Cape Peninsula
University of Technology

The "Suitcase Hypothesis" – can eddies provide a pathway for gene flow between Madagascar and KwaZulu-Natal?

by

Samantha Angelique Natasha Ockhuis

Thesis submitted in fulfilment of the requirements of for the degree
Master of Technology: Oceanography
in the Faculty of Applied Science
at the Cape Peninsula University of Technology

Supervisors: Dr Jenny Huggett and Dr Conrad Sparks
Co-supervisor: Dr Gavin Gouws

Cape Town
Date submitted: November 2016

DECLARATION

I, **Samantha Angelique Natasha Ockhuis**, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

_____   _____

Signed   **Date**

ABSTRACT

Similarities in marine fauna found off the coasts of southern Madagascar and KwaZulu-Natal (KZN, South Africa) led to the development of the "Suitcase Project", with the aim of establishing whether eddies that form off southern Madagascar may package and transport biological material, as if in a suitcase, across the Mozambique Channel. In pursuit of this question, sampling was conducted on the southern Madagascan shelf and along a transect across a cyclonic eddy which originated off the southern tip of Madagascar, between the 15th and 23rd of July 2013. Bongo nets (300 and 500 μm-mesh) and a neuston net (900 μm-mesh) were used to collect zooplankton within the upper 200 m and at the surface, respectively. Samples were sorted for meroplankton (larval stages of fish and benthic invertebrates) under a stereo microscope, particularly seeking species known to be common to both the east coasts of Madagascar and South Africa and, thus potential indicators of connectivity between these regions. Larvae of crabs, rock lobster, and fish were used for DNA barcoding. Zooplankton biovolume and abundance were compared between the eddy core, eddy periphery and outer regions of the eddy, as well as stations from the Madagascan shelf. Mean neuston biovolume on the Madagascan (0.08 mL m^{-3}) was not significantly higher than that in the eddy (0.06 mL m^{-3}). Mean bongo biovolume in the upper 200 m was much higher on the Madagascan shelf (0.62 mL m^{-3}) than in the eddy (0.16 mL m^{-3}) although only 2 stations were sampled on the shelf. Highest biovolume in the eddy was recorded in the west eddy zone (0.25 mL m^{-3}) and west outer zone (0.23 mL m^{-3}), which was not statistically significantly higher than the eddy core (0.12 mL m^{-3}) and east eddy zone (0.17 mL m^{-3}). Meroplankton was comprised of coastal origin taxa and was most abundant on the shelf and in the eddy perimeters. Larval goat-fish, *Parupeneus fraserorum* was identified, a newly described mullid, and has been recorded on both the coasts of Madagascar and KZN, SA. Larvae of coastal invertebrate species identified, include the squat lobster *Allogalathea elegans* and camel shrimp *Rhynchocinetes durbanensis*. Other larval fish identified, but not found in high abundance include the families of reef associated fishes, for example: Apogonidae, Labridae, Pomacentridae, Priacanthidae, Serranidae and Sparidae. Higher zooplankton biovolumes, larval abundances and reef-associated larval assemblages found on the Madagascan shelf and in the periphery of the cyclonic eddy compared to the core in this study provide support for the suitcase hypothesis that planktonic organisms are entrained within eddies as they propagate south-westwards of the Madagascan shelf. However, further studies are required to determine whether planktonic larvae are able to cross the Mozambique Channel and reach the KZN coast in time to settle.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

LIST OF ACRONYMS

AC (Agulhas Current)

ACEP (African Coelacanth Ecosystem Programme)

ANOVA (Analysis of Variance)

ASCLME (Agulhas and Somali Currents Large Marine Ecosystems Programme)

KZN (KwaZulu-Natal)

BOLD (Barcode of Life Data Systems)

COI (cytochrome *c* oxidase subunit I)

CTD (Conductivity Temperature Depth)

DNA (Deoxyribonucleic acid)

EACC (East African Coastal Current)

K2P (Kimura 2-parameter distance)

MC (Mozambique Channel)

MEGA (Molecular Evolutionary Genetics Analysis)

MESOBIO (Influence of mesoscale dynamics on biological productivity at multiple trophic levels in the Mozambique Channel)

NEMC (North East Madagascar Current)

NJ (Neighbour joining)

PCR (Polymerase chain reaction)

PLD (Pelagic larval duration)

SA (South Africa)

SEC (South Equatorial Current)

SEMC (South East Madagascar Current)

SICC (South Indian Countercurrent)

SST (Sea Surface Temperature)

SSHA (Sea Surface Height Anomaly)

Sv (Sverdrup): Measurement of volume transport – i.e. 15 x 106 / m^3 = 15 Sv

SWIO (South West Indian Ocean)

WBC (Western Boundary Currents)

WIO (Western Indian Ocean)

CHAPTER 1: INTRODUCTION

1.1 Introduction

Mesoscale eddies are features of ocean circulation, with horizontal spatial scales of 10 – to 1000 km and time scales of 1 to 3 years, which may entrain planktonic organisms, influencing their distribution and acting as vectors of transport (Rodrıguez *et al.* 1999, Mackas and Galbraith 2002, Batten and Crawford 2005, Holliday *et al.* 2011). Through the clockwise rotation of cyclonic eddies in the southern hemisphere, a vertical upwelling flow is caused in their interiors which causes a divergence (outward flow) of surface waters and subsurface nitrate-rich water is pushed upwards into the euphotic zone (Bakun 2006), resulting in increased primary production in the cores of eddies (Yentsch and Phinney 1985, Falkowski *et al.* 1991, McGillicuddy and Robinson 1997, McGillicuddy *et al.* 1998). In anticyclonic eddies, the opposite occurs; anticlockwise rotation and convergence cause surface waters to be pushed downwards (downwelling) (Bakun 2006). Eddies both cyclonic and anticyclonic may also entrain productive water of coastal origin and, as a consequence, eddies can function as nursery grounds for organisms such as fish larvae (Rodrıguez *et al.* 1999, Govoni *et al.* 2010).

Mesoscale eddies are found in all oceans (Rhines 2001), but are particularly prominent at the ends of Western Boundary Currents (WBC). The Agulhas Current (AC) is the largest WBC in the global ocean (Bryden *et al.* 2005) and the only WBC with frequent mesoscale activity in its source region, generated by two zones of high mesoscale variability: the Mozambique Channel (MC) (Schouten *et al.* 2002) and the South East Madagascar Current (SEMC) (Siedler *et al.* 2009).

A number of recent studies conducted on mesoscale eddies in the MC (Barlow *et al.* 2014, Halo, *et al.* 2014a, Hancke *et al.* 2014, Huggett 2014, Lamont *et al.* 2014, Lebourges-Dhaussy *et al.* 2014, Marsac *et al.* 2014, Ménard *et al.* 2014, Potier *et al.* 2014, Ternon, Roberts, *et al.* 2014a) demonstrated the origins and importance of eddies in the largely oligotrophic system of the MC, where they provide mechanisms by which the physical energy of the ocean system can be converted to trophic energy to support biological processes and food webs (Bakun 2006, Godø *et al.* 2012).

The biota on the south-east coast of South Africa (SA) comprises species of both subtropical and tropical origin (Teske *et al.* 2011). The coastal environment on the south-east coast of SA is profoundly influenced by the warm, southward-flowing AC, which could explain the presence of tropical biota on the southeast coast of SA in this region (Figure 1) (Teske *et al.*

2011). The disjunct distribution of certain subtropical species on the coasts of both KZN and Madagascar is less easily explained. These species include several fishes of the family Sparidae (S. Fennessy, personal communication) as well as various, non-endemic invertebrates, including the brown mussel *Perna perna* (Berry 1978), the shallow water spiny lobster *Panulirus homarus* (Charbonnier and Crosnier 1961), the deep-water spiny lobster *Palinurus delagoae* (Gopal *et al.* 2006), the ghost crab *Ocypode madagascariensis* (Jackson *et al.* 1991), the estuarine crabs *Scylla serrata* and *Varuna litterata* (Petrocci and Lipton 1994), and the corals *Acropora austera* and *Platygyra daedalea* (M. Schleyer, ORI, unpublished data). Additionally, southern Madagascan gammarid amphipods (Ledoyer 1982) have striking similarities morphologically to coastal and marine amphipods found in northern KZN (F. Mackay, ORI, unpublished data).
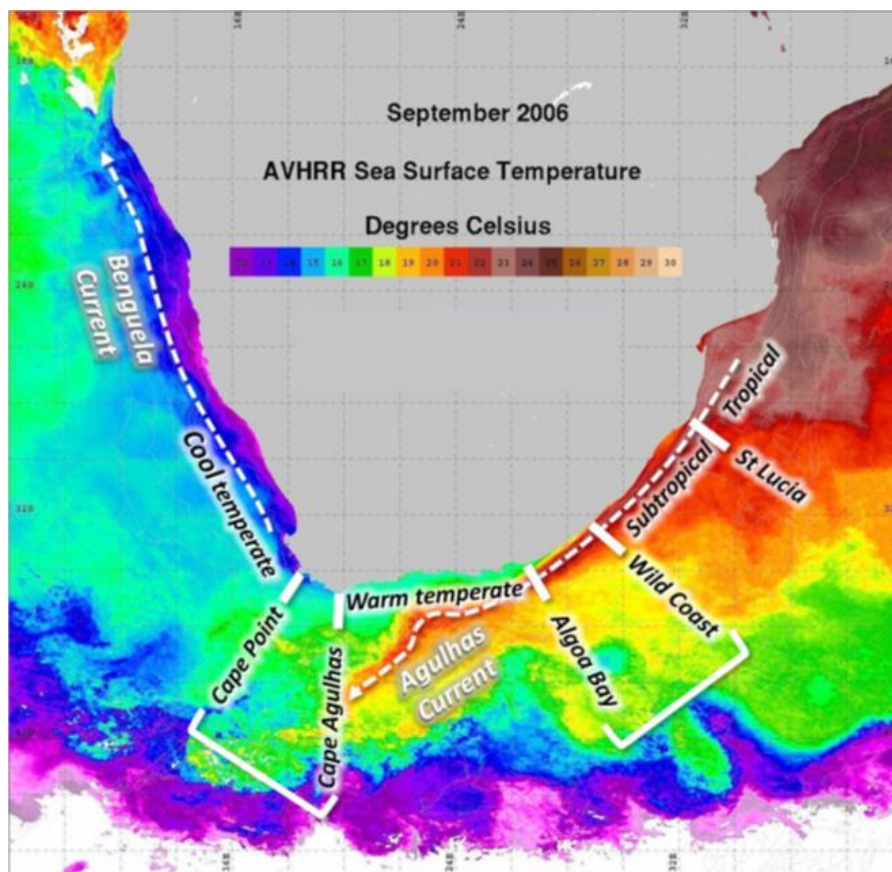
Figure 1 Sea Surface Temperature (SST) of the South African coastal region. The coastal region is divided into four major marine biogeographic provinces (cool temperate, warm temperate, subtropical and tropical) by the two major boundary currents: the cold Benguela Current on the west coast and the warm Agulhas Current on the south-east coast. Each of these provinces has its own characteristic assemblages of species (Source: Teske *et al.* 2011).

