LARVAL ASSEMBLAGES IN INTERTIDAL HABITATS: THE USE OF ARTIFICIAL AND NATURAL MICROHABITATS

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Abstract

Coastal habitats, and more specifically, intertidal habitats, host a unique range of biodiversity and are key areas for many fish and invertebrate species across one or more of their life stages. This is due to the provision of microhabitats which offer an escape from harsh environmental stressors and predation as well as increased food supply, hence increasing chances of survival. Due to the growing human population however, coastal habitats are being replaced by artificial structures (jetties, seawalls, piers, breakwaters) which partially or heavily fragment the natural environment through urbanisation-related expansion processes. These coastal infrastructures also have different physical properties from the natural environment and therefore tend to support different biological assemblages and can potentially alter the existing biodiversity and its functionality. The overall aim of this project was therefore to evaluate the use of artificial and natural intertidal microhabitats by fish and invertebrate larvae along the South African, Eastern Cape coastline.

As independent case studies, fieldwork was conducted at an urban (Port Alfred Marina) and rocky shore (Kenton-on-Sea) site. Within each of these study sites, two replicated sheltered subsites were selected, which represented microhabitats. Samples were collected from these replicated microhabitats from September 2019 to February 2020 using light traps which targeted phototactic larval species, as well as a portable pump, for photo-neutral/negative taxa. All samples were preserved onsite in 99% ethanol and specimens were later counted and identified in the laboratory to the lowest possible taxonomic level using a stereomicroscope. Additionally, DNA barcoding was conducted on selected larval taxa for verification of morphological identification as well as contributing to the field of larval taxonomy through development of public database records. The barcoding technique was effective in positively identifying 96% and 58% of fish and invertebrate larvae sampled, respectively (overall identification success of 86%), to either family, genus or species level.

Results of microhabitat use indicate higher larval abundances associated with artificial structures as compared to natural structures, with significant differences between the selected microhabitats within the rocky shores and the marina respectively, across months. High numbers of several early stage taxa were observed within the selected microhabitats in the marina, with *Pinnotheres* sp. (zoea) (Family: Pinnotheridae) being the most abundant invertebrate larval taxon collected at the artificial microhabitats of jetties and vertical walls. Fish larvae of *Omobranchus woodi* (preflexion) and *Etrumeus whiteheadi* (postflexion) were the most dominant at the selected artificial microhabitats within the marina. The DNA barcoding tool used in the current study to verify morphological identification proved to be instrumental in the accuracy of the reliable data collection of the early life stages present in these habitats. These results suggest that artificial structures may provide refugia for the vulnerable very early life stages of species and, in turn, play a potential facilitative role in reproductive and population connectivity which could result in replenishment of natural populations. It is therefore possible that these urban habitats could be considered as hubs for maintenance of coastal biodiversity.

Dedication

"It always seems impossible until it's done."

—Nelson Mandela.

This thesis is dedicated to my mum, Roshendree Reddy, and my dad, Koobendran Reddy. Thank you for being my anchor and keeping me grounded throughout the challenges I have faced in life and showing me what it is to be fearless and always follow my dreams and aspirations no matter what!

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CHAPTER 1: General Introduction

Habitat selection is a universal process amongst all organisms and influences most of an individual's succeeding lifestyle (Orians and Wittenberger, 1991). Habitat selection implies making an active choice in selecting a site to live in (Gutiérrez, 1998) and has generally been measured as usage relative to availability or as usage versus non-usage (Mayor et al., 2009). Habitat selection is a multifaceted process which can be broadly divided into two stages: (1) locating an area with suitable biological, chemical and physical conditions for an organism to make use of or occupy and (2) finding a smaller area within this suitable habitat that could be a potential territorial zone or home range (Piper, 2011). Habitat selection could potentially integrate a variety of direct and indirect evolutionary and ecological processes at multiple spatial scales, such as inter- and intra-specific interactions amongst species, density-dependence, resource distributions and spatio-temporal differences amongst individuals (Stamps, 2001; Morris, 2003). By influencing relationships among the distributions of species, habitat selection could link these ecological processes across local and regional scales, as local change of species composition across space and time could govern variation in trophic structure across complex habitats (Resetarits, 2005). Habitat selection could, therefore, have important effects on regulation of populations and structuring of ecological communities, which could, in turn, further impact the overall foundation and preservation of biodiversity (Morris, 1996; Morris, 2003).

In the marine realm, many fish and invertebrate species have complex life cycles (Harmelin-Vivien et al., 1995; Jenkins and Hawkins, 2003; Hoey and McCormick, 2004; Jenkins, 2005; Rey et al., 2019), with stage-structured life histories (Lecchini et al., 2017), which encompass a dispersive planktonic larval phase and a relatively sedentary benthic adult phase (Ohman et al., 1998; Jenkins, 2005; Lecchini et al., 2017), with each stage occupying different habitats (Grantham et al., 2003; Jenkins, 2005; Anger, 2001; Wilson et al., 2016; Rey et al., 2019). The transition between this pelagic environment and the benthic environment (also termed the

recruitment process) signifies a crucial period in the ontogeny of marine fish and invertebrates (Lecchini, 2005; Lecchini et al., 2017), as it represents a period in an organism's life in which it experiences a radical shift from one habitat to another, with changes in environmental conditions that would require relatively rapid adaptation from a pelagic to a benthic lifestyle (Lecchini, 2005; Lecchini et al., 2017; Rey et al., 2019). During this transition period, just preceding recruitment and hence habitat selection, pelagic larval stages are exposed to a number of factors that increase mortality and limit the abundance of species (Lecchini, 2005). Larval trait-mediated effects (linked to phenotypic and genetic variability), moreover, are shaped throughout the ontogenetic cycle of species and, therefore, could influence fitness (such as growth and survival) of subsequent life stages of a particular species (Shima and Swearer, 2009; Giménez, 2010; Rey et al., 2019). Habitat selection by dispersing species could, therefore, influence population and source/sink dynamics as well as overall population persistence (Stamps et al., 2005; Marshall and Morgan, 2011; Fey et al., 2019) as early stages represent a bottleneck in an organism's life cycle in terms of survival, fitness and overall adult population dynamics (Cowen and Sponaugle, 2009). It is, therefore, crucial to identify and understand the aspects which regulate habitat selectivity of dispersers during the stage of searching for different habitats to disperse to or settle in (Stamps et al., 2005).

For marine fish and invertebrate species with dispersive capabilities, larvae are generally able to assess abiotic and biotic factors which determine their 'searching/exploratory' behaviour during the larval dispersal stage and final settlement (Bell, 1991; McMahon and Matter, 2006). Searching behaviour can be broadly influenced by four factors: (1) the abilities and traits of an animal, which determine their perspective and mobility skills (Bell, 1991); (2) abiotic factors which can determine availability of resources; (3) inter- and intra-competition, which can influence the amount/type of risks generated in obtaining such resources as well as other structural features regulating availability of shelter or possibly creating obstacles to searching

(Bell, 1991) and; (4) internal factors such as physiological and metabolic costs incurred during the search of a suitable habitat type (Bell, 1991; Stamps et al., 2005). Organisms are adapted to detect abiotic and biotic conditions that are linked to the success of reproduction and overall survival rates. During habitat selection, they respond by remaining in habitats which offer the necessary cues to enable these processes, while still being able to continue exploring different habitats if these cues are not detected, hence, searching through areas that could potentially be unsuitable until favourable cues are identified (McMahon and Matter, 2006). Suitable habitats linked to further growth and overall survival of early life stages are generally those which offer increased food availability, increased chances of predator avoidance and refuge (McMahon and Matter, 2006).

Organisms are often confronted with a trade-off between availability of food and predator avoidance and this can be resolved through shifts in levels of activity and selection of habitats (Peckarsky, 1993; Alofs and Polivka, 2004). Individuals directly benefit from foraging through input of energy and indirectly through increased growth, hence, selecting an appropriate habitat which provides such amenities can be a crucial behaviour that determines an organism's fitness (Alofs and Polivka, 2004). On the other hand, habitats which offer sheltered areas are generally more complex, thus offering increased living spaces which can provide protection from predation (Vorsatz, 2020). This aspect can be related to the textural discontinuity hypothesis (TDH), which proposes that naturally discontinuous habitats can influence the size of an organism that is able to efficiently utilise a space (Holling, 1992), thus creating niche partitioning (Kadmon and Allouche, 2007). This, in turn, can allow smaller organisms to occupy areas which are not accessible by their larger predators (Vorsatz et al, 2021a), thus offering temporary refuge for prey items either by remaining undiscovered or inaccessible to predators (Diehl, 1993). As an example, only fish with body sizes smaller than structural openings present in habitat patches, such as crevices, can exploit these areas for shelter, thus

excluding predators of larger size classes (Nash et al., 2013). Availability of food and safety from predators are therefore complementary resources and heterogeneity/complexity provided by habitat patches or microhabitats allows organisms to use these distinct topographical features, with the ability to offer resources and maximise fitness (Alofs and Polivka, 2004).

Habitat complexity at the macrohabitat level can modify environmental factors at small spatial scales, thus, creating microhabitats (Vorsatz et al, 2021a). Macrohabitats are distinguishable units comprising the minimum area in which organisms carry out all their biological functions during a standard life cycle (Morris, 1987), while microhabitats form smaller distinct topographical features (Martins et al., 2010). Microhabitats are generally characterised by a suite of chemical and physical properties (Morris, 1987), which can offer protection and shelter from environmental extremes and predation as well as food supply (Aguilera et al., 2014; Adams et al., 2004).

Habitat structure and complexity of natural ecosystems allows movement and transport of early life stages of organisms across microhabitats and can play a crucial role in determining the diversity, richness and identity of species existing in biological communities (Connell and Glasby, 1999; Godbold et al., 2011). The integrity of naturally occurring habitats, however, has been increasingly threatened by human mediated impacts, with modification of natural substrata being a common occurrence (Connell and Glasby, 1999), especially in coastal regions, where its rich array of resources has attracted humans for centuries (Erlandson and Fitzpatrick, 2006; Neumann et al., 2015). During recent decades, development and utilisation of coastal areas due to socio-economic activities have increased substantially, and consequently, coasts have undergone immense environmental and structural change (Martínez et al., 2007; Neumann et al., 2015). Changes related to anthropogenic activities include: degradation due to pollution, introduction of alien species, over-fishing, unsustainable aquaculture activities and modification of coastal geomorphology and hydrology (Airoldi and

Beck, 2007). In addition to these sources, habitat loss (Airoldi and Beck, 2007) and fragmentation (Hagen et al., 2012) from land reclamation schemes, habitat alteration and overall coastal development are also evident and widespread anthropogenic related actions (Airoldi and Beck, 2007; Perkins et al., 2015). These activities have been mainly linked to the growing human population within coastal areas (Lai et al., 2015; Aguilera et al., 2016; Todd et al., 2019). The loss of natural habitat is predicted to intensify as the human population continues to grow, especially in developing countries (Perkins et al., 2015). In Africa, the total human population is expected to increase to approximately 2.5 billion by the year 2050 (Guneralp et al., 2017) and in countries such as South Africa, the total human population (as at 2015) is estimated to be around 49.32 million, with 21.2% situated in the province of KwaZulu-Natal and 13.5% in the Eastern Cape Province, both situated within the Western Indian Ocean region, with 40% of this total, living within 100 km of the coastline (Celliers and Ntombela, 2015). The total population in South Africa has further increased to 60.53 million in 2021 (O'Neill, 2021). For coastal African countries and others which are experiencing socio-economic growth, the human population, urbanisation and increased migration toward the coastline will only continue to rapidly increase and this will exacerbate change and modification of coastal systems (Hinkel et al., 2012).

Intertidal habitats, such as natural rocky shores have been negatively impacted by these anthropogenic activities related to population growth and urbanisation and these impacts are predicted to be intensified by climate change, sea level rise and stormier seas (Firth et al., 2013; Neumann et al., 2015; Perkins et al, 2015; Firth et al., 2020; Foti et al., 2020). These events will increase the risk of land inundation, thus escalating the need and demand for construction of protective structures/armouring along coastlines (Firth et al., 2013; Spalding et al., 2014). Ultimately, the interaction between the impacts of land reclamation and coastal development, along with measures to mitigate climate change, will drive continued building of engineered

artificial coastal structures (Bulleri and Chapman, 2010; Dugan et al., 2011), which replace or fragment natural habitats (Strain et al., 2017; Aguilera et al., 2020). Since these artificial infrastructures are generally associated with lowered abundance, increase of invasive and/or extinction of species (Bulleri and Chapman, 2004; Bulleri, 2005; Moreira et al., 2006; Lee and Li, 2013; Ravinesh and Bijukumar, 2013; Aguilera et al., 2014; Wetzel et al., 2014), and made up of different substrata which are less structurally complex (Aguilera et al., 2016), hardening of the coastline could further influence dispersal and settlement of early life stages of fish and invertebrate species, potentially affecting overall adult population dynamics (Cowen and Sponaugle, 2009). It is therefore of utmost importance to understand the function of artificial substrates as potential surrogate habitats for natural surfaces in relation to use by early stage coastal species (Connell and Glasby, 1999; Jebakumar et al., 2021).

One of the main challenges when addressing the diversity of early life stages is the taxonomic identification of marine larval species due to the lack of distinguishable morphological characteristics generally present in their adult counterparts as well as features being different amongst the various developmental stages (Pradillon et al., 2007; Steinke et al., 2016b; Kusbiyanto et al., 2020). By forming an integrated approach in connectivity studies which encompasses both molecular techniques (such as DNA barcoding) and traditional morphological methods, it can accelerate traditional identification techniques by combining genetic markers in addition to morphological characteristics for species delineation and contribute to ecology and taxonomy and could potentially lead to development of new and unanticipated information (Larsen, 2001; Sheth and Thaker, 2017).

1.1 Aims and Objectives

The overall aim of this thesis was to evaluate the use of artificial and natural intertidal microhabitats by fish and invertebrate larvae along the South African, Eastern Cape coastline.

The first objective of the study was to determine the species composition and abundance of early life stages of fish and invertebrate larvae that are associated with coastal artificial infrastructure and a naturally occurring area within a rocky shore environment, which were conducted as independent case studies. In addition to this first objective, the relationship between larval assemblages and environmental parameters collected over the sampling period was also determined for the respective study sites. The second, complementary objective was to identify the fish and invertebrate larvae collected to the lowest possible taxonomic level using the DNA barcoding technique, which was used together with morphological identification to assess larval diversity as well as contribute to the field of larval taxonomy.

1.2 Thesis structure

This thesis is divided into four chapters comprising of a general introduction (Chapter 1) which provides an overview of the importance of (micro)habitat selection amongst biological communities in natural ecosystems and its relevance for different ontogenetic stages of marine fish and invertebrates as well as highlighting threats of anthropogenic activities to these natural coastal systems. Chapter 2 focuses on the composition and abundance of fish and invertebrate larval assemblages associated with microhabitats at independent case study sites representing an artificial environment and a naturally occurring environment. Chapter 3 examines the diversity of the larval assemblages collected at the selected study sites using the DNA barcoding technique as an identification tool and its relevance for ecologically based studies. Lastly, Chapter 4 provides a synthesis around the results of the study and highlights the importance of artificial structures as potential surrogates of naturally occurring microhabitats within the framework of habitat selection.

CHAPTER 2: The potential role of urban and natural intertidal microhabitats in determining the composition and abundance of fish and invertebrate larval assemblages

2.1 Introduction

Coastal habitats play a pivotal role in sustaining a unique range of biodiversity (Skilleter and Loneragan, 2002; Aguilera et al., 2014) as they are highly productive areas which support many different fish and invertebrate species across one or more of their life stages (Seitz et al., 2014; Henseler et al., 2019). These habitats, therefore, form ecologically important structural components of the geographic areas they occupy by sustaining vital functions such as feeding, survival, growth, reproduction, spawning and migration of marine species (Seitz et al., 2014; Henseler et al., 2019; Lefcheck et al., 2019). The sustenance of these functions can be attributed to the variability in the structural complexity, hydrodynamics and nature of the substrate within these coastal habitats (Koch, 2001), which generally improve habitat quality and connectivity and hence, help determine overall species composition and maintenance of biodiversity (Seitz et al., 2014, Henseler et al., 2019).

The unique and distinct characteristics that represent coastal habitats underpins its most important and perhaps, most cited function as nursery areas (Litvin et al., 2018; Lefcheck et al., 2019). In its simplest form, nurseries can be defined as areas in which early life stages of fish species occur at higher abundances, are able to evade predation more efficiently and sustain faster growth rates as compared to other habitats (Beck et al., 2001), therefore, playing a key role in the survival of these larval and juvenile stages (Beck et al., 2001; Strydom, 2003; Kruger and Strydom, 2010). Similarly, many coastal environments are also recipients of invertebrate larvae as they culminate in finding suitable adult habitat, therefore, increasing rates of post-settlement growth and survival (Jenkins, 2005; Pineda and Reyns, 2018). These coastal environments, overall, generally provide favorable conditions (Lipcius et al., 2007; Litvin et al., 2018) by ameliorating the surrounding physico-chemical properties through substrate heterogeneity (Evans et al., 2021), and, in turn, generate microhabitats (Strydom, 2008; Aguilera et al., 2014; Evans et al., 2021), which are distinguishable topographical structures

(Martins et al., 2010). These include rock pools, crevices, tide pools and gullies (Strydom, 2008; Aguilera et al., 2014), that are defined by physical and chemical variables which affect the allocation of time and energy used by an individual during its life cycle within its home range (Morris, 1987; Vorsatz et al., 2021b).

In the marine environment, especially intertidal rocky shores, complexity of rock surfaces can create areas with increased surface area, enhanced nutrient cycling by retention, increased sedimentation and humidity levels, decreased exposure to sunlight and temperature and dissipate the energy of wave action, thus offering a vast variety of microhabitats and niches with varying microclimates, available and accessible for colonisation and shelter from predators (Kostylev et al., 2005; Tokeshi and Arakaki, 2012; Hanlon et al., 2018), low tide desiccation stress (Gosselin and Chia, 1995; Lathlean et al., 2017) and thermal refuge (Garrity, 1984; Lathlean et al., 2017). These microhabitats are generated through the complexity of hard and soft substratum which are naturally occurring along rocky shores (Sebens, 1991). Hard substrata can comprise of solid rocky surfaces of different types which usually contain varying sizes of crevices and holes created by erosion and weathering by natural processes over time (Moschella et al., 2005; Pinn et al., 2005; Martins et al., 2016). There are also differences in orientation patterns in relation to other surfaces (Sebens, 1991) which can alter water flow (Connell, 1999) and exposure to light and temperature (Thorson 1964) which could potentially affect the transfer of food resources and propagules within the ecosystem (Sebens, 1991). Biogenic accumulation on hard substrata, moreover, leads to provision of secondary surfaces through the attachment and growth of benthic organisms and accompanying deposition of calcium carbonates (Hewitt et al., 2005; Lindsey et al., 2006). Soft substrata comprise grains of different sizes and texture which are made up of organic and inorganic materials which can differ in their chemical compositions. The composition of this substrata, additionally, can constantly be locally modified by resident organisms, which can alter the input of organic

matter and agitate particles (Sebens, 1991). Habitat heterogeneity can be beneficial to species as it allows coexistence by partitioning of habitats amongst different species within a specific area, therefore, variation in local surroundings can bring about higher overall diversity (Munguia et al., 2011; Bulleri et al., 2016). For marine organisms with complex life cycles, habitat heterogeneity additionally can provide the habitat necessary for each life stage, therefore, promoting population maintenance within a regional landscape (Munguia et al., 2011).

In addition to the vast ecological value of coastal habitats, these systems also hold important economic and social value for the human population by providing goods and services attained via ecosystem functions and biodiversity (Costanza et al., 1997; Cardinale et al., 2009; Aguilera et al., 2020). Human beings have not been unresponsive to the variety of opportunities and benefits provided by coastal habitats and are highly drawn to them, hence making the coast one of the most preferred locations for residential, commercial and tourist activities (Martinez et al., 2007), with over 60% of the world's largest cities as well as 40% of the world's population being situated within 100 km of the coast (Bulleri and Chapman, 2010; Bishop et al., 2017; Todd et al., 2019). This is predicted to further expand as over 75% of people will inhabit areas within 100 km of the coast by the year 2025, which will inevitably lead to an expansion in the development of coastal infrastructure (Bulleri and Chapman, 2010). Urbanisation is generally considered one out of many anthropogenic pressures that currently contributes the most to habitat loss from local to regional scales (Selfati et al., 2018; Wang et al., 2019). Due to the increase in urbanisation, together with the growing human population, many coastal habitats are being replaced by artificial structures for a range of purposes, such as coastal armouring/protection (e.g. breakwaters, seawalls, groynes, bulkheads, riprap revetments) (Bulleri and Chapman, 2004; Aguilera et al., 2016; Bishop et al., 2017), residential, recreational

and commercial activities (e.g. marinas, waterfront estates, boat ramps, jetties, piers, pontoons, mooring buoys, pilings) (Aguilera et al., 2014; Bishop et al., 2017; Selfati et al., 2018).

Artificial structures fragment natural habitats by partially or entirely removing them through urbanisation-related expansion processes (Strain et al., 2017; Aguilera et al., 2020) and can therefore alter the existing biodiversity through direct subtraction of space and indirect disruption of the functioning of natural ecosystems, which result from changes in local and regional connectivity (Aguilera et al., 2014; Dafforn et al., 2015). These disruptions to habitat connectivity can create physical barriers which can modify movement patterns of species and their resources, further resulting in functional alteration of trophic transfer regimes and fitness (LaPoint et al., 2015; Bishop et al., 2017). Artificial structures, moreover, differ in many aspects when compared to natural habitats, hence influencing the physical successful establishment of specific species, these include properties such as: substrate composition and complexity, orientation/slope, age, surface area, mobility of some structures (such as buoys) and disturbance through maintenance/repair operations on the structures themselves (Bulleri and Chapman, 2010; Airoldi and Bulleri, 2011; Chapman and Underwood, 2011; Bishop et al., 2017). Artificial structures placed in a coastal habitat, additionally, can modify and change availability of light, flow of water, wave energy, sediment dynamics, depositional and geomorphic processes as well as alter mediating, among others, predator-prey interactions (Bishop et al., 2017, Heery et al., 2017). Properties as such, can further manifest in altered crucial ecological processes such as reproduction, spawning activities, larval recruitment, competition and predation (Bulleri and Chapman, 2010), with repercussions on the establishment of altered compositions of assemblages, generally dominated by non-indigenous species (Moschella et al., 2005; Chapman, 2006; Page et al., 2019). Native biodiversity can, hence, be threatened due to lowered fitness in the modified environment and the severe changes in the population dynamics of local communities (Pinochet et al., 2020).

For many coastal marine species, the larval dispersal stage implies the spread of larvae from the source of spawning to final settlement and recruitment into the adult habitats (Pineda et al., 2007), thus driving exchange of individuals among subpopulations (D'Aloia et al., 2015), which, in turn, can be a major determinant of adult population dynamics (Grosberg and Levitan, 1992; Gillanders et al., 2003; Jenkins and Hawkins, 2003; Levin, 2006; Cowen and Sponaugle, 2009). Ecological persistence of species populations hence requires an adequate supply of propagules, which is determined by the rate of larval survival, driven in turn, by biological and physical factors as well as a combination of the two (Pineda et al., 2007; Cowen and Sponaugle, 2009; Treml et al., 2015). Biological drivers include processes that affect larval production, growth, development, behaviour and overall survival (Pineda, 2007; Cowen and Sponaugle, 2009; Treml et al., 2015). Physical drivers comprise of environmental properties such as hydrodynamics, temperature, salinity, upwelling as well as aspects of local habitat structure (Treml et al., 2015; Oricchio et al., 2016; Airoldi et al., 2005). While bio-physical drivers include the intricate interaction between certain early life history traits and their surrounding environmental physical properties that operate at both spatial and temporal scales (Airoldi et al., 2005; Cowen and Sponaugle, 2009; Fobert et al., 2019). These bio-physical interactions are vital as selection by early life stages of species for adult/nursery habitat is based on a combination of responses to environmental gradients, physiological factors, distribution of predators and prey, foraging success and density of competitor species (Baltz et al., 1998; Baltz and Jones, 2003), all of which determine larval settlement/recruitment success. If larvae cannot locate suitable habitat to settle, they continue to swim/explore/being transported in the water until this suitable location is found/or time to settle "expires" (Doyle, 1975; Hadfield et al., 2001; Hadfield and Paul, 2001; Botello and Krug, 2006; Pinochet et al., 2020). Most early life stages of marine fish species have a lecithotrophic life stage and are negatively-buoyant (Pinochet et al., 2020), and marine invertebrate larvae can possess the ability to delay

metamorphosis until specific physical and/or chemical cues are detected (Hadfield and Paul, 2001; Elkin and Marshall, 2007), therefore, continuous 'searching/exploring' within the water column can reduce metabolic energy (Elkin and Marshall, 2007; Botello and Krug, 2006; Igulu et al., 2011). This can then result in decreased energy available for initial growth necessary for post-settlement as well as increased mortality rates which can then compromise the dynamics of their adult populations (Elkin and Marshall, 2007; Igulu et al., 2011; Pinochet et al., 2020).

During the exploration phase that precedes settlement/recruitment, competent larvae may not be able to distinguish between artificial and natural structures (Bulleri and Chapman, 2010), therefore potentially settling on either structure in response to specific settlement cues/stimuli (Rodriguez et al., 1993; Hadfield and Paul, 2001; Arvedlund and Kavanagh, 2009). If larvae cannot sense these cues due to their absence, they are able to extend their larval stage and delay settlement (Victor, 1986), hence expending energy which can potentially increase risk of larval mortality as well as influence post metamorphosis survival rates (Pechenik et al., 1998; Botello and Krug, 2006). Additionally, the structural heterogeneity offered by microhabitats in natural environments as compared to artificial structures, which lack such structural complexity, could potentially impact post-settlement survival rates of fish and invertebrate early life stages associated with such habitats (Bulleri and Chapman, 2010). It is therefore possible that availability of certain substrate (in relation to settlement cues) could either increase or decrease the overall supply of larvae to a population. Determining the degree of larval assemblage associated with artificial and natural coastal habitats is, therefore, imperative to understand the biological and ecological implications of anthropogenic and the associated biophysical changes in a context of potential habitat loss and/or provision.

This study, therefore, aimed to assess the species composition and abundance of benthic invertebrate and fish larval assemblages associated with an artificially constructed environment (Port Alfred Marina) and a natural rocky shore environment (Kenton-on-Sea) along the Eastern

Cape (South Africa) coastline as independent case studies. The objective was to determine the degree of microhabitat use for early life stages of fish and invertebrate larvae within coastal areas respectively associated with artificially constructed vertical walls and jetties within an urban setting and rock pools and gullies within a natural rocky shore setting. In addition to this objective, the relationship between larval assemblages and environmental parameters collected over the sampling period was also determined for the respective study sites.

2.2 Materials and Methods

2.2.1 Study sites

Two study sites, which represented either a naturally or artificially constructed coastal environment, situated on the south coast of South Africa were selected. Although study sites were treated as independent case studies, for the artificial environment selected, flora and fauna were dominantly, but not exclusively similar to, the intertidal species commonly present in the surrounding natural rocky shore environments (Kruger and Strydom, 2010). Within each of these two study sites, two further sheltered subsites were selected as replicates; these subsites will hereafter be referred to as 'microhabitats' for both the natural and artificial environment, so that subsequent sections of this chapter are easier to follow. The replicated microhabitats selected in the natural environment were rock pools and gullies, while jetties and vertical walls were chosen for the artificial environment.

2.2.1.1 Kenton-on-Sea – Natural environment

The Kenton-on-Sea (33° 41' 42.7" S, 26° 39' 40.5" E) rocky shore coastline falls within close proximity to three permanently open estuaries, namely: the Kariega Estuary, which enters the sea directly at Kenton-on-Sea; the Bushman's Estuary, which is approximately 3 km to the west and the Kowie Estuary, which is approximately 25 km east along the coastline. The natural

environment site is made up of Aeolian dune rock (Fraser, 2003), which forms several wave-cut platforms which extend outward into the sea (van der Walt, 2019). These platforms are characterised by further erosional features that have formed over time, such as rock pools and gullies. For the current study, two gullies and two rock pools within this rocky coastline were selected, namely: Sydney's Hope gully (33° 41' 44.91" S, 26° 39' 56.16" E); Shelley Bay gully (33° 41' 44.59" S, 26° 40' 0.048" E); a large rock pool (33° 41' 43.98" S, 26° 39' 57.64" E) and a smaller rock pool (33° 41' 44.95" S, 26° 39' 57.64" E) situated on a wave-cut platform adjacent to Sydney's Hope gully (Figure 2.1).

Sydney's Hope gully (Figure 2.2A) is a subtidal gully which is shielded by wave-cut rocky walls on either side. This gully is exposed to the force from the oncoming waves during high tide, however, the rocky walls provide some protection/shielding from the direct force of wave energy, while at low tide, the waters are generally calmer.

Adjacent to Sydney's Hope gully is a wave-cut platform which contains rock pools of various sizes. The west and south sides are open to the sea and covered by mussel beds, while the north and east sides are shielded by elevated wave-cut rocky walls (van der Walt, 2019), which provide a degree of shelter/protection to the rock pools within this area. During high tide, the wave-cut platform is completely inundated and open to the sea, while at low tide, it is closed off from this access. Within this site, the selected large rock pool (approximately 15 m in length; Figure 2.2C) as well as the smaller rock pool (approximately 6 m in length; Figure 2.2D), were approximately 38 m apart. Due to logistical challenges, rock pools of the same approximate size could not be located at the chosen study site.

Shelley Bay gully (Figure 2.2B) is situated east alongside the above-mentioned sites which is divided by raised wave-cut rocky walls and is approximately 97 m apart from Sydney's Hope gully. Shelley Bay is surrounded by rocky walls which form a semi-circular shape around it,

which offers some protection/shielding against direct force from oncoming waves during high tide.

