17.65) ns	44.50 (10.53)	45.75 (5.37)
Turbidity (NTU)	6.23 (9.90)	5.21 (2.90) *	8.85 (9.20) *	6.22 (6.10)
pH	7.85 (0.84)	7.88 (0.44)	7.95 (0.20)	7.83 (0.64)
Dissolved Oxygen (mg.L^{-1})	6.89 (6.23) ns	7.21 (2.95)	7.61 (1.95)	7.12 (2.79)
Dissolved Oxygen (%)	95.97 (89.10) ns	102.32 (43.20)	103.05 (15.40)	103.92 (34.20)
Total Rainfall (mm)	43.80 / 714.00	68.50 / 447.40	43.80 / 714.00	99.10 / 564.60

4.4.2 Morphological differences

Large morphological differences of *Gichristella aestuaria* larvae were observed between 2015 and 2016 (Table 4.2). The *G. aestuaria* larvae collected from the Nahoon and Xhora Estuaries, both mangrove estuaries, had a significantly larger length, dry weight, body depth, myomere height and eye diameter in 2016 than those collected in 2015 (Table 4.2). Larval length did not correlate with temperature ($rs = 0.04$, $N = 793$, $P > 0.05$). Although the larvae were sampled at very similar dates and moon phases in the two sampling years, larvae in 2016 seem to be further developed in mangrove estuaries in 2016.

Table 4.2: Morphological differences of *Gilchristella aestuaria* larvae within the four sampled estuaries during summer, 2015 and 2016. The average and range as well as the sample size (N) is given. Significance codes that denote the following: *** $P < 0.0001$, ** $P < 0.001$; * $P < 0.003$, and ns is non-significant.

	Mangrove		**Non-mangrove**	
	Nahoon	**Xhora**	**Gonubie**	**Qora**
2015	(N = 100)	(N = 98)	(N = 99)	(N = 99)
Standard Length (mm)	10.81 (9.49) ***	9.19 (5.61)	11.14 (2.90) ***	9.25 (10.42)
Dry weight (mg)	1.79 (11.09)	0.40 (1.66)	1.47 (1.94)	0.57 (4.03)
Body Depth at Pectoral Fin Base (mm)	0.88 (2.06) ***	0.55 (0.68)	0.80 (0.52) ***	0.58 (0.94)
Myomere Height Anterior of Anal fin (mm)	0.72 (1.89) *	0.40 (0.64)	0.72 (0.48) *	0.43 (1.09)
Eye Diameter (mm)	0.51 (1.11) ***	0.33 (0.66)	0.52 (0.29) ***	0.37 (0.64)
2016	(N = 100)	(N = 99)	(N = 98)	(N = 100)
Standard Length (mm)	13.99 (8.34) ***	15.30 (7.83) ***	10.94 (7.09)	9.20 (7.45)
Dry weight (mg)	5.67 (10.31) ***	6.95 (14.04) ***	1.71 (3.62)	0.65 (2.81)
Body Depth at Pectoral Fin Base (mm)	1.65 (1.66) ***	1.93 (2.39) ***	0.94 (1.01)	0.62 (1.02)
Myomere Height Anterior of Anal fin (mm)	1.33 (1.51) ***	1.44 (1.80) ***	0.75 (1.22)	0.46 (0.69)
Eye Diameter (mm)	0.87 (0.90) *	1.02 (1.16) ***	0.56 (0.87)	0.36 (0.72)

4.4.3 Larval density

The mean density of *G. aestuaria* larvae was significantly higher in the Qora Estuary in 2015 than in 2016 with a mean ± range of 122.95 ± 230.59 (number.100 m^{-3}) and 30.18 ± 60.32 (number.100 m^{-3}), respectively (Figure 4.2). Larvae were at a higher density in the Qora Estuary than in the Nahoon Estuary during 2015 and 2016. The larvae in the Xhora Estuary (44.97 ± 101.23 number.100 m^{-3}) were significantly denser than in the Nahoon Estuary (5.97 ± 10.35 number.100 m^{-3}) and Qora Estuary (30.18 ± 60.32 number.100 m^{-3}) in 2016 (Figure 4.2). Larval density did not differ among mangrove and non-mangrove estuaries during 2015 and 2016 ($U = 212.50$, $N1 = 24$, $N2 = 24$, $P > 0.003$). The larval densities correlated negatively with temperature ($rs = -0.52$, $N = 793$, $P < 0.05$) and turbidity ($rs = -0.52$, $N = 793$, $P < 0.05$), however it did not correlate with the standardised RNA:DNA ratio (*sRD*) (Table 4.4).