There is no distinct westward-flowing ocean current, nor an eastward–flowing one linking South Africa and Madagascar in the opposite direction, which could possibly explain this similarity in fauna and the distributions of these species. One possibility is that these populations could have been common to both Madagascar and South Africa before the splitting up of the two landmasses over geological times (McLoughlin 2001). However, several studies on mesoscale turbulence from the southern Madagascan coast (Lutjeharms *et al.* 1981, Gründlingh 1995, Biastoch and Krauss 1999, Chapman *et al.* 2003, Schouten *et al.* 2003, Quartly and Srokosz 2004, Quartly *et al.* 2006, Siedler *et al.* 2009) led to an alternative explanation - that westward-propagating eddies could provide oceanic connectivity between the south-east coast of Madagascar and the east coast of South Africa. This gave rise to the suitcase concept (Marsac et al. 2014), which proposes that eddies could provide a mechanism to transport planktonic larvae of the above-mentioned species from Madagascar across the Southwest Indian Ocean (SWIO) Mozambique Basin to the KZN coast. The analogy used was that of biological material of Madagascan shelf origin being "packed up" in an eddy, like clothes in a suitcase, which is then transported across the SWIO to the KZN coast, where the contents of the suitcase are subsequently released.

Once planktonic larvae are incorporated within the cyclonic eddies, the physical processes within the eddies influence the biological characteristics, with cyclonic eddies characterised by the upwelling of cooler water from depths, and subsequent nutrient and phytoplankton enrichment (Bakun 2006). Eddies may also modify the biological environment with regard to food availability, larval prey and predators within the eddy (Muhling *et al.* 2007). Landry *et al.* 2008 recorded higher zooplankton biomass within a cyclonic eddy in response to elevated primary production. (Mullaney and Suthers 2013) also recorded greater biomass within an eddy compared to the adjacent shelf in the East Australian current, with enhanced growth rates found in the larval fish *Sardinops sagax* in the eddy ~ 5 mm longer and ~ 10 days older. Retention time of planktonic larvae within an eddy is also an important factor to consider. For example, using a particle-tracking model, Condie and Condie 2016 found that eddy retention times of plankton ranged from 5 to 67 days, with plankton residing near the surface experiencing shorter retention times than those residing in the same eddy at depth. Thus plankton undergoing vertical migration have longer retention times compared to plankton that do not undergo vertical migration. Eddies clearly have the potential to retain and support planktonic larvae.

Should eddies make the journey across the MC, how would they release their biological contents onto the KZN coast? (Braby 2014) found that eddies dissipate upon reaching the KZN coast, with more eddies near the coast than in the open ocean. It was further suggested that eddies interact with the AC and the continental shelf, which could cause

eddies to become weak and dissipate. This could provide a mechanism for biological material entrained within an eddy to be released onto the continental shelf. Braby (2014) further shows how the interaction of a cyclonic eddy with the KZN coast causes the eddy to become elongated and to progress down the AC. This was also recorded by (Morris *et al.* 2013) and suggests another way in which biological material could be released onto the KZN coast.

The Suitcase Project is a registered project under ACEP (African Coelacanth Ecosystem Programme) and is divided into two components, the oceanography component and shore-based component. The oceanography component includes several sub-projects focussing physical parameters, primary productivity, phytoplankton pigments and zooplankton in coastal waters of southern Madagascar and in a cyclonic eddy in the Mozambique Basin. This thesis fits within the oceanography component, testing whether mesoscale eddies are potential vectors of gene flow between KZN, South Africa and Madagascar. The shore-based component focussed on the population genetics of organisms common to both regions. Seaweeds, limpets, barnacles, corals, mussels, prawns, lobsters and fishes from estuaries, rocky shores and reefs in the region of Fort Dauphin, the main town in south-east Madagascar were collected. Equivalent biological material from South African fauna has also been collected to enable scientists to start determining whether there is a genetic connection between the two areas.

1.2 Hypothesis

It is hypothesised that eddies are able to entrain fish and invertebrate larvae from the southeast Madagascan coast and transport these westwards across the MC to the east coast of SA. This hypothesis will be explored by examining the following questions:

- Can eddies entrain planktonic larvae of fish and invertebrate species from the southeast Madagascan coast?
- Would eddies be able to transport planktonic larvae to the east coast of Africa within a reasonable time before they undergo metamorphose/settle/swim away/sink/die?

1.3 Objectives of the research

The aim of this thesis is to investigate the "suitcase hypothesis", proposed by Marsac et al. (2014) whereby meroplankton (invertebrate and fish larvae) may be entrained by mesoscale eddies and transported across the Mozambique Channel. The objectives are as follows:

- To investigate the abundance and taxonomic composition of meroplankton along the southern Madagascan shelf.
- To investigate the abundance and taxonomic composition of meroplankton within the eddy core, annulus and outer regions.
- To compare the abundance and taxonomic composition of meroplankton along the southern Madagascan shelf to meroplankton within the eddy.
- To use DNA barcoding to identify key species which can be linked to adult species found on both the KZN and Madagascan coasts.

CHAPTER 2: LITERATURE REVIEW

2.1 Circulation of the Southwest Indian Ocean

The circulation of the SWIO is characterised by high variability with frequent mesoscale activity, which largely results from the interaction between the South Equatorial Current (SEC) and the Madagascan landmass (Penven *et al.* 2006). The SEC flows from east to west and, as it approaches Madagascar at 20°S, then bifurcates into the North East Madagascar Current (NEMC) and South East Madagascar Current (SEMC) (Ridderinkhof *et al.* 2010). The NEMC flows north and around Cape Amber, the northern tip of the island, contributing to the northward East African Coastal Current (EACC), as well as flowing into the MC (Figure 2) (Swallow *et al.* 1988). The SEMC flows southward and retroflects when it reaches Cape St. Marie, casting off eddies which become entrained within the Agulhas Current (AC) (Lutjeharms *et al.* 1981, Lutjeharms 1988a, Di Marco et al. 2000, Lutjeharms and Machu 2000, Quartly and Srokosz 2004). Furthermore, the mesoscale variability of the AC is related to flow through the MC (Schouten et al. 2002) and from the SEMC (Stramma and Lutjeharms 1997).
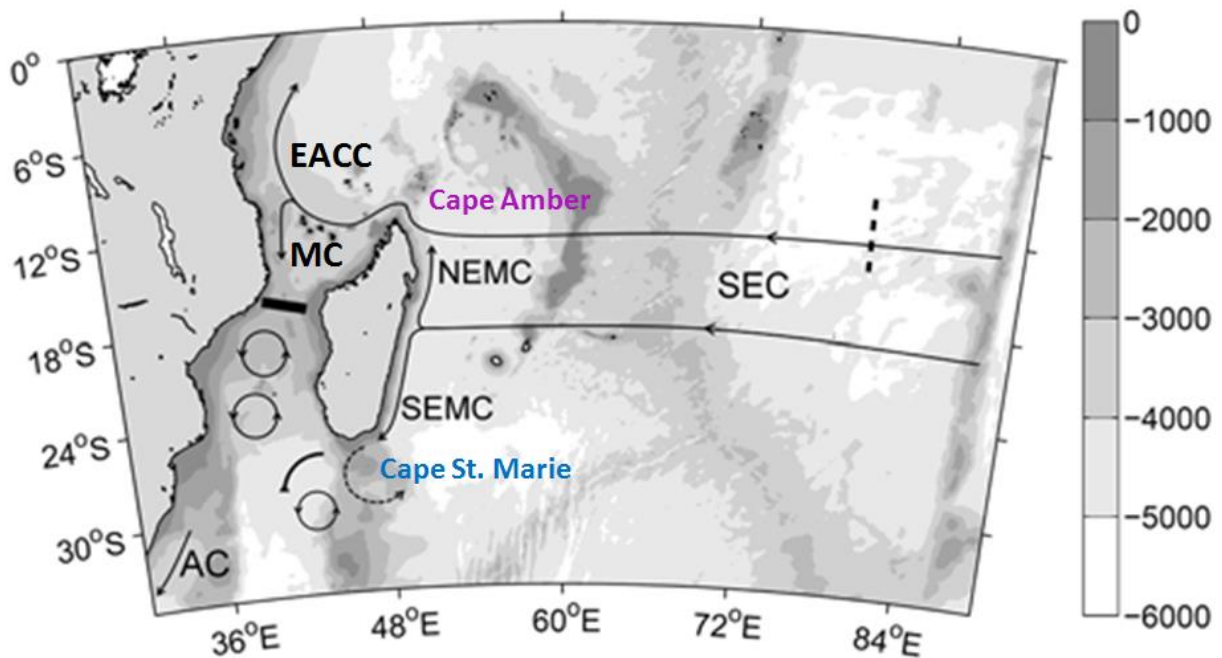
Figure 2 Bathymetry and major circulation features of the Southwest Indian Ocean (Source: Ridderinkhof *et al.* 2010).