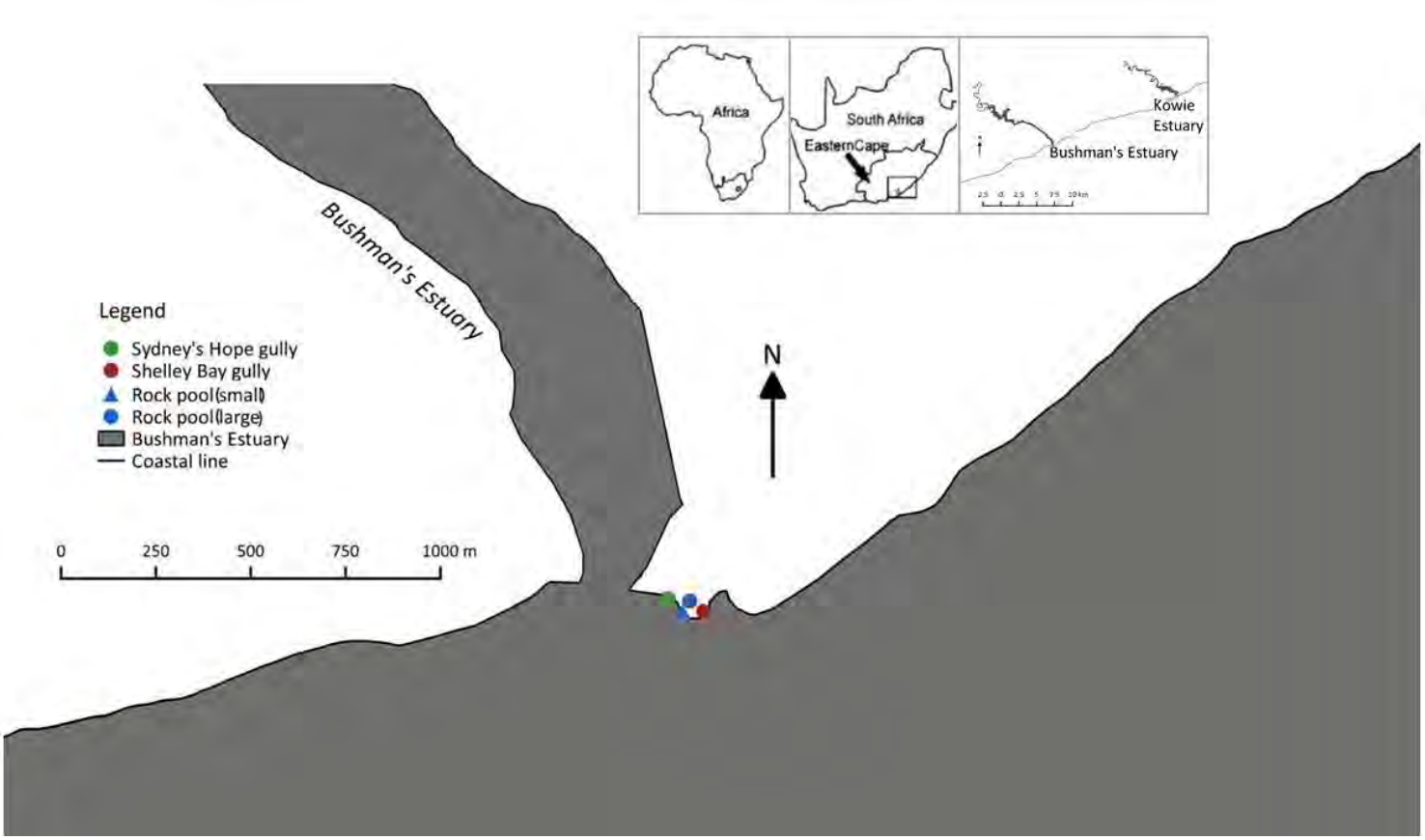


Figure 2.1. Map of the study sites at Kenton-on-Sea showing trap deployment locations at selected natural microhabitats.

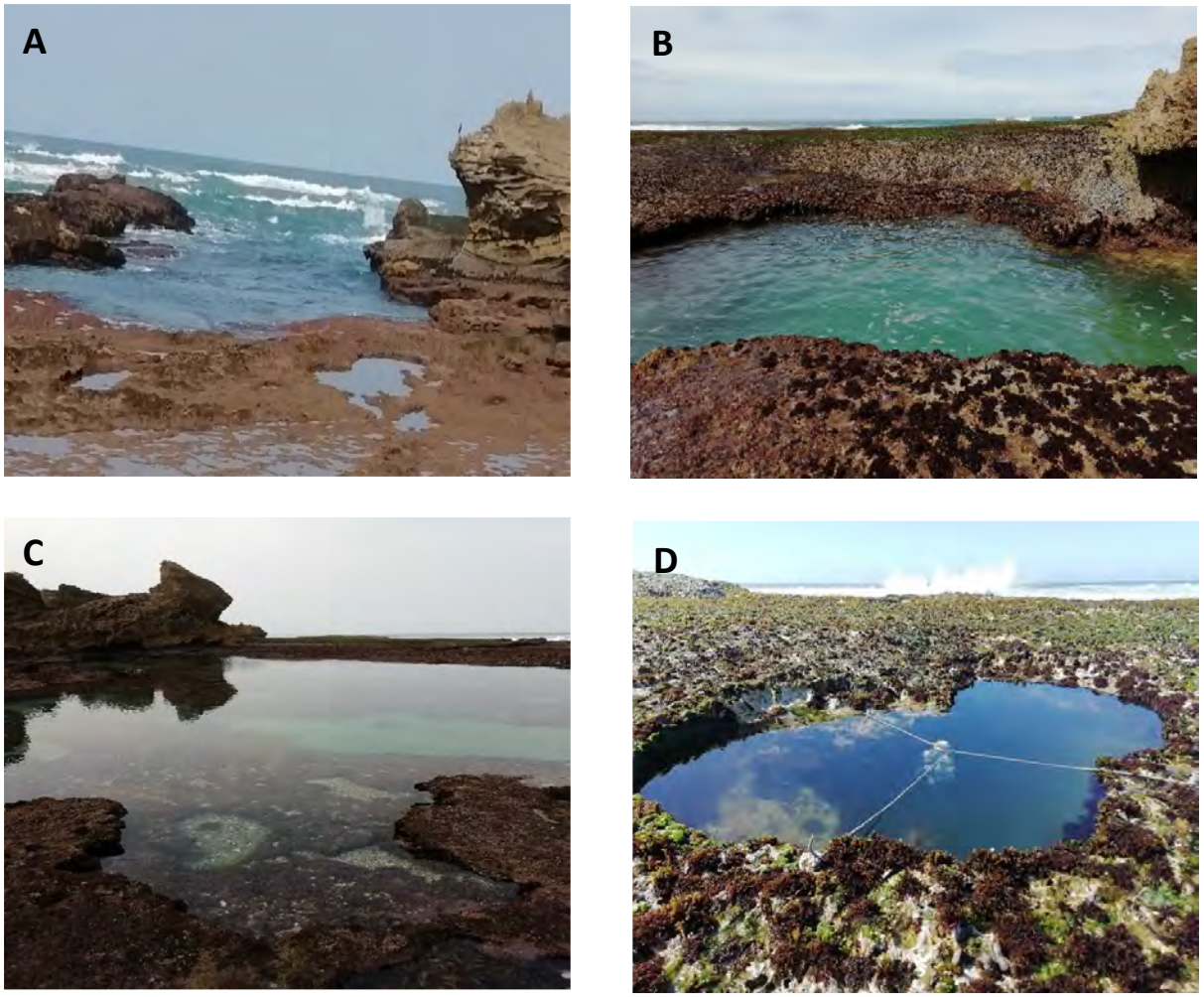


Figure 2.2. Photographs depicting the natural microhabitats at Kenton-on-Sea which were selected for deployment of light traps. (A) Sydney's Hope gully. (B) Shelley Bay gully. (C) Rock pool (large). (D) Rock pool (small), with deployed light trap.

2.2.1.2 Royal Alfred Marina – Artificial environment

The Royal Alfred Marina (hereafter referred to as the Port Alfred Marina) (33° 35' 41.39" S, 26° 53' 44.02" E) is situated at the mouth of the Kowie Estuary (Figure 2.3). The marina was constructed in 1989 by a private developer (Cock, 2018) and is characterised by artificially constructed walls which make up the entrance and channels of the marina (Kruger and Strydom, 2010). The artificial sides along the marina entrance and channels are made up of

steep vertically packed stoned walls at a 60° incline which drops into the sandy estuary bed (Kruger and Strydom, 2010).

The marina is an exclusive development which consists of 360 plots, each with its own waterfront. Property value generally ranges from 5 to14 million ZAR (Cock, 2018). Most of the properties are holiday homes which lends itself to low overall occupancy year around (Cock, 2018), but also leads to periodic levels of high activity during holiday periods (such as December) (Janson, 2020). Adjacent to the marina, a small boat harbour (33° 35' 37.03" S, 26° 53' 31.27" E) provides jetties (Figure 2.4B) for approximately 300 small boats (Cock, 2018) which marina residents also utilise for mooring of their personal crafts.

Within the Port Alfred Marina, approximately 450 m from the mouth of the estuary, two vertical stone packed walls which form the banks of the estuary were selected (Figure 2.4A). These two sites were situated alongside marine properties with the street names: 'The Spithead' and 'Sir Francis Drake's Court' (hereafter referred to as Spithead and Francis Drake, respectively). Spithead (33° 36' 1.62" S, 26° 53' 47.80" E) and Francis Drake (33° 35' 57.23" S, 26° 53' 50.60" E) are approximately 150 m apart and both face the mouth of the estuary (Figure 2.3) and, therefore, experience daily influxes and mixing of fresh and saltwater which can be relatively turbulent during high tide.

Within the small boat harbour, two jetties were selected, namely: Jetty B (33° 35' 38.41" S' 26° 53' 30.66" E) and Jetty D (33° 35' 37.04" S' 26° 53' 31.40" E), which are approximately 50 m apart (Figure 2.3). This area is approximately 1.2 km from the mouth of the estuary and therefore experiences low influx and mixing of fresh and saltwater. There is, however, some boat activity which leads to turbulent waters at periodic intervals.

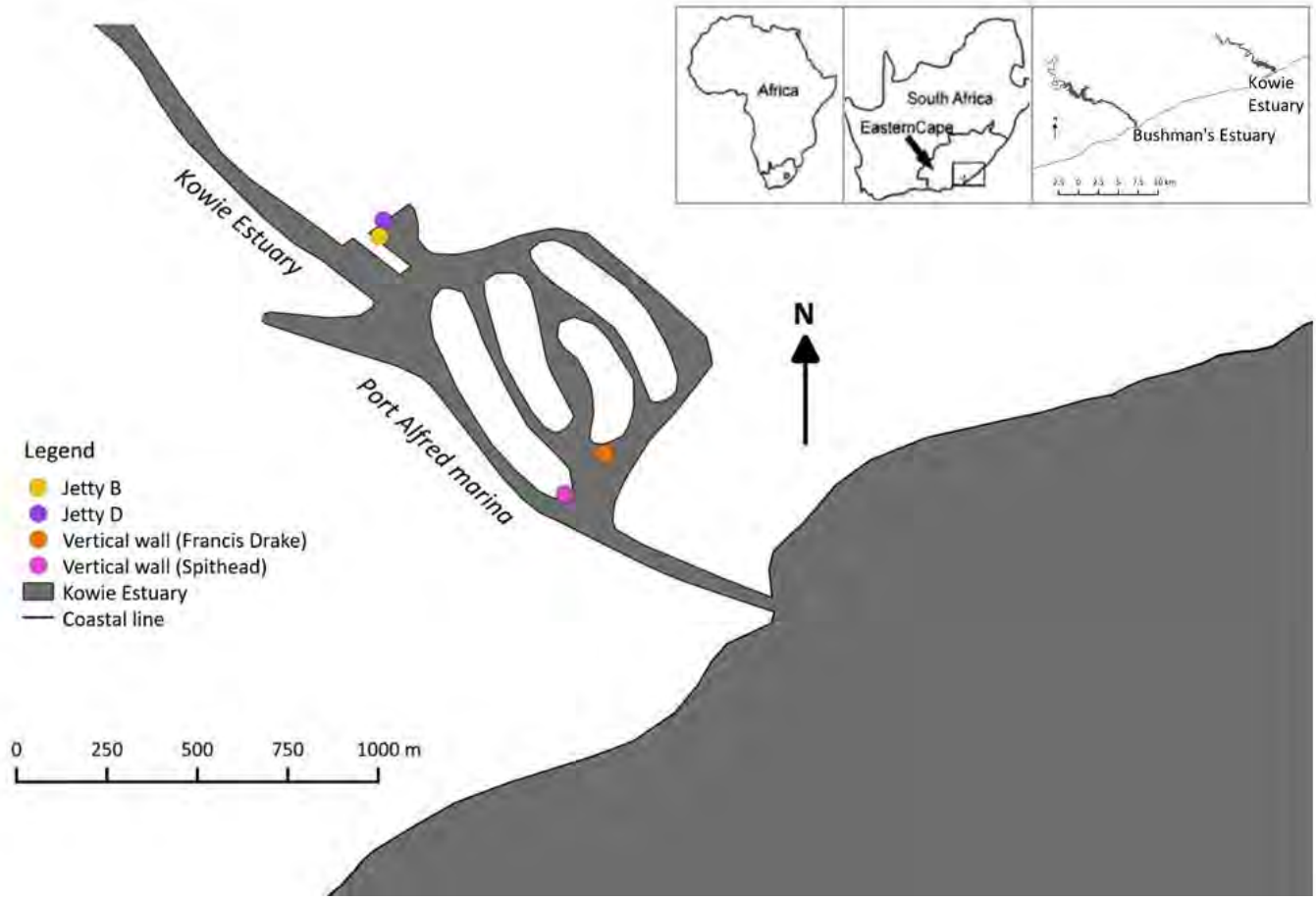


Figure 2.3. Map of the study sites at the Port Alfred Marina showing trap deployment locations at selected artificial microhabitats.

Figure 2.4. Photographs depicting the artificial microhabitats at Port Alfred Marina which were selected for deployment of light traps. (A) Vertical walls (Spithead and Francis Drake). (B) Jetties (B and D).

2.2.2 Sample collection

Samples were collected from the selected microhabitats at both Kenton-on-Sea and the Port Alfred Marina using light traps (based on the design by Chan et al., 2016; Figure 2.5A and B). The light trap design was modified to allow more effective use and sampling in the rocky shore environment. This was done by adding weights (in the form of fishing sinkers) which were

securely attached to the body of the light trap using cable ties in order to ensure that the traps remained submerged and in an upright position for the duration of sampling during high tide conditions and turbulent water flow.

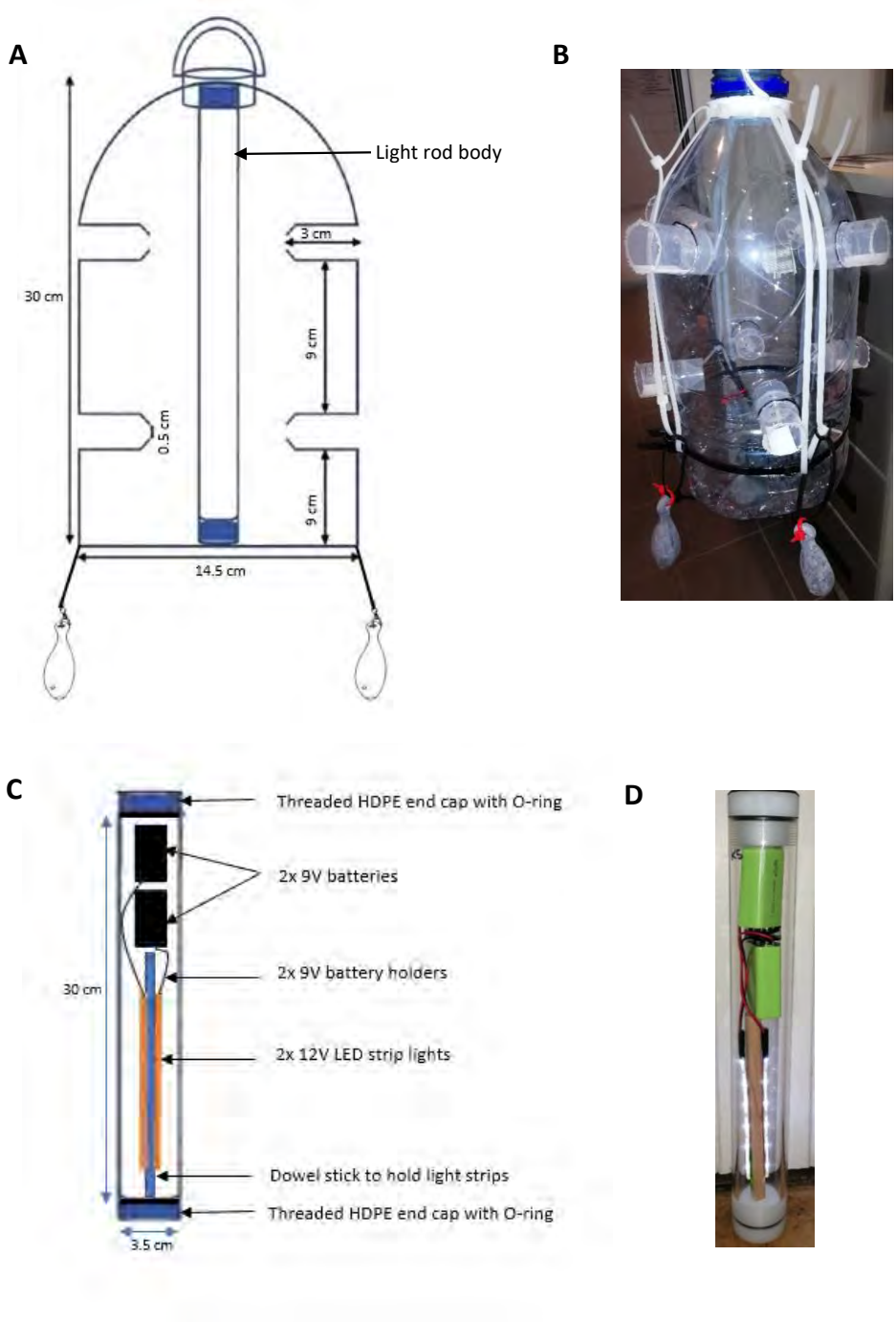


Figure 2.5. Modified light traps used to collect larval samples (based on the design by Chan et al., 2016). (A) Illustration of the design and dimensions of the light trap. (B) Photograph of the light trap body. (C) Illustration of the light rod body and electrical circuit set-up. (D) Photograph of the light rod.

Furthermore, the light rod of the trap was also modified. This was done by using transparent acrylic tubes with threaded HDPE end caps on both ends with O-ring seals (manufactured by Plastics by Graymaur (Pty) Ltd). By screwing both end caps onto the tube, the O-rings were compressed, which created a waterproof seal. The electrical circuit of the light rod included two 12 V cool white LED strip lights cut into 10 cm strips which were powered by two 9 V NiMH rechargeable batteries connected in a parallel circuit. The light strips were held in place by attachment to a wooden dowel positioned in the center of the light rod (Figure 2.5C and D).

Light traps use a passive sampling technique which have proven to be effective in collecting data on larval abundance (Hickford and Schiel, 1999). The light traps targeted and collected larval taxa at night which were attracted to the light source (phototactic species). Traps were deployed during the new moon (to maximize the effectiveness of the sampling technique) spring tides, for approximately 24 hours, over two consecutive nights for a six-month period (September 2019 to February 2020), which equated to three days of sampling per month. Light traps were always deployed underwater and, therefore, were always submerged in the water column. Sampling months were selected based on the peak spawning season for most coastal species of fish and invertebrates within the region (Strydom, 2005; Porri et al. 2006; Hodgson, 2010; Kruger and Strydom, 2010). Two traps were deployed at each replicated microhabitat and positioned at least 5 m apart to prevent sample overlap. This was based on light intensity measurements of the light rod using a lux meter (Lutron LX-101). All traps were deployed using polysteel rope which were secured to the body of the trap. At Kenton-on-Sea, multiple ropes were tied to eyebolts which were drilled into the surrounding rock surfaces (Figure 2.6A), while at the Port Alfred Marina, the traps were attached using a single rope tied to the sturdy structures already present in the area (Figure 2.6B).

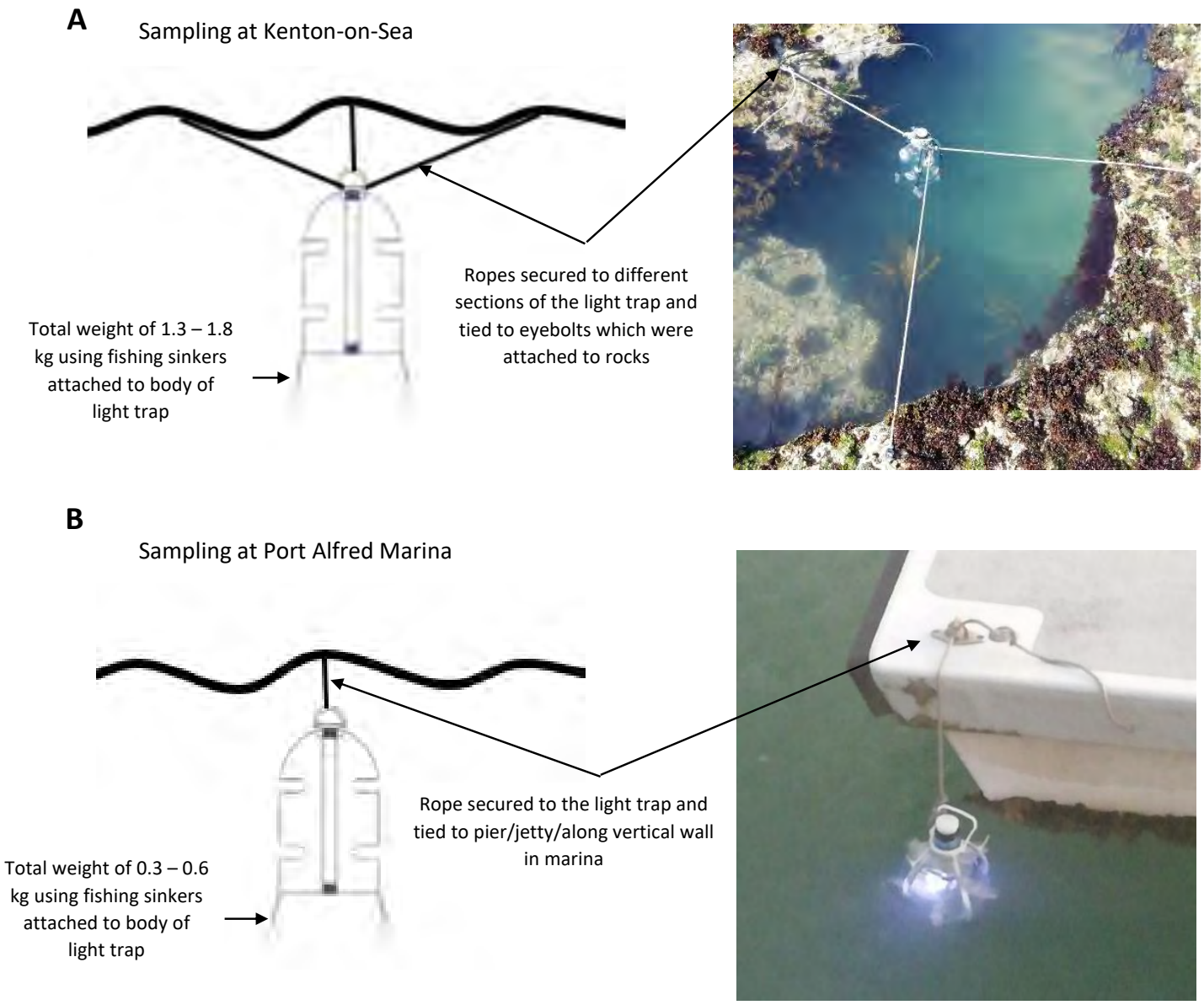


Figure 2.6. Diagram and photograph demonstrating the set-up and attachment of light traps using polysteel rope at (A) Kenton-on-Sea and (B) Port Alfred Marina.

On the first day of a sampling month/trip, traps were deployed during the morning low tide at Kenton-on-Sea and thereafter at the Port Alfred Marina. A total of eight traps were placed at each of these study locations (two traps placed within each selected microhabitat area). On the following day of sampling (during the morning low tide), traps were emptied by transferring the collected sample from the light trap into a 25 L bucket (with no spillage), thereafter, samples

were passed through a 65 µm mesh sieve and specimens collected were transferred into a 500 ml sample bottle. Collected samples were then immediately preserved using 99.9% ethanol. The rechargeable batteries in the light rod were then removed and replaced with fully charged batteries (to ensure that the light intensity during the second night of sampling was standardized) and the traps were redeployed. On the third day of the sampling month/trip, the emptying and transferal process followed as during sampling day two and then all traps were removed.

In order to account for larval taxa which were not phototactic, a portable rechargeable pump (TruePower; 12 V, flow rate, 760 $L.h^{-1}$) (Porri et al., 2021) was used to collect 100 L of water passed through a 65 µm mesh sieve at each selected microhabitat within the vicinity of the trap deployment area. This was done on day one (at low tide during first deployment of light traps) and two (at low tide during redeployment of light traps) of each sampling month and the samples collected were preserved as per above.

2.2.3 Sorting, counting and identification

In the laboratory, larval specimens were counted and identified using a Leica Zoom 2000 stereomicroscope and, for specimens which required more detailed observation at higher resolutions, an Olympus SZX16 stereomicroscope (model SZX2-ILLB) was used. In order to morphologically identify specimens to their lowest possible taxonomic level, published larval descriptive keys were used for fish (Neira et al., 1998; Leis and Carson-Ewart, 2000; Rodriguez et al., 2017) and invertebrate larvae (Paula, 1996; Rice and Tsukimura, 2007; Martin et al., 2014; Weiss, 2017; Bento and Paula, 2018).

Two species of zoeae were identified as too abundant to be counted individually in the samples collected at the jetties from the Port Alfred Marina. As such, these samples were first sorted by removing and then identifying and counting all other species present in the sample. Then, any

debris found in the sample was also removed and discarded. The sample with the remaining two zoeae species were then sub-sampled using a Motodo plankton splitter (Motoda, 1959) until approximately 100 individuals of the most dominant amongst these two species had been enumerated (Shanks and Shearman, 2009).

2.2.4 Environmental variables

Temperature was recorded at each of the eight selected microhabitats over the entire duration of the sampling period (September 2019 to February 2020) using 27 mm EnvLoggers v2.4 (Electric Blue, Tools for Environmental Monitoring and Biologging) encapsulated in hard acrylic, which were enclosed in a casing constructed from a centrifuge tube and cable ties for deployment purposes (Figure 2.7). One EnvLogger was positioned within the immediate vicinity of the light trap deployment locations at each microhabitat and temperature data was downloaded on the third day of each sampling month. The loggers use Near Field Communication (NFC) technology to connect to any smartphone with the EnvLogger Viewer software/app by 'tapping' the logger with a phone (Figure 2.8). In addition, during the three sampling days each month, pH (Aqualytic) and salinity (Hanna Instruments) were also measured and recorded *in situ* using handheld portable devices when each trap was deployed on day one and retrieved on days two and three.

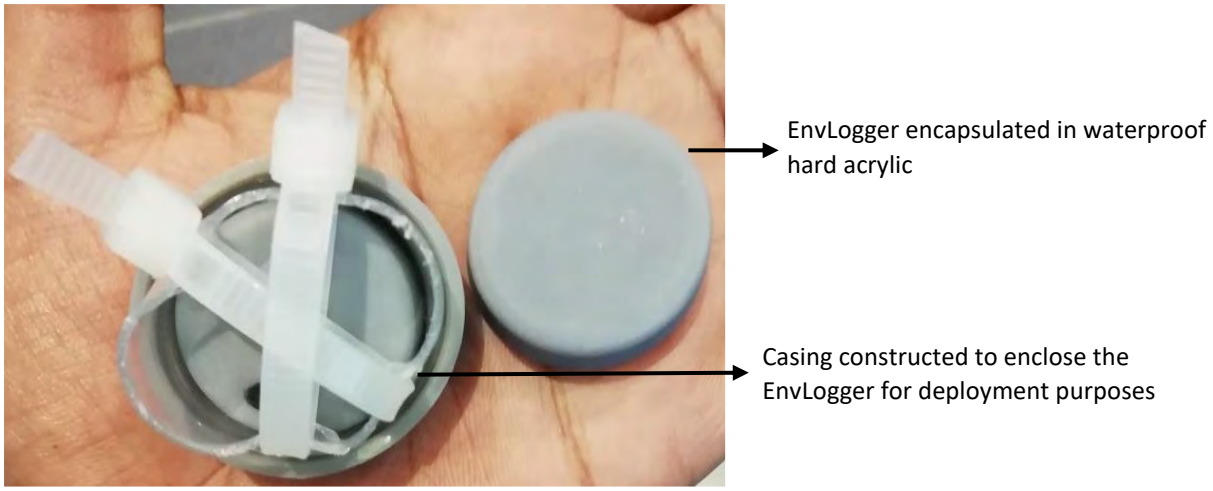


Figure 2.7. EnvLogger v2.4 used to record temperature at trap deployment locations.

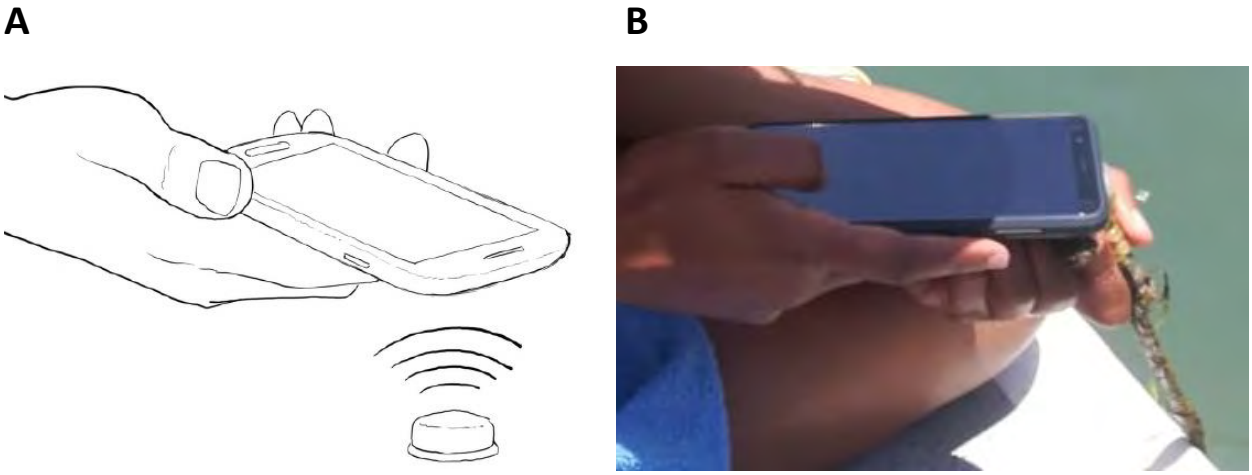


Figure 2.8. Connecting and downloading data from an EnvLogger by 'tapping' the logger with a smartphone (EnvLogger System, User manual rev. 11 June 2019).