4.4.4 Nutritional condition

There were significantly more DNA and RNA per larva in the two mangrove estuaries in 2016 (Table 4.3). The larvae in the Xhora Estuary in 2016 had a significantly lower DNA/DW and RNA/DW value and thus were in a better nutritional condition than the larvae in all the other estuaries (Table 4.3).

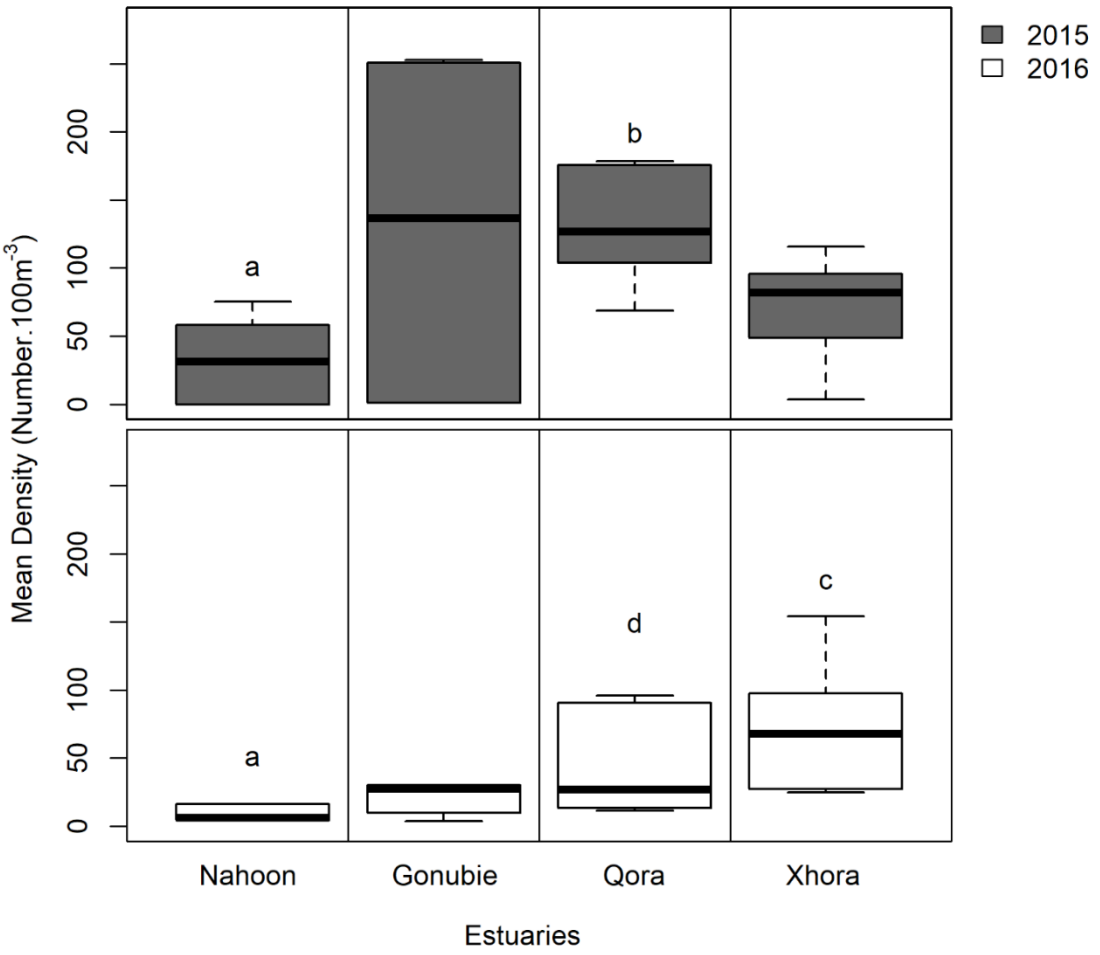

Figure 4.2: Larval *Gilchristella aestuaria* density in mangrove and non-mangrove estuaries on the south east coast of South Africa during summer 2015 (grey) and 2016 (white). (Median, interquartile, minimum and maximum are given, with lettering denoting $P < 0.003$)