2.2 The South East Madagascar Current

The SEMC is a narrow WBC, 120 km in width, reaching as deep as 1100 m with typical velocities of 1.1 m.s^{-1} (Nauw *et al.* 2008), which are approximately half that of the fast flowing AC at 2 m.s^{-1} (Lutjeharms 2006a) Early studies depicted the SEMC as flowing directly westwards into the AC (Gründlingh 1985, 1987, 1993, Lutjeharms 1988b), but subsequent studies found that the SEMC retroflects as it reaches the southern tip of Madagascar, shedding off eddies (Lutjeharms 1988a). Quartly *et al.* (2006) concluded that no persistent retroflection exists and that the SEMC alternates between periods of retroflection and no retroflection.

The area south-east of the SEMC near 25° S is characterised by strong disturbances in the flow field, propagating from east to west across the South Indian Ocean until they arrive at the eastern side of Madagascar (Siedler *et al.* 2009). Schouten *et al.* (2002) detected these disturbances to be Rossby waves, whereas Quartly *et al.* (2006) considered them more likely to be a train of propagating eddies. Using altimetry data Morrow *et al.* (2004) observed that anticyclonic eddies generated south of 25°S in the Leeuwin Current off Australia head in a west-north-westward direction (towards Madagascar), which could be a source of eddies in this region. Another large-scale oceanographic feature just south of the SEMC is the South Indian Ocean Countercurrent (SICC), described by Siedler *et al.* (2006) and Palastanga *et al.* (2007). The SICC is a shallow, eastward-flowing current, transporting ~10 Sv (1 Sv = 1 Sverdrup = 10^6 m^3.s^{-1}) down to 800 m (Siedler *et al.* 2006), compared to the AC transporting ~ 70 Sv (1 Sv = 10^6 m^3) in the upper 2000 m (Lutjeharms 2006a) It lies above the deep-reaching, westward-flowing SEC around the latitude of the high variability band at 25°S (Palastanga *et al.* 2007) (Figure 3), and is an essential component of the near-surface subtropical circulation in the South Indian Ocean (Siedler *et al.* 2006).

Siedler *et al.* (2009) identified two branches in the SEMC, using satellite observations and numerical model data. South of Madagascar, the SEMC separates into one branch toward the AC and into a second branch, retroflecting and connecting to the SICC, contributing 40% of the volume. This interpretation is not supported by recent *in situ* hydrographic data (Ridderinkhof *et al.* 2013). Halo *et al.* (2014b) suggested that a connection exists between the SEMC and SICC through the shedding of anticyclonic eddies. *In situ* hydrographic observations and satellite altimetry data revealed that, after the SEMC separates from the Madagascan coast, it propagates south-westwards and breaks into a series of contra-rotating eddies, known as dipoles (Ridderinkhof *et al.* 2013). Anticyclones are generated south-west of Madagascar, caused by the interaction of the SEMC with the Madagascar

Ridge, leading to an anticyclonic recirculation, whereas cyclones are generated as lee eddies inshore of the SEMC as it separates from the southern Madagascan shelf (de Ruijter *et al.* 2004, Ridderinkhof *et al.* 2013).
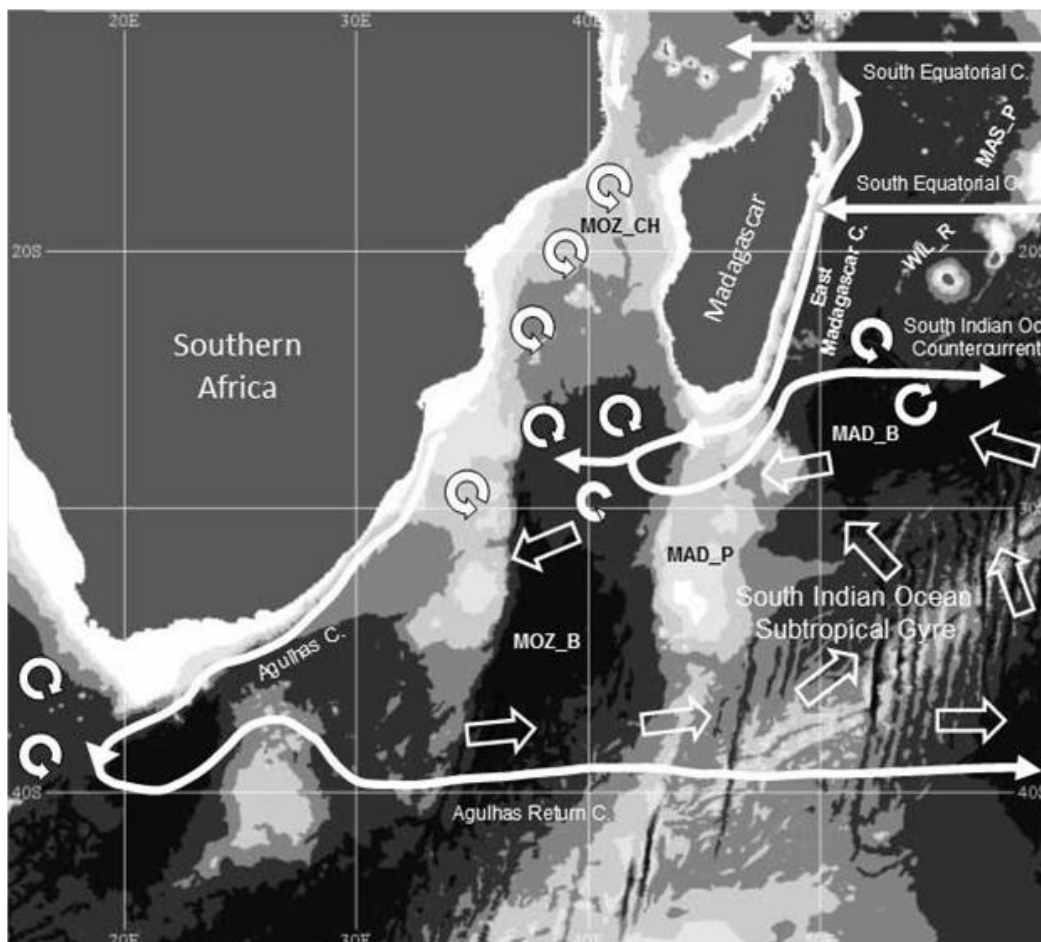
Figure 3 Schematic of the South East Madagascar Current (SEMC) and surface currents in the South West Indian Ocean (SWIO). MOZ_CH is the Mozambique Channel, MOZ_B is the Mozambique Basin, MAD_P is the Madagascar Plateau, MAD_B is the Madagascar Basin, WIL_R is the Wilshaw Ridge and MAS_P is the Mascarene Plateau. Thin solid arrows indicate currents and thick open arrows indicate the subtropical gyre. Eddies are indicated by rotating arrows. (Source: Siedler *et al.* 2009)

2.3 Mesoscale variability off southern Madagascar

Sea surface temperature (Di Marco *et al.* 2000), ocean colour (Lutjeharms and Machu 2000) and hydrographic data (Machu *et al.* 2002) have shown the southern coast of Madagascar to be a very productive area with year-round coastal upwelling events, which are mainly wind driven. Using satellite altimetry and hydrographic data, both cyclonic and anti-cyclonic eddies have been observed to form south of Madagascar, which then propagate into the northern AC (De Ruijter *et al.* 2004, Ridderinkhof *et al.* 2013). These mesoscale eddies are generated at two regions on the southern Madagascan coast, the south-east and south-west, with distinctly different mesoscale activity and eddy characteristics (Halo *et al.* 2014b). Formation of eddies is seasonal, with more eddies formed in winter than in summer, and with mesoscale activity highly impacted by seasonality in the southeast region but only slightly affected in the south-west region (Halo *et al.* 2014b). The south-east region eddies are formed in the upper ocean (0-300 m) and the south-west region eddies at intermediate depths (800-2000 m) (Halo et al. 2014b). Cyclonic eddies tend to form directly south and southwest of the Madagascan coast, whereas anticyclonic eddies form slightly offshore (Halo *et al.* 2014b). De Ruijter *et al.* (2004) and Ridderinkhof *et al.* (2013) recorded between four and six mesoscale eddies per annum originating south of Madagascar, while Halo *et al.* (2014b) reported between six and thirteen mesoscale eddies per annum in this area. The latter study used more precise algorithms to identify mesoscale eddies, hence obtaining higher estimates.