2.2.5 Data analysis

2.2.5.1 Environmental data

The data collected for temperature, pH and salinity were analysed separately to explore possible differences among microhabitat and month at each study site using SigmaPlot 12.5. Normality of distributions and homogeneity of variances were tested using a Kolmogorov-Smirnov test and Levene's test, respectively. These tests showed a violation of the assumptions of either normality or both normality and homogeneity for the three environmental variables under analysis at both study sites. A Kruskal-Wallis test was hence conducted in place of the parametric tests to test for differences in temperature, pH and salinity among the factors microhabitat and month. Skewness of data make parametric tests less robust and powerful as the central measure of tendency is the mean, while performing non-parametric tests is more robust to non-normal and skewed distributions which are more accurately represented by the median (Pappas and DePuy, 2004; Grech and Calleja, 2018). Pairwise comparisons were thereafter conducted using Tukey post-hoc tests for the significant interaction between microhabitat and month.

2.2.5.2 Species assemblage structure

Multivariate statistical analysis and ordination plots were conducted to assess patterns in assemblage structure using PRIMER V6.1.16 with PERMANOVA+ V1.0.6. Permutational Multivariate Analysis of Variance (PERMANOVA) is widely used and accepted across fields such as ecology, as it allows for robust and flexible analysis of the dataset obtained (Anderson, 2001; Anderson et al., 2008; Anderson and Walsh, 2013). Flexibility arises from the ability to base the analysis on a resemblance measure with different options to choose from, while the robustness arises from distribution free inferences which is achieved by using permutations to obtain p-values (Anderson, 2001; Anderson et al., 2008; Anderson, 2014). This non-

parametric, rank based approach is therefore highly suitable for ecological data (such as measures of abundance of species) which are likely over-dispersed and zero-inflated (Anderson et al., 2008), which is the case for the data in the current study.

Prior to statistical analysis, data were log (x+1) transformed. This type of transformation is useful when data contain a high degree of variation, are extremely spread and contain zeros, which is generally the case for count data (McCune and Grace, 2002) and is suitable for the nature of the current study. Higher values are compressed, while lower values are spread by expressing values as orders of magnitude (McCune and Grace, 2002). This allows all species in the dataset (both common and rare) to have a balanced influence on the outcome of the analysis.

All statistical analyses conducted were based on a zero-adjusted Bray-Curtis resemblance matrix. The standard Bray-Curtis characterises cells as 'undefined' for two samples which contain no species (for count data, this would be denoted by zero), and for count data which contain an abundance of zeros (common for ecological datasets), this can result in a resemblance matrix which contains a surplus of 'undefined' cells which can prevent further meaningful analysis (Clarke et al., 2006; Clarke and Gorley, 2006). The zero-adjusted Bray-Curtis coefficient reduces this problem in an analogous manner by adding a constant of 1 of log (x+1) transformed data (to account for x=0), which leads to two samples which would be previously characterised as 'undefined' as being 100% similar (Clarke and Gorley, 2006). This is appropriate when samples have a value of zero for the same reason/cause, rather than from random occurrences (Clarke et al., 2006; Clarke and Gorley, 2006). Additionally, this zero-adjusted version has shown to have no effect on the regular functioning of the Bray-Curtis resemblance matrix when a moderate quantity of data is available for all samples (Clarke et al., 2006), which is the case for the current study data.

Separate statistical analyses were conducted for each broad taxonomic group (fish and invertebrates) as well as each sampling technique used (light traps and pump) for each case study (Kenton-on-Sea and Port Alfred Marina). Ordination plots derived from Principal Coordinates Analysis (PCO) were first constructed by microhabitat across months to visualise the assemblage structure. PERMANOVA analyses were then performed to test for significant main effects and interactions among the factors microhabitat (4 levels) and month (6 levels) using 999 permutations (permutation of residuals under a reduced model) and Type III sums of squares partitioning. To test if data for microhabitats and months differed in their within-type dispersion, a complementary analysis to test for homogeneity of multivariate dispersion (PERMDISP, Anderson, 2006) was conducted. Post-hoc pairwise tests were also carried out to test for significant differences between microhabitat and month when a significant interaction (p<0.05) microhabitat X month resulted from the main PERMANOVA. When the number of unique permutations were low (<100) and, therefore, would not provide a robust and reliable test result (Anderson et al., 2008), Monte Carlo simulations (Anderson and Robinson, 2003) were applied (Anderson et al., 2008).

A Similarity Percentages analysis (SIMPER) was performed to identify which taxa most contributed to the dissimilarities between microhabitat and month which showed significant differences (cut off criteria selected was 50% of the cumulative contribution of the dissimilarities). Although the cut-off criteria selected falls within the lower spectrum, it is a broadly and successfully applied limit for SIMPER analysis (e.g. Soto et al., 2017; Streich et al., 2017; Furness and Unsworth, 2020).

2.2.5.3 Environmental correlates of assemblage structure

Distance-based linear models (DistLM) were conducted to analyse the relationship between larval assemblage and the environmental data collected over the sampling period. This analysis

allows predictor variables to be fit individually or together in pre-determined sets (Anderson et al., 2008). Prior to running the model, Variance Inflation Factors (VIF) were used to detect for multicollinearity amongst environmental variables, with VIF values >2.5 suggesting high levels of multicollinearity (Graham, 2003; Senaviratna and Cooray, 2019). This was conducted to prevent autocorrelations amongst the predictor variables that could potentially reduce the power of the model being used. DistLM were based on 999 permutations with a step-wise selection procedure and Akaike Information Criterion (AIC) using the zero-adjusted Bray-Curtis resemblance matrices for invertebrate and fish larval assemblages respectively. The p-values (p<0.05) obtained by permutations from marginal tests were used to determine if there were significant correlations between the larval assemblage and each of the environmental variables (temperature, pH and salinity). Distance-based redundancy analysis (dbRDA) is a non-parametric multivariate regression analysis and the plot derived was used to visualise and provide a graphical representation of the relationship between significant environmental predictor variables and the larval assemblage.

2.3 Results

2.3.1 Port Alfred Marina

2.3.1.1 Environmental variables

Water temperature over the sampling period at the Port Alfred Marina ranged from 14.6 – 25.3 °C at the jetties (B and D) with an average of 19.9 °C, while the average at the vertical walls (Spithead and Francis Drake) was 18.3 °C with a range of 11.8 – 26.5 °C (Figure 2.9A). The pH ranged from 7.54 – 8.37 at the jetties with an average of 8.12 and 7.73 – 8.37 with an average of 8.15 at the vertical walls (Figure 2.9B). Salinity at the jetties ranged from 30 – 46 with an average of 37 and 30 – 48 with an average of 38 at the vertical walls (Figure 2.9C).

There was an overall significant effect of the interaction between microhabitat and month for the environmental variable temperature (Table 2.1). The outcome of the Tukey post-hoc tests for this significant effect revealed that during the months of September, November and December 2019, there were significant differences between the jetties (B and D) and vertical walls (Spithead and Francis Drake), with no intra-microhabitat differences (Figure 2.9A). In October 2019 and January 2020, the temperature at the jetties differed from records at the vertical walls, with further intra-microhabitat differences (jetties in October 2019 and vertical walls in January 2021). In February 2020, there were significant differences in temperature across each microhabitat (Figure 2.9A)

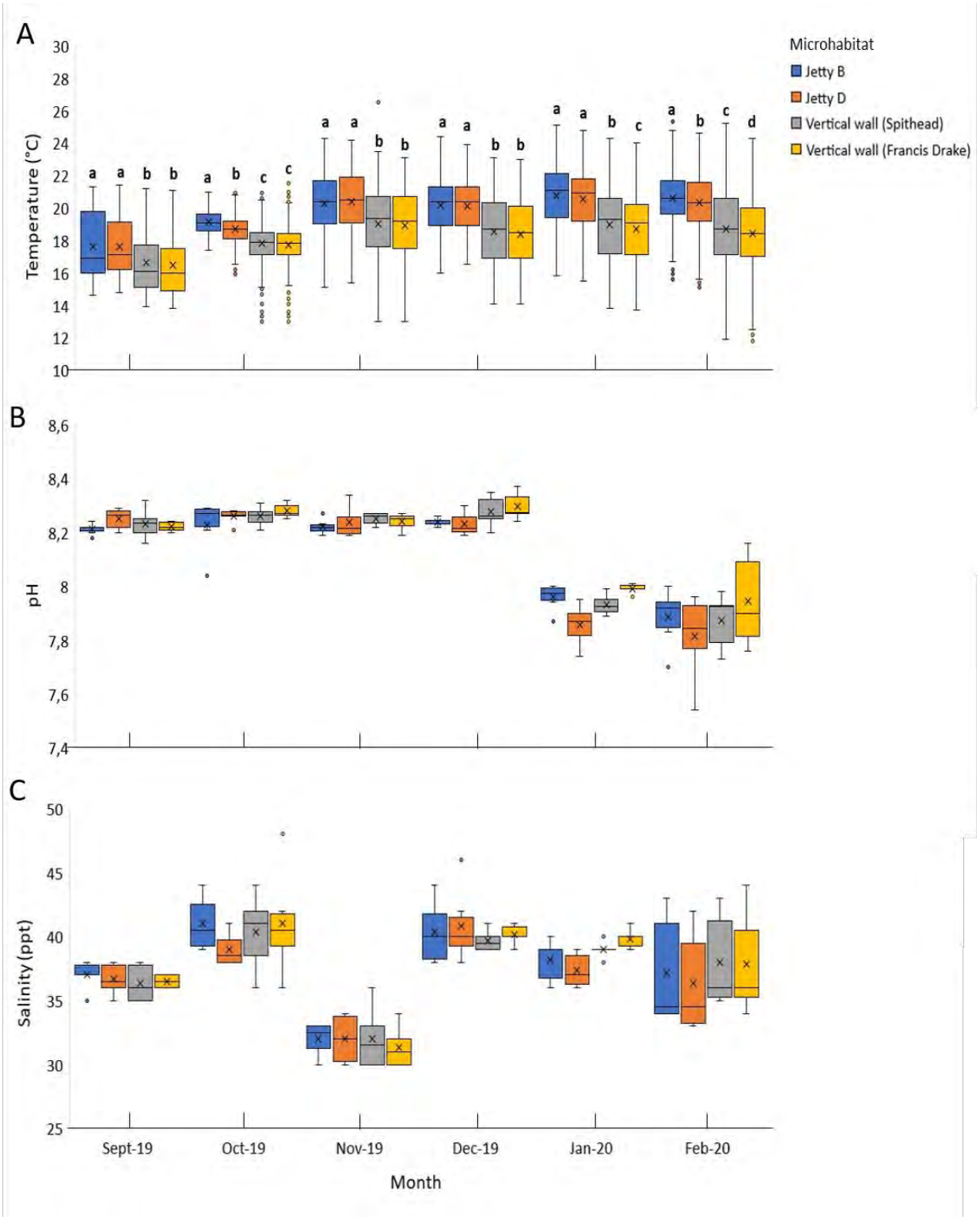


Figure 2.9. Categorised boxplots of (A) temperature; (B) pH; (C) salinity at the Port Alfred Marina for each microhabitat from September 2019 to February 2020. Quartiles 1 and 2 are represented by the lower and upper boundaries of each box, respectively. Horizontal lines within each box indicates the median and the '×' indicates the mean. Vertical lines or 'whiskers' represent the maximum and minimum values and the circles/dots show outliers. Letters displayed above boxplots indicate pairwise comparisons conducted using Tukey post-hoc tests for the significant interaction between microhabitat and month.

Table 2.1. Results from the Kruskal-Wallis test for the factors microhabitat and month which indicates the effects of single factors and the interaction between them for (a) temperature, (b) pH and (c) salinity at the Port Alfred Marina. The associated sum of square values (SS), degrees of freedom (df), F-values and p-values with significant results (p<0.05) shown in bold are reported.

Factor	df	SS	F	p-value
(a) Temperature				
Microhabitat (Mi)	3	7144,601	700,781	**<0,001**
Month (Mo)	5	8752,671	515,106	**<0,001**
Mi×Mo	15	433,708	8,508	**<0,001**
Residuals	15067	51203,651		
Total	15090	70886,256		
(b) pH				
Microhabitat (Mi)	3	0,0557	3,802	**0,012**
Month (Mo)	5	3,732	152,883	**<0,001**
Mi×Mo	15	0,0877	1,198	0,283
Residuals	120	0,586		
Total	143	4,462		
(c) Salinity				
Microhabitat (Mi)	3	11,354	0,648	0,586
Month (Mo)	5	1192,035	40,802	**<0,001**
Mi×Mo	15	43,438	0,496	0,939
Residuals	120	701,167		
Total	143	1947,993		

There were no significant differences in pH or salinity for the interaction between microhabitat and month at the Port Alfred Marina (Table 2.1b and c). There were, however, significant differences in pH among months and microhabitats (Table 2.1b), with lower pH values during the months of January and February 2020 (Figure 2.9B). In addition, there was a significant difference among months for salinity (Table 2.1c), with lower salinity readings during November 2019 as compared to the other months (Figure 2.9C).

2.3.1.2 Larval abundance and distribution amongst microhabitats

Overall, the total number of invertebrates collected in the light traps at the Port Alfred Marina across all microhabitats sampled were 2 728 162 and was made up of 20 taxa, with the zoea of the *Pinnotheres* sp. (pea crab) being the most common across both microhabitats (Table 2.2).

The total number of invertebrate larvae collected at the jetties (B and D) was 2 725 295, comprising of 16 taxa (Table 2.2), of which the zoea of the *Pinnotheres* sp. was the most abundant, contributing 94% of the total invertebrates collected, followed by the zoea of the crown crab, *Hymenosoma orbiculare* (4.5%) (Table 2.2). Jetty D had the highest number of *Pinnotheres* sp. zoea, making up 84% of the total 94% collected at both jetties B and D (Figure 2.10). At the vertical walls (Spithead and Francis Drake), a total of 2 867 invertebrates were collected, comprising of 17 taxa, with the zoea of *Pinnotheres* sp. being the most common (27.6%), followed closely by the megalopa of the same genus (25.9%) and the cyprid of *Cirripedia* sp. (23.9%) (Table 2.2). The highest number of the zoea of *Pinnotheres* sp. was collected at Spithead (17.6%), while the dominant species collected at Francis Drake was the megalopa of the same genus (21.9%) (Table 2.2).

Table 2.2. Invertebrate larval abundance by total number per species, per microhabitat and as a percentage (%) of the total catch collected (September 2019 to February 2022) in the light traps at the Port Alfred Marina.

			Jetties				Vertical walls			
Developmental stage	**Family**	**Taxon**	**Total counts**	**Jetty B %**	**Jetty D %**	**Total %**	**Total counts**	**Spithead %**	**Francis Drake %**	**Total %**
Zoea	Pinnotheridae	*Pinnotheres* sp.	2561697	9,6152	84,3818	93,9971	792	17,58	10,05	27,62
	Pinnotheridae	*Pinnixa* sp.	1284	0,0096	0,0375	0,0471	3	0,07	0,03	0,10
	Hymenosomatidae	*Hymenosoma orbiculare*	123968	1,0604	3,4884	4,5488	237	1,74	6,52	8,27
	Plagusiidae	*Guinusia chabrus*	5808	0,1105	0,1026	0,2131	16	0,07	0,49	0,56
	Upogebiidae	*Upogebia africana*	1182	0,0102	0,0332	0,0434	0	0	0	0
	Palaemonidae	*Palaemon pacificus*	1362	0,0301	0,0199	0,0500	1	0	0,03	0,03
	Xanthidae		13913	0,1929	0,3176	0,5105	16	0,28	0,28	0,56
	Porcellanidae		15	0,0004	0,0001	0,0006	0	0	0	0
	Leucosiidae		171	0,0026	0,0037	0,0063	23	0,14	0,66	0,80
Megalopa	Pinnotheridae	*Pinnotheres* sp.	1692	0,0348	0,0273	0,0621	742	3,98	21,9	25,88
	Hymenosomatidae	*Hymenosoma orbiculare*	862	0,0137	0,0179	0,0316	186	2,65	3,84	6,49
	Camptandriidae	*Danielella edwardsii*	641	0,0111	0,0124	0,0235	104	0,87	2,76	3,63
	Varunidae	*Cyclograpsus punctatus*	11	0,0003	0,0001	0,0004	3	0,07	0,03	0,10
	Diogenidae	*Diogenes brevirostris*	9	0,0001	0,0002	0,0003	0	0	0	0
	Portunidae		5	0,0001	0,0001	0,0002	23	0,31	0,49	0,80
	Leucosiidae		93	0,0019	0,0015	0,0034	23	0,07	0,73	0,80
	Polybiidae		0	0	0	0	1	0	0,03	0,03
		Unidentified megalopa sp.1	0	0	0	0	3	0	0,1	0,10
		Unidentified megalopa sp.2	0	0	0	0	2	0	0,07	0,07
		Unidentified megalopa sp.3	2	0	0,0001	0,0001	0	0	0	0
		Unidentified megalopa sp.4	0	0	0	0	5	0	0,17	0,17
		Unidentified megalopa sp.5	0	0	0	0	1	0	0,03	0,03
Cyprid		*Cirripedia*	12580	0,2116	0,2500	0,4616	686	8,55	15,38	23,93

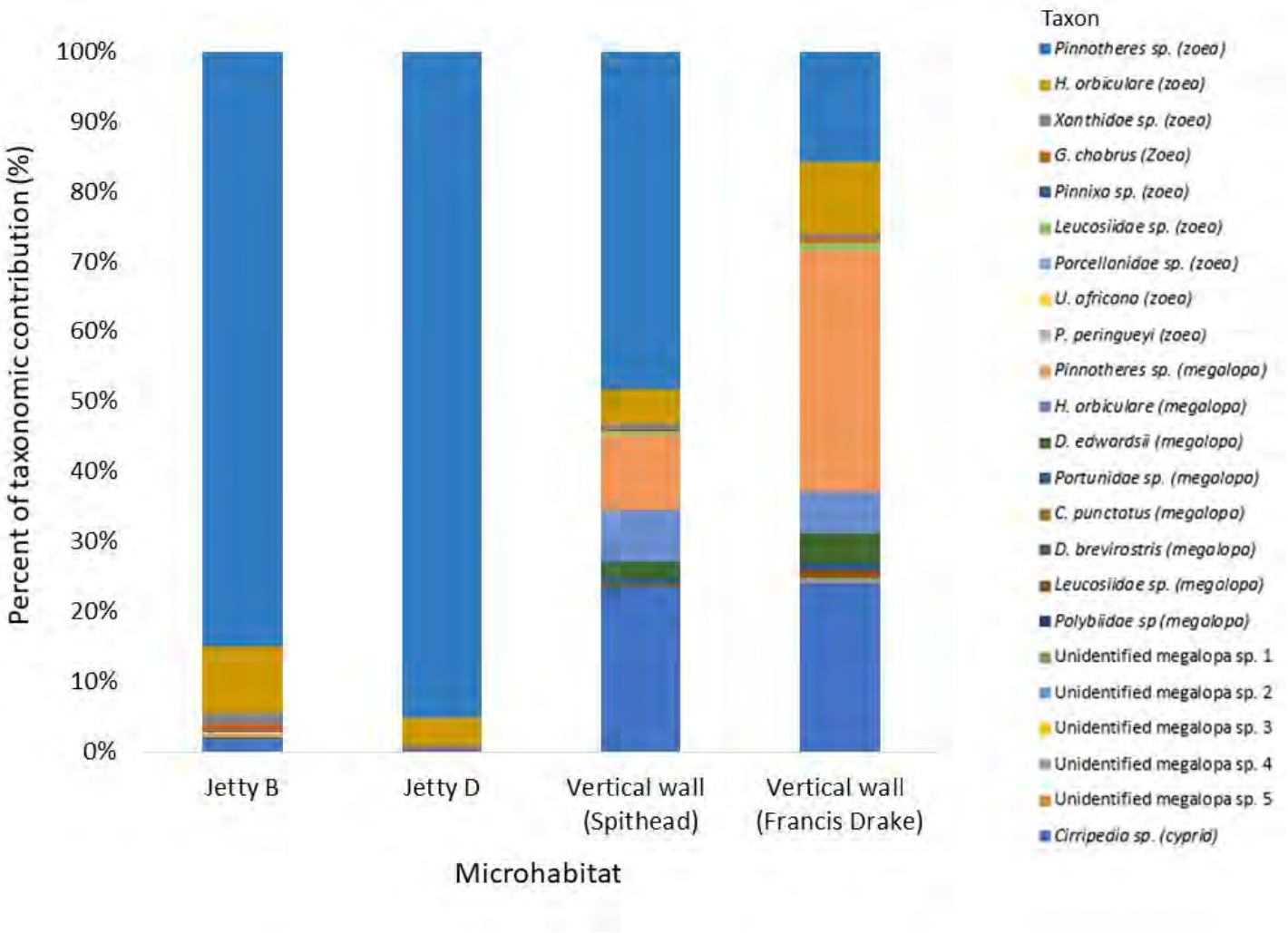


Figure 2.10. Percentage contribution of invertebrate larval species collected in light traps at the Port Alfred Marina among the selected microhabitats from September 2019 to February 2020. Developmental stages of species are indicated in brackets.

Overall, the total number of fish larvae collected in the light traps at the Port Alfred Marina across all microhabitats sampled was 712, comprising 18 taxa (Table 2.3). At the jetties (B and D), the total number of fish larvae sampled was 200, with the kappie blenny, *Omobranchus woodi* (preflexion), the round herring, *Etrumeus whiteheadi* (postflexion) and gobies, *Caffrogobius* sp. (preflexion) being the most dominant, making up 28%, 27.5% and 18.5% of the total fish larvae sampled, respectively (Figure 2.11). Jetty B was dominated by *E. whiteheadi*, while *O. woodi* was most common at jetty D. At the vertical walls (Spithead and

Francis Drake), 512 fish larvae were collected, of which *E. whiteheadi* and the European anchovy, *Engraulis encrasicolus* (both postflexion) were the most common, comprising 56.6% and 34.6% of the total fish larvae sampled, respectively, with Francis Drake making up majority of the catch of these species (Figure 2.11).

Table 2.3. Fish larval abundance by total number per species, per microhabitat and as a percentage (%) of the total catch collected (September 2019 to February 2020) in the light traps at the Port Alfred Marina.

			Jetties				Vertical walls			
Developmental stage	**Family**	**Taxon**	**Total counts**	**Jetty B %**	**Jetty D %**	**Total %**	**Total counts**	**Spithead %**	**Francis Drake %**	**Total %**
Postflexion	Sparidae	*Diplodus capensis*	6	1,5	1,5	3	7	0,4	1	1,4
	Sparidae	*Rhabdosargus holubi*	10	1,5	3,5	5	0	0	0	0
	Dussumieriidae	*Etrumeus whiteheadi*	55	22	5,5	27,5	290	2,9	53,7	56,6
	Engraulidae	*Engraulis encrasicolus*	1	0,5	0	0,5	177	0,2	34,4	34,6
	Clupeidae	*Sardinops sagax*	1	0,5	0	0,5	0	0	0	0
	Haemulidae	*Plectorhinchus chubbi*	1	0,5	0	0,5	30	0,0	5,9	5,9
	Monodactylidae	*Monodactylus falciformis*	1	0,5	0	0,5	0	0	0	0
	Samaridae	*Samariscus triocellatus*	1	0	0,5	0,5	0	0	0	0
	Clinidae		0	0	0	0	1	0,2	0	0,2
	Trichiuridae	*Trichiurus lepturus*	1	0	0,5	0,5	0	0	0	0
	Blenniidae	*Parablennius sp.*	1	0,5	0	0,5	0	0	0	0
	Soleidae	*Solea turbynei*	1	0	0,5	0,5	0	0	0	0
	Gobiesocidae		1	0	0,5	0,5	0	0	0	0
		Unidentified fish sp.1	0	0	0	0	3	0	0,6	0,6
Flexion	Atherinidae	*Atherina breviceps*	16	5,5	2,5	8	0	0	0	0
Preflexion	Blenniidae	*Omobranchus woodi*	56	10	18	28	3	0	0,6	0,6
	Gobiidae	*Caffrogobius sp.*	37	11,5	7	18,5	1	0,2	0	0,2
	Gobiidae	*Psammogobius knysaensis*	11	1	4,5	5,5	0	0	0	0

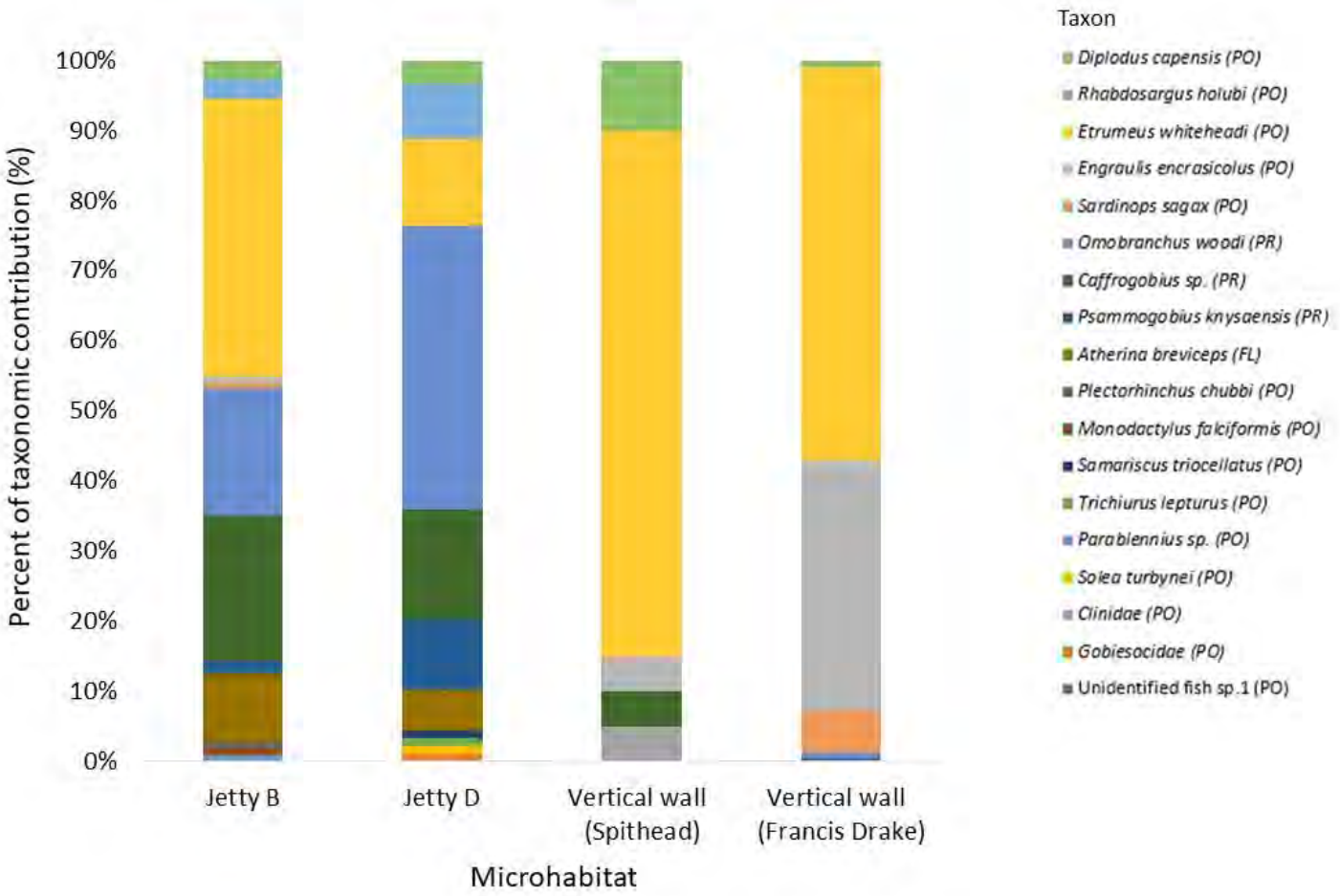


Figure 2.11. Percentage contribution of fish larval species collected in light traps at the Port Alfred Marina among the selected microhabitats from September 2019 to February 2020. Developmental stages of species are indicated in brackets (PO: Postflexion, F: Flexion, PR: Preflexion).

Overall, the total number of invertebrates collected using the pump at the Port Alfred Marina across all microhabitats sampled were 72 (with 47 collected at the jetties and 25 collected at the vertical walls) and were made up of five taxa (Table 2.4). Three of these taxa were only collected using the pump method and not collected in light traps, namely: Ascidiacea (planktonic larva), *Cirripedia* sp. (nauplius) and Bivalvia (veliger). The most abundant taxon collected at the jetties (B and D) was the *Cirripedia* sp. (nauplius), which was most dominant at Jetty D. At the vertical walls (Spithead and Francis Drake), *Cirripedia* sp. (cyprid) were the most abundant, which dominated at Francis Drake (Figure 2.12).

Table 2.4. Invertebrate larval abundance by total number per species, per microhabitat and as a percentage (%) of the total catch collected (September 2019 to February 2020) using a rechargeable pump at the Port Alfred Marina.