The larvae in the Qora Estuary in 2015 and 2016 as well as the Xhora Estuary in 2015 had the highest DNA/DW and RNA/DW values and thus the larvae in these estuaries were in the worst nutritional condition (Table 4.3). The *sRD* values revealed that *G. aestuaria* larvae within the Nahoon Estuary in 2015 were in a significantly better nutritional condition than in all the other estuaries (Figure 4.3). In 2015, the *G. aestuaria* larvae in the Xhora Estuary were in a significantly lower nutritional condition than all the other estuaries, while in 2016, larvae in the Gonubie Estuary were in the lowest nutritional condition (Figure.4.3). Larvae in the Qora Estuary in 2016 had a significantly greater *Gi* than all other estuaries, while the larvae in the Gonubie Estuary in 2016 had the lowest *Gi* values (Figure 4.3). Spearman-rank correlations revealed that larval dry weight and myomere height showed the strongest positive correlation with *sRD*, while turbidity and temperature showed the strongest negative correlations (Table 4.4). The RNA residual index indicated that larvae in the Nahoon Estuary in 2016 were in the best nutritional condition than the rest of the estuaries (Figure 4.3). Larvae in the Gonubie Estuary during both 2015 and 2016 were in the worst nutritional condition (Figure 4.3). The

post-flexion larvae had a higher *sRD* in the mangrove estuaries than in the non-mangrove estuaries (Figure 4.4); as well as a higher *Gi* in the mangrove estuaries than in the non-mangrove estuaries (Figure 4.4). Larvae during the flexion stage had similar *sRD*, however they had a significantly higher *Gi* in the non-mangrove estuaries (Figure 4.4). The *sRD* and *Gi* of pre-flexion stages were similar in the mangrove and non-mangrove estuaries in 2015 and 2016 (Figure 4.4).

Table 4.3: Nucleic acid concentrations of *Gilchristella aestuaria* larvae within the four sampled estuaries during summer, 2015 and 2016. The average and range as well as the sample size (N) is given. Significance codes that denote the following: *** $P < 0.0001$, ** $P < 0.001$; * $P < 0.003$, and ns is non-significant.

	Mangrove		**Non-mangrove**	
	Nahoon	**Xhora**	**Gonubie**	**Qora**
2015	(N = 100)	(N = 98)	(N = 99)	(N = 99)
LePecq DNA (ug)/larva	7.86 (30.11) **	3.13 (9.45)	6.60 (5.12) **	3.73 (19.48)
RNA (ug)/larva	13.88 (51.69) **	4.86 (17.30)	11.04 (9.49) **	6.51 (37.01)
RNA/Dry Weight (mg)	10.03 (12.19)	13.59 (27.69) ***	7.76 (9.96)	13.02 (21.80) ***
DNA/Dry Weight (mg)	5.75 (7.06)	8.67 (10.72)	4.63 (6.37)	7.77 (11.17)
2016	(N = 100)	(N = 99)	(N = 98)	(N = 100)
LePecq DNA (ug)/larva	21.53 (28.96) ***	22.40 (36.33) ***	8.69 (15.4)	4.57 (13.23)
RNA (ug)/larva	37.54 (57.75) ***	37.40 (58.00) ***	11.98 (21.03)	7.80 (24.01)
RNA/Dry Weight (mg)	7.10 (14.08)	5.90 (7.43) *	7.69 (11.62)	13.29 (14.99) **
DNA/Dry Weight (mg)	4.09 (7.55)	3.73 (6.89) *	5.52 (7.23)	8.03 (10.30)

Table 4.4: Spearman-rank correlations of the studied variables with the standardised RNA:DNA ratio (*sRD*) of *Gilchristella aestuaria* larvae sampled from the four studied estuaries in 2015 and 2016. (Bold values = $P < 0.05$).

	Spearman r_s	*P* - value
Standard Length (mm)	**0.09**	**0.01**
Body Depth at Pectoral Fin Base (mm)	**0.09**	**0.01**
Myomere Height Anterior of Anal Fin (mm)	**0.11**	**0.00**
Eye Diameter (mm)	0.07	0.05
Dry Weight (g)	**0.13**	**0.00**
Larval Density	0.06	0.12
Temperature (°C)	**-0.21**	**0.00**
Salinity	0.00	0.97
Turbidity (NTU)	**-0.23**	**0.00**
pH	0.01	0.83
Dissolved Oxygen (mg l^{-1})	**-0.08**	**0.03**
Dissolved Oxygen (%)	0.00	0.90