Using a numerical model and observational data, Halo *et al.* (2014b) showed that nearly all eddies to the south of Madagascar were highly nonlinear, suggesting that they can trap and transport biological material over considerable distances (Robinson 1983), and are thus potential vectors of planktonic larvae between Madagascar and Africa. The nonlinear properties of these mesoscale eddies was also observed by Quartly and Srokosz (2004), where chlorophyll *a* features (indicative of the phytoplankton community) were visualised using ocean colour data, and the eddies were shown to propagate in a south-westerly direction from southern Madagascar towards the African coast, carrying chlorophyll *a* rich waters offshore away from the Madagascan coast (Quartly and Srokosz 2004) (Figure 4).
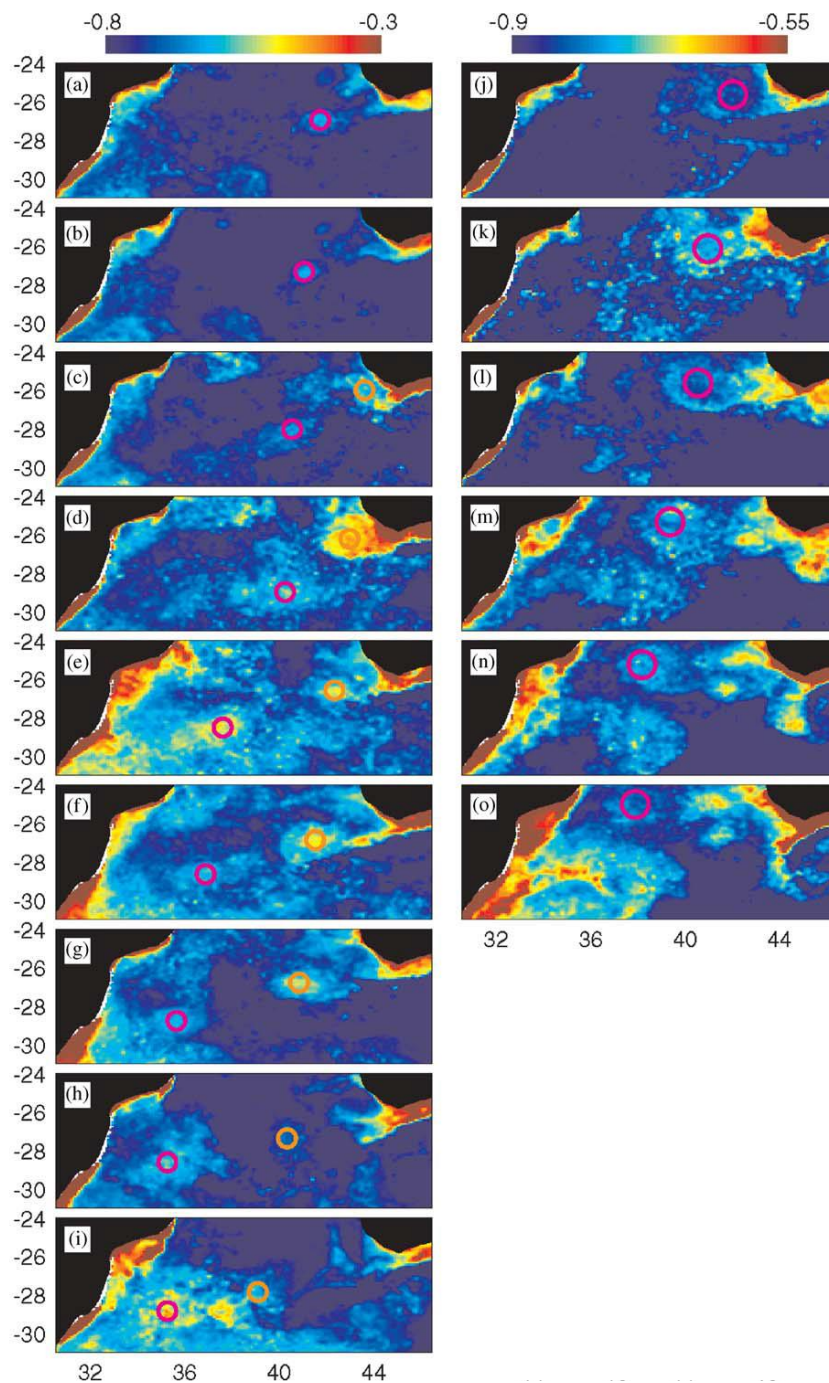
Figure 4 Cyclonic eddies southwest of Madagascar made visible by high chlorophyll *a* concentration around their peripheries. One chlorophyll *a* feature (pink circle) appears and propagates in a south-westerly direction towards Durban (4a-4i). The second chlorophyll *a* feature (orange circle) can be seen (4c-4i) and also proceeds in a south-westerly direction. The second series (4j-4o) shows images of a cyclonic eddy heading south-westwards. (Source: Quartly and Srokosz 2004).

2.4 The role of mesoscale eddies in retention and transport of plankton

Plankton comes from the Greek "planktos", meaning that which is passively drifting or wandering (Lalli and Parson 1993), and refers to marine organisms that live in the water column and are incapable of swimming against a current, i.e. are passively transported by currents in the sea. There are two types of plankton, phytoplankton, the microscopic plants and zooplankton, the microscopic animals of the ocean. Zooplanktons are capable of movement, but not capable of making their way against a current and are thus zooplanktons are subject to ocean currents and oceanographic processes.

Oceanographic processes influence larval distribution and transport, which are vital for recruitment dynamics and variability in marine populations (Nakata *et al.* 2000, Hare *et al.* 2002, Werner and Quinlan 2002, Govoni 2005). Plankton studies associated with mesoscale eddies in the SWIO are scarce, with only few studies recorded in the MC. Recently Huggett, (2014) described zooplankton biomass and composition associated with mesoscale eddies in the MC, while Lebourges-Dhaussy *et al.* (2014) investigated the size composition of zooplankton associated with these eddies.

On a global scale, the importance of mesoscale eddies in relation to retention (Sale 1970, Hamner and Hauri 1981, Lee *et al.* 1994) and transport (Limouzy-Paris *et al.* 1997, Yeung *et al.* 2001) of planktonic larvae has been widely reported. For example, the retention mechanism of eddies enhanced survival rates of sardine larvae in the California Current, where sardine larvae distributions in relation to environmental conditions were investigated (Logerwell and Smith 2001). Observations indicated more 'survivor' sardine larvae offshore associated with mesoscale eddies than inshore, where chlorophyll biomass and zooplankton were highest. On the Great Barrier Reef, tidal eddies occurring in close proximity to One Tree Island retained reef fish larvae in the vicinity of the reef, increasing their chances of returning to their natal reef (Burgess *et al.* 2007). In the Florida Current, eddies retained fish larvae of snappers and groupers, and also provided a source of food for survival of these larvae (Lee *et al.* 1994). Short-lived eddies (2 to 4 weeks) increased recruitment of fish larvae in the East Australian Current where fish larvae were retained in eddies for a sufficient time to complete their larval cycle and return to the coast (Mullaney and Suthers 2013). Furthermore, zooplankton were retained within an eddy of coastal origin in the North Pacific, avoiding predation by not undergoing diel vertical migration during the day (Mackas and Galbraith 2002, Mackas *et al.* 2005).

With respect to transport of plankton, mesoscale eddies in the Gulf of Alaska (Shelikof Strait) have been shown to be important for retention and transport of larval fish during their pelagic

phase, whilst providing a nursery ground and subsequently enhancing recruitment success of walleye pollock (Canino *et al.* 1991, Bailey *et al.* 1996, Hermann *et al.* 1996, Kendall *et al.* 1996). Mesoscale eddies propagating along the continental shelf of the Gulf of Alaska influenced larval fish growth, survival and subsequent recruitment, with implications for the timing and extent of larval distribution in this region (Atwood *et al.* 2010). Limouzy-Paris *et al.* (1997) demonstrated how spin-off eddies of the Florida Current act as a transport mechanism, where eddies transported fish larvae back to their inshore settlement sites across a strong frontal boundary.

2.5 Importance of the pelagic larval phase and dispersal

Most marine animals have a planktonic larval stage (Riginos *et al.* 2011), which increases the opportunities for long-distance dispersal. Dispersal varies among marine organisms, with the majority of marine species spawning planktonic eggs from which larvae hatch, but there are a few marine invertebrates, as well as fish, that do not have a pelagic larval phase at all (Riginos and Victor 2001). Dispersal of planktonic larvae plays a fundamental role in structuring the populations of fish and invertebrates (Kinlan and Gaines 2003). (Shanks *et al.* 2003) initially suggested that pelagic larval duration (PLD) may be a strong predictor of dispersal distances. However, subsequent research showed that PLD is in fact a weak indicator of dispersal distances and that other factors such as larval behaviour and mesoscale oceanography can influence larval dispersal considerably (Shanks 2009, Weersing and Toonen 2009).

Dispersal of marine species over long distances is not unusual and is aided by lengthy planktonic larval phases (Shanks 2009). In the SWIO, studies on gene flow and ocean connectivity are limited, with only few studies documented in this area. Spiny lobsters (Palinuridae) have a flat-bodied crystalline larval phase, the phyllosoma, which is adapted for dispersal in oceanic waters (Booth and Phillips 1994, Palero and Abello 2007), making them excellent models for studying the influence of ocean processes on gene flow. The rock lobster *Panulirus homarus* occurs off both the SA east coast and the southern Madagascan coast (Berry 1974). *P. homarus* has a phyllosoma larval stage lasting up to 6 months, making it an ideal species to survive being transported across the Mozambique Channel. Interestingly, the scalloped spiny lobster *Panulirus homarus rubellus* appears to have distinct lineages for the southeast coast of Africa and Madagascar, suggesting an absence of contemporary gene flow across the Mozambique Channel (Reddy *et al.* 2014). It is also shown that the African lineage appears to be ancestral, and the source population for the

Madagascan clade, which would negate the likelihood of eddies in facilitating genetic connectivity between the modern South African and Madagascan populations.

The giant mud crab *Scylla serrata* is widely distributed throughout mangrove habitats in the Indo-West Pacific region (MacNae 1969). These crabs would also be good candidates to survive the channel crossing as they also have a long larval stage. For example, a larval stage duration of 62 days (roughly 2 months) at a water temperature of 25°C has been recorded for *Scylla serrata* (Nurdiani and Zeng (2007).

The brown mussel *Perna perna* is widely distributed in the tropical and subtropical regions of the Indian and Atlantic Oceans and also extends into the Mediterranean (Berry 1978). *Perna perna* also has a long planktonic larval phase of around 15 to 20 days with two stages: a dispersal stage (the actively swimming veliger) and a recruitment stage (the pediveliger), (Hicks and Tunnell 1995). However, settlement can be delayed by three months, depending on food availability and (low) water temperatures (Teske *et al.* 2007). Just like the above mentioned crustaceans, *P. perna* has a long-lived planktonic stage ranging from 1-3 months, which makes the brown mussel a possible candidate for surviving the journey across the MC.

As with invertebrates, a long larval phase for fish would be required for cross-channel eddy transport. However, in fish other factors, such as larval behaviour and distribution (Leis 1991), swimming speeds and orientation (Stobutzki and Bellwood 1994, 1997, Leis and Carson-Ewart 1997), should be considered along with larval duration. In the Western Indian Ocean (WIO), only a few studies on genetic connectivity in fishes have been conducted. In one study, a prolonged pelagic larval phase (30 days) and strong swimming ability enhanced larval dispersal for the snapper *Lutjanus kasmira* over distances up to 4000 km, suggesting high gene flow within the WIO (Muths *et al.* 2012). The parrot fish *Scarus ghobban* was shown to have a high dispersal range of at least 2500 km (Visram *et al.* 2010), and a study on the genetic structure of the grouper *Epinephelus merra* revealed high gene flow in the WIO (Muths *et al.* 2015). A hydrodynamic model revealed that reefs in the WIO are connected by larval dispersal, which is mainly driven by ocean currents (Crochelet and Lagabrielle 2012). Given the above examples of reef fish species showing high gene flow in the WIO, it should be possible for larvae of some fish species to survive the journey across the MC.