			Jetties				Vertical walls			
Developmental stage	**Family**	**Taxon**	**Total counts**	**Jetty B %**	**Jetty D %**	**Total %**	**Total counts**	**Spithead %**	**Francis Drake %**	**Total %**
Zoea	Pinnotheridae	*Pinnotheres* sp.	2	2,13	2,13	4,26	0	0	0	0
	Upogebiidae	*Upogebia africana*	0	0	0	0	2	0	8	8
Veliger		Bivalvia	0	0	0	0	5	12	8	20
Nauplius		*Cirripedia*	36	8,51	68,09	76,60	0	0	0	0
Planktonic larvae		Ascidiacea	3	2,13	4,26	6,39	3	8	4	12
Cyprid		*Cirripedia*	6	2,13	10,64	12,77	15	20	40	60

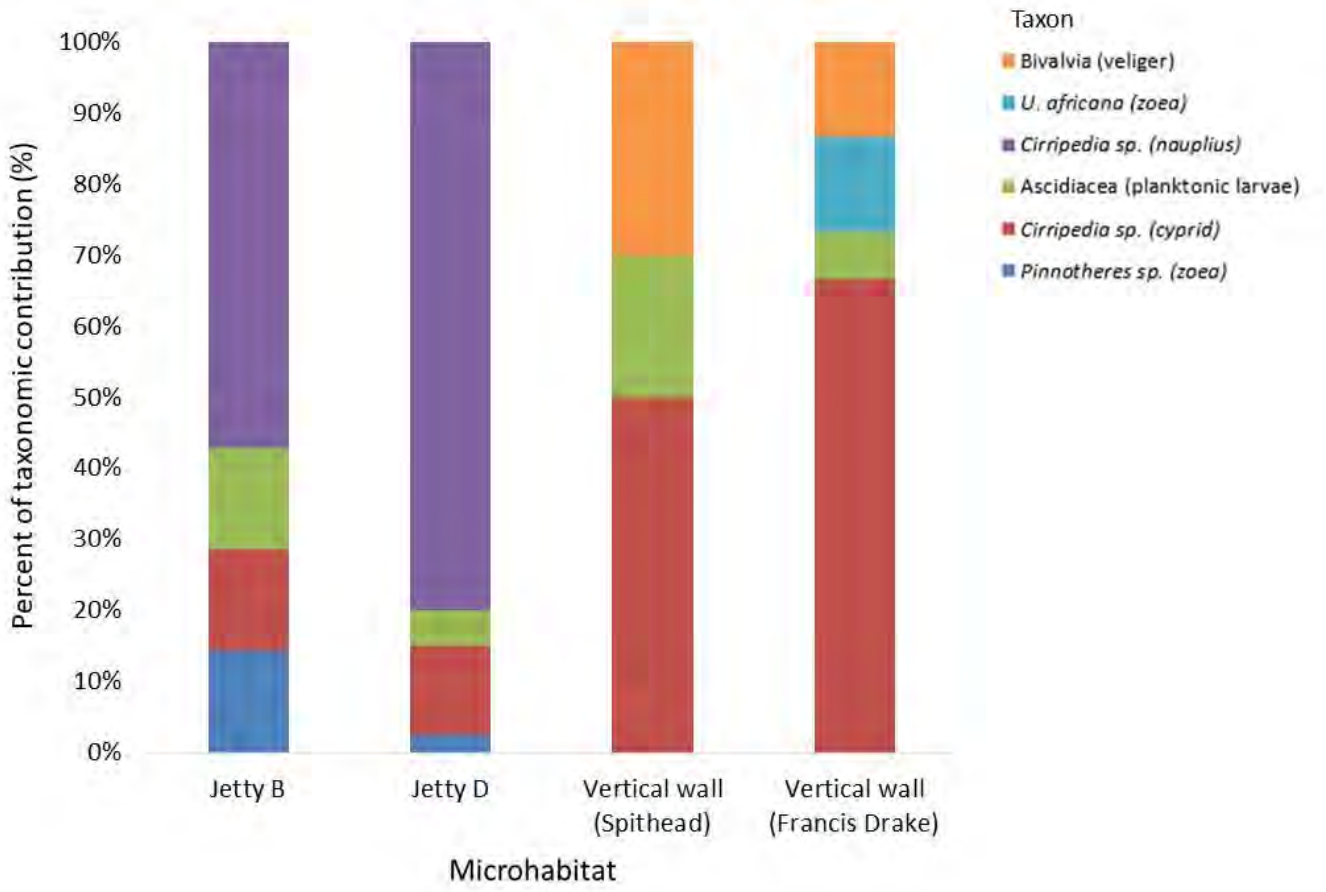


Figure 2.12. Percent contribution of invertebrate larval species collected using a rechargeable pump at the Port Alfred Marina among the selected microhabitats from September 2019 to February 2020. Developmental stages of species are indicated in brackets.

Initial visualisation of the invertebrate and fish larval assemblages collected in light traps by ordination plots indicate separation of the jetties (B and D, which pooled together) from the vertical walls (Spithead and Francis Drake, more scattered than jetties), with 67.5% and 65.8% of the total variation being explained for the invertebrate and fish larval assemblage data, respectively (Figures 2.13A and B). For the invertebrate larval assemblage collected using the pump, visualisation of data is less clear, with overlap between the microhabitat samples, which is likely due to the presence of many zeros and generally low numbers in the dataset. Over 50%

of the variability of data is, however, explained by the x and y axes, with some separation of jetties B and D from the rest of the sampled microhabitats (Figure 2.13C).

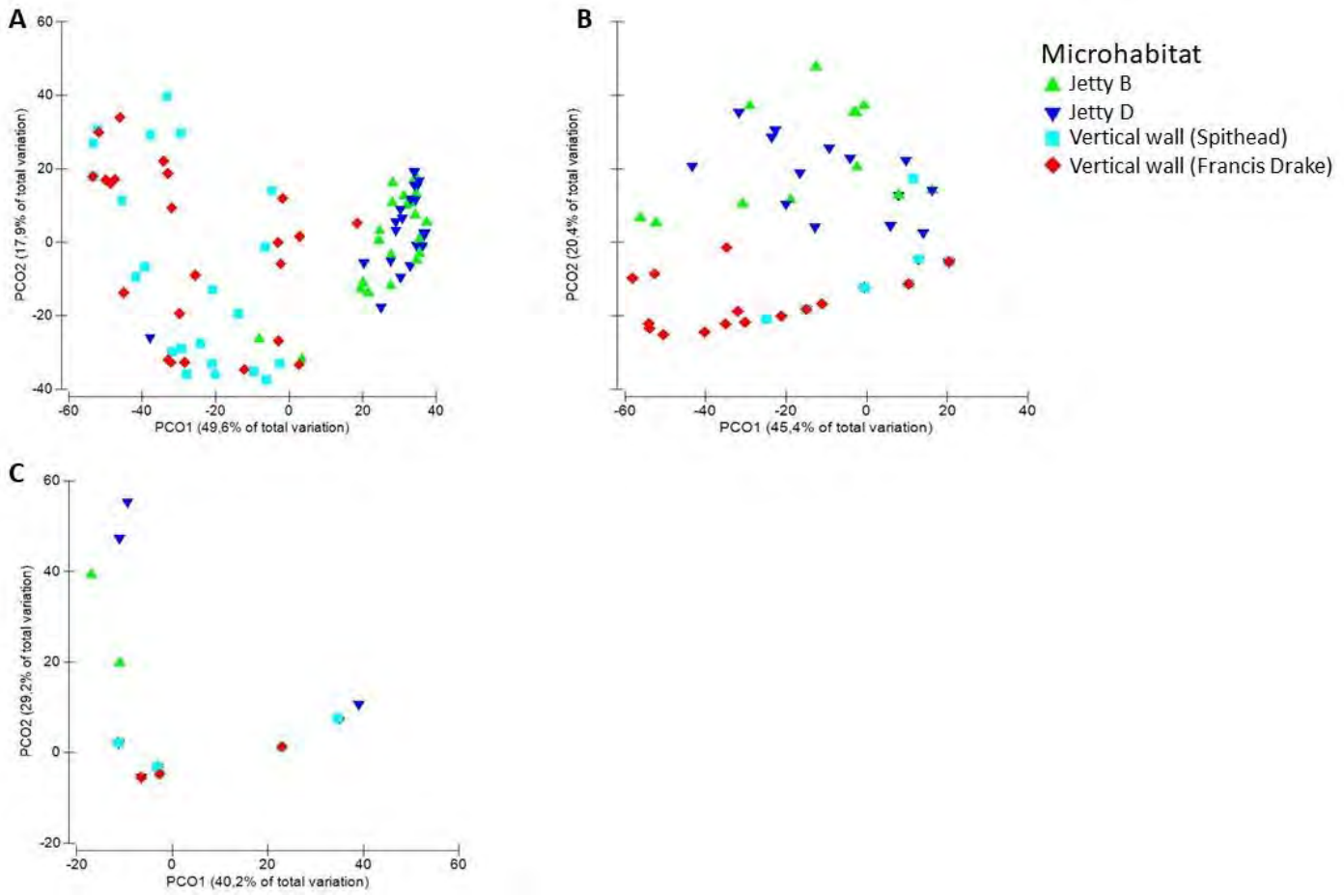


Figure 2.13. Ordination plots derived from Principal Coordinates Analysis (PCO) of (A) invertebrate larval assemblages collected in light traps, (B) fish larval assemblages collected in light traps and (C) invertebrate larval assemblages collected using a rechargeable pump at the Port Alfred Marina among the selected microhabitats from September 2019 to February 2020.

Results from the PERMANOVA (with complementary PERMDISP to assess heterogeneity of data) indicate significant differences (p<0.05) among microhabitats and months, as well as significant interactions between microhabitat and month in both invertebrate and fish larval assemblages collected using the light traps and invertebrates collected by the pump (Table 2.5). In general, results of PERMDISP show significant differences (p<0.05) for microhabitats

within a given month, except among microhabitats for invertebrate larvae collected using the pump (Table 2.5). Even if this shows some degree of heterogeneity, with dispersion within each group (centroid), PERMANOVA is a robust test, especially given the independent and balanced nature of the data (Anderson 2005).

Table 2.5. Results of the PERMANOVA and PERMDISP for the factors microhabitat and month indicating the effects of single factors and their interaction of Log (x+1) transformed species abundance data of (a) invertebrate and (b) fish larvae collected in light traps and (c) invertebrate larvae collected using a pump at the Port Alfred Marina. The associated degrees of freedom (df), sum of square values (SS), Pseudo-F values, unique permutations and p-values with significant results (p<0.05) shown in bold are reported.

	PERMANOVA					PERMDISP	
Factors	**df**	**SS**	**Pseudo-F**	**P-value**	**Unique perms**	**F**	**p-value**
(a) Invertebrates (Light trap)							
Microhabitat (Mi)	3	80898	37.068	**0.001**	997	46.752	**0.001**
Month (Mo)	5	37375	10.275	**0.001**	999	9.168	**0.001**
Mi×Mo	15	34951	3.203	**0.001**	998		
Residuals	72	52370					
Total	95	2.056E5					
(b) Fish (Light trap)							
Microhabitat (Mi)	3	17623	7.630	**0.001**	998	16.115	**0.001**
Month (Mo)	5	23003	5.984	**0.001**	998	12.800	**0.001**
Mi×Mo	15	21457	1.861	**0.002**	997		
Residuals	72	55360					
Total	95	1.174E5					
(c) Invertebrates (Pump)							
Microhabitat (Mi)	3	1466.8	1.849	**0.039**	997	1.337	0.545
Month (Mo)	5	3084.1	2.332	**0.003**	999	12.021	**0.001**
Mi×Mo	15	6496.1	1.637	**0.005**	999		
Residuals	72	19044					
Total	95	30091					

For the invertebrate larvae collected in light traps, pairwise comparisons indicated that during the months of October 2019, December 2019, January 2020 and February 2020, the jetties (B and D) were significantly different to the vertical walls (Spithead and Francis Drake) and there

were significant differences across all microhabitats except between the vertical walls in September 2019 and the jetties in November 2019 (Table 2.6a). For the invertebrate larvae collected using the pump, there was only a significant difference during the month of November 2019 between jetty D and Francis Drake (Table 2.6c). For the fish larvae, within the month of October 2019, the vertical walls (Spithead and Francis Drake) were significantly different from each other, and from jetty D, as well as jetty B being significantly different from Francis Drake, while in November 2019 the jetties (B and D) were significantly different to the vertical walls (Spithead and Francis Drake) (Table 2.6b).

Table 2.6. Pairwise comparisons between microhabitat and month for (a) invertebrate and (b) fish larvae collected in light traps and (c) invertebrate larvae collected using a pump at the Port Alfred Marina. Monte Carlo p-values are reported (p-value (MC)) due to low number of unique permutations. N.S – Not significant, * - Significant (p<0.05).

		Light trap				Pump	
		Invertebrates (a)		Fish (b)		Invertebrates (c)	
	Post hoc pairwise comparisons of microhabitats	**Unique perms**	**p-value (MC)**	**Unique perms**	**p-value (MC)**	**Unique perms**	**p-value (MC)**
Sept-19	Jetty B vs Jetty D	35	*	6	N.S	N/A	N.S
	Jetty B vs Spithead	35	*	3	N.S	N/A	N.S
	Jetty B vs FDrake	35	*	5	N.S	N/A	N.S
	Jetty D vs Spithead	35	*	6	N.S	N/A	N.S
	Jetty D vs FDrake	35	*	6	N.S	N/A	N.S
	Spithead vs FDrake	35	N.S	2	N.S	N/A	N.S
Oct-19	Jetty B vs Jetty D	35	N.S	35	N.S	11	N.S
	Jetty B vs Spithead	35	*	18	N.S	8	N.S
	Jetty B vs FDrake	35	*	35	*	6	N.S
	Jetty D vs Spithead	35	*	25	*	6	N.S
	Jetty D vs FDrake	35	*	35	*	4	N.S
	Spithead vs FDrake	35	N.S	25	*	3	N.S
Nov-19	Jetty B vs Jetty D	35	N.S	35	N.S	5	N.S
	Jetty B vs Spithead	35	*	18	*	2	N.S
	Jetty B vs FDrake	35	*	35	*	7	N.S
	Jetty D vs Spithead	35	*	18	*	5	N.S
	Jetty D vs FDrake	35	*	35	*	11	*
	Spithead vs FDrake	35	*	11	N.S	7	N.S
Dec-19	Jetty B vs Jetty D	35	N.S	13	N.S	N/A	N.S
	Jetty B vs Spithead	25	*	18	N.S	N/A	N.S
	Jetty B vs FDrake	25	*	18	N.S	2	N.S
	Jetty D vs Spithead	25	*	18	N.S	N/A	N.S
	Jetty D vs FDrake	25	*	18	N.S	2	N.S
	Spithead vs FDrake	18	N.S	18	N.S	2	N.S
Jan-20	Jetty B vs Jetty D	35	N.S	3	N.S	N/A	N.S
	Jetty B vs Spithead	35	*	2	N.S	4	N.S
	Jetty B vs FDrake	35	*	3	N.S	N/A	N.S
	Jetty D vs Spithead	35	*	3	N.S	4	N.S
	Jetty D vs FDrake	35	*	6	N.S	N/A	N.S
	Spithead vs FDrake	25	N.S	3	N.S	4	N.S
Feb-20	Jetty B vs Jetty D	35	N.S	8	N.S	2	N.S
	Jetty B vs Spithead	35	*	4	N.S	2	N.S
	Jetty B vs FDrake	35	*	8	N.S	4	N.S
	Jetty D vs Spithead	35	*	3	N.S	4	N.S
	Jetty D vs FDrake	35	*	8	N.S	11	N.S
	Spithead vs FDrake	35	N.S	4	N.S	6	N.S

The SIMPER results revealed that the invertebrate larval species (collected using the light traps) which contributed most consistently to the dissimilarity among microhabitats across all months was, overall, the zoea of *Pinnotheres* sp., however, during the month of September, the zoea of *H. orbiculare* contributed a higher percentage of the dissimilarity between jetty B versus Spithead and Francis Drake (Figure 2.14). During the month of January 2020, *Cirripedia* sp. showed an increase in its contribution to the dissimilarity between Jetty D versus Spithead and Francis Drake (Figure 2.14). Overall, the zoeal stages of *Pinnotheres* sp., *H. orbiculare*, the rock crab, *Guinusia chabrus* and Xanthidae sp. contributed most to the dissimilarity between jetties and vertical walls (Figure 2.14). During September 2019, the zoeal stages of shrimps, *Upogebia africana* and *Palaemon peringueyi* drove the differences between jetties B and D, while in November 2019, the megalopal stage of *Pinnotheres* sp. contributed most to the dissimilarity between the vertical walls Spithead and Francis Drake (Figure 2.14).

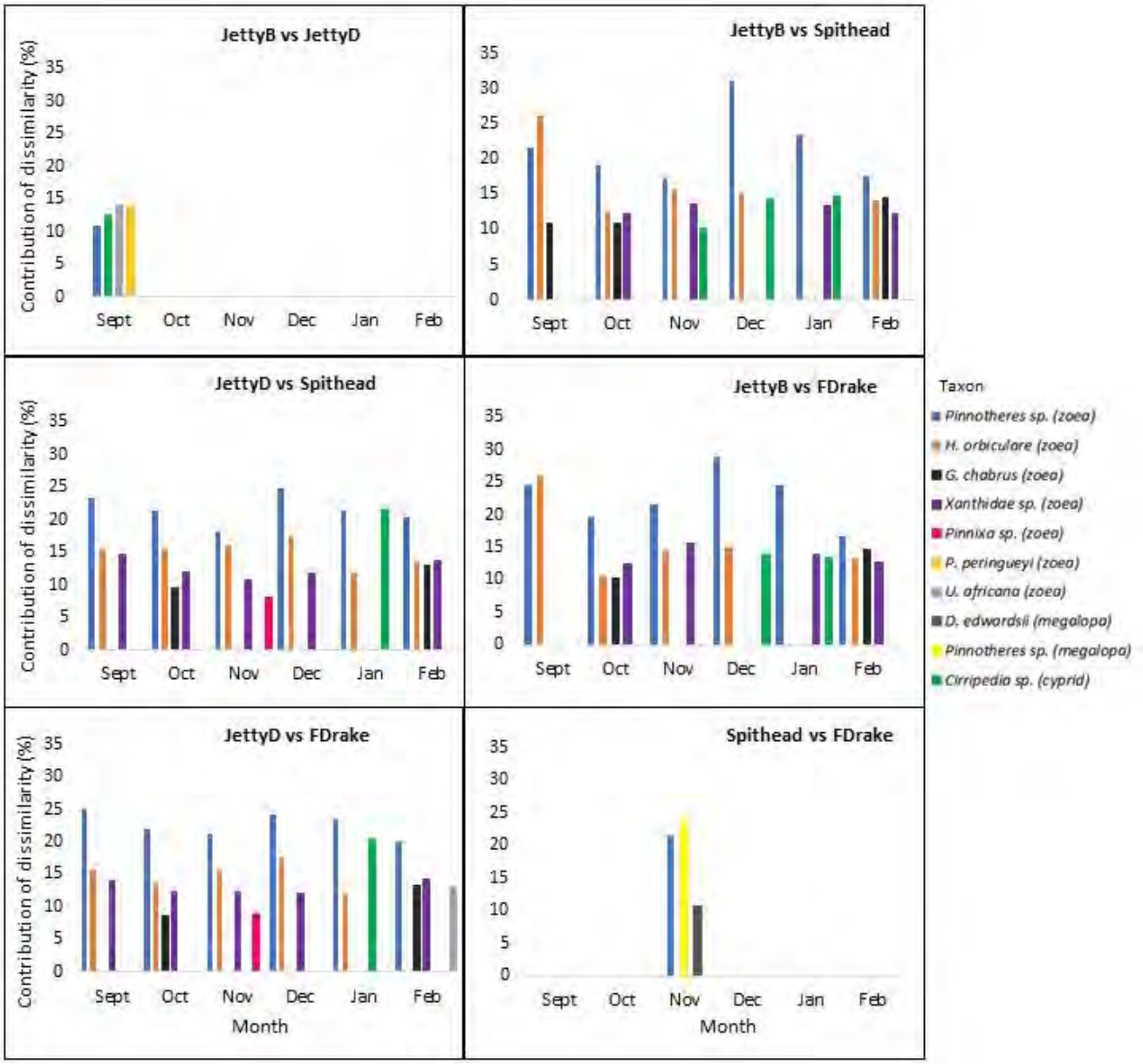


Figure 2.14. Results of SIMPER analyses of significant PERMANOVA pairwise tests (of the microhabitat X month significant interactions) indicating invertebrate larval taxa (collected using light traps) contributing most (50% cut-off) to the dissimilarity among significantly different microhabitats and month at the Port Alfred Marina. Developmental stages of species are indicated in brackets.

For the fish larvae (collected using the light traps), the SIMPER analysis revealed that four taxa contributed most to the dissimilarities between microhabitats during the months of October and November 2019 (Figure 2.15). During October, differences of jetties B and D versus Francis

Drake as well as between Spithead and Francis Drake were driven by *E. whiteheadi* and *E. encrasicolus*, while *O. woodi* and *E. whiteheadi* drove changes between jetty D and Spithead (Figure 2.15). During November, *O. woodi* and *Caffrogobius* sp. contributed most to the dissimilarities between Spithead and jetties B and D respectively, while *O. woodi* and *E. whiteheadi* contributed most to the dissimilarities between Francis Drake and jetties B and D, respectively (Figure 2.15).

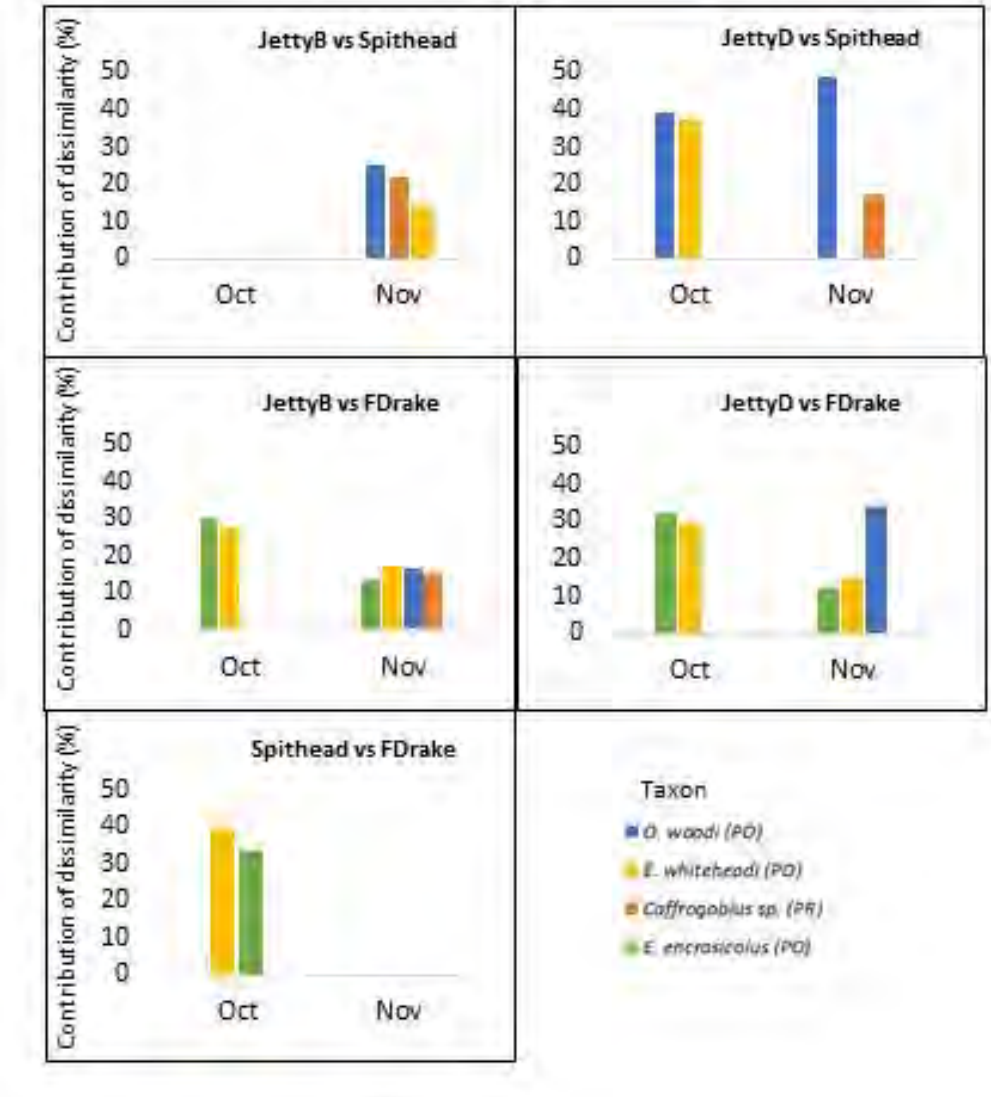


Figure 2.15. Results of SIMPER analyses of significant PERMANOVA pairwise tests (of the microhabitat X month significant interactions) indicating fish larval taxa (collected using light traps) contributing most (50% cut-off) to the dissimilarity among significantly different microhabitats and month at the Port Alfred Marina. Developmental stages of species are indicated in brackets (PO: Postflexion, F: Flexion, PR: Preflexion.

2.3.1.3 Environmental variables and correlation with larval assemblage structure

Multicollinearity VIF values for all environmental variables (temperature, salinity and pH) were <2.5, therefore, all were included in the succeeding analysis. The results of the Marginal Tests from the DistLM revealed significant correlations (p<0.05) of the environmental variables temperature and pH and the invertebrate and fish larval assemblage collected using the light trap (Table 2.7a and b). Additionally, tests indicated that there were no significant correlations with environmental variables and the invertebrate larval assemblage collected using the pump (Table 2.7c). The dbRDA plot for the invertebrate larvae collected using the light traps and the environmental predictor variables explained 17.2% of the total variation, with temperature and pH shown to be positive predictors of the jetty assemblage as well as the vertical walls assemblage, although some samples from the vertical walls falling outside the range of the environmental predictors (negative relationship) (Figure 2.16A). For the fish larvae collected using the light traps, temperature and pH was shown to have a positive relationship on the majority of the fish larval assemblage collected at the jetty, with assemblages from the vertical walls mainly having a negative relationship to both these environmental variables. The total variation explained by the vectors, however, was only 11% (Figure 2.16B).

Table 2.7. Results of Marginal Tests from the DistLM indicating the correlations between (a) invertebrate and (b) fish larvae collected in light traps and (c) invertebrate larvae collected using a pump and each of the environmental variables at the Port Alfred Marina. Corresponding R^2 and AIC values are presented for each group and the associated degrees sum of square values (SS), Pseudo-F values and p-values obtained by permutation with significant results (p<0.05) shown in bold are also reported.

Variable	SS	Pseudo-F	p-value
(a) Invertebrates (Light trap) (R^2 = 0.172, AIC = 724.18)			
Temperature	26370	13.83	**0.001**
pH	7651.8	3.63	**0.01**
Salinity	1556.6	0.72	0.553
(b) Fish (Light trap) (R^2 = 0.110, AIC = 677.27)			
Temperature	6251.1	5.29	**0.001**
pH	5718	4.81	**0.002**
Salinity	1152.9	0.93	0.451
(c) Invertebrates (Pump) (R^2 = 0, AIC = 553.77)			
Temperature	114.18	0.36	0.834
pH	494.19	1.57	0.147
Salinity	186.93	0.59	0.681

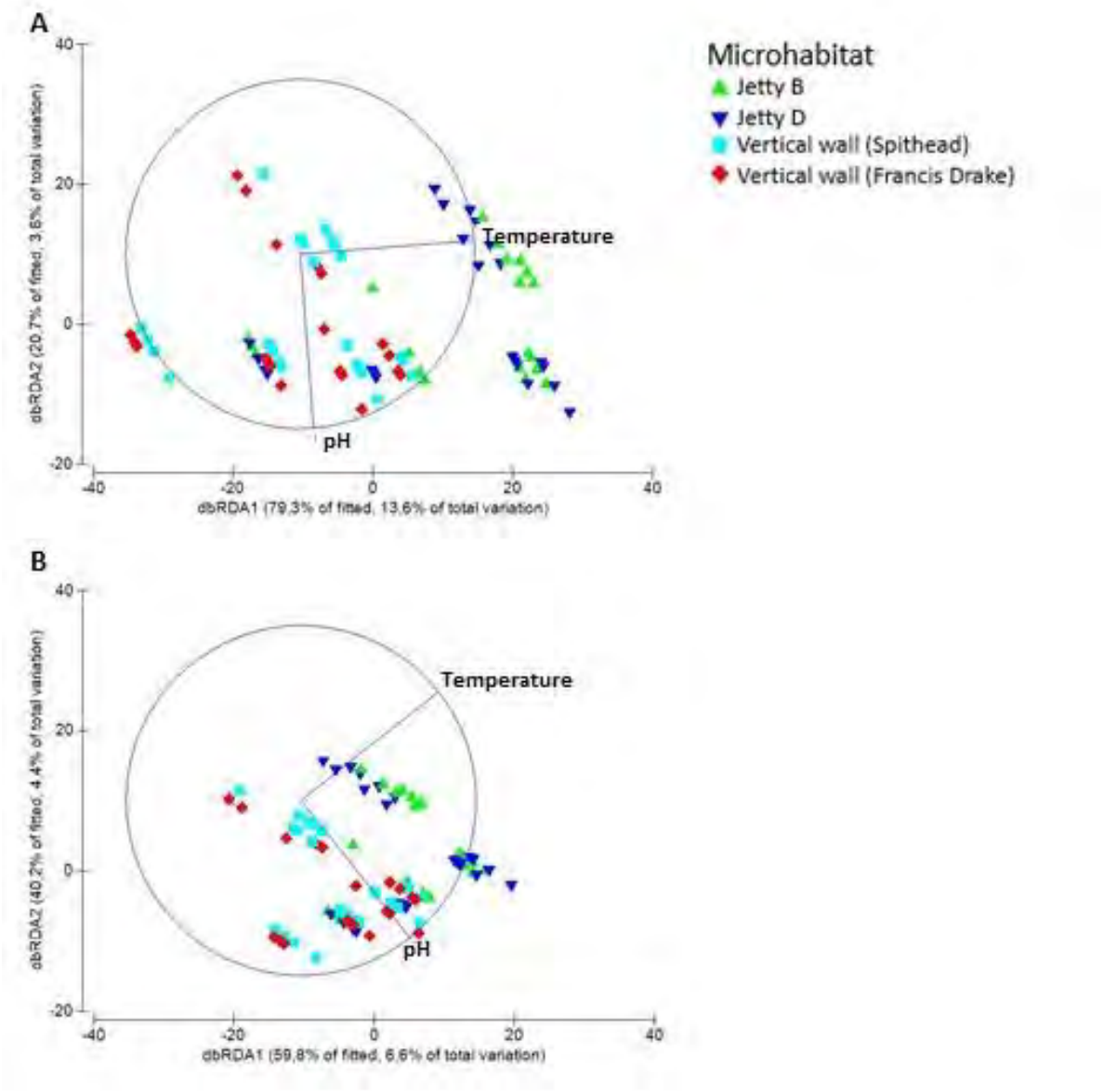


Figure 2.16. Plots derived from the Distance-based redundancy analysis (dbRDA) indicating the relationship between environmental variables which significantly correlated with (A) invertebrate and (B) fish larval assemblage structure collected in light traps at the Port Alfred Marina. The length and direction of vectors indicate the direction and strength of the relationship between the environmental predictor variables and the larval assemblage.