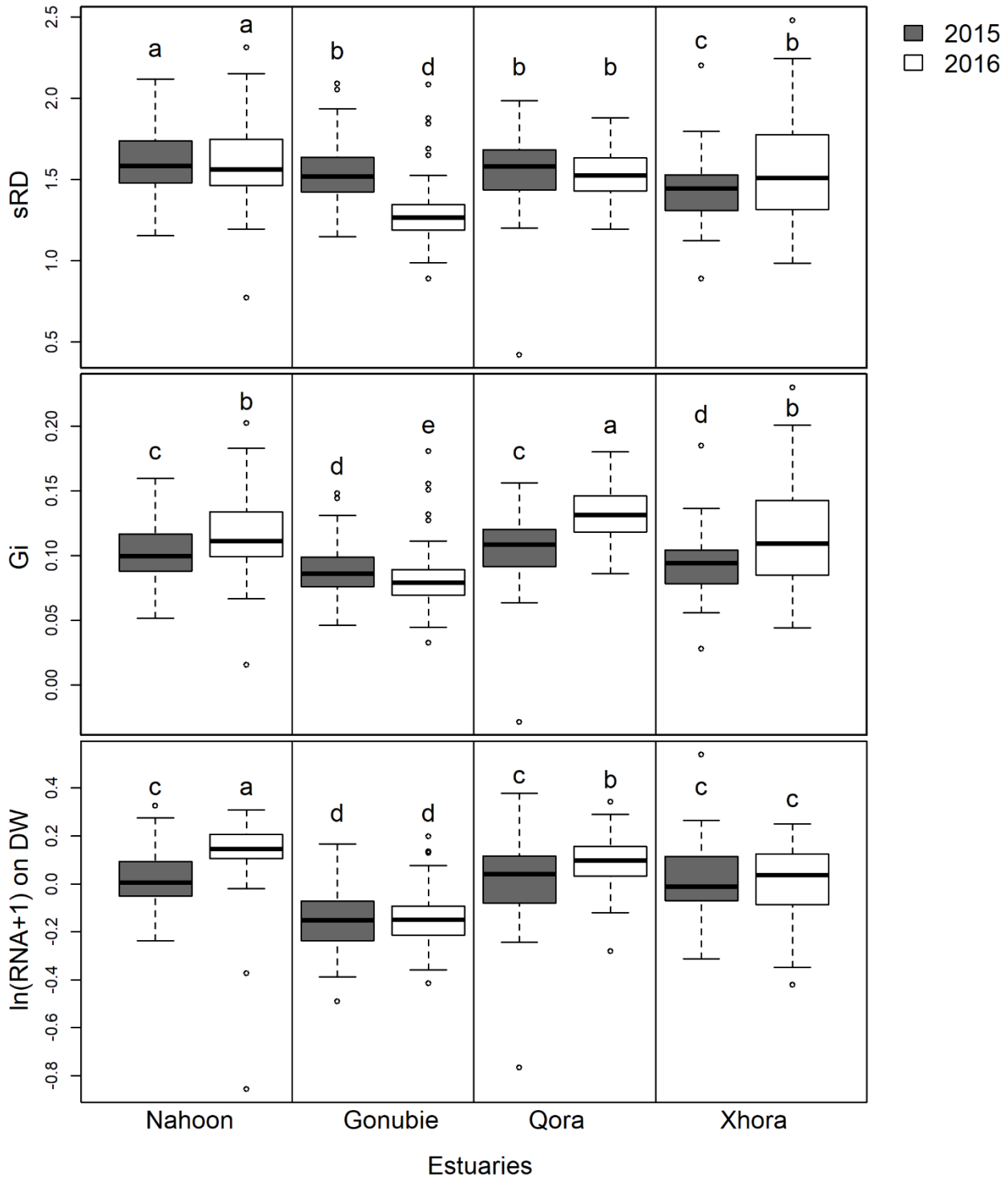

Figure 4.3: Nutritional condition of larval *Gilchristella aestuaria* in mangrove and non-mangrove estuaries on the south east coast of South Africa during summer, 2015 (grey) and 2016 (white). Indices include: standardised RNA:DNA ratio, instantaneous growth rate (*Gi*) and residual RNA index on dry weight. (Median, interquartile, minimum, maximum and outliers are given, with lettering denoting $P < 0.003$)