2.5 DNA Barcoding as an identification tool

Traditionally, microscopic analysis has been used to identify plankton. Using morphological characteristics only, it can be difficult and sometimes impossible to identify the early stages of species, for example, larval phases such as the zoea and megalopa of decapods (Bucklin *et al.* 2011). It also becomes difficult when dealing with cryptic species (species that are morphologically similar but genetically distinct) (Hebert *et al.* 2004a) and species where the larvae have not been identified, characterised or described yet (Bucklin *et al.* 2011). For a long time, molecular systematists have been able to identify species based on DNA sequences or genetic data (Avise 1994), but it was Hebert *et al.* (2003a) who popularised this in terms of (and established the phrase) "DNA-barcoding". DNA barcoding uses a single mitochondrial cytochrome *c* oxidase subunit I (COI) gene fragment (for most animal taxa) to accurately identify known taxa and assign unknown individuals to species, thus enhancing the discovery of new species (Hebert *et al.* 2003a, Stoeckle 2003). Hebert *et al.* (2003a) proposed the compilation of an online library of DNA barcodes that would be linked to named specimens, and in 2004 the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007) was launched. The goal of this online library was to develop DNA barcoding as a global standard for the identification of biological species (Savolainen *et al.* 2005).

DNA barcoding studies at a global scale have shown this method to be effective in identifying meroplanktonic taxa such as decapods. For example, a barcoding study on megalopa larvae of three sympatric crab species in the southeastern Pacific Ocean resulted in the identification and description of diagnostic characters, which now allow species to be distinguished (Pardo *et al.* 2009). This method has been particularly effective with fish species that undergo ontogenetic changes during the larval phase, which makes identification difficult to achieve and requires high levels of expertise (Caterino and Tishechkin 2006, Pegg *et al.* 2006). DNA barcoding has also been a successful tool in identifying fish larvae, and linking adult and larval fish. For example, DNA barcoding was used to identify fish larvae in Antarctica (Webb *et al.* 2006), and fish eggs and larvae off the Great Barrier Reef (Pegg *et al.* 2006). In other studies, it proved an effective tool for matching young stages and adults of fish (Valdez-Moreno *et al.* 2010, Baldwin *et al.* 2011). Bivalve larval stages are not easily distinguishable due to their similar appearance during their early development (Garland and Zimmer 2002). However, molecular techniques showed 92 % overall accuracy when used to identify commercially important bivalve species in New England (Hare *et al.* 2000).

The COI gene is the barcoding standard fragment for most animal taxa (Hebert *et al.* 2003a). It can be used to identify a species across all larval and life stages (e.g., eggs and larvae of marine fishes) (Savolainen *et al.* 2005), and to distinguish between cryptic species. Furthermore, universal primers for this gene are robust and have proven to be useful for species from a broad range of metazoan phyla (Folmer *et al.* 1994). In comparison to other genes, COI appears to possess phylogenetic signal that is useful over a broad range of taxonomic levels (Folmer *et al.* 1994).

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials and Methods

Sampling was conducted during a 24-day cruise between the 16th and the 22nd of July 2013 on the RV *Algoa*, as part of the African Coelacanth Ecosystem Programme (ACEP). The RV *Algoa* sailed from Durban on Monday 8 July 2013 to undertake a study of a cyclonic eddy which had originated off the southern tip of Madagascar, and to collect comparative samples from the southern Madagascan coast. The eddy was first detected on the 16th of June 2013 as an enclosed, coherent eddy in the south-eastern Mozambique Channel and was a month old when it was sampled during this cruise (Figure 5). Eddy life-time from the SWIO region ranges from 90-183 days (Halo *et al.* 2014b) A more detailed description of the eddy formation and its movement is presented in Noyon *et al.* (in revision). En route to Fort Dauphin in Madagascar a series of stations was sampled along a transect running offshore to inshore towards the east of Fort Dauphin. On the southern Madagascan shelf a series of 24 stations was sampled from east to west. A course was then set to sail to the eddy (in the Mozambique Basin) and a set of 25 stations, 18.5 km apart along a transect from northeast to southwest was sampled (Figure 5).
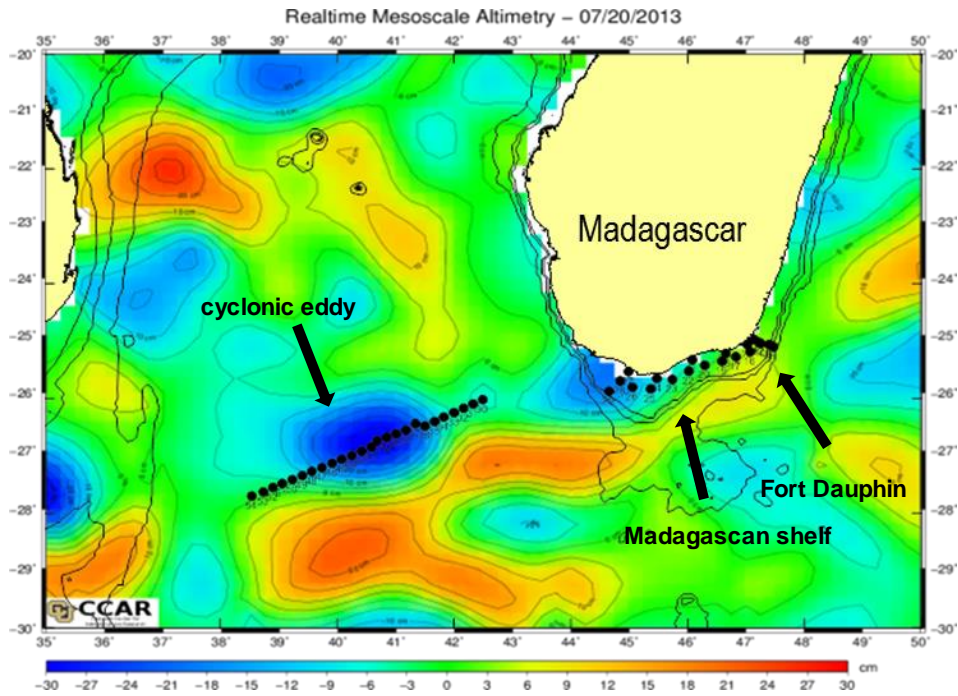
Figure 5 Zooplankton sampling stations on the Madagascan shelf and along a transect through a cyclonic eddy in the Mozambique Basin. Station positions are superimposed on a real-time map of sea surface height anomaly (SSHA), where strong negative anomalies (in blue) indicate cyclonic features, and strong positive anomalies (in red) indicate anticyclonic features.

3.1.1 Environmental parameters sampling

CTD casts were conducted using a Seabird 911+ CTD-F (measuring conductivity, temperature, depth-fluorescence and oxygen), fitted with a 12-bottle Niskin (5L) rosette system. Physical parameters were sampled from the surface to a depth of 1000 m. Vertical fluorescence profiles were converted to chlorophyll *a* equivalents (mg m^{-3}) by calibration against discrete chlorophyll *a* concentration of samples collected at several depths at each station (Noyon *et al*. in revision). Contour maps of sea surface temperature (°C) and chlorophyll *a* concentration (mg m^{-3}) on the Madagascan shelf, as well as vertical profiles through the cyclonic eddy, and the horizontal distribution of zooplankton biovolume (mL m^{-3}) were visualised using Surfer (Golden Software 2011).

3.1.2 Zooplankton sampling

Oblique bongos with 300 & 500 μm mesh nets (0.25 m^2 mouth area) were deployed to a depth of 200 m to sample meroplankton (bivalve, echinoderm, decapod and fish larvae) in the upper 200 m or down to 5 m above the bottom at shallower depths (< 200 m). Bongo nets were towed obliquely through the water column at 2 knots to maximise the volume of water filtered and thus zooplankton catches. Volume filtered was calculated from flowmeters mounted in the mouth of the net. Only samples from the 500 μm Bongo net were analysed in this study. A Neuston net fitted with 900 μm mesh was towed at the surface for 15-20 minutes at 2 knots to collect neustonic plankton. Due to time constraints following a period of bad weather, only two Bongo net hauls (500 μm) were made on the Madagascan shelf, one to the west (station 20) and one to the east (station 26) (Figure 6). Samples were preserved in ethanol (99%) for later extraction of meroplankton, as well as microscopic and genetic analysis.

3.1.3 Eddy Zonation

The approach used to divide the eddy into zones was derived from Noyon *et al.* (in revision), who considered chlorophyll *a* to be a better parameter than temperature to characterise the eddy structure. Vertical profiles of temperature indicated weak doming (elevation) of isotherms through the eddy, with no clear distinction between the eddy core and edges (Figure 7a). The chlorophyll *a* profiles indicated elevated chlorophyll *a* concentrations (>0.2 ug/l) in the eddy (stations 38 to 51), compared to the area outside the eddy (stations 30 to 37, and 52 to 54), with the highest concentrations in the eddy core (stations 44 to 47) (Figure 7b). From this, the different zones in the eddy were derived: namely, Outside Eddy (E-Out), East Eddy (E-Ed), Core, West Eddy (W-Ed) and West Outer (W-Out).

3.1.4 Microscope analysis

In the laboratory, samples from Bongo and Neuston nets were poured into a measuring cylinder, settled overnight and the volume was recorded. Biovolume (mL m^{-3}) was calculated by dividing the settled volume (mL) by the volume filtered through the net (m^3; Table 1). The whole sample was sorted under a stereo microscope and meroplankton were picked out and placed into vials for further microscope identification and later DNA barcoding. For this study a few selected stations were analysed from both the Bongo and Neuston nets. Stations were selected to represent the Madagascan shelf, the region outside of the eddy, and the different zones within the eddy. On the Madagascan shelf, stations were selected from both the east shelf (station 20) and the west shelf (stations 26 and 28). Along the eddy transect one station was selected from both the Bongo and Neuston nets respectively. Stations selected include: station 30, outside of the eddy (E-Out), stations 39 and 40 in the east eddy zone (E-Ed), stations 44 and 46 in the eddy core (Core), and stations 49 and 50 in the west eddy zone (W-Ed) (Figure 6).