2.3.2 Kenton-on-Sea

2.3.2.1 Environmental variables

Water temperature over the sampling period at Kenton-on-Sea ranged from 12.7 – 37.4 °C at the gullies (Sydney's Hope and Shelley Bay) with an average of 18.3 °C, while the average at

the rock pools (large and small) was 18.4 °C with a range of 13.5 – 38.5 °C (Figure 2.17A). The high occurrence of outliers in the temperature dataset could be a result of loggers being thrown out of the water column during periods of extreme hot weather. It is therefore possible that higher temperature records may be due to loggers being exposed to the sun on the rocks for a period of time before being pushed back into the water column. The pH ranged from 7.63 – 8.46 at the gullies with an average of 8.21 and 7.40 – 8.77 with an average of 8.23 at the rock pools (Figure 2.17B). Salinity at the gullies ranged from 29 – 45 with an average of 36 and 29 – 45 with an average of 37 at the rock pools (Figure 2.17C).

There was an overall significant effect of the interaction between microhabitat and month for the environmental variable temperature (Table 2.8). The outcome of the Tukey post-hoc tests for this significant effect revealed that during the month of October 2019, the large rock pool was significantly different from the small rock pool and both the gullies, while in December 2019, Sydney's Hope gully was different from Shelley Bay gully and both the rock pools (Figure 2.17A). During January 2020, there were intra-microhabitat differences within the gullies and rock pools along with inter-microhabitat differences between the gullies and rock pools (Figure 2.17A). In February 2020, the temperature at the two gullies significant differed, with further inter-microhabitat differences between Sydney's Hope gully and the small rock pool as well between the large rock pool and Shelley Bay gully (Figure 2.17A).

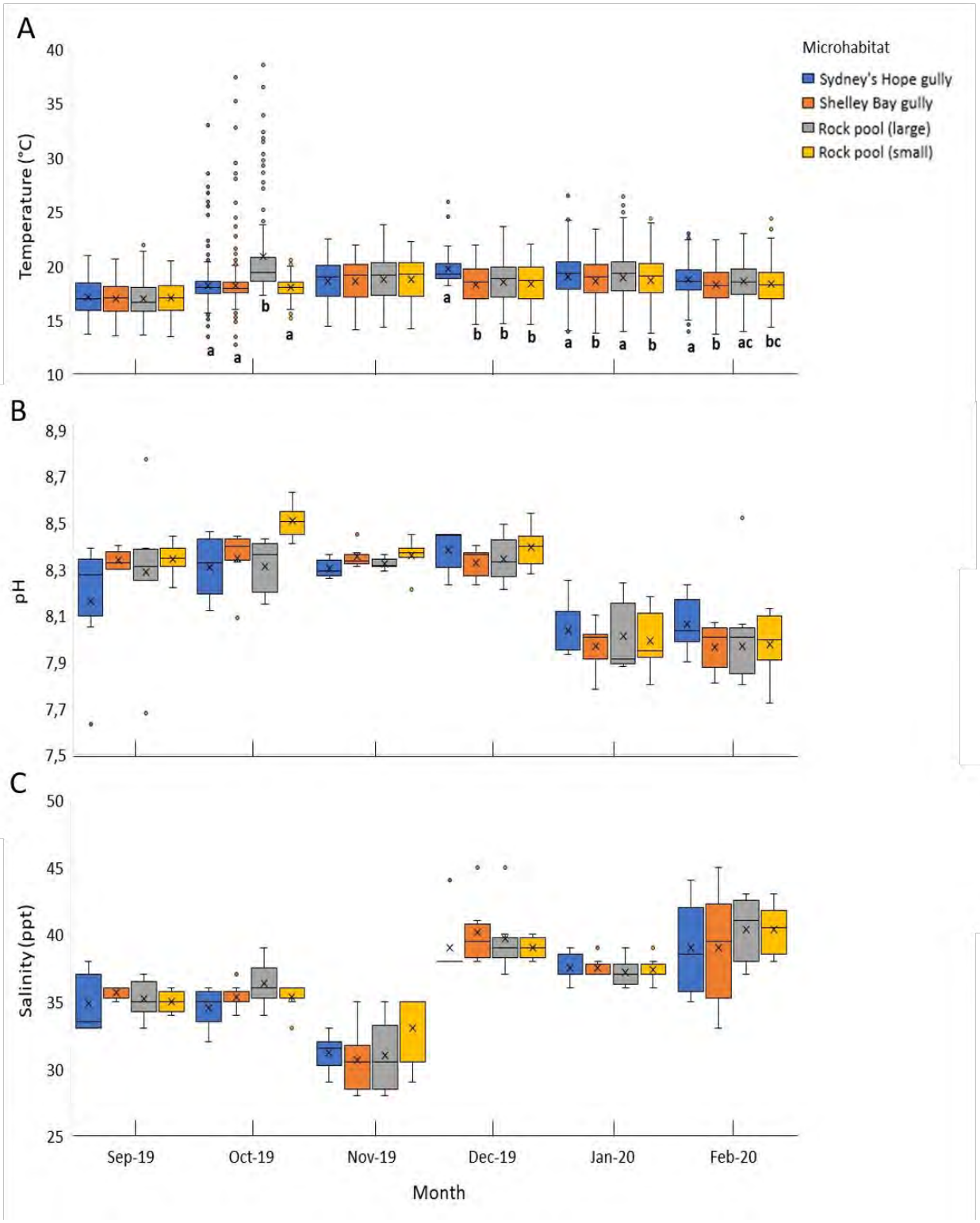


Figure 2.17. Categorised box plots of (A) temperature; (B) pH; (C) salinity at Kenton-on-Sea for each microhabitat from September 2019 to February 2020. Quartiles 1 and 2 are represented by the lower and upper boundaries of each box, respectively. Horizontal lines within each box indicate the median and the '×' indicate the mean. Vertical lines or 'whiskers' represent the maximum and minimum values and the circles/dots show outliers. Letters displayed below boxplots indicate pairwise comparisons conducted using Tukey post-hoc tests for the significant interaction between microhabitat and month.

Table 2.8. Results from the Kruskal-Wallis test for the factors microhabitat and month which indicate the effects of single factors and the interaction between them for (a) temperature, (b) pH and (c) salinity at Kenton-on-Sea. The associated sum of square values (SS), degrees of freedom (df), F-values and p-values with significant results (p<0.05) shown in bold are reported.

Factor	df	SS	F	p-value
(a) Temperature				
Microhabitat (Mi)	3	687,125	68,182	**<0,001**
Month (Mo)	5	3653,143	217,496	**<0,001**
Mi×Mo	15	1093,234	21,696	**<0,001**
Residuals	14384	51203,651		
Total	14407	53136,121		
(b) pH				
Microhabitat (Mi)	3	0,0747	0,719	0,580
Month (Mo)	5	3,740	28,805	**<0,001**
Mi×Mo	15	0,332	0,638	0,876
Residuals	114	2,961		
Total	143	7,188		
(c) Salinity				
Microhabitat (Mi)	3	10,000	0,470	0,758
Month (Mo)	5	973,048	36,569	**<0,001**
Mi×Mo	15	41,167	0,387	0,991
Residuals	114	606,667		
Total	143	1809,000		

There were no significant differences in pH or salinity for the interaction between microhabitat and month at Kenton-on-Sea (Table 2.8b and c). There were, however, significant differences in pH among months (Table 2.8b), with lower pH values during the months of January and February 2020 (Figure 2.17B). There was also a significant difference in salinity among months (Table 2.8c), with lower salinity levels during the month of November 2019 (Figure 2.17C).

2.3.2.2 Larval abundance and distribution amongst microhabitats

Overall, the total number of invertebrates collected in the light traps at Kenton-on-Sea across all microhabitats sampled was 27, made up of nine taxa, with the most specimens being collected at the gullies (Table 2.9). This total excluded the small rock pool, as no specimens were collected in the traps through the duration of sampling (statistical analysis was also conducted excluding the small rock pool, which yielded the same results). At the gullies

(Sydney's Hope and Shelley Bay), the total number of invertebrate larvae collected was 20, which were made up of seven taxa (Table 2.9), of which the megalopa of the Portunidae sp. were the most dominant, contributing 35% of the total invertebrates collected, followed by the megalopa of *Pinnotheres* sp. (25%) and the cyprid of *Cirripedia* sp. (15%) (Table 2.9). Sydney's Hope gully had the highest number of *Cirripedia* sp. cyprid, while the megalopa of Portunidae sp. dominated at Shelley Bay gully (Figure 2.18). At the large rock pool, a total of seven invertebrate larvae were collected, comprising of five taxa (Table 2.9). The zoea of Xanthidae sp. and the megalopa of *Pinnotheres* sp. were the most common, followed by the zoea of the angular crab, *Goneplax rhomboides*, megalopa of *Hymenosoma orbiculare* and *Cirripedia* sp. cyprid (Figure 2.18).

Only two fish larvae were collected throughout the sampling period at Kenton-on-Sea, one at Shelley Bay gully and one at the large rock pool (Table 2.9).

Table 2.9. Invertebrate and fish larval abundance by total number per species, per microhabitat and as a percentage (%) of the total catch collected (September 2019 to February 2020) in the light traps at Kenton-on-Sea.

			Gullies				Rock pools			
Developmental stage	**Family**	**Taxon**	**Total number counts**	**Sydney's Hope gully %**	**Shelley Bay gully %**	**Total %**	**Total number counts**	**Rock pool (large) %**	**Rock pool (small) %**	**Total %**
					Invertebrates					
Zoea	Hymenosomatidae	*Hymenosoma orbiculare*	1	5	0	5	0	0	0	0
	Plagusiidae	*Guinusia chabrus*	1	5	0	5	0	0	0	0
	Xanthidae		0	0	0	0	2	28,6	0	28,6
	Goneplacidae	*Goneplax rhomboides*	0	0	0	0	1	14,3	0	14,3
Megalopa	Pinnotheridae	Pinnotheres sp.	5	5	20	25	2	28,6	0	28,6
	Hymenosomatidae	*Hymenosoma orbiculare*	2	10	0	10	1	14,3	0	14,3
	Portunidae		7	0	35	35	0	0	0	0
Cyprid		*Cirripedia*	3	15	0	15	1	14,3	0	14,3
Veliger		Bivalvia	1	0	5	5	0	0	0	0
					Fish					
Postflexion	Clinidae	*Clinus cottoides*	1	0	100	100*	0	0	0	0
Postflexion	Gobiesocidae		0	0	0	0	1	100	0	100*

***Indicates collection of a single individual**

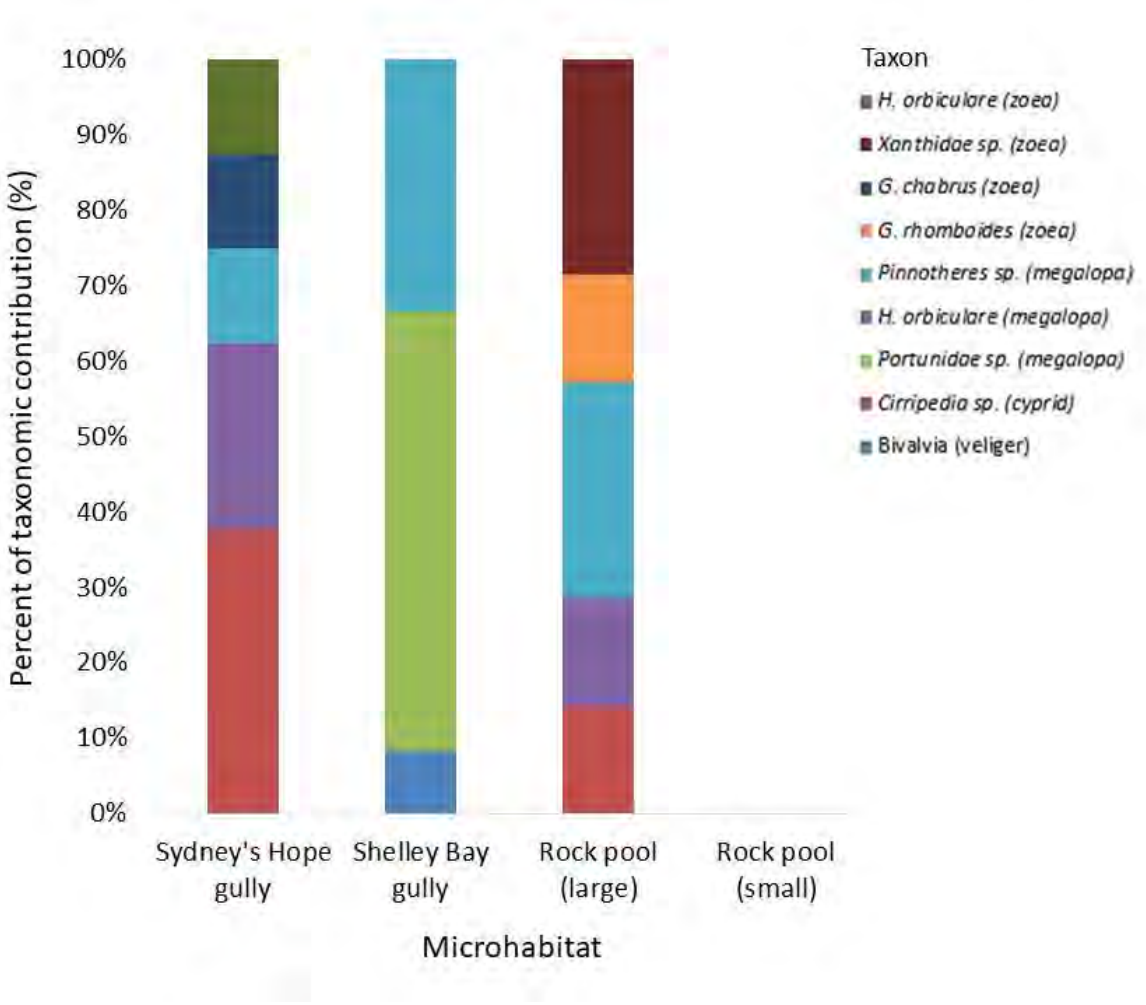


Figure 2.18. Percentage contribution of invertebrate larval species collected in light traps at Kenton-on-Sea among the selected microhabitats from September 2019 to February 2020. Developmental stages of species are indicated in brackets.

Overall, the total abundance of invertebrates collected using the pump at Kenton-on-Sea across all microhabitats sampled were 224 (with 50 collected at the gullies and 174 collected at the rock pools) and were made up of three taxa (Table 2.10). Two of these taxa were only collected using the pump method and not collected in light traps, namely: ascidians (planktonic larva) and *Cirripedia* sp. (nauplius). The most abundant taxon collected at the gullies was the *Cirripedia* sp. (cyprid), which was most dominant at Sydney's Hope gully. At the rock pools (large and small), *Cirripedia* sp. (nauplius) were the most abundant, which dominated at the large rock pool (Figure 2.19).

Table 2.10. Invertebrate larval abundance by total number per species, per microhabitat and as a percentage (%) of the total catch collected (September 2019 to February 2020) using a rechargeable pump at Kenton-on-Sea.

		Gullies				Rock pools			
Developmental stage	**Taxon**	**Total number counts**	**Sydney's Hope gully %**	**Shelley Bay gully %**	**Total %**	**Total number counts**	**Rock pool (large) %**	**Rock pool (small) %**	**Total %**
Veliger	Bivalvia	10	14	6	20	16	8,62	0,57	9,19
Nauplius	*Cirripedia*	7	2	12	14	144	68,97	13,79	82,76
Planktonic larvae	Ascidiacea	12	8	16	24	7	1,15	2,87	4,02
Cyprid	*Cirripedia*	21	22	20	42	7	1,15	2,87	4,02

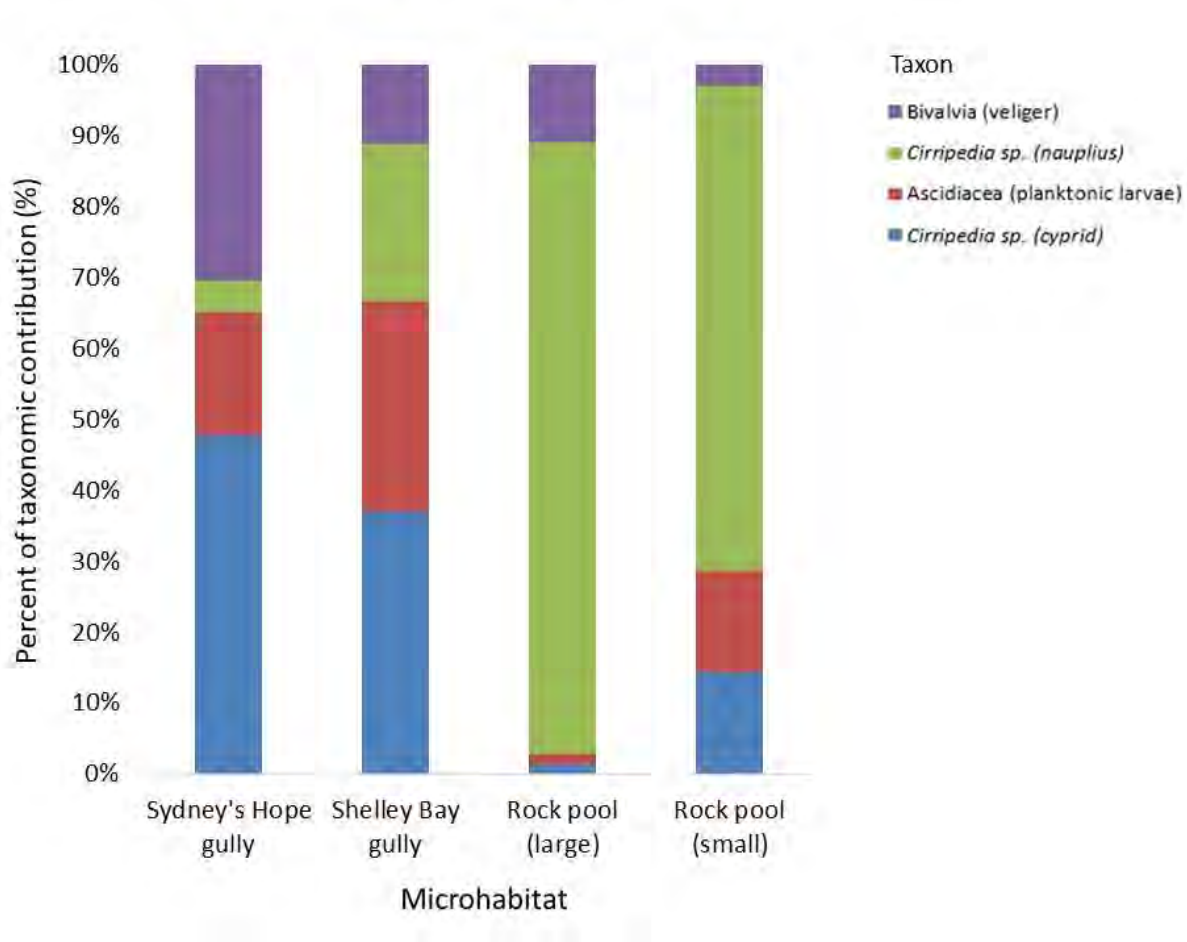


Figure 2.19. Percent contribution of invertebrate larval species collected using a rechargeable pump at Kenton-on-Sea among the selected microhabitats across the six- month sampling period. Developmental stages of species are indicated in brackets.

Initial visualisation of the invertebrate larval assemblage collected in light traps by ordination plots are not entirely clear, with overlap between the microhabitat samples across the six-month sampling period. The total variability of the data explained, however, is 70% with some separation of Shelley Bay gully from the rest of the sampled microhabitats (Figure 2.20A). For the invertebrate larval assemblage collected using the pump, ordination plots explain 74.5% of the total variation and indicate a slight separation of the large rock pool (scattered) from the rest of the microhabitats, with the small rock pool and Shelley Bay gully pooling together (Figure 2.20B).

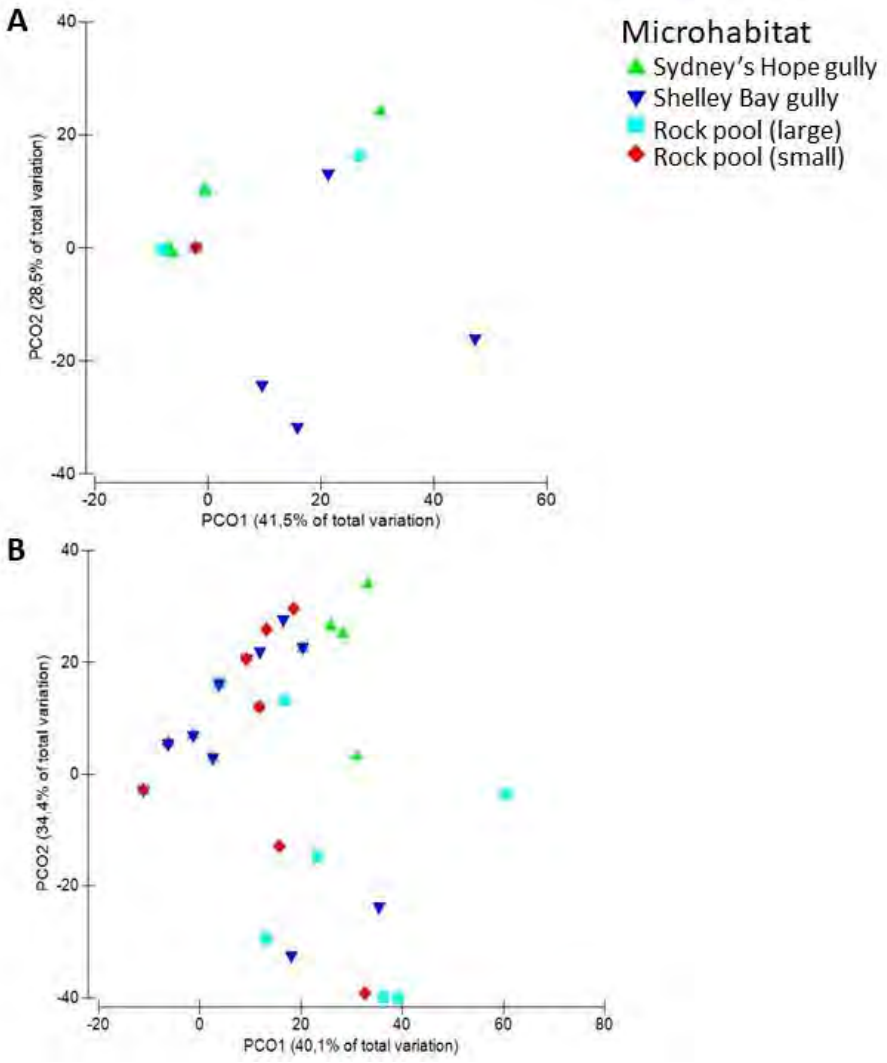


Figure 2.20. Ordination plots derived from Principal Coordinates Analysis (PCO) of (A) invertebrate larval assemblages collected in light traps and (B) invertebrate larval assemblages collected using a rechargeable pump at Kenton-on-Sea among the selected microhabitats from September 2019 to February 2020.

Results from the PERMANOVA (with complementary PERMDISP to assess heterogeneity of data) indicate significant differences (p<0.05) only among microhabitats, with no significant interaction between microhabitat and month for the invertebrate larval assemblages collected using the light traps (Table 2.11a). For the invertebrate larval assemblages collected using the pump, significant differences were revealed among microhabitats and months, with no significant interaction between microhabitat and month (Table 2.11b). In general, results from

PERMDISP indicate significant differences (p<0.05) for microhabitats comparisons within a given month, except among months and microhabitats for invertebrates collected using the light trap and pump, respectively (Table 2.11).

Table 2.11. Results of the PERMANOVA and PERMDISP for the factors microhabitat and month indicating the effects of single factors and their interaction of Log (x+1) transformed species abundance data of (a) invertebrate larvae collected in light traps and (b) invertebrate larvae collected using a pump at Kenton-on-Sea. The associated degrees of freedom (df), sum of square values (SS), Pseudo-F values, unique permutations and p-values with significant results (p<0.05) shown in bold are reported.

	PERMANOVA					PERMDISP	
Factors	**df**	**SS**	**Pseudo-F**	**P-value**	**Unique perms**	**F**	**P-value**
(a) Invertebrates (Light trap)							
Microhabitat (Mi)	3	1078.90	2.445	**0.012**	999	9.056	**0.005**
Month (Mo)	5	980.63	1.334	0.172	998	2.959	0.163
Mi×Mo	15	2908.70	1.318	0.082	998		
Residuals	72	10590					
Total	95	15558					
(b) Invertebrates (Pump)							
Microhabitat (Mi)	3	2469.70	2.218	**0.026**	999	0.212	0.962
Month (Mo)	5	19920	10.733	**0.001**	997	35.857	**0.001**
Mi×Mo	15	9831.70	1.766	0.051	999		
Residuals	72	26726					
Total	95	58947					

For the invertebrate larvae collected in light traps, pairwise comparisons among microhabitats indicated intra-microhabitat differences between Shelley Bay gully and all other microhabitats (Table 2.12a). For the invertebrate larvae collected using the pump, there was a significant difference between Sydney's Hope gully and rock pools (Table 2.12b).

Table 2.12. Pairwise comparisons among microhabitats for (a) invertebrate larvae collected in light traps and (b) invertebrate larvae collected using a pump at Kenton-on-Sea. The p-values obtained by permutations are reported (p-value (perm)). N.S – Not significant, * - Significant (p<0.05).

	(a) Light trap		(b) Pump	
Post hoc pairwise comparisons of microhabitats	**Unique perms**	**p-value (perm)**	**Unique perms**	**p-value (perm)**
Sydney's Hope gully vs Shelley Bay gully	999	*	999	N.S
Sydney's Hope gully vs Large rock pool	997	N.S	999	*
Sydney's Hope gully vs Small rock pool	999	N.S	999	*
Shelley Bay gully vs Large rock pool	999	*	999	N.S
Shelley Bay gully vs Small rock pool	998	*	998	N.S
Large rock pool vs Small rock pool	999	N.S	998	N.S

The SIMPER results revealed that the invertebrate larval species taxon (collected using the light traps) which contributed most to the dissimilarity between microhabitats were the *Pinnotheres* sp. and Portunidae sp. megalopal stages (Figure 2.21). For the invertebrate larvae collected using the pump, the species which drove the dissimilarities between Sydney's Hope gully and the large rock pool were *Cirripedia* sp. (nauplii and cyprid), while Ascidiacea planktonic larvae and *Cirripedia* sp. (cyprid) contributed most to the dissimilarities between Sydney's Hope gully and the small rock pool (Figure 2.22).

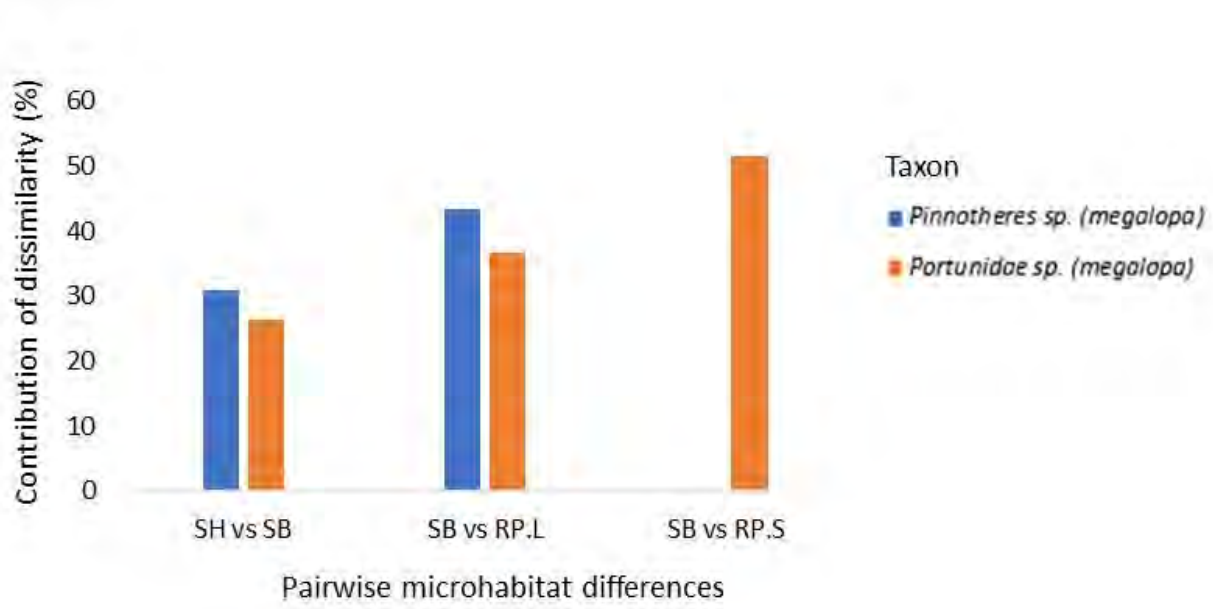


Figure 2.21. Results of SIMPER analyses of significant PERMANOVA pairwise tests among microhabitats indicating invertebrate larval taxa (collected using light traps) contributing most (50% cut-off) to the dissimilarity among significantly different microhabitats at Kenton-on-Sea. Developmental stages of species are indicated in brackets.

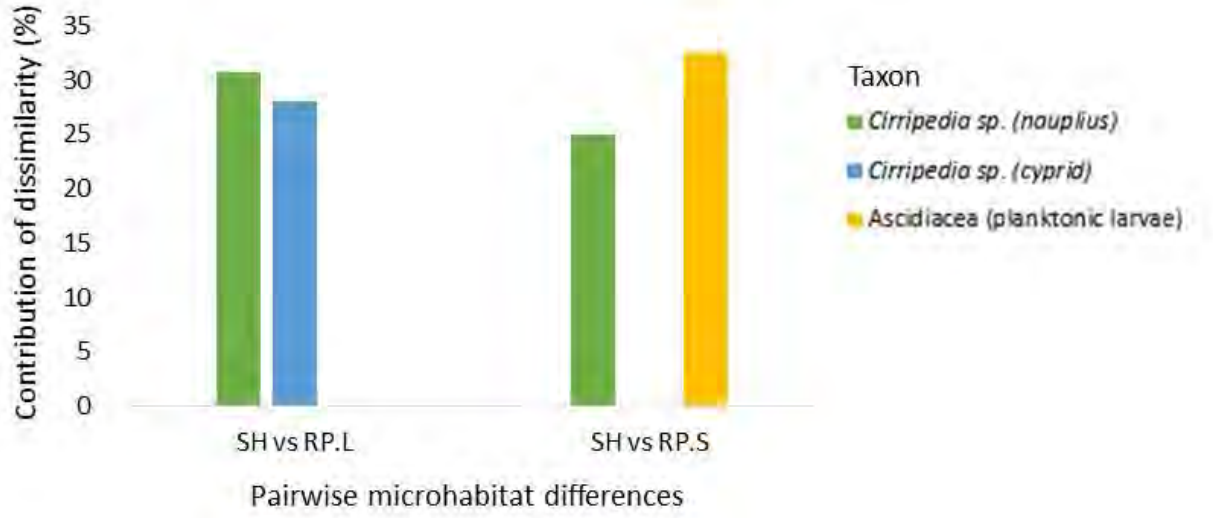


Figure 2.22. Results of SIMPER analyses of significant PERMANOVA pairwise tests among microhabitats indicating invertebrate larval taxa (collected using the pump) contributing most (50% cut-off) to the dissimilarity among significantly different microhabitats at Kenton-on-Sea. Developmental stages of species are indicated in brackets.