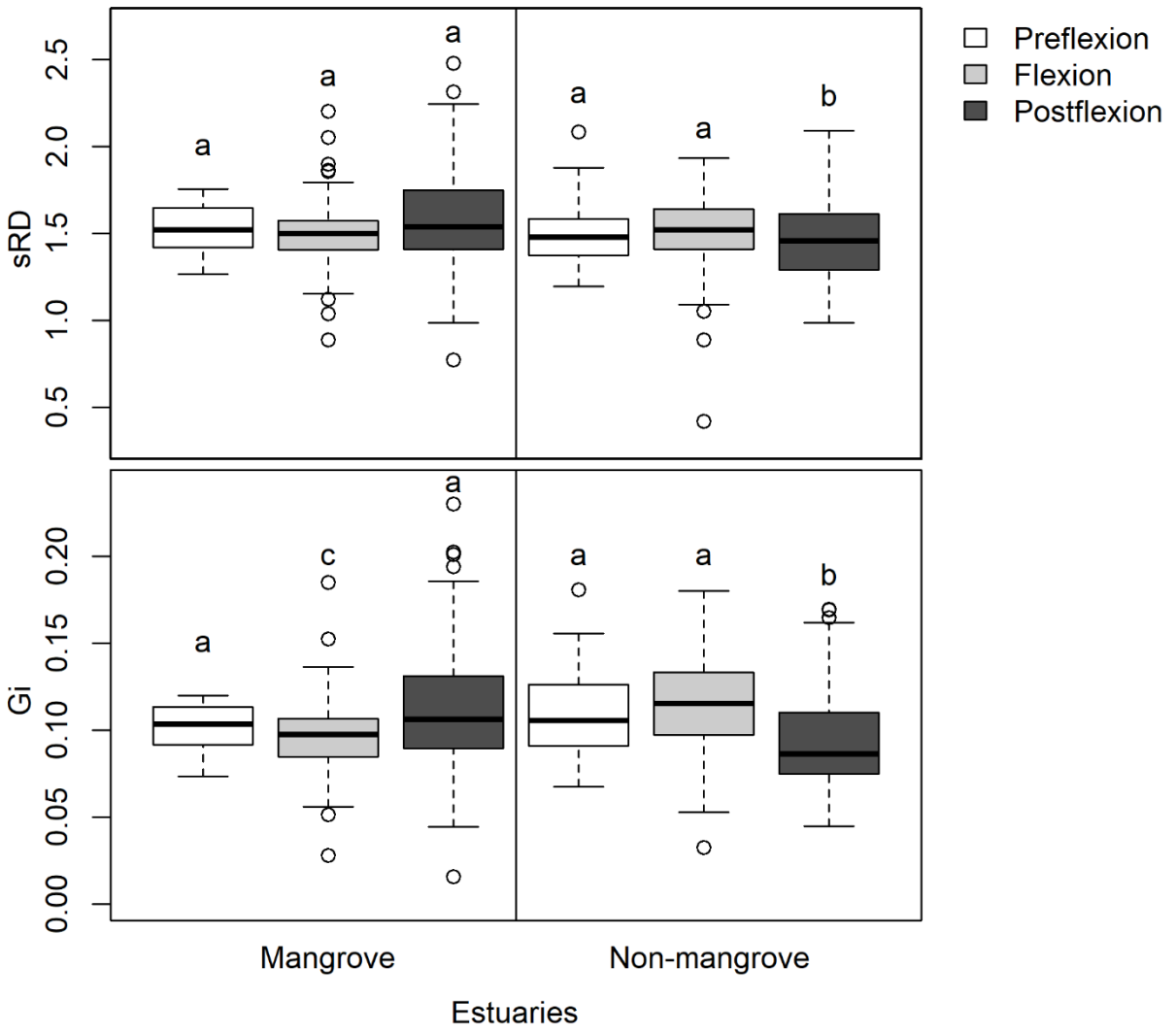

Figure 4.4: Nutritional condition of larval stages of *Gilchristella aestuaria* in mangrove and non-mangrove estuaries on the south east coast of South Africa. Indices include: standardised RNA:DNA ratio and instantaneous growth rate (*Gi*). (Median, interquartile, minimum, maximum and outliers are given, with lettering denoting $P < 0.003$)

4.5 DISCUSSION

The standardised RNA:DNA (*sRD*) values indicated that *G. aestuaria* larva differed in nutritional condition between the two years and the four estuaries of this study. The *sRD* values indicated that *G. aestuaria* larva within the Nahoon Estuary (mangrove) were in the best nutritional condition during both the sampling years. In 2016, the larvae in the Gonubie Estuary (non-mangrove) were in the worst nutritional condition than all the other estuaries. However, in 2015 the larvae in the Xhora Estuary (mangrove) were in the worst nutritional condition. As a result, the *sRD* index indicated that the larvae were in a better nutritional condition in the mangrove estuaries in 2016 only, mainly due to the low *sRD* values seen in the Gonubie in 2016. The *sRD* values obtained in this study were much higher

than the findings of Costalago *et al.* (2015) suggesting that the estuaries closer to the warm temperate-subtropical boundary provide better feeding conditions for larvae than those farther south. Costalago *et al.* (2015) found that salinity and the abundance of zooplankton were the major factors that influenced the condition of *G. aestuaria* larvae. However, in this study, salinity was not correlated to *sRD* despite being significantly different in the Nahoon and Gonubie estuaries in 2015. The only physico-chemical variables that correlated to *sRD* were turbidity, temperature, and to a lesser extent, dissolved oxygen.

Larval density was found to be similar in mangrove and non-mangrove estuaries during both sampling years, suggesting that competition pressure for food was similar and thus probably not influencing the nutritional condition values found in this study. Overall densities were higher in 2015 than in 2016, however the larvae were of an earlier development stage and thus more likely to be in higher numbers. The larval densities correlated negatively with temperature and turbidity, however no correlation was found with *sRD*.