Meroplankton were identified to the lowest taxonomic level where possible under a stereo microscope, using guides such as those of (Gurney 1942, Brownell 1979, Conway *et al.* 2003, Richards 2005). In particular, larvae of potential key indicator adult species, i.e. similar fauna that are known to occur on both the east coasts of Madagascar and South Africa, were sought. The extracted larvae were used for DNA barcoding to confirm microscopic identification. The abundance of meroplanktonic groups on the Madagascan shelf and through the cyclonic eddy was calculated and visualised using Microsoft Excel. The Pearson product-moment correlation was used to test for relationships between zooplankton biovolume and environmental parameters when variance was homogenous amongst samples and data were normally distributed. Otherwise, a Spearman's rank correlation was calculated. For the neuston net, near-surface environmental parameters (from the CTD) considered were temperature at 7 m, temperature at 100 m, temperature at the depth of maximum fluorescence, fluorescence at 7m and fluorescence at 100 m. To compare the zooplankton biovolume in the different zones (Madagascan shelf and along the eddy transect), a one-way Analysis of Variance (ANOVA) was used, followed by Tukey HSD post hoc tests when variance was homogenous amongst samples (Levene's test) and data were normally distributed, otherwise a Kruskal-Wallis test was performed. Statistical analyses were conducted using STATISTICA version 7.0.
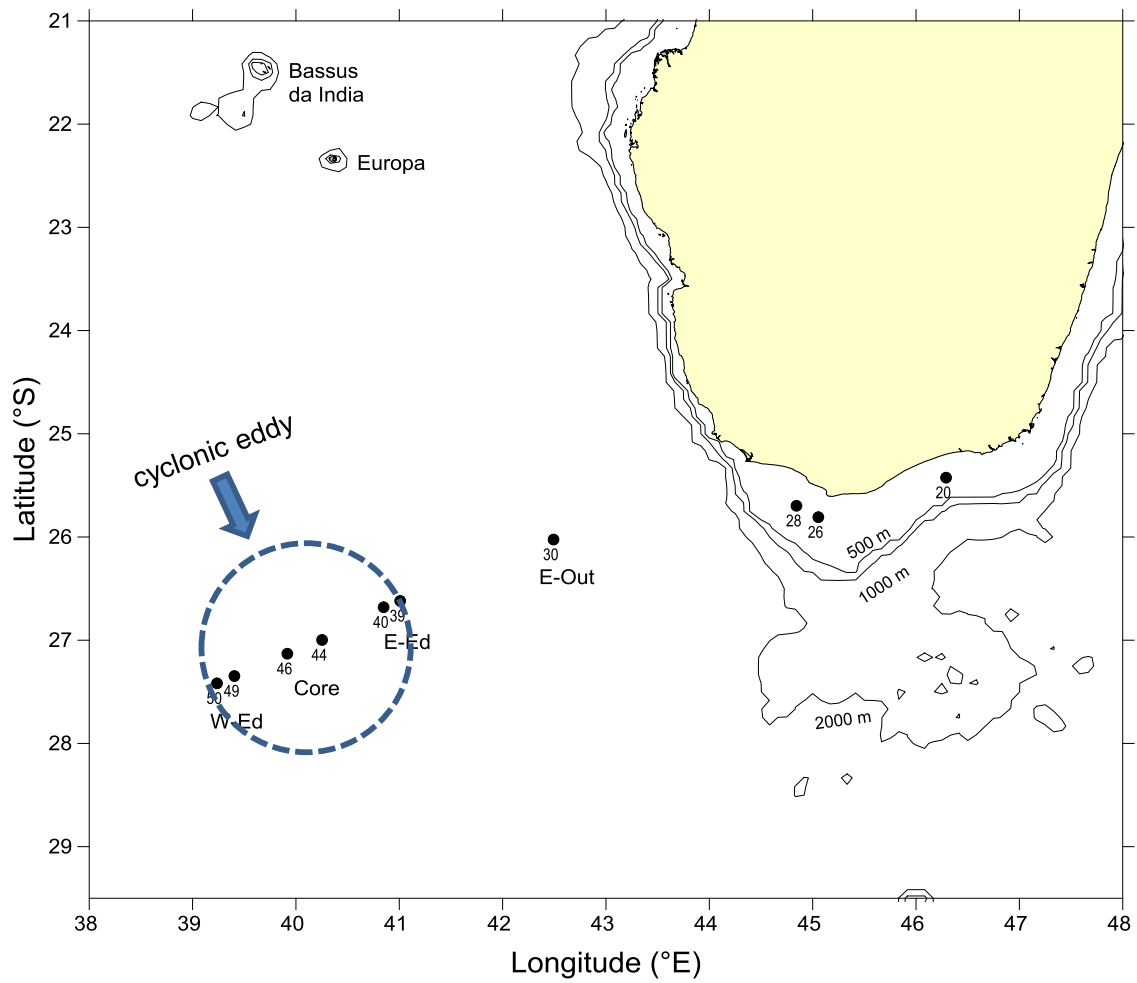
Figure 6 Stations (Bongo and Neuston nets) used for this study on the Madagascan shelf, station 26 and 28 on the west shelf and station 20 on the east shelf. Stations along the eddy transect: East Outer, station 30: East Eddy, stations 39 and 49; Core, stations 44 and 46; West Eddy, stations 49 and 50.
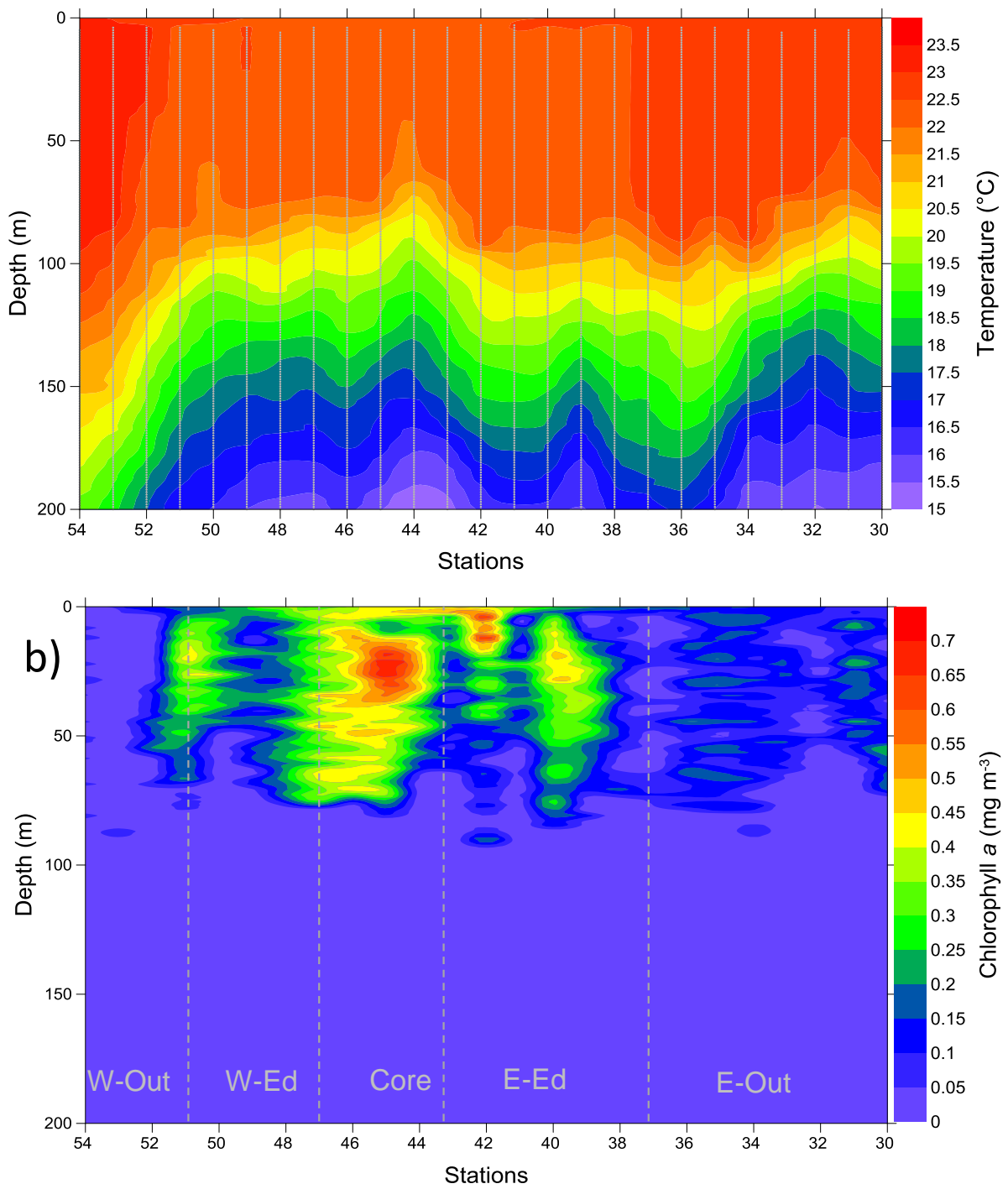
Figure 7 (a) Thermal structure, where grey vertical dashed lines indicate stations sampled, and (b) chlorophyll *a* concentrations (mg m^{-3}) along the cyclonic eddy transect down to 200 m deep. The zones are indicated as East Outer (E-Out), East Eddy (E-Ed), Core, West Eddy (W-Ed) and West Outer (W-Out).

3.1.5 DNA Barcoding

A standardised gene region, a fragment of the mitochondrial DNA cytochrome *c* oxidase subunit I (COI) gene, was used to identify meroplankton to the lowest taxonomic level possible. The primary reason for the selection of the COI gene in this study is that it can clearly identify and delimit conspecifics, and thus distinguish between different species from the same genus (Hebert *et al.* 2003a).