2.3.2.3 Environmental variables and correlation with larval assemblage structure

Multicollinearity VIF values for all environmental variables (temperature, pH and salinity) was <2.5, therefore, all were included in the succeeding analysis. The results of the Marginal Tests from the DistLM revealed no significant correlations of the environmental variables and the invertebrate larval assemblage collected using the light traps or the pump (Table 2.13a and b). This is likely due to the overall low numbers of the specimens collected during the sampling period using these two methods.

Table 2.13. Results of Marginal Tests from the DistLM indicating the correlations between (a) invertebrate larvae collected using light traps and (b) invertebrate larvae collected using a pump and each of the environmental variables at Kenton-on-Sea. Corresponding R^2 and AIC values are presented for each group and the associated sum of square values (SS), Pseudo-F values and p-values obtained by permutation with significant results (p<0.05) shown in bold are also reported.

Variable	SS	Pseudo-F	p-value
(a) Invertebrates (Light trap) (R^2 = 0, AIC = 490.45)			
Temperature	155.77	0.95	0.405
pH	234.94	1.44	0.214
Salinity	165.87	1.01	0.423
(b) Invertebrates (Pump) (R^2 = 0, AIC = 458.62)			
Temperature	2828.6	0.74	0.401
pH	3141.3	1.29	0.221
Salinity	4047.9	1.93	0.138

2.4 Discussion

The species composition and abundance of fish and invertebrate larval assemblages indicated temporally driven inter- and intra-microhabitat differences at the artificially constructed Port Alfred Marina and naturally occurring rocky shores at Kenton-on-Sea. While full orthogonal comparisons cannot be made between the selected independent case study sites, it should be acknowledged that, overall, low abundances of both invertebrate and fish larval species at different life stages were found at the rocky shores when compared to the Port Alfred Marina.

Although few individuals of fish and invertebrate larvae were collected at the natural rocky shore, it nevertheless validates the presence of larval taxa at varying developmental stages and use of these sheltered microhabitat areas by these larval taxa (Vorsatz et al., 2021b) at two independent study sites.

At the natural site, Shelley Bay gully supported the highest larval species abundance and composition, inclusive of one out of the two fish larval specimens collected, as well as being significantly different from the rest of the microhabitats. Shelley Bay was more sheltered than the other natural microhabitat site selected, as part of it was surrounded by a cave-like structure of the rocky platform enclosing the gully on one side as compared to Sydney's Hope gully, which did not have a similar partially enclosed area. Compared to the rock pools, there was a higher abundance and diversity of larvae at the gullies, this pattern could have been partially due to increased water owing to the permanent opening. Although the study aimed at selecting microhabitats with similar characteristics of a sheltered nature, some natural differences were inevitably observed, hence, the more sheltered space available at one gully as compared to the other gully and rock pools. Megalopa of *Pinnotheres* sp. and Portunidae sp. drove dissimilarities between Shelley Bay gully and the rest of the microhabitats at the natural site in Kenton-on-Sea. Large aggregations of mussel beds were observed along the rocky shore surrounding the gully and rock pool microhabitats and members of the class Bivalvia are commonly found to host the small symbiotic crabs of the family Pinnotheridae (Campos, 1996; Hsueh, 2003; Salgado-Barragán, 2015; De Gier and Becker, 2020), which could explain the presence of megalopal stage larvae present in the water column. While the megalopal stages of the family Portunidae could not be identified to a lower taxonomic level, it is common for most species of estuarine decapods to migrate to offshore coastal waters for their developmental from megalopal to adult stages (Papadopoulos et al., 2002), with higher salinities and

intermediate to warm temperatures playing an important role during larval development (Alberts-Hubatsch et al, 2016).

At the urban site located at the Port Alfred Marina, both megalopal and zoeal stages of invertebrate species were found in high numbers, with abundance at the jetties being significantly higher in comparison to the vertical walls. Estuarine systems can be considered as sheltered extensions of the adjacent coastal environment, often characterised by salinities typical of the marine environment, whilst offering calmer and shallower waters as compared to the high energy coastline (Cyrus and Forbes, 1996), which can favour retention of early life stages within the mouth of the estuary at the marina. Release of larvae by decapod species is maximal soon after the new or full moon, during which strong ebb tide current facilitates rapid transport of larvae to coastal waters (Christy and Morgan, 1998; Papadopoulos et al., 2002). Within the conditions of the estuarine environment along with the urban settings, however, this natural flow regime could be restricted due to construction of artificial structures which are specifically designed to partially or completely enclose an area to create a sheltered location for small boat harbours, which, in turn, leads to restricted flow conditions as a result of the increased number of hard structures and vessels within a relatively small vicinity (Rivero et al., 2013; Martin et al., 2005; Bouchoucha et al., 2016). An altered flow regime could favour active retention of early life stages of invertebrate taxa by providing conditions with less tidal forcing, hence, allowing larvae to benefit from the reduced current speed at the bottom waters by expending less energy on vertical movement, thus delaying mass export to offshore waters (Vorsatz et al., 2021b). Although not investigated during the current study, reduced speed of surface water flow, hence, more ‘stagnant’ water was observed at the jetties during deployment and collection of light traps as compared to the vertical wall habitat, which was possibly due to construction of the high walls enclosing the location of the jetty vicinity and surrounding area as well as the distance of the jetties from the mouth of the estuary (approximately 1.2 km).

The combination of both these factors could have led to tidal currents being greatly dissipated before reaching the jetties. In contrast, the vertical walls were situated facing the mouth of the estuary, thus experiencing stronger effects of tidal influences, with current velocity and wave height being greater during the spring tide, which is expected during this time, as tidal exchange rates are increased (Janson, 2020 for a local insight). This setting would therefore facilitate seaward transport of larvae, hence, potentially explaining the much lower numbers of (especially) the zoeal stages of invertebrates at the walls as compared to the jetties.

Invertebrate zoea were, overall, found in higher abundances as compared to the megalopal stages, with *Pinnotheres* sp. of zoea found in extremely high numbers at the jetties, which inevitably drove the overall dissimilarities between this site and the vertical walls. Overall, the zoea of *Pinnotheres* sp. were found in high abundances throughout the six-month sampling period compared to all other taxa, however, during the months of October and November, the abundance noticeably increased. In some species of invertebrates, breeding can occur in low levels year-round with distinctive peaks during spring (Hodgson, 2010), which can be characterised by a rise in water temperatures (Kalejta and Hockey, 1991; Hodgson, 2010). Some species belonging to the family Pinnotheridae, additionally, have been shown to display excessive swarming behaviour when exposed to light, such as the pea crabs *Pinnotheres bidentatus* (Hsueh, 2001) and *Tritodynamia horvathi* (Takahashi et al., 1999) and is considered a seasonal activity related to reproduction (Takahashi et al., 1999; Campos, 2016). Furthermore, most larvae are released at night, which is a common occurrence in brachyuran crabs, and is recognised to be in response to avoidance of planktivorous fish in an attempt to increase chances of survival by reduction in rate of predation (Christy and Morgan, 1998; Pereira et al., 2000; Papadopoulos et al., 2002).

When megalopal stages of invertebrates reach competence, their ultimate goal is to find a suitable and favourable habitat for settlement and further development (Forward et al., 2001;

Papadopoulos et al., 2002) and this is generally in response to different chemical and physical exogenous and/or endogenous cues (Forward et al., 2001; Hadfield and Paul, 2001; Epifanio and Cohen, 2016). Megalopal stages have stronger swimming abilities as compared to their earlier counterparts, zoeal stages (Queiroga et al., 2007) and these cues could potentially stimulate increased swimming activity and vertical migration into waters with higher flow rates, thus allowing the megalopa to 'explore' different areas before final settlement and metamorphosis (Doyle, 1975; Papadopoulos et al., 2002; Saigusa et al., 2003). In relation to this aspect, brachyuran crabs are able to delay their final settlement and metamorphosis in response to mainly chemical cues, such as extreme variation in environmental factors and water quality conditions (Forward et al., 2001). Within the marina settings, the physico-chemical environment can be altered as compared to natural environments, with increased levels of contamination from chemical pollutants as a result of boating related activities (Bulleri and Chapman, 2004; Di Franco et al., 2011; Rivero et al., 2013; Bouchoucha et al., 2016). This could delay metamorphosis of invertebrates, potentially explaining high abundance of larval stages present at the jetties as compared to the vertical walls, as the jetties experience reduced water flushing capacity. It is also worth noting, however, since both zoeal and megalopal stages of the same taxa (*Pinnotheres* sp. and *Hymenosoma orbiculare*) were found in high numbers at the jetties, it is possible that species are completing development within this urban microhabitat, indicating that these early stages could be managing in the water conditions at the jetties. Conversely, since zoeal stages were found in much higher numbers than megalopal stages, it is also probable that, together with natural mortality rates, a bottleneck is reached, where the metamorphosis of some larval stages is unable to reach completion due to surrounding abiotic factors. Given that water quality conditions were not measured in the current study, however, full inferences of the scenarios of completed larval development and

potential bottlenecks cannot be made and would therefore require further investigation with *in situ* and laboratory based experiments.

Since light traps specifically select for positively phototactic fish and invertebrate larval species with relatively strong swimming capabilities as well as certain developmental stages (due to shifts in phototaxis during these different stages) (Doherty, 1987; McLeod and Costello, 2017), a portable rechargeable pump was used as a second sampling method to also target more passive larval taxa. By using a sampling technique that accounted for photo-neutral/negative larval taxa, a more complete picture of the overall larval assemblage at differing life stages present within the selected study areas could be collected.

During the current study, larval taxa of certain developmental stages collected at both the marina and rocky shores, that were abundant in the pump samples, were not present in the light trap samples, namely: Ascidiacea (planktonic larva) and *Cirripedia* sp. (nauplius). Both early and late stage nauplius have been shown to exhibit some form of phototactic behaviour, with later stage nauplii being more phototactic than earlier stages, however, this has been observed at only certain wavelengths of light intensity (Barnes, 1972; Lang et al., 1979; Bingham and Young, 1993), with positive phototactic responses declining at decreasing light intensities (Bingham and Young, 1993). Larval stages of some barnacle species, moreover, show some form of diurnal variation, hence, higher numbers of these larvae could be expected to be active in the water column during the day rather than at night (Pyefinch, 1949). This, in turn, could explain high and exclusive abundance of nauplii collected using the pump at both the rocky shore and marina (as pumping was conducted during the day as opposed to light traps only sampling at night). Veliger larvae of the class Bivalvia were collected only using the pump at the marina and not the light trap, which is expected, as young veliger larvae tend to display photonegative responses (Dobrestov and Miron, 2001). If photopositive reactions are detected in certain species, the swimming ability of these stages may not be strong enough to actively

seek the light source, especially against turbulent conditions (Barile et al., 1994). At the rocky shores, however, veliger larvae were collected using the pump as well as the light trap, this occurrence could be due to the photonegative response combined with a negative geotactic response, which result in accumulation of veliger larvae closer to the surface of the water column at night (Bayne, 1964; Barile et al., 1994; Fuchs and DiBacco, 2011), which is where light traps were deployed. Larvae could, therefore, have semi-opportunistically entered the trap by current flow.

At the Port Alfred Marina, fish larvae were found in preflexion, flexion and postflexion developmental stages, with abundance at the vertical walls being significantly higher in comparison to the jetties. Marine transient species of *Etrumeus whiteheadi* and *Engraulis encrasicolus,* as well as estuarine spawners *Omobranchus woodi* and *Caffrogobius* sp. were the main drivers for the dissimilarities during the months of October and November, as some of these species were found in higher or lower abundances as compared to the others. Density of larval fish are generally higher in spring/summer months as this is coupled with warmer temperatures and the peak spawning season for many coastal species (Strydom, 2005; Kruger and Strydom, 2010). Artificial structures within marina's are often associated with adult, juvenile and larval stage fishes (Martin et al., 2005; Clynick et al., 2008; Bouchoucha et al., 2016). Fish can actively seek shelter in areas which are similar to their body size to prevent predation from larger bodied animals (Adams and Ebersole, 2009) and it is possible that artificial structures in the form of pilings, vertical walls and jetties provide such protection as well as sources of food (Clynick, 2007; Bouchoucha et al., 2016). Increased fish diversity (juveniles and adults) is generally associated with urban systems in closer proximity to the ocean, with marinas located nearer to mouths of estuaries having increased fish abundance, which could be attributed to larval supply and food accessibility (Clynick, 2007). The vertical walls at the Port Alfred Marina were both located close to the mouth of the estuary, which

could explain the higher abundances of fish larvae present. Structures which are vertically orientated, moreover, are able to host plankton over a wider depth range as they extend further down the water column, providing a greater surface area for food availability, whilst providing escape from potential predators (Clynick, 2007).

While only two taxa of postflexion fish larvae were sampled at the natural occurring rocky shore environment at Kenton-on-Sea, one of these, the clinid, *Clinus cottoides* is a carnivorous species and live-bearer with no planktonic larval phase and is endemic to South Africa, with adults commonly found in tide pools along the rocky shore habitat (Zsilavecz, 2005). The postflexion larvae of this species is, therefore, seldomly encountered during plankton surveys, unlike numerous other endemic South African fish species (von der Heyden et al., 2008). This highlights the potential methodological importance of using these microhabitat-tailored light traps as a sampling technique in these environments to account for species that may otherwise not be encountered by other methods which are commonly used in these types of dynamic environments due to the life history traits of some fish species.

At the open coast rocky shores, wave exposure, tidal regimes and current velocity are generally intense and variable as compared to estuarine systems, which are effectively more sheltered as protected from direct wave action (Cyrus and Forbes, 1996; Leonard et al., 1998). High tidal currents and increased wave action can decrease the catch efficiency of traps due to the inability of larvae to swim toward and enter the trap against the fast-flowing water, even though they may be attracted to the light (Thorrold, 1992; Strydom, 2003; Chicharo et al., 2009). A significant and negative relationship between increasing current speed and decreasing catch rate of early life stages of species by light traps have been shown in previous studies which sampled in fore-reef sites (Anderson et al., 2002) and oil/gas platforms situated off the Gulf of Mexico (Lindquist and Shaw, 2005; Shaw et al, 2007). Additionally, currents can affect the stability and sampling effectiveness of the traps, even if they are firmly secured, by

rotating/spinning the trap, which will inevitably impede/delay entry into as well as retention within the traps (Shaw et al., 2007; McLeod and Costello, 2017) and could also cause external damage of the trap body/frame. During the current study, it was observed that, upon retrieval of traps, the rope used to secure them were often twisted and entangled and, in some instances, led to the rope being cut, resulting in complete loss of traps. Moreover, wave action and the prevailing currents, especially during high tide and adverse weather conditions, can cause resuspension of sediments, particles and biological matter from surrounding invertebrate communities, which can decrease overall water clarity (Hickford and Schiel, 1999; Lindquist and Shaw, 2005). This can also affect the intensity of light emitted from the lighting source in the trap by hindering transmission of certain wavelengths (Gallegos et al., 1990). Contrastingly, at the Port Alfred Marina, during the entire duration of the sampling period, no traps were damaged or lost, indicating calmer conditions, which could have led to increased catch efficiency.

Environmental variables collected over the duration of the current study at both study sites showed differences between microhabitat and month. Temporal differences are expected due to seasonal fluctuations and physical position of the estuarine system in relation to the coastal water source (Davis et al., 2009). Temperature and pH were correlated with both fish and invertebrate larval assemblages, which has also been shown in previous studies (e.g., González-Gordillo and Rodríguez, 2003; Queiroga et al., 2007; Nemeth, 2009; Kruger and Strydom, 2010; Strydom, 2015; Espinel-Velasco et al., 2018). Events such as time of year, spawning season, lunar cycles and day patterns influence reproductive cycles of fish and invertebrate species, with environmental variables varying at each of these scales (Sheaves, 2006; Nemeth, 2009). Fish and invertebrate species rely on the environmental cues associated with these events for timing of larval release which offer maximum dispersal capabilities and survival

rates of eggs and early larval stages (Arvedlund and Kavanagh, 2009; Bouchoucha et al., 2016; Oricchio et al., 2016).

Although many studies have shown that artificial structures are associated with overall reduced and/or altered species composition and abundance as compared to natural habitats (Bulleri and Chapman, 2004; Bulleri, 2005; Lee and Li, 2013; Aguilera et al., 2014), others have also revealed the potential importance of these structures in hosting native faunal assemblages, especially in relation to marinas (Cyrus and Forbes, 1996; Martin et al., 2005; Bouchoucha et al., 2016; Oricchio et al., 2016) and this is the case for this research, with an increased abundance of fish and invertebrate larvae being present in the urban microhabitats along the marina setting. While habitat heterogeneity has been coupled with natural structures through the provisioning of microhabitats which act as refuge for early stage fish and invertebrate species (Cyrus and Forbes, 1996; Martins et al., 2010; Sheaves et al., 2014, Evans et al., 2021), it is also plausible that certain features linked to artificial structures can also provide such novel habitats (Oricchio et al., 2016). With the vast majority of natural coastal habitats being continuously replaced by urban related artificial infrastructure, it is essential to ensure coastal urban settings can sustain early stage functionality and connection with adjacent naturally occurring shorelines, hence functioning as a potential source population for replenishment of natural populations. It is, therefore, imperative that changes related to marinas and addition of further artificial structures undergo stringent ecological impact assessments, so that the current larval assemblages utilising these areas can be maintained for potential supply to adult populations as well as connectivity to natural populations.

CHAPTER 3: Identification of fish and invertebrate larvae using the DNA barcoding technique, with a particular focus on the Port Alfred Marina, Eastern Cape, South Africa

3.1 Introduction

The coastline of South Africa hosts an abundance of estuarine and shallow water marine habitats which serve several vital ecosystem functions (Beck et al., 2001). High levels of both primary and secondary productivity, which allow sustenance of a diverse array of fish and invertebrate species, occur in these coastal systems (Beck et al., 2001; von der Heyden, 2009). Owing to this productivity and diversity, these habitats also serve as nursery areas for early life stages of certain species (Beck et al., 2001). These habitats, therefore, form an important link between maintenance of larval/juvenile stages and adult populations (Gillanders et al., 2003).

The importance of the early life stages of marine fish and invertebrate species becomes clear when we take into consideration that every current adult was, at one time, a barely visible egg and then larva, with only a small fraction of these surviving the initial months of life (Fuiman and Werner, 2002). These early life stages, however, play such a critical role in the sustainability and structuring of the adult populations (Fuiman and Werner, 2002; Levin, 2006; Greer et al., 2016). The extent of successful larval dispersal and survival is, therefore, a key determinant of the dynamics within such populations (Cowen and Sponaugle, 2009).

The fluid nature of the marine environment provides resident populations with a variety of means to disperse among and within populations (Cowen and Sponaugle, 2009). For most coastal invertebrate and fish species, the larval stage is mainly associated with a planktonic dispersal phase (Becker et al., 2007; Cowen and Sponaugle, 2009). Patterns of larval dispersal, distribution and overall survival, therefore, have a direct significance for recruitment and regulation of populations (Bradbury and Snelgrove, 2001). By understanding which coastal populations act as sources or sinks, how areas are connected by larval exchange and the dispersal abilities of colonising species, applications for management, conservation and restoration strategies/practices can be improved (Levin, 2006; Cowen and Sponaugle, 2009;

Vanderklift et al., 2020). This can be done by optimising the design of marine protected areas (MPAs) through maximising location, size and configuration (Levin, 2006; Cowen and Sponaugle, 2009; Krueck et al., 2017), which can then facilitate maximisation of fishery yields of commercially valuable fish and invertebrate species as well as protection of threatened species (Levin, 2006; Cowen and Sponaugle, 2009). Furthermore, since the presence of invasive species can disrupt the production, transport and settlement (in the case of invertebrates) of the early life stages of indigenous species (Rius et al., 2009), information on spatial scales of larval dispersal is also a useful tool to monitor and potentially control the spread of invasive species (Neubert and Caswell, 2000; Levin, 2006; Becker et al., 2007). In addition, larval transport is also a key factor of population connectivity as it influences the spatial scales of dispersal (broad or restricted) (Pineda et al., 2007). Changes in hydrodynamics, through global climate change can, therefore, further impact species at the population level by influencing larval transport and recruitment patterns (Harley et al., 2006; Kirby et al., 2008).

Hence, it is evident that the larval stages of species form an interactive and pivotal component of the structuring and maintenance of marine ecosystems by buffering, connecting and driving the dynamics of their adult populations (Adams et al., 2012). In addition, their central role in transport and recruitment can aid in other fields of scientific research (Heimeier et al., 2010). Scientific research based on these early life history processes, however, all require one essential element: accurate taxonomic identification of the different stages, which can be a challenging undertaking for both fish and invertebrate larvae (Heimeier et al., 2010; Pegg et al., 2006).

Accurate taxonomic identification of larvae requires trained taxonomists, who identify specimens based on morphological characteristics. This method requires a particular level of expertise in examining morphological traits and generally leans toward a narrow specialisation in identification of specimens belonging to a specific group of taxa (Raupach and Radulovici,

2015). While it can be advantageous to have this specialised expertise, with the limited number of taxonomists available worldwide, few of which are still regularly involved in species identification (MacLeod et al., 2010), routine and correct identification of a diverse array of larval species can be challenging and time-consuming (Ko et al., 2013; Raupach and Radulovici, 2015). The main reasons for this are that: most larvae bear little to no resemblance to their adult counterparts (Pradillon et al., 2007; Tang et al., 2010); there are minimal morphological characteristics to distinguish between species due to limited development of key features (Steinke et al., 2016b; Kusbiyanto et al., 2020); different larval stages belonging to the same species can have different morphologies (Ko et al., 2013; Kusbiyanto et al., 2020); and larvae belonging to different species can show similar morphology (Valdez-Moreno et al., 2010; Ko et al., 2013; Kusbiyanto et al., 2020). Also, due to their small size, larvae are very fragile, which often leads to specimens being badly damaged during collection. Furthermore, larvae also tend to lose pigmentation patterns and shrink during the fixation process. All these challenges further complicate potential morphological identification and adds to the time-consuming nature of this technique (Webb et al., 2006; Valdez-Moreno et al., 2010; Fazhan et al., 2021). These circumstances may, therefore, lead to species misidentification, inconsistencies and uncertainties in some identifications which can all result in unreliable data collection (Ko et al., 2013; Raupach and Radulovici, 2015; Kusbiyanto et al., 2020).

These limitations in morphologically based identification techniques, together with the declining ensemble of available specialised taxonomists, along with the lack (South Africa) or paucity of larval identification keys/guides has prompted the need for an alternative and new approach for taxonomic identification of species, one such approach being DNA-based identification systems, commonly known as DNA barcoding (Hebert et al., 2003). The key aspect of the DNA barcoding technique is the use of a standard mitochondrial DNA sequence which corresponds to a single homologous gene region that can be amplified by polymerase

chain reaction (PCR) and is able to differentiate between a wide selection of taxonomic groups (Neigel et al., 2007). The cytochrome c oxidase subunit I (COI), found in the mitochondrial DNA (mtDNA), meets all these requirements and has proven to be a reliable and rapid means of identifying species, as demonstrated in previous studies (Ward et al., 2005; da Silva et al., 2011; Jeffery et al., 2011; Weis et al., 2014). Additionally, COI sequences have already been established for a relatively large number of species (especially model species) and made available on public online databases (Neigel et al., 2007).

The basis of identifying specimens using DNA barcoding is matching an unknown individual's COI sequence to one or more positively identified sequences (Neigel et al., 2007; Zhang and Hanner, 2011). The effectiveness of this method, however, is dependent on the availability of extensive public databases were COI sequences of a wide selection of organisms (which ideally, have been screened for morphological taxonomic accuracy and linked to a voucher specimen) can be deposited (Neigel et al., 2007; Weigand et al., 2019). There are now well organised initiatives in place to develop such databases which have allowed for a more streamlined process for depositing COI sequences and other DNA barcoding resources, and as a result, databases are continuously updated with new sequences emerging from DNA barcoding studies (Neigel et al., 2007). These databases all fall under one international organisation: the Consortium for the Barcode of Life (CBOL) (Costa and Carvalho, 2007; Neigel et al., 2007). The Barcode of Life Data (BOLD) system is one such database (Ratnasingham & Hebert, 2007; https://www.boldsystems.org). The BOLD system is an online workbench for coordinating, depositing, storing and analysing data from DNA barcoding projects (Ratnasingham & Hebert, 2007). This system consists of a database of sequence records which contains an identification engine which allows comparison between an uploaded unknown sequence and those available in the database (Neigel et al., 2007; Hubert et al., 2008). DNA barcoding, overall, provides a consistent framework for organising specimens along with

their sequence data and life history information for systematic research (DeSalle and Goldstein, 2019).

It is evident that DNA barcoding can be an effective tool to bridge the gap between the difficulties encountered during morphological identification of larval specimens and increasing the success rate of identifying these samples to the lowest taxonomic level. This will decrease the probability of misidentification of fish and invertebrate larval stages and accelerate collection of morphological, ecological and geographical data (Bucklin et al., 2011), which can lead to improved understanding in research related to spawning, hatching, transport of larvae, recruitment and the utilisation of nursery areas (Swartz et al., 2008; Burrows et al, 2018). Incorrect identification could mislead our views on these important processes, with negative repercussions, on, e.g., management of species.

Utilisation of the DNA barcoding technique offers an improved means of accurate identification of the early life stages. In turn, the barcoding information obtained can aid in the development of public database records of COI sequences. The overall data obtained on larval diversity, moreover, could potentially act as a scientific basis for improved species and ecosystem management and conservation efforts. This study, therefore, aims to identify both fish and invertebrate larvae collected from coastal habitats in the Eastern Cape, South Africa, to the lowest possible taxonomic level using the mitochondrial COI gene as a DNA barcoding marker. The information presented on the larval assemblages and diversity of Chapter 2 was based on morphological identification which was mainly confirmed by the results obtained from this DNA barcoding chapter. Given the necessary details of the experimental design and specific investigation in relation to the microhabitat use, however, this chapter (Chapter 3) follows the larval assemblage one (Chapter 2).

3.2 Materials and Methods

3.2.1 Sample collection

Larval specimens used for this study were sorted and removed from the original samples collected from the Port Alfred Marina and Kenton-on-Sea rocky shore (Eastern Cape, South Africa), as described in Chapter 2, Materials and Methods. While all identified taxonomic groups collected were barcoded, the current study, however, mainly focuses on the larval species collected at the marina as it covered the broadest range of taxa from both fish and invertebrate larvae during this research.

3.2.2 Sorting and image capture

Fish and invertebrate specimens belonging to different taxonomic groups were identified and selected from larval samples and individually stored in 1.5 ml Eppendorf tubes containing 99.9% ethanol. Each of these individual specimens was assigned an identification code based on their morphological taxonomic grouping and were further allocated unique numbers to distinguish between individuals belonging to the same morphological group. The number of individuals selected per taxonomic grouping to be barcoded was based on the success rate of the sequencing process, with more individual specimens being barcoded if the success rate was low, so that a minimum of three larval sequences per taxon could be obtained. Images of each specimen were then captured at the NRF-SAIAB Aquatic Ecophysiology Research Platform, using an Olympus SZX16 stereomicroscope (model SZX2-ILLB) with an attached camera (model DP27) linked to the Olympus Stream Essentials image analysis software (v2.2). An Olympus KL 1600 LED gooseneck was used as an additional light source. A minimum of four images per specimen were captured at all possible angles (specimen facing left and right). In certain instances, distinguishing features were also zoomed in, and images captured for

morphological identification purposes. To gain a clearer and more focussed image of some specimens, the focus stacking option (part of the image analysis software) was used. This involves automatically merging several images captured at slightly different focal points to obtain the resultant sharper image.

3.2.3 DNA extraction, polymerase chain reaction (PCR), purification and sequencing

Genetic analyses, from extraction through to sequencing were conducted using the facilities and equipment provided by the NRF-SAIAB Aquatic Genomics Research Platform. DNA was extracted from the whole specimen for the zoeal, cyprid and early megalopal stages of the invertebrate larvae (0.5 – 5 mm), as well as the preflexion and flexion stages of the fish larvae (<5 mm). For the older megalopal stages, which were larger in size (>5 mm) and the postflexion stage fish larvae (>10 mm), half the specimen was used.

The salting out protocol (Sunnucks and Hales, 1996) was followed for the DNA extraction process. The DNA quality and concentration were measured using a Nanodrop 1000 spectrophotometer (Thermo Scientific, USA).

A partial fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (COI) was amplified by PCR with different sets of primers depending on the specimen used (fish or invertebrate) and the reaction success. For the fish larvae, FishF1/Fish R1 (Ward et al., 2005) primers were used for all taxa, apart from the sardine, *Sardinops sagax* and the beltfish, *Trichiurus lepturus*, which used the degenerate universal metazoan primers dgLCO1490/dgHCO2198 (Meyer, 2003). For the invertebrate larvae, Crust DF1/Crust DR1 (Steinke et al., 2016a) were used for all taxa (and all life stages), except for *Hymenosoma orbiculare* (zoea), which used dgLCO1490/dgHCO2198 (Meyer, 2003). All reactions were performed in a 25 µl volume containing 12.5 µl Taq (2x Master Mix, RED, 1.5 mM $MgCl_2$), 6.5 µl of molecular grade water, 0.5 µl each of the forward and reverse primer (concentration

10 µM) and 5 µl of DNA template (DNA concentration <100 ng/µl). During instances when DNA concentration was above 100 ng/µl (mainly for fish larvae), samples were diluted to obtain concentrations <100 ng/µl. The PCR thermal protocols were as follows:

- Fish F1/Fish R1 primer set: initial denaturation at 94°C for 1 minute, followed by 5 cycles of 94°C for 30 seconds, 50°C for 40 seconds, 72°C for 1 minute and 35 cycles of 94°C for 30 seconds, 54°C for 40 seconds, 72°C for 1 minute, with a final extension step of 72°C for 10 minutes (Ivanova et al., 2007).
- Crust DF1/Crust DR1 primer set: initial denaturation at 94°C for 2 minutes, followed by 5 cycles of 94°C for 40 seconds, 45°C for 40 seconds, 72°C for 1 minute and 35 cycles of 94°C for 40 seconds, 51°C for 40 seconds, 72°C for 1 minute, with a final extension step of 72°C for 5 minutes (Steinke et al., 2016a).
- dgLCO1490/dgHCO2198 primer set: initial denaturation at 95°C for 2 minutes, followed by 35 cycles of 95°C for 1 minute, 48°C for 1 minute, 72°C for 1 minute and a final extension step of 72°C for 7 minutes (Meyer, 2003).