Large morphological differences in *G. aestuaria* larvae were observed between 2015 and 2016. The *G. aestuaria* larvae collected in 2016 had a significantly larger length, body depth, myomere height, and eye diameter in the mangrove estuaries than those collected in 2015. These morphological differences correlated with each other, but did not correlate with any of the physico-chemical variables measured, such as temperature or turbidity. There was a weak, albeit significant, positive correlation between the morphological variables and *sRD*. The strongest positive correlation was larval dry weight and myomere height. Larval size and age can have a major influence on the nutritional condition and growth (Clemmesen, 1994; Esteves et al., 2000; Teodósio et al., 2017). Larger larvae are more developed, enabling them to swim faster and also feed on larger, more selected prey (Pepin and Penney, 1997; Bochdansky et al., 2008; Silva et al., 2014). The larger larvae have also survived for longer allowing the smaller and younger larvae in poorer condition to die, which can result in a biased sample of larvae in a better condition (Clemmesen, 1994). The effect of size and age can be avoided by using the RNA residual index (Suthers et al., 1996; Chícharo et al., 1998) The RNA residual index, however, only slightly differed from the *sRD* index, indicating that, for both the sampling years, the larvae in the Gonubie were in the worst condition.

The lower *sRD* and residual RNA values measured in the Gonubie Estuary coincided with increased turbidity. This increased turbidity was unlikely to be a consequence of differing freshwater inputs, as the salinity was similar in the sampled estuaries and could have been driven by wind events or anthropogenic disturbance. Turbidity is known to have a positive effect on young fishes in terms of

abundances (Blaber et al., 1981; Snow et al., 2000; Strydom et al., 2002); however, this might only be due to increased protection from visual predators during early life. Despite the benefits for predation avoidance, feeding success may be inhibited under these conditions, as it is known that planktivorous larvae rely on their vision for prey capture and successful feeding (O'Brien, 1979; Utne-Palm, 2002).

The surface temperatures of the Qora Estuary were significantly warmer than all the other estuaries in 2016. As RNA:DNA ratios are sensitive to temperature, the multi-species growth model by Buckley et al. (2008) was used to determine the instantaneous growth rates (*Gi*) of the larvae. As protein synthesis is more efficient at higher temperatures (Buckley et al., 2008), larvae in warmer estuaries are able to grow faster with less RNA. Thus, the larvae in the Qora Estuary would have low *sRD* values but high *Gi* values, which could explain the high growth rates found in the Qora estuary in 2016, as well as the similar *Gi* values found between mangrove and non-mangrove estuaries. However, the temperatures in this study are close to the upper limit of the Buckley et al. (2008) model, hence we needed to use multiple indices when making comparisons.

Comparing the condition of the larvae according to their growth stages revealed that post-flexion larvae had higher *sRD* and *Gi* values in mangrove estuaries than in non-mangrove estuaries. Mangroves have been found to be sediment sinks decreasing turbidity within the estuary channel, which might favour larger larvae (Furukawa and Wolanski, 1996; Wolanski et al., 1998). As the larvae reach the post-flexion stage, the ontogenic changes that occur generally result in improved swimming ability and are thus sufficiently developed to benefit from any advantage that mangrove habitats may provide, such as decreased turbidity. Decreased turbidity, however can negatively impact early stage larvae by increasing predation pressures (Wolanski et al., 1998; Teodósio et al., 2016). Large variation was observed in the condition of post-flexion larvae. One might expect a decrease in variation as the weaker members of the cohort are removed from the population, however larvae spend a much longer time in the post-flexion stage than the earlier stages and therefore larvae in different cohorts may be grouped, which may account for this variation.

This study found that temperature and turbidity are the main factors impacting the nutritional condition of larvae found in warm temperate estuaries and that the presence of mangroves may only provide limited advantages to post-flexion larvae possibly due to increased feeding success. However, a suite of factors are likely at play in warm temperate estuaries that govern nutritional condition and growth in resident larvae. This study is the first of its kind and more is yet to be understood in terms of feeding environment dynamics, prey species selection and how habitat quality influences the

survival of larval fishes. The use of RNA/DNA ratios is a valuable method to explore feeding environment dynamics and larval fish survival in estuaries, however this method only gives the nutritional condition of the larvae a few days prior to sampling, reflecting a snapshot of the nutritional condition of larvae in these estuaries. Due to the dynamic nature of estuaries it is recommended that future studies include more frequent sampling events to reveal changes over a longer temporal scale, and combine sampling with otolith analyses to account for any age-related effects. Isotopes can also be used to supplement the nucleic acid indices to evaluate the food web and more accurately link ecosystem attributes to nutritional condition of fish larvae in estuaries.