3.1.5.1 DNA isolation

Total DNA was extracted from single individuals in a sample using a commercially-available kit. Initially, DNA was extracted from decapod and bivalve larvae, using the GeneJet Genomic DNA Purification Kit (ThermoScientific), according to the specifications of the manufacturer. Next, DNA from fish larvae was extracted using the "salting out" method (Sunnucks and Hales 1996). Attempts to extract DNA from bivalve larvae were unsuccessful using either extraction method.

3.1.5.2 Amplification and sequencing

The gene of interest was replicated by Polymerase Chain Reaction (PCR) using universal primers LCOI1490 (10 µM) and HCOI2198 (10 µM) (Folmer et al. 1994). These primers were successful with the prawn larvae, but failed to work with shrimp and crab larvae. In these cases, degenerate primers were used: dgLCO1490 (10 µM) and dgHCO2198 (10 µM) (Meyer 2003). Primers Fish F1 (50 µM) and Fish R1 (50 µM) (Ward *et al.* 2005) were initially used for the fish larvae, but when visualizing PCR success on 1% agarose gels, double bands were observed. A switch was made to primers (50 µM) VR1-T1 and (50 µM) VF2-T1 (Ivanova *et al.* 2007), which worked successfully for most fish larvae. For those that failed, the amplification was repeated using the degenerate (Meyer 2003) primers. For larvae such as the phyllosoma and fish larvae, which failed to work with above mentioned primers, the mitochondrial 16S ribosomal RNA gene was amplified using primers 16Sar (10µM) and 16Sbr (10µM) (Palumbi 1996).

The PCR consisted of a master mix of 12.9 µL MilliQ H_2O, 2.5 µl 10 x buffer, 3.0 $MgCl_2$ (25 mM), 2.5 µL dNTP (10 mM), 0.5 µL of each primer, 0.1 µL Taq (5 U/µL) and 3 µL of extracted DNA. PCR reactions were performed in a 96-well gradient thermal cycler (Applied

Biosystems Veriti cycler). Amplifications were made using a cycling profile that included an initial step of 2 min at 95°C, followed by 35 cycles of 30 s at 95 °C, 40 s at 48 °C and 1 min at 72°C, followed by 10 min at 72°C. The shrimp and crab larvae failed to be amplified initially with this thermo-cycling process, and, using optimization, the suitable annealing temperature was determined to be 46°C for the larvae of both groups. PCR products were visualized after electrophoresis in an 1% agarose gel, stained with ethidium bromide, under UV light and then purified using Exonuclease I – Shrimp Alkaline Phosphate (Exo/SAP ThermoFisher Scientific) (Werle *et al.* 1994). A reaction mixture of 10 µL, consisting of 0.5 µl Exonuclease I and 2 µl FastAP^{TM} Thermosensitive Alkaline Phosphatase/Shrimp Alkaline Phosphatase and PCR product, was incubated at 37°C for 15 minutes and the reactions were stopped by heating the mixture at 85°C for 15 minutes. Purified products were then sequenced using Sanger/Big Dye version 3.1 fluorescently-labelled terminator chemistry, using a 10 µL reaction with 1 µL BigDye reaction mix, 2 µL of 5X sequencing buffer, 1 µL of primer at 1 µM , up to 3µL PCR product and ddH$_2$O to make up to 10 µl. The thermocycling conditions were as follows: an initial step of 2 min at 95 °C and 25 cycles of 10 s at 95 °C, 5 s at 50 °C and 4 min for 60°C, and then maintained at 4 °C. These cycle-sequencing products were purified using EDTA/NaOAc/EtOH precipitation and frozen prior to sequencing. Purified products were then sequenced using SAIAB's in-house sequencer, an ABI 3500 Genetic Analyzer, following the manufacturer's instructions. There were initial attempts using this sequencer and the quality of some sequences was not good. Due to time constraints, samples were sent off to a commercial sequencing facility (Macrogen, Seoul, South Korea) for sequencing.

Sequences were edited and trimmed using software Chromas Lite version 2.1 (Technelysium PTY Ltd, South Brisbane, Queensland, Australia, 2012) and then submitted, for identification, to Barcode of Life Data Systems (BOLD: http://www.boldsystems.org). BLAST (Altschul *et al.* 1990) searches on GenBank (http://www.ncbi.nlm.nih.gov) were used to assign identities to unknown species which could not be matched using BOLD. COI DNA sequences generated in this study of fish larvae were aligned using ClustalX v.2.1 (Larkin *et al.* 2007) software. Sequence divergences were calculated using the Kimura two-parameter (K2P) distance model (Kimura 1980) and neighbour-joining (NJ) trees (Saitou and Nei, 1987) were constructed, based on K2P distances, in MEGA v.6 (Tamura *et al.* 2013) to provide a graphic representation of the divergence patterns and distance relationships. The analysis of genetic distances was complemented by downloading related sequences from BOLD/GenBank for comparison with unknown sequences. To test the reliability and robustness of the relationships depicted in the tree, it was bootstrapped using 1000 replicates (Felsenstein 1985).

CHAPTER 4: RESULTS

4.1 Environmental variables

Vertical sections of temperature along the eddy transect indicated weak doming of the isotherms with slightly cooler water (22 - 22.5°C) in the upper 100 m associated with the eddy, but could this not be used to clearly distinguish between the core and outer regions of the eddy (Figure 7a). Chlorophyll *a* concentrations along the eddy transect gave a clearer indication of the eddy structure, and were highest in the core (0.5 - 0.7 mg m^{-3}) and intermediate in the eddy periphery (0.3 - 0.5 mg m^{-3}) (Figure 7 b).

Cool near-surface water temperatures (<22 °C) were recorded inshore on the southwest shelf and southeast shelf of Madagascar, with warmer water of the SEMC (23-24°C) farther offshore off the southeast coast (Figure 8a). Near-surface chlorophyll *a* concentrations on the Madagascan shelf were highest at station 26 on the southwest shelf (1.08 mg m^{-3}) and station 12 on the southeast shelf (1.06 mg m^{-3}) (Figure 8b).

4.2 Zooplankton biovolume

Mean Neuston biovolume collected on the Madagascan shelf was 0.08 ± 0.04 mL m^{-3} (Table 1). Mean neuston biovolume in the eddy was 0.06 ± 0.03 mL m^{-3}, with maxima in zones E-Ed (0.08 ± 0.03 mL m^{-3}) and W-Out (0.07 ± 0.03 mL m^{-3}). Mean Neuston biovolume in the eddy was not significantly different from that on the shelf (ANOVA; p>0.05). Using the bongo net, mean zooplankton biovolume in the upper 200 m was much higher on the Madagascan shelf (0.63 mL m^{-3}) than in the eddy (0.16 ± 0.08 mL m^{-3}) although only 2 stations were sampled on the shelf (Table 1). Highest mean biovolumes in the eddy were recorded in zones W-Ed (0.25 ± 0.07 mL m^{-3}) and W-Out (0.23 ± 0.14 mL m^{-3})), but these were not significantly higher than eddy zones E-Ed (0.17 ± 0.06 mL m^{-3}) and Core (0.12 ± 0.08 mL m^{-3}) (ANOVA, p>0.05).

Highest neuston biovolume on the Madagascan shelf was associated with relatively cool water (<22°C), and with high chlorophyll *a* concentrations to the west (1.08 mg m^{-3}), but not on the eastern shelf. In the eddy, there were no clear patterns, and highest neuston biovolumes were associated with intermediate chlorophyll *a* concentrations. A significant relationship was found between neuston biovolume and fluorescence at 7 m (Spearman rank r=0.42, p<0.05).
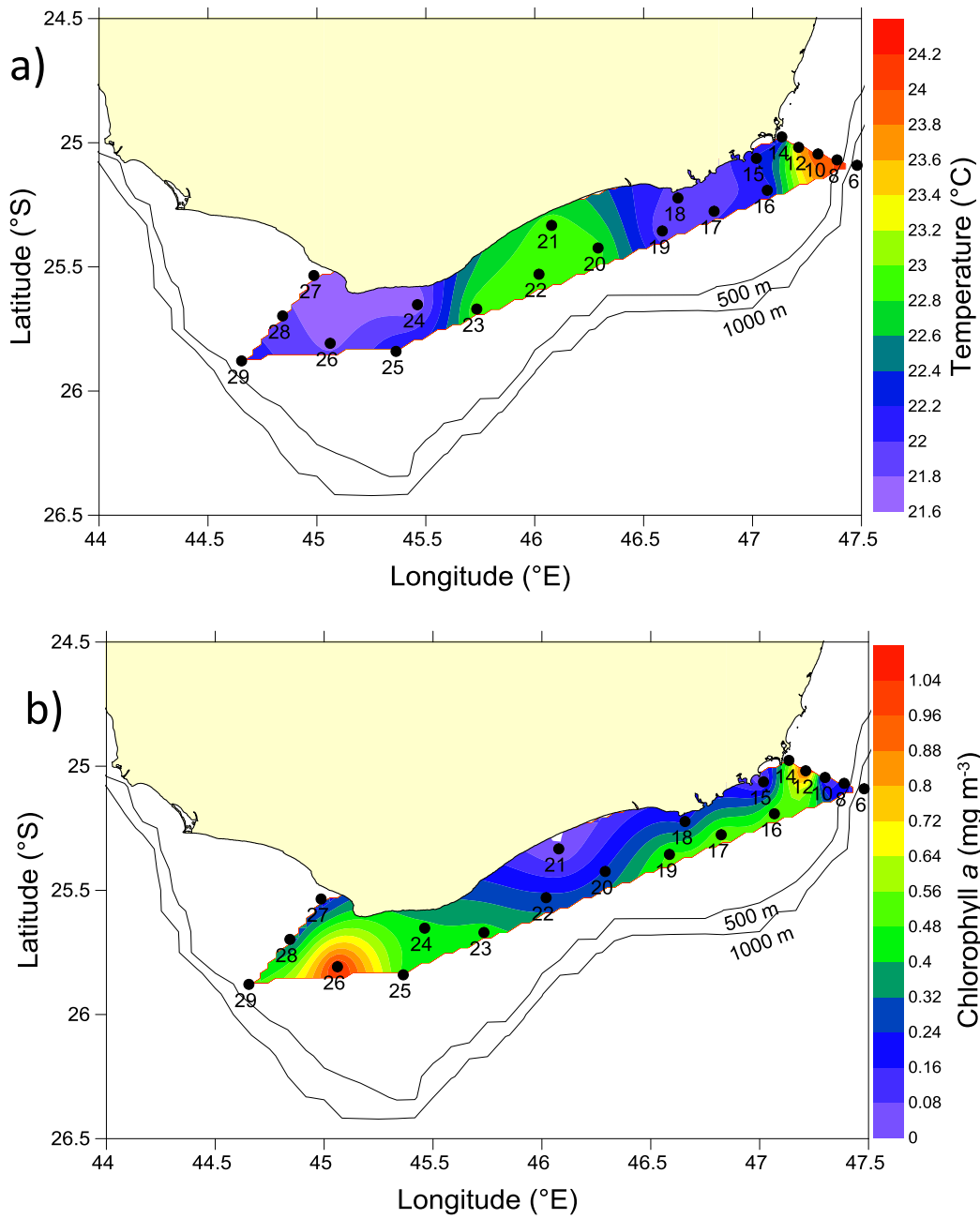
Figure 8 (a) Near-surface (7 m) temperature (° C) and (b) near-surface (7 m) chlorophyll a concentration (mg m^{-3}) on the southern Madagascan shelf.