For the invertebrate larvae, the reaction success of the PCR was not always optimal (products produced bands that were faint/light) (Figure 3.1). In these instances, the protocols for the Crust DF1/Crust DR1 and dgLCO1490/dgHCO2198 primer sets were adjusted by adding 2 extra cycles to the stage initially containing 35 cycles (total of 37 cycles).

All PCR products were loaded into wells of precast 1% agarose gel, electrophoresed at 100 V for 30 minutes and visualised under ultraviolet (UV) light. Products which produced distinct single bands which were bright/most intense were considered successful and selected for sequencing (Hajibabaei et al., 2005; Kress and Erickson, 2012) (Figure 3.1). These selected PCR products were thereafter purified using an Exonuclease I-Shrimp Alkaline Phosphate (Exo/SAP) method. This methodology allows removal of unincorporated nucleotides and

excess primers from the PCR reaction (Werle et al., 1994). Purified products were sequenced using BigDye V3.1 (Applied Biosystems) terminator chemistry in the forward and reverse direction and then precipitated using the ethanol-EDTA precipitation protocol (manufacturer recommended). This method assists in removing unincorporated dye-labelled terminators (Sambrook and Russel, 2001). Lastly, samples were analysed using an ABI-Hitachi 3500 Genetic Analyser (Applied Biosystems) following the manufacturers protocol.

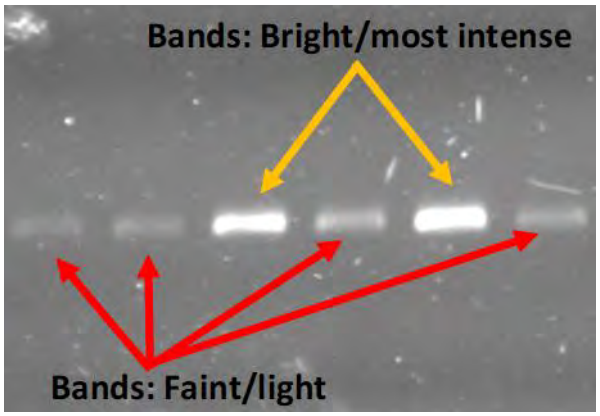


Figure 3.1. Image of PCR gel electrophoresis captured under UV light comparing PCR products which produced faint/light bands and bright/most intense bands.

3.2.4 Data analysis

All sequences were first observed using SnapGene Viewer 5.2.2 (from Insightful Science; available at snapgene.com) as an initial quality check. Forward sequences were then manually edited using SeqMan Pro (DNASTAR, Lasergene, 7.2) and saved in a FASTA format.

The Barcode of Life Data (BOLD) System (Ratnasingham & Hebert, 2007; https://www.boldsystems.org) was used as an online tool for sequence comparison and identification. Using BOLD, FASTA formatted sequences (in the forward orientation, primer direction 3'-5') were entered into the identification engine, with the submitted sequence then compared against the available reference database to find the closest/nearest matching species,

which is displayed as a percentage probability of 0 to 100%. A total of three sequences per larval taxon were entered into BOLD for comparison to further ensure quality control of selected sequences (unless only one specimen was available for a particular taxon). For the current study, specimens were recognised to the species, genus and/or family levels if the similarity/match percentages were >98% (Ko et al., 2013; Brandao et al., 2016; Azmir et al., 2017). Sequences with similarity/matches <98% were classified as failed identifications using the BOLD system.

Publicly available sequences with a >98% similarity/match were downloaded from the BOLD reference database (two sequences for each positively identified sequence from the current study). Using the software package MEGA 7 (Kumar et al., 2016), these two sequences retrieved from BOLD, together with the edited forward sequences from the current study (three per taxon) were aligned by ClustalW and an unrooted neighbor-joining tree of Kimura two-parameter distance was created. This phylogenetic method provided a graphical representation of the placement of taxon groups to confirm positive identification as well as strengthen and further verify matching of unknown COI sequences to known reference sequences from the BOLD System. The bootstrap method (1000 replicates) was used to evaluate the 'confidence' level of each clade (Felsenstein, 1985).

3.3 Results

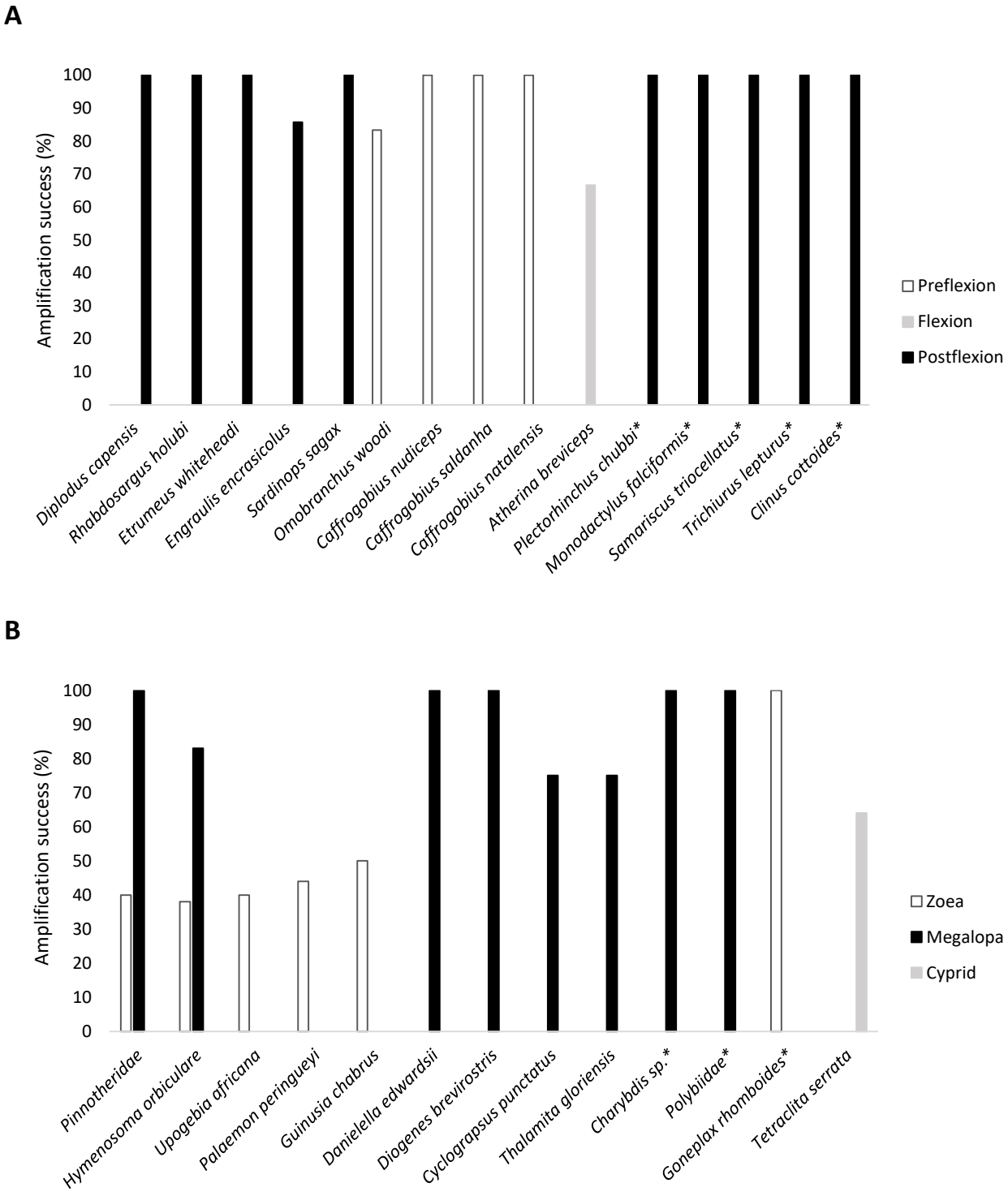
DNA was extracted from a total of 67 fish larvae and 241 invertebrate larvae belonging to different life stages. DNA was successfully extracted from 66 fish and 156 invertebrate larvae and produced successful PCR products by using either primer sets detailed in Section 3.2.3. and COI sequences were obtained for 53 fish and 99 invertebrate larvae and are thus presented in this chapter. Using the BOLD Systems, 51 fish and 57 invertebrate larvae sequences (forward) generated a >98% match/similarity. This in turn resulted in the positive identification of 12 fish families, 13 fish genera and 15 fish species and 12 invertebrate families, 11 invertebrate genera and 10 invertebrate species (Table 3.1).

Table 3.1. Range of match/similarity (>98%) for successfully amplified fish and invertebrate larvae sequences on BOLD. A total of three sequences per species were used for comparison against the database, unless only one specimen was collected (indicated by *). The last column indicates the number of successfully amplified reverse sequences for each forward sequence. The thick bottom line indicates the break between the fish and invertebrate larvae information.

BOLD Systems Identification	Family	Developmental stage	Total no. of successful forward sequences	BOLD Systems similarity (%)	Total no. of successful reverse sequences
Diplodus capensis	Sparidae	Postflexion	4	100	4
Rhabdosargus holubi	Sparidae	Postflexion	4	100	4
Etrumeus whiteheadi	Dussumieriidae	Postflexion	5	99.62 - 100	5
Engraulis encrasicolus	Engraulidae	Postflexion	6	99.81 - 100	6
Sardinops sagax	Clupeidae	Postflexion	5	99.28 - 100	5
Omobrancus woodi	Blenniidae	Preflexion	5	99.79 - 100	5
Caffrogobius nudiceps	Gobiidae	Preflexion	5	99.40 - 100	5
Caffrogobius saldanha	Gobiidae	Preflexion	4	99.62 - 100	4
Caffrogobius natalensis	Gobiidae	Preflexion	4	99.60 - 99.62	4
Atherina breviceps	Atherinidae	Flexion	4	99.81 - 100	4
*Plectorhinchus chubbi**	Haemulidae	Postflexion	1	100	1
*Monodactylus falciformis**	Monodactylidae	Postflexion	1	100	1
*Samariscus triocellatus**	Samaridae	Postflexion	1	100	1
*Clinus cottoides**	Clinidae	Postflexion	1	99.79	1
Trichiurus cf. lepturus var 2*	Trichiuridae	Postflexion	1	100	1
Pinnotheridae	Pinnotheridae	Zoea	6	99.81 - 100	2
Palaemon peringueyi	Palaemonidae	Zoea	3	98.36 - 99.81	1
Upogebia africana	Upogebiidae	Zoea	3	99.81 - 100	3
Hymenosoma orbiculare	Hymenosomatidae	Zoea	6	99.03 -100	3
Guinusia chabrus	Plagusiidae	Zoea	3	98.60 - 100	1
*Goneplax rhomboides**	Goneplacidae	Zoea	1	99.01	1
Danielella edwardsii	Camptandriidae	Megalopa	5	99.40 - 99.80	4
Pinnotheridae	Pinnotheridae	Megalopa	6	99.82 - 100	3
Hymenosoma orbiculare	Hymenosomatidae	Megalopa	10	99.81 - 100	3
Diogenes brevirostris	Diogenidae	Megalopa	3	99.24 - 99.81	3
Cyclograpsus punctatus	Varunidae	Megalopa	3	99.20 - 100	0
Thalamita gloriensis	Portunidae	Megalopa	3	99.22 - 99.61	2
*Charybdis sp.**	Portunidae	Megalopa	1	100	1
Polybiidae*	Polybiidae	Megalopa	1	100	1
Tetraclita serrata	Tetraclitidae	Cyprid	3	99.38 - 99.81	2

*indicates only one specimen was collected during the entire sampling period of the study.

Amplification success (when a successful PCR product produced a good quality COI sequence) of samples with a >98% match on the BOLD System (from Table 3.1) was, overall, higher for the fish as compared to the invertebrate taxa (Figure 3.2). Twelve out of 15 positively identified fish taxa, had an amplification success of 100%, which also included five taxa that had only one specimen each collected. The lowest amplification success was 65%, belonging to the species the cape silverside, *Atherina breviceps* (Figure 3.2A). Six out of 12 positively identified invertebrate taxa had an amplification success of 100%, with five of these belonging to the megalopa life stage and one to the zoea life stage (only one specimen collected). Amplification success of the remaining zoeal life stages fell within the lowest range, which were between 38 to 50% and the only taxon with a cyprid life stage (the volcano barnacle, *Tetraclita serrata*) was 64% (Figure 3.2B).



*indicates only one specimen was collected during the entire sampling period of the study.

Figure 3.2. Amplification success of mitochondrial gene, cytochrome oxidase subunit 1 (COI) as a percentage for each species of (A) fish larvae and (B) invertebrate larvae which generated a >98% match/similarity on BOLD. Pinnotheridae and *Hymenosoma orbiculare* were the only groups with two different life stages collected (zoea and megalopa).

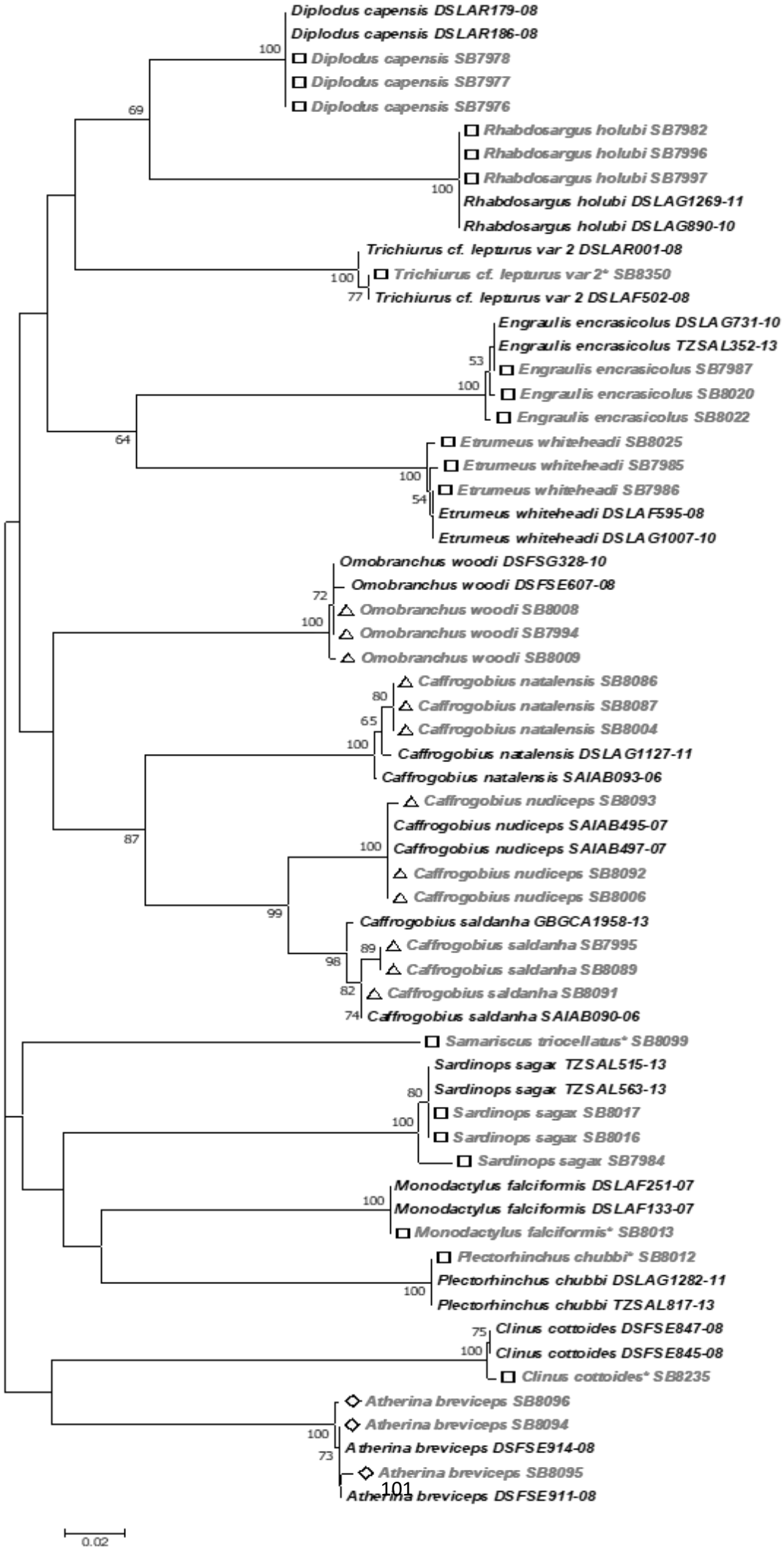
For the fish larval samples that had failed sequences and consequently had to be excluded from this chapter, nine out of 13 belonged to four different taxa (Table 3.2). Additionally, only one (K.UF3) fish larval sequence could not be positively matched to any taxonomic level on BOLD (closest similarity was below the probability percentage used in this study) (Table 3.2). For the invertebrate larvae, two taxa had all failed PCR products, while three taxa belonging to the megalopal stage (only one specimen each collected) had failed COI sequences. In addition, six invertebrate taxa could not be positively matched to any taxonomic level on BOLD (Table 3.2). All these samples, therefore, failed to be identified by DNA barcoding. Some of these taxa were, however, identified morphologically (Table 3.2 and Chapter 2).

Table 3.2. Fish and invertebrate larval samples which failed to be identified by DNA barcoding. Unique ID codes of unidentified fish/invertebrate specimens used for the unique taxonomic assignment. Amplification success of sequences as a percentage for one fish larva and six groups of invertebrate larval taxa are indicated in brackets under the 'Successful forward sequences' column. The last column indicates the level of morphological identification achieved for each of these taxa.

Unique ID code	Successful PCR's	Successful forward sequences	BOLD Systems similarity (%)	Morphological identification (from Chapter 2)
Fish (G.P.K)	Yes	No	N/A	*Psammogobius knysaensis*
Fish (B1)*	Yes	No	N/A	*Parablennius* sp.
Fish (Sole 1 N)*	Yes	No	N/A	*Solea turbynei*
Fish (SH.UF4)*	Yes	No	N/A	Clinidae
Fish (K.UF3)*	Yes	Yes (100%)	81 - 82	Gobiesocidae
Zoea (UDZE)	No	No	N/A	*Pinnixa* sp.
Zoea (UDZD)	No	No	N/A	Porcellanidae
Megalopa (S MU5)*	Yes	No	N/A	No morphological identification
Megalopa (N MU2)*	Yes	No	N/A	No morphological identification
Megalopa (D MU2)*	Yes	No	N/A	No morphological identification
Zoea A (UDZA)	Yes	Yes (78%)	84 - 85	Xanthidae
Zoea B (UDZB)	Yes	Yes (25%)	83 - 84	No morphological identification
Zoea C (UDZC)	Yes	Yes (40%)	82 - 83	Leucosiidae
Megalopa (NOVU3)	Yes	Yes (33%)	83	No morphological identification
Megalopa (S MU1)	Yes	Yes (100%)	90 - 91	Leucosiidae
Megalopa (D MU1)	Yes	Yes (100%)	82 - 85	No morphological identification

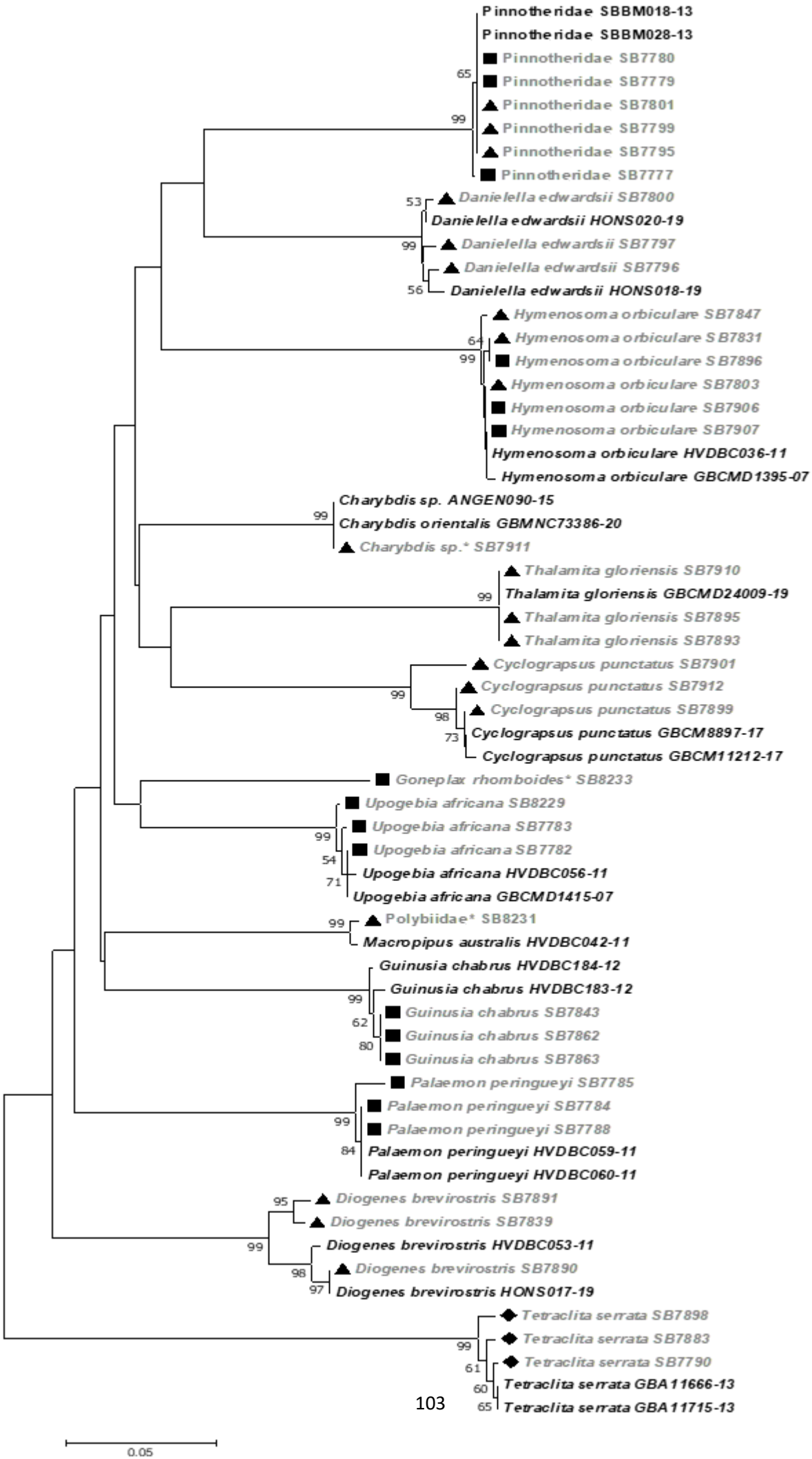
*indicates only one specimen was collected during the entire sampling period of the study.

Neighbor-joining phylogenetic trees (unrooted) were constructed for both fish and invertebrate larvae using sequences that were obtained in this study (three per taxon, referred to in Table 3.1), together with the three highest hit reference sequences retrieved from BOLD (Appendix, Table A1). As showed in both the fish and invertebrate neighbor-joining trees, all samples formed distinct clusters that grouped together with their respective reference sequences. Fish larvae sequences grouped into 15 distinct clusters, with low-high bootstrap values (53-100%), providing support to the species identification (Figure 3.3). The lowest bootstrap values were associated with the groupings of *Engraulis encrasicolus* (53%) and *Etrumeus whiteheadi* (54%) (Figure 3.3). Invertebrate larvae sequences grouped into 13 distinct clusters, with low-high bootstrap support (53-99%) (Figure 3.4). The lowest bootstrap values were associated with the groupings of *Upogebia africana* (54%) and *Danielella edwardsii* (53% and 56%) (Figure 3.4). The low bootstrap within groupings/clusters could be due to intraspecific variation, however, this was not the objective of the current study, and, as such, was not further examined, although this can make for additional analysis in future work. A single photograph was selected (of specimens from the current study) for each of these clusters (based on quality and clarity) and showcased in Table 3.3 so that a link could be created between the positively identified sequence and the actual larval specimen at its specified life stage.



*indicates only one specimen was collected during the entire sampling period of the study.

Figure 3.3. Neighbor-joining tree of fish larvae based on COI sequences, showing the placement of 63 specimens belonging to 12 families, including reference sequences. A total of three sequences per species (unless indicated by *) were used for samples from the current study and two sequences per species were retrieved from BOLD for comparison (in the case of *Samariscus triocellatus*, sequences were not publicly available on BOLD). Grey sample names and ID codes represent specimens from the current study and shapes appearing before codes indicate life stages: square(□)= postflexion, triangle(Δ)= preflexion, diamond(♢)= flexion. Black sample names and ID codes represent sequence data from BOLD. Bootstrap values over 50% are represented on the internal nodes of the tree. The scale bar represents 0.02 sequence divergence.

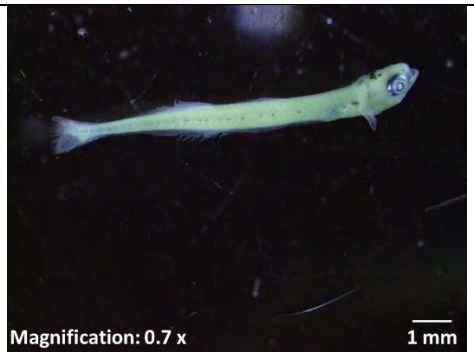


*indicates only one specimen was collected during the entire sampling period of the study.

Figure 3.4. Neighbor-joining tree of invertebrate larvae based on COI sequences, showing the placement of 61 specimens belonging to 12 families, including reference sequences. A total of three sequences per species (unless indicated by *) were used for samples from the current study and two sequences per species were retrieved from BOLD for comparison (in the case of *Goneplax rhomboides*, sequences were not publicly available on BOLD and only one sequence was available for Polybiidae and *Thalamita gloriensis*). Grey sample names and ID codes represent specimens from the current study and shapes appearing before codes indicate life stages: square(■)= zoea, triangle(▲)= megalopa, diamond(◆)= cyprid. Black sample names and ID codes represent sequence data from BOLD. Bootstrap values over 50% are represented on the internal nodes of the tree. The scale bar represents 0.05 sequence divergence.

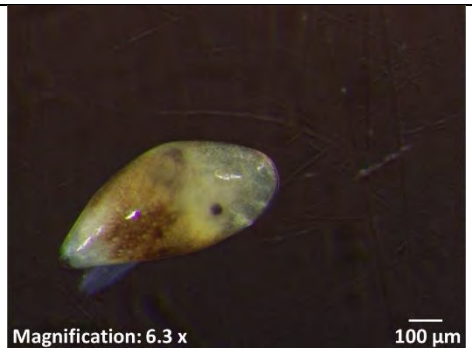
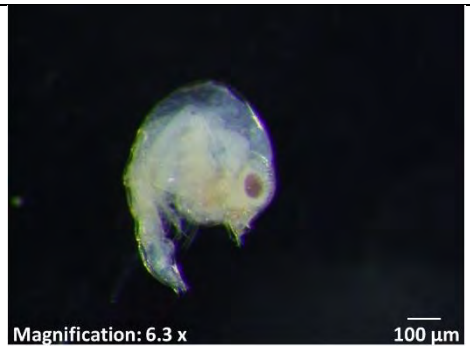
Table 3.3. Images and final BOLD identification (>98%) of specimens used for DNA barcoding from the current study. Life stages of species collected are indicated under the last column in brackets. The break in the table indicates the end of the fish larvae and beginning of invertebrate larvae.

Sample ID code	**Specimen image**	**BOLD identification (>98% match/similarity**
SB8004		*Caffrogobius natalensis* (Preflexion)
SB8006		*Caffrogobius nudiceps* (Preflexion)
SB8091		*Caffrogobius saldanha* (Preflexion)
SB7994		*Omobranchus woodi* (Preflexion)

SB8094		*Atherina breviceps* (Flexion)
SB7976		*Diplodus capensis* (Postflexion)
SB7997		*Rhabdosargus holubi* (Postflexion)
SB8013		*Monodactylus falciformis* (Postflexion)
SB8012		*Plectorhinchus chubby* (Postflexion)

SB8099		*Samariscus triocellatus* (Postflexion)
SB8235		*Clinus cottoides* (Postflexion)
SB7987		*Engraulis encrasicolus* (Postflexion)
SB7986		*Etrumeus whiteheadi* (Postflexion)
SB7984		*Sardinops sagax* (Postflexion)

SB8350		*Trichiurus cf. lepturus* var 2 (Postflexion)

SB7790		*Tetraclita serrata* (Cyprid)
SB7896		*Hymenosoma orbiculare* (Zoea)
SB7799		Pinnotheridae (Zoea)

SB7862		*Guinusia chabrus* (Zoea)
SB7784		*Palaemon peringueyi* (Zoea)
SB7782		*Upogebia Africana* (Zoea)
SB8233		*Goneplax rhomboides* (Zoea)
SB7801		Pinnotheridae (Megalopa)

SB7796		*Danielella edwardsii* (Megalopa)
SB7847		*Hymenosoma orbiculare* (Megalopa)
SB7912		*Cyclograpsus punctatus* (Megalopa)
SB7890		*Diogenes brevirostris* (Megalopa)
SB7911		*Charybdis sp.* (Megalopa)

SB7893			*Thalamita gloriensis* (Megalopa)
SB8231			Polybiidae (Megalopa)

3.4 Discussion

The majority of larval fish and invertebrates used for DNA barcoding during the current study were collected from the Port Alfred Marina, Eastern Cape, South Africa (only one larval fish species [the klipfish, *Clinus cottoides*] and one larval invertebrate species [*Goneplax rhomboides*] were collected at the first study site, Kenton-on-Sea). The marina forms part of the Kowie Estuary, which is considered an important nursery area for both marine and estuarine fish species (Kruger and Strydom, 2010). Similarly, many species of invertebrate larvae are transported between estuarine and coastal environments (Dame and Allen, 1996) as they search for suitable habitat for final settlement (Pineda and Reyns, 2018). This study provides the first report of overall taxonomic composition of larvae present at this site using the DNA barcoding technique as an identification tool.