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Chapter 5: Synthesis and Conclusions

Mangrove habitats in tropical climates are critical nursery habitats to fishes during their early life history stages which contribute to these habitats having substantial fisheries value (Costanza et al., 1997; Cocheret De La Morinière et al., 2004; Dahlgren et al., 2006; Nagelkerken et al., 2010). Although their value to juvenile fishes in terms of feeding and refuge are widely cited, warm temperate systems are comparatively poorly studied (Faunce and Serafy, 2006; Blaber, 2007; Sheaves, 2017). The isolated studies in warm temperate mangrove systems generally found that many of the species utilizing mangrove habitats were equally abundant in alternative habitats such as mudflats and salt-marsh creeks (Clynick and Chapman, 2002; Smith and Hindell, 2005; Payne and Gillanders, 2009). Most recently, a concurrent study found that small fish assemblages were similar between warm temperate mangrove and non-mangrove estuaries in South Africa (Muller, 2017). However, the results of this study indicated that although densities of postflexion *Gilchristella aestuaria* and their prey species were similar between mangrove and non-mangrove estuaries, mangrove presence appeared to influence the feeding ecology and subsequent growth of the late larval stages.

The nursery quality of a specific habitat is not only linked to the abundance and diversity of juvenile fishes utilising the habitat, but is also linked to the growth, feeding success and increased survival which leads to the successful recruitment to adult habitats (Beck et al., 2001). A few recent studies on the nursery role of warm temperate mangroves found no differences in juvenile fish abundance and diversity (Clynick and Chapman, 2002; Smith and Hindell, 2005; Payne and Gillanders, 2009; Muller, 2017). However, it is apparent from the findings of the thesis that the density and distribution of the fish larvae (Chapter 2) were determined by the interactive effects of both physico-chemical and biological variables.

The nutritional condition and growth of *G. aestuaria* larvae were found to be related to multiple environmental factors coupled to freshwater inflow in warm temperate South African estuaries (Costalago et al., 2015). The estuaries in the present study, were more saline when compared to most other warm temperate estuaries, which is probably a result of their relatively small catchment sizes, yet permanently open mouth conditions. As the study estuaries are therefore relatively nutrient limited, evident from the low larval and zooplankton densities seen when compared to other warm temperate estuaries (Chapter 2 and 3), the resulting productivity as a consequence of freshwater inflow, may have a larger contribution to the system than nutrient inputs by mangrove habitats. Warm temperate mangroves have been found to be less productive than tropical mangroves (Komiyama et al., 2008).

Thus, factors coupled with river flow are probably the main driving factors impacting larval *G. aestuaria* densities.

The spatial and temporal variation of larval densities are not only affected by abiotic factors such as temperature, the growth and survival of larvae are also affected by biotic factors such as prey density and predator-prey interactions (O'Brien, 1979; Clemmesen, 1994; Esteves et al., 2000). Previous studies have found that *G. aestuaria* abundance is positively correlated with copepod densities which are their main prey (Whitfield, 1999). This was supported here (Chapter 3), as postflexion *G. aestuaria* densities positively correlated to the three dominant Copepoda species in the study estuaries. The diet of *G. aestuaria* mainly consisted of the same dominant Copepoda species, which included: *Pseudodiaptomus hessei*, *Paracartia longipatella*, and *Acartiella natalensis*. Previous studies found that at least 50% of the dietary requirements of *G. aestuaria* comprised of *P. hessei*, which attains high abundances during periods of increased river inflow in the permanently open, warm temperate Sundays Estuary (Wooldridge and Bailey, 1982; Whitfield and Harrison, 1996). In this study, however, *P. hessei* only contributed to 15.41% of the stomach volume. Densities of *P. hessei* have been found to correlate with flooding or after strong freshwater inflow events (Wooldridge and Melville-Smith, 1979; Wooldridge and Bailey, 1982). Despite similar *P. hessei* densities, rainfall and salinity gradients measured in this study, a larger volume of *P. hessei* were consumed by postflexion larvae in mangrove estuaries when compared to non-mangrove estuaries. The larger size of *P. hessei* compared to the other Copepoda prey species may offer more nutritional value which may be the reason for larvae actively selecting these larger prey species. The larvae in this study also showed other selective feeding behaviours by preferring to feed on *A. natalensis*, despite similar densities of *P. longipatella* observed in the two northern estuaries, Qora and Xhora. This selective feeding behaviour contrasts other studies further south that found larval *G. aestuaria* to be general planktivores, feeding on the dominant prey available (Whitfield and Harrison, 1996; Froneman and Vorwerk, 2003; Strydom et al., 2014; Costalago et al., 2016). Only one study found selective feeding behaviour by late stage larvae of *G. aestuaria* where they actively fed on Copepoda eggs (Strydom et al., 2014). The density and spatial distribution of larval *G. aestuaria* not only depend on prey density and environmental variables, but was also influenced by competition with predatory mysid species. Juvenile *Rhopalophthalmus terranatalis* and adult *Mesopodopsis wooldridgei* readily prey on the copepod *P. hessei*, which is a dominant prey item of *G. aestuaria* (Wooldridge and Webb, 1988; Strydom et al., 2014). Larval *G. aestuaria* densities negatively correlated with *M. wooldridgei*. As these two species are of similar size, it is likely that they are competing for the same prey resource. Predatory crab larvae, in particular *Hymenosoma orbiculare*, also was negatively correlated with preflexion *G. aestuaria* densities, which is most likely due to predation.