Larvae were successfully identified from a variety of phyla to the lowest possible taxonomic level by using the DNA barcoding technique. During the current study, this technique was effective in successfully identifying 96% and 58% of fish and invertebrate larvae collected in the field (overall identification success of 86%), respectively, to either family, genus or species level. This result falls within the range (43 – 98%) of several globally based studies which used DNA barcoding as a tool to identify larval fish species in various aquatic environments (Ko et al., 2013; Ardura et al., 2016; Ayala et al., 2016; Azmir et al., 2017; Wibowo et al., 2017; Valdez-Moreno et al., 2010; Panprommin et al., 2020). For the invertebrate larvae, the identification success rate surpassed the range (22 – 36%) of other international studies based on identifying crustacean larvae using DNA barcoding in marine environments (Barber and Boyce, 2006; Webb et al., 2006; Heimeier et al., 2010), while it fell within the lower range (60 – 93%) of other global DNA barcoding based larval crustacean studies in marine (Tang et al., 2010; Brandao et al., 2016) and estuarine (Kusbiyanto et al., 2020) waters. Most DNA barcoding studies focus on either fish or invertebrate larvae from a specific aquatic system, while other studies tend to concentrate on specific taxonomic groups (Walczynska et al., 2019; Pardo et al., 2009; Victor et al., 2009; Hubert et al., 2010; Pappalardo et al., 2015), but limited studies focus on both fish and invertebrates from a range of taxonomic groups.

Attaining high concentrations of good quality DNA is the vital first step in determining the success rate of PCR's and, therefore, achieving successful DNA barcodes (Hajibabaei et al., 2005; Hellberg et al., 2014; Athanasio et al., 2016). Typically, a small quantity of DNA is required for barcode analysis and, therefore, the amount of tissue used for the extraction process is usually minimal (Hajibabaei et al., 2005). This is generally the comparative size of a matchstick head (Sunnucks and Hales, 1996) or a pencil head (Hajibabaei et al., 2005). Differences in overall amplification success between the fish and invertebrate larvae during the current study could, therefore, be due to the amount of muscle tissue available for the DNA

extraction process. For the fish larvae, there was normally enough muscle tissue, even for the smaller specimens, in which case the whole specimen was used. For the invertebrate zoeal and cyprid life stages, however, this was not the case, and this could be due to the chitinous exoskeleton of these crustaceans which can make the digestion process during DNA extraction problematic as this tissue can be very tough and, therefore, difficult to completely dissolve, hence yielding less DNA during extraction (Li et al., 2011; Athanasio et al., 2016). Additionally, the exoskeleton of most crustaceans is largely made up of polysaccharide chitins (Chen et al., 2008), which is known to contain PCR inhibitors (Schrader et al., 2012). The inhibitors contain substances that can have negative effects on the success rate of PCR amplification (Schrader et al., 2012; Nielsen et al., 2019). Coupled with the extremely minute size of these life stages (approximately 1 mm), along with having to use the whole specimen during the extraction process, the DNA concentrations were typically low and PCR amplification success varied amongst species. Furthermore, successful PCR amplification of the phylum Decapoda has also proven to be challenging in some instances (Eischeid et al., 2016). Low DNA concentrations could be addressed by using a larger amount of tissue (Penafiel et al., 2019), which could not be generated in the case of these early life stages as the whole specimen had to be used. Although the megalopal stages also possess a chitinous exoskeleton, which is more calcified (Xue et al., 2013), these life stages are larger in size and their muscle fibres are more developed (Xue et al., 2013) as compared to their earlier counterparts, zoeal stages. Additionally, during the current study, fish and crustacean specific primers along with universal primers were utilised with a standard reaction composition, however, primers can still be designed for specific taxon groups or several mixed primers could be used with variations in reaction compositions and/or thermal cycling (Heimeier et al., 2010; Eischeid et al., 2016). This could enhance overall amplification success of different species (Heimeier et al., 2010).

The next critical step in DNA barcoding is built on determining a match between a voucher specimen and its corresponding DNA sequence (Costa and Carvalho, 2007). To achieve this, morphological identification is a mandatory step, and in the absence of this, a species description cannot be allocated to the sequence (Barber and Boyce, 2006). The success of DNA barcoding, thus, is dependent on the joint work between taxonomists and the DNA barcoding community (Ardura et al., 2013; Raupach and Radulovici, 2015). Accurate and consistent identification of taxonomic groups at the early life stages, however, can be very challenging and sometimes, impossible, even for highly trained and experienced taxonomists (Wibowo et al., 2017). Mass scale efforts in DNA barcoding initiatives conducted on specific groups of animals within a certain region (more commonly on adult individuals) (Fagg et al., 2021), which include georeferencing with high quality images of individuals (Gratton et al., 2017), can allow taxonomists to accurately identify adult individuals rather than the early life stages and sequences of adult specimens can later be linked to their larval counterparts as the DNA barcoding databases expand. Examples of such mass scale efforts conducted in South Africa include studies conducted by Steinke et al (2016b) on fish species and Fagg et al (2021) on invertebrate species. Larval sequences for some common/model species have, therefore, become available on the BOLD system (with attached images) and have been matched to their adult counterparts.

For the invertebrate larvae that could not be amplified and the sequences that did not find a significant match on the BOLD system, the zoeal developmental stages were morphologically identified to family and genus level, while only one megalopal developmental stage was identified to the family level, the rest could not be identified using morphological methods. The different developmental stages of invertebrate larvae often do not show any specific diagnostic characters among one another and/or their adult stage, but instead can exhibit very cryptic morphologies (Anger, 2006; Pardo et al., 2009; Becker et al., 2011; Raupach and

Radulovici, 2015). As an example, crustacean larvae belonging to the infraorder Brachyura require detailed dissection of appendages and observation of the features revealed. This can be time-consuming and involves availability of comprehensive larval descriptive keys (Brandao et al., 2016). As a result, there is a lack of reference/comparative sequences available on public databases (Webb et al., 2006). The invertebrate larval taxa which had successful sequence amplifications, but no significant match on the BOLD system, could, therefore, have no reference sequences that were uploaded on the database, reducing success of resolution. Another scenario that is possible (but less likely), is that these sequences correspond to an undescribed species (Webb et al., 2006; Brandao et al., 2016).

Fish represent the most comprehensively sampled and researched group of marine metazoans in comparison to others (Bucklin et al., 2011), however, there are still inherent difficulties in accurately morphologically identifying their early life stages to the species level (Victor et al., 2009; Ko et al., 2013; Sachithanandam and Mohan, 2018). For the larval fish taxa that could not be amplified during the current study, two fish larvae were morphologically identified to species level, and the other two were identified to genus and family level, respectively. In contrast, the taxa with successful sequences were all identified to the species level using the DNA barcoding technique. In a study conducted by Ko et al (2013), it was revealed that morphological identification to the species level amongst different taxonomists and laboratories were relatively low and differed significantly, this is also exacerbated by a lack of descriptive keys available for fish larval species (Azmir et al., 2017), which is especially true for South African species (Somana, 2019). It should be noted, however, that there has been an increased number of larval sequences (with attached images) being deposited into the BOLD system, as expertise in morphological identification of larval specimens expands amongst taxonomists. The DNA barcoding technique has, nevertheless, proved to be extremely beneficial in larval fish species identification to the lowest possible taxonomic level. In the

current study, only one fish larval sequence failed to find a significant match on the BOLD system. During a mass scale study conducted by Steinke et al (2016b), over 1000 species of South African fish were successfully barcoded and deposited in the BOLD system, which was made up of both adults and larvae, contributing significantly to the representation of this taxonomic group in the database and leading to most species being identified successfully to species level during the current study. This reinforces that mass scale studies increase geographic coverage and make a significant contribution to identification of the early life stages in DNA barcoding databases. The fact that even one specimen could not be significantly matched on BOLD, however, highlights the need to further update the reference library of COI sequences on the database for fish.

This study used COI molecular sequences to identify species into the lowest possible taxonomic level, preferably to species level. While species identification using a single molecular marker (as compared to using multiple markers) could, in some cases, prevent identification to species level, the COI mitochondrial marker was strategically selected due to three main reasons. These reasons were as follows: (1) 'universal' primers were successfully able to amplify a sizeable region of this gene, which lends itself the ability to be used for species from a broad range of taxa (Folmer et al., 1994); (2) when compared to other mitochondrial markers, COI lacks overlap between interspecific and intraspecific variation, which is critical for accuracy of barcode gene regions (Meyer and Paulay, 2005); and (3) the evolution of the COI gene is fairly rapid which leads to high phylogenetic resolution and allows discrimination of closely related species (Bucklin et al., 2011). There have also been several studies which have successfully barcoded larval crustacean species using the COI gene region and reported clear clustering of monophyletic groups with their reference sequences (Heimeier et al., 2010; Tang et al., 2010; Brandao et al., 2016; Kusbiyanto et al., 2020). The same can be said for larval fish species, with several studies highlighting the reliability of using the COI marker for

species level identification (Pegg at al., 2006; Valdez-Moreno et al., 2010; Ko et al., 2013; Ayala et al., 2016; Azmir et al., 2017; Nuryanto et al., 2017).

Verification of molecular based taxonomic identification was based on matching unknown COI sequences to known reference sequences and phylogenetic analysis during the current study. The phylogenetic approach provides a reliable compromise between accuracy in identification and the ability to individually assess identification confidence levels by considering the vastly heterogenous species coverage in the public reference databases (Heimeier et al., 2010). The main strengths of such an approach are two-fold: (1) reasonably powerful tests can be used (such as the bootstrapping method) to reveal the probability of an unknown sequence matching a reference sequence, irrespective of the level of taxon identification and; (2) the phylogenetic trees are represented in a graphic context which allows simple visual assessment of the placement and confidence levels of each taxon (Heimeier et al., 2010; DeSalle and Goldstein, 2019). All samples in the current study (separate neighbor-joining trees constructed for fish and invertebrate larval samples) formed distinctive groups with their reference sequences, providing two pieces of information. Firstly, the sample assignment into specific taxonomic levels as provided by the BOLD System results is strengthened, as specimens which form single distinctive clades are considered to be part of the same taxon (Xu et al., 2015). Secondly, it provides further verification that the mitochondrial COI gene is indeed a reliable DNA barcoding marker for identification of marine fish and invertebrate species (Kusbiyanto et al., 2020).

All fish and invertebrate species identified through DNA barcoding in the present study are currently found in South African waters and distributed within the Eastern Cape (World Register of Marine Species; https://www.marinespecies.org), with the exception of the invertebrate megalopal stage larvae belonging to the family Portunidae (*T. gloriensis*). This

species has a native distribution in the Indo West Pacific and known non-native distribution in the Mediterranean Sea (Crosnier, 1962). Known vectors of *T. gloriensis* include shipping and maritime traffic (Brockerhoff and McLay, 2008). Brachyuran crabs are a globally successful group of invaders, with the Portunidae family making up the highest number of this statistic (Brockerhoff and McLay, 2011; Swart et al., 2018), with records of some species within this family having range extensions in South Africa (Ma and McQuaid, 2021). While there could be specific traits associated with the early life stages of the portunids which make them successful invaders, such as long larval developmental time, high rate of dispersal and high reproductive potential, these have not yet been confirmed and remain uncertain (Swart et al., 2018). The species *T. gloriensis* was positively identified with a similarity percentage of >99% on the BOLD system and according to the results of this match, 'this identification is solid unless there is a very closely allied congeneric species that has not yet been analysed' (https://www.boldsystems.org/index.php/IDS_IdentificationRequest). Although there were no detections of adult *T. gloriensis* in the area, there have been cases where larval stages of invasive invertebrate species were detected before their adult stages (Marco-Herrero et al., 2018). It is, therefore, possible that this species could be a potential invader in the initial stages of establishment (bearing in mind that presence of larvae is an indication of recent reproduction), however, this will require further investigation that was beyond the scope of the current study.

During the current study, five species that were collected and barcoded from the Port Alfred Marina represent commercially and/or recreationally important fish species within South Africa which spend their early life stages in estuarine systems, namely: the sparids: blacktail, *Diplodus capensis* and cape stumpnose, *Rhabdosargus holubi*, as well as the clupeoids: *Etrumeus whiteheadi*, *Engraulis encrasicolus* and *Sardinops sagax*. Assessment of these important species is essential in maintaining their fishery yields and quantification and

classification of their early life stages is an effective method of monitoring recruitment processes (Wibowo et al., 2017). Larval stages of fish are the most vulnerable and tend to have the highest mortality rate and are, thus, considered the primary determining factor of recruitment (Bergenius et al., 2002; Costalago et al., 2018). The ability to predict these recruitment patterns would, therefore, significantly advance fisheries management (Huggett et al., 1998). The key requirement for this is to accurately identify species (both adults and early life stages) (Swartz et al., 2008; Valdez-Moreno et al.,2010). Misidentification of the early life stages in particular, can misinform understanding of important aspects, such as: speciation; niche-partitioning; diversity; and other ecosystem attributes (Swartz et al., 2008). DNA barcoding offers an effective, reliable and rapid system for identification of these specimens by non-taxonomists, irrespective of the life stage (Krishnamurthy and Francis, 2012; Trivedi et al., 2016). Shearer and Coffroth (2006) measured recruitment success of Caribbean scleractinian coral recruits between two different reef habitats using genetic identification methods and found that there was mostly exclusive recruitment from two species and that there was higher recruitment in one of the reef habitats as compared to the other. Valdez-Moreno et al (2010) provided new and original information on the spawning times and areas for a commercially important fish species in the Mexican Caribbean by connecting/matching collected eggs and larvae to adults by using barcodes. These types of findings can assist in fisheries research by improving management efforts in certain areas.

The current study has provided accurate identifications to the lowest possible taxonomic level using the DNA barcoding method for both fish and invertebrate larval stages present within a specified geographic region and proved to be both fast and reliable. Each of these barcoded specimens are supported by good quality images/photographs that can potentially be linked to their adult counterparts that have been previously deposited in the BOLD system. An extensive database of COI sequences for all species that are linked to expertly identified adult voucher

specimens which are then correlated with their early life stages (larvae/juvenile) would facilitate major progression in invasive species management (Bezeng and van der Bank, 2019), the fishing industry (Becker et al., 2011), larval distribution patterns which relates to spawning grounds (Burrows et al., 2018) and overall can contribute significantly to ecological conservation and management initiatives (Karahan et al., 2017).

CHAPTER 4: General Discussion

Some of the most severe and irreversible human alterations to natural ecosystems have been steered by urbanisation-related processes, and while the impacts of urbanisation have been investigated at length on land, they also have extreme impacts on ocean ecosystems due to the growing number of people occupying coastal areas (Bishop et al., 2017; Todd et al., 2019). On the other hand, however, the marine realm has received less attention (Airoldi et al., 2021). Coastal cities, where artificial and naturally occurring environments adjacent to coastlines interface (Zari et al., 2019), are rapidly increasing and their related infrastructure is spreading into oceans because of the growing need for space for humans and their associated economic and social activities (Bulleri and Chapman, 2010). Meeting the needs and wants of the growing human population migrating towards coastal areas, while still protecting and sustaining the natural functionality of coastal systems and the diverse biodiversity they support, has become one out of many significant challenges of our time (Airoldi et al., 2021).

Within this framework, and to contribute to advancing knowledge of the biological and ecological repercussions of artificially constructed urbans settings on the natural environments they fragment and/or replace, this thesis aimed to investigate the role that natural and urban microhabitats play on the early life stages of fish and invertebrate species, which are the foundation of adult populations and form the bulk of the cohort that will directly determine habitat selection. This scope was accomplished by analysing the composition and distribution of larvae amongst selected microhabitats as well as determining the relationship between these larval assemblages and ambient environmental variability within each habitat. Additionally, since identification of larval species formed a crucial component of the current study, as with most diversity-based studies, DNA barcoding was used as a complementary tool to increase the success rate of identifying larval species.

Main findings of this research indicate that vertical walls and jetties within the urban site, identified as potential sheltered microhabitats that somehow resemble the natural structural complexity of rocky shores, are important for the early ontogeny of fish and invertebrates. Marinas may, therefore, act as novel habitats for early stage diversity and this may largely depend on the fact that some of their components (as jetties and vertical walls) are always submerged, hence, offering new and unique hard substrata where none formerly existed (Gracia et al., 2021). Additionally, due to the semi-enclosed nature of marinas, hydrodynamic and other environmental processes (such as temperature, pH and salinity) are modified in response to reduced wave action and tidal flushing capacity (Ming et al., 2010; Bouchoucha et al., 2016; Megina et al., 2016). This could further facilitate the retention of early life stages, by acting as a barrier/buffer and, hence, delaying the export to open waters (Vorsatz et al, 2021b).

Development of biological communities on artificial substrates begins with initial settlement of epibiotic assemblages such as algal and sessile invertebrate species which can provide important food sources as well as shelter and protection from predation (Clynick et al., 2007; Andersson et al. 2009). This potential for enhanced protection is foundational for the colonisation and establishment of additional mobile fish and invertebrate species (Moshella et al., 2005; Wehkamp, 2012). Such artificial, urban settings could, therefore, provide suitable conditions linked to improved growth and overall survival of species, thus making urban coastal armouring, and associated provision of microhabitats, possible surrogates, if not enhancers of natural surfaces.

4.1 Habitat selection on urban coastal infrastructures: a hub for replenishment of natural populations?

Coastal urban regions refer to aquatic-based areas, generally characterised by a substantial amount/coverage of artificial infrastructures and/or irreversible modification of resident and

surrounding seafloor features (such as marinas and armoured coastlines) as well as being in close proximity or directly surrounded by metropolitan settings with a relatively high population count (Airoldi et al., 2021). This is the case for the Port Alfred Marina, located within an estuarine coastal system, surrounded by the town of Port Alfred with an estimated population of 65 000 residents (Cock, 2018). Since coastal systems are generally linked to high productivity and provide habitats for a variety of fish and invertebrate species (Vasconcelos et al., 2010), it is critical to determine if urban coastal microhabitats have the potential to facilitate habitat selection and host high biodiversity, hence acting as hubs for replenishment of adjacent natural populations. The results from this research suggests so.

The urban (micro)habitats in the Port Alfred Marina presented a large abundance and diversity of larval species. The presence of larval stages generally indicates recent reproduction and the possibility of completed larval development within the marina, due to the occurrence of zoeal and megalopal stages of the same two taxa (*Pinnotheres* sp. and *Hymenosoma orbiculare*). These novel habitats could, therefore, be linked to provision of settlement cues related to successful reproduction and overall survival. Organisms could, therefore, respond to these cues by remaining in these habitats. It is, hence, probable that these urban coastal infrastructures are reliable recipients of habitat selection for early life stages. Furthermore, genetically and phenotypically inherited larval traits connected to successful settlement cues could play a role in early life stages continually selecting and recruiting to urban habitats. Consistent selection of a specific habitat could be advanced through a phenomenon referred to as Natal Habitat Preference Induction (NHPI), in which experience with settlement cues in the natal habitat determines habitat selection of individuals, thus increasing the likelihood that an individual will favourably select a habitat which has comparable/similar stimuli to that of its natal habitat (Davis and Stamps, 2004), and in this case, it could be the urban microhabitats. Neither direct measures of the dynamics of habitat selection, nor estimates of adult stages were evaluated

during the current study. Further studies on the adult presence, settlement and recruitment patterns of early life stages as well their molecular profiles, could, therefore, assist in revealing if the large abundance of fish and invertebrate larvae at urban habitats could, in fact, serve as replenishment populations to the adjacent natural habitats.

Species which can exploit resources (including provision of microhabitats) made available by the urban settings and which are also capable of performing crucial ecological processes can form an important component in sustaining ecosystem functioning and diversity in natural habitats that have been partially or completely replaced by urban infrastructure (Hobbs et al., 2006, Mayer-Pinto et al., 2018). The resulting increased diversity and potential functional redundancy are crucial factors which may allow urban ecosystems to withstand further anthropogenic disturbances (Olds et al., 2016) and remain in a relatively stable state (Biggs et al., 2020).

The degradation of the structural complexity/heterogeneity of many coastal habitats derived from urbanisation has often been associated with structural and functional differences of biological communities as compared to the natural marine environments (e.g., Mayer-Pinto et al., 2018). The current study has, however, highlighted that larval abundances and diversity can still thrive in urban settings. Although the high numbers of larval assemblages found in the Port Alfred Marina were not compared to the natural environment adjacent directly adjacent to the estuary, this result still indicates that a relatively stable ecosystem remains in place with potential functional redundancy through the provisioning of structures in the form of artificially constructed microhabitats.

Caution is, however, advised as both biological diversity and functional redundancy are particularly sensitive to continued landscape alteration (Olds et al., 2018), and if ecosystem function deteriorates, the system can transition from an overall steady to an unstable state,

possibly resulting in permanent decline of the species thriving within that system (Alberti and Marzluff, 2004). Additionally, the novel habitats created by urban coastal infrastructure have been continuously shown to facilitate the establishment and spread of invasive species (Glasby et al., 2007; Long et al., 2011; Airoldi et al., 2015). Although a heavy presence of larval stages of invasive species was not observed in this study, strong active-based intervention measures in coastal urban settings are necessary to incorporate economic, social- and environmentally-mediated objectives, (Airoldi et al., 2005; Morris et al., 2019) in order to preserve and possibly enhance the biodiversity currently occupying these spaces, especially in connection to functionality of early stage processes and diversity.

Major intervention measures currently making headway in actively maintaining and enriching biodiversity in coastal urban settings include two approaches. Firstly, greening grey infrastructure or ecological engineering schemes. These practices involve designing low impact and multifunctional artificial coastal structures which mimic the features present in natural habitats (such as microhabitats) (Chapman and Underwood, 2011; Firth et al., 2013; Firth et al., 2016; Waltham and Sheaves, 2018; Loke et al., 2019; Evans et al., 2021). This can encourage habitat selection, and, therefore, settlement and recruitment of early life stages of species. Secondly, habitat restoration initiatives which encompass various forms of interventions such as remediation, rehabilitation and reallocation of systems impacted by artificial infrastructures. These procedures aim to repair and/or enhance the natural state of the system by facilitating positive species interactions (Geist and Hawkins, 2016; Abelson et al., 2020) and potentially improving settlement and overall post-settlement survival. The result of these facilitative processes can increase fitness of the resultant adult population, ultimately ensuring biological redundancy within the urban system. These broadly based intervention strategies are crucial in conserving early life stages of species still thriving in coastal urban habitats by improving processes related to their successful settlement and recruitment, hence,

improving chances of their survival to adult stages. The conservation of natural populations in these novel habitats can then enhance its tolerance to growing anthropogenic pressures from the level of molecular to ecosystem functions, thus, preserving the ability of these systems to provide key functions and services to both humans and overall aquatic biodiversity (Airoldi et al., 2021).

In conclusion, since coastal urbanisation will continue to be a common phenomenon occurring on a global scale, it is essential to not only understand and comprehend the consequences of the proliferation of coastal artificial structures, but also recognise the potential benefits and creation of novel habitats that could support early life stages of native species similar to that of natural habitats, which was highlighted in the current study. It is, hence, of key relevance to maintain a focus on the processes that drive onset of coastal early life stage population use of urbanised habitats when managing the construction and development of future coastal infrastructure. While this could prove challenging, especially in relation to accurately collecting taxonomic information on early life stages of species, the current study also highlighted that DNA barcoding can be used as a consistent and effective tool for this purpose, hence allowing for reliable data collection on the early life stages present in these habitats. The results from dedicated environmental impact assessments can inform managers of urban settings who are working toward implementation and execution of initiatives designed with the goal of sustaining and/or increasing the biodiversity of organisms present within these areas whilst simultaneously increasing the invasive resistance of artificial structures (Mayor-Pinto et al., 2018). Although these strategies have major potential in mitigating the impacts of artificial structures on marine biodiversity while still maintaining the needs of the growing human populations socio-economic needs, they require clear and precise aims with a solid foundation built on a profound understanding of artificially created urban habitats. This knowledge is essential in balancing the emerging conflict between providing human services and sustaining

the delicate equilibrium created within coastal urban habitats. This will ensure that early stage functionality and diversity within these coastal urban systems can persist, therefore, providing connections to natural systems and potentially acting as a source of replenishment to natural populations to support the recovery of foundation species, thus, serving as hubs for maintenance of coastal biodiversity.

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Chapter 3 Appendix

Table A1: Information for reference sequences retrieved from BOLD used in the construction of the neighbor-joining tree for comparison against fish and invertebrate larvae sequences from the current study. The thick bottom line indicates the break between the fish and invertebrate larvae information.

BOLD ID	Locality	Photo available	Life stage	Taxonomy
DSFSE914-08	South Africa, Eastern Cape, East Kleinemonde	No	Information unavailable	*Atherina breviceps*
DSFSE911-08	South Africa, Eastern Cape, East Kleinemonde	No	Information unavailable	*Atherina breviceps*
DSLAG1127-11	South Africa, KwaZulu-Natal, Durban	No	Information unavailable	*Caffrogobius natalensis*
SAIAB093-06	South Africa, Western Cape, Knysna estuary	Yes	Adult	*Caffrogobius natalensis*
SAIAB497-07	South Africa, Eastern Cape, Keiskamma River	No	Information unavailable	*Caffrogobius nudiceps*
SAIAB495-07	South Africa, Eastern Cape, Keiskamma River	No	Information unavailable	*Caffrogobius nudiceps*
SAIAB090-06	South Africa, Western Cape, Knysna estuary	Yes	Adult	*Caffrogobius saldanha*
GBGCA1958-13	Information unavailable	No	Information unavailable	*Caffrogobius saldanha*
DSFSE847-08	South Africa, Eastern Cape, Kelly's Beach	No	Information unavailable	*Clinus cottoides*
DSFSE845-08	South Africa, Eastern Cape, Kelly's Beach	No	Information unavailable	*Clinus cottoides*
DSLAR179-08	South Africa, KwaZulu-Natal, Park Rynie	Yes	Eggs/larvae	*Diplodus capensis*
DSLAR186-08	South Africa, KwaZulu-Natal, Park Rynie	Yes	Eggs/larvae	*Diplodus capensis*
DSLAG731-10	South Africa, KwaZulu-Natal, Park Rynie	Yes	Eggs/larvae	*Engraulis encrasicolus*
TZSAL352-13	South Africa, KwaZulu-Natal, Park Rynie	Yes	Larvae	*Engraulis encrasicolus*
DSLAF595-08	South Africa, KwaZulu-Natal, Park Rynie	Yes	Eggs/larvae	*Etrumeus whiteheadi*
DSLAG1007-10	South Africa, KwaZulu-Natal, Park Rynie	No	Information unavailable	*Etrumeus whiteheadi*
DSLAF251-07	South Africa, KwaZulu-Natal, Park Rynie	Yes	Eggs/larvae	*Monodactylus falciformis*
DSLAF133-07	South Africa, KwaZulu-Natal, Park Rynie	Yes	Eggs/larvae	*Monodactylus falciformis*
DSFSG328-10	South Africa, KwaZulu-Natal, Illovo estuary	No	Information unavailable	*Omobranchus woodi*
DSFSE607-08	South Africa, KwaZulu-Natal, Illovo estuary	Yes	Adult	*Omobranchus woodi*

DSLAG1282-11	South Africa, KwaZulu-Natal, Durban	Yes	Eggs/larvae	*Plectorhinchus chubbi*
TZSAL817-13	South Africa, KwaZulu-Natal, Park Rynie	Yes	Larvae	*Plectorhinchus chubbi*
DSLAG1269-11	South Africa, KwaZulu-Natal, Park Rynie	Yes	Larvae	*Rhabdosargus holubi*
DSLAG890-10	South Africa, KwaZulu-Natal, Park Rynie	Yes	Larvae	*Rhabdosargus holubi*
TZSAL515-13	South Africa, KwaZulu-Natal, Park Rynie	Yes	Larvae	*Sardinops sagax*
TZSAL563-13	South Africa, KwaZulu-Natal, Park Rynie	Yes	Postflexion/juvenile	*Sardinops sagax*
DSLAF502-08	South Africa, KwaZulu-Natal, Park Rynie	Yes	Eggs/larvae	*Trichiurus cf. lepturus* var 2
DSLAR001-08	South Africa, KwaZulu-Natal, Park Rynie	Yes	Eggs/larvae	*Trichiurus cf. lepturus* var 2
GBA11666-13	Information unavailable	No	Information unavailable	*Tetraclita serrata*
GBA11715-13	Information unavailable	No	Information unavailable	*Tetraclita serrata*
GBCM8897-17	Information unavailable	No	Information unavailable	*Cyclograpsus punctatus*
GBCM11212-17	Information unavailable	No	Information unavailable	*Cyclograpsus punctatus*
HONS020-19	South Africa, Western Cape, Knysna, Thesen Island	Yes	Adult	*Danielella edwardsii*
HONS018-19	South Africa, Western Cape, Knysna, Thesen Island	Yes	Adult	*Danielella edwardsii*
HONS017-19	South Africa, Western Cape, Knysna, Train Bridge	Yes	Adult	*Diogenes brevirostris*
HVDBC053-11	South Africa, Eastern Cape, Addo	Yes	Adult	*Diogenes brevirostris*
HVDBC036-11	Namibia, Cunene	Yes	Adult	*Hymenosoma orbiculare*
GBCMD1395-07	Information unavailable	No	Information unavailable	*Hymenosoma orbiculare*
HVDBC059-11	South Africa, Eastern Cape, Addo	Yes	Adult	*Palaemon peringueyi*
HVDBC060-11	South Africa, Eastern Cape, Addo	Yes	Adult	*Palaemon peringueyi*
HVDBC056-11	South Africa, Eastern Cape, Addo	Yes	Adult	*Upogebia africana*
GBCMD1415-07	Information unavailable	No	Information unavailable	*Upogebia africana*
HVDBC183-12	South Africa, Western Cape, Betty's Bay	Yes	Adult	*Guinusia chabrus*
HVDBC184-12	South Africa, Western Cape, Betty's Bay	Yes	Adult	*Guinusia chabrus*

SBBM018-13	South Africa, Eastern Cape, Transkei	Yes	Megalopa	Pinnotheridae
SBBM028-13	South Africa, Eastern Cape, Transkei	Yes	Megalopa	Pinnotheridae
ANGEN090-15	India, Gujarat	Yes	Adult	*Charybdis sp.*
GBMNC73386-20	Bangladesh	No	Information unavailable	*Charybdis orientalis*
GBCMD24009-19	Australia	No	Information unavailable	*Thalamita gloriensis*
HVDBC042-11	Namibia, Cunene	Yes	Adult	*Macropipus australis*