The nutritional condition and growth of preflexion and flexion stage *G. aestuaria* larvae were similar in mangrove and non-mangrove estuaries, however postflexion larvae had significantly higher standardised RNA:DNA (*sRD*) values in mangrove estuaries suggesting that mangrove estuaries were better food patches for these larvae when compared to non-mangrove estuaries (Chapter 4). The nutritional condition of larvae in this study were much higher than the findings of Costalago *et al.* (2015) suggesting that the estuaries closer to the warm temperate-subtropical boundary provide better feeding conditions and growth for larvae than those farther south where water temperatures are slightly lower. In this study, preflexion and flexion stage larvae were in a similar nutritional condition regardless of mangrove presence. Costalago *et al.* (2015) found that salinity and the abundance of zooplankton were the major factors that influenced the condition of *G. aestuaria* larvae. However, in this study, salinity was not correlated to the *sRD* because salinities of the study estuaries were relatively similar. The only physico-chemical variables that correlated to *sRD* were turbidity, temperature, and to a lesser extent, dissolved oxygen. Large morphological differences in *G. aestuaria* larvae were observed between 2015 and 2016. The *G. aestuaria* larvae collected in 2016 had a significantly larger length, body depth, myomere height, and eye diameter in the mangrove estuaries than those collected in non-mangrove estuaries. Morphological plasticity has been previously observed in adult *G. aestuaria* (Blaber et al., 1981; Strydom and Whitfield, 2000). It was found that adult *G. aestuaria* had smaller eyes and fed mostly on calanoid Copepoda in the more turbid St Lucia estuarine lake compared to other estuaries that were less turbid and had lower prey densities (Blaber et al., 1981). In the less turbid estuaries, the adult *G. aestuaria* had larger eyes and were selectively feeding on larger sized prey species such as mysids (Blaber et al., 1981). In this study, the two mangrove estuaries were slightly less turbid in 2016 and may be a reason for the larger body sizes seen within these systems. Mangroves have been found to be sediment sinks decreasing turbidity within the estuary channel, which might favour larger larvae (Furukawa and Wolanski, 1996; Wolanski et al., 1998). As the larvae reach the postflexion stage, the ontogenic changes that occur generally result in improved swimming ability and are thus developed enough to make use of any advantage that mangrove habitats may provide, such as decreased turbidity. Decreased turbidity, however can negatively impact early stage larvae by increasing predation pressures (Wolanski et al., 1998; Teodósio et al., 2016).

In conclusion, mangrove habitats acted as sediment sinks, slightly reducing the turbidity of these estuaries resulting in postflexion larvae actively selecting larger, more nutritious prey which increased their growth rate when compared to other postflexion larvae found in non-mangrove estuaries. This can be related to the optimal foraging theory as larvae maximised their energy gain while expending

the least amount of energy on the cost of foraging within mangrove estuaries when compared with non-mangrove estuaries (Fortier and Harris, 1989). Therefore it is here suggested that mangrove estuaries are better food patches for postflexion larvae of this important mid-trophic estuarine species when compared with non-mangrove estuaries. This study is the first of its kind assessing the value of mangroves in driving fodder fish populations. Thus, it is recommended that future studies assessing fish nursery habitats should not only focus on fish abundance and diversity but should include a suite of factors, which include predator-prey interactions as well as nutritional condition of early stage fishes. This study is limited by sampling frequency and thus, due to the dynamic nature of estuaries, future studies should sample more frequently or should consider including isotopes which can be used to supplement the nucleic acid indices to evaluate the food web and more accurately link ecosystem attributes to nutritional condition of fish larvae in estuaries. This would give a more holistic view of the possible intrinsic factors driving food patch dynamics in order to identify and assess important fish nurseries.

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