Table 1 Mean (± standard deviation) of settled zooplankton biovolume (mL m^{-3}) on the Madagascan shelf (east and west) and the eddy zones (E-Outer, E-Ed, Core, W-Ed, W-Out), collected with the Neuston and Bongo nets.

Zones	Neuston biovolume (mL m^{-3})	n	Bongo biovolume (mL m^{-3})	n
East shelf	0.07 ± 0.04	8	0.57*	1
West shelf	0.09 ± 0.05	7	0.68*	1
Mean shelf	**0.08 ± 0.04**	**15**	**0.63***	**2**
E-Out	0.05 ± 0.03	7	0.12 ± 0.04	8
E-Ed	0.08 ± 0.03	6	0.17 ± 0.06	6
Core	0.05 ± 0.05	4	0.12 ± 0.08	4
W-Ed	0.06 ± 0.01	4	0.25 ± 0.07	4
W-Out	0.06 ± 0.03	3	0.23 ± 0.14	3
Mean eddy	**0.06 ± 0.03**	**24**	**0.16 ± 0.08**	**25**

* Only 2 stations sampled to east and the west on the Madagascan shelf with the Bongo net.
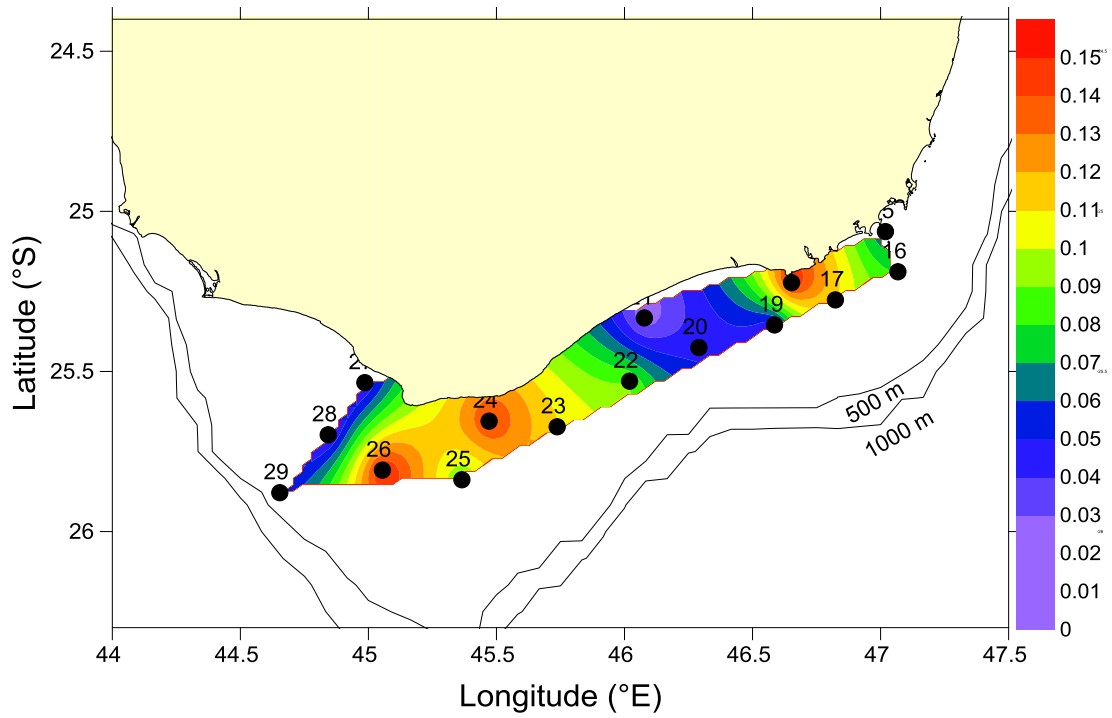
Figure 8 Contour plot of zooplankton biovolume (mL m^{-3}) on the southern continental shelf of Madagascar collected with the Neuston net.
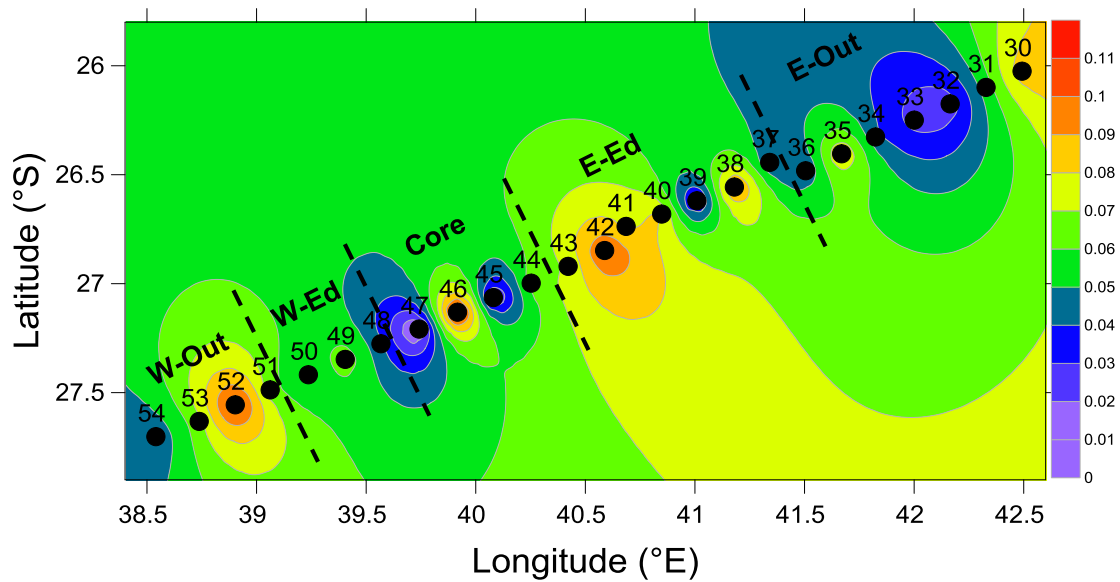
Figure 9 Contour plot of zooplankton biovolume (mL m^{-3}) along the eddy transect collected with the Neuston net.
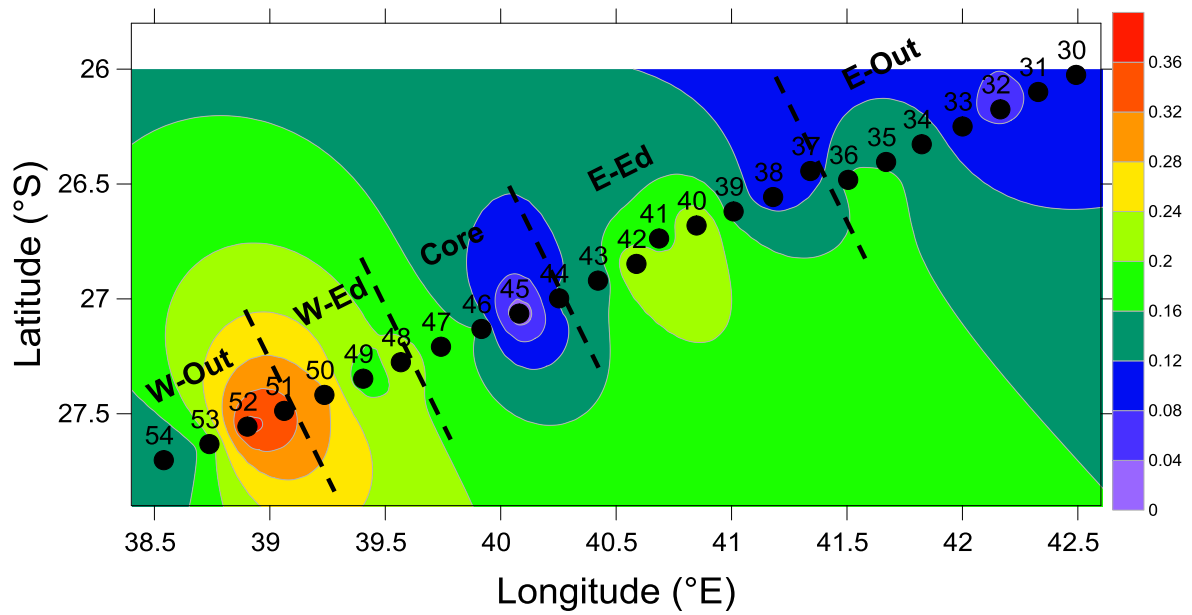
Figure 10 Contour plot of zooplankton biovolume (mL m^{-3}) along the eddy transect collected with the Bongo net (500 μm).

4.3 Meroplankton communities

Morphological identification of decapod larvae was difficult given the lack of available reference data, especially for the SWIO. Thus, identification of invertebrate larvae was mainly to higher taxonomic groups. There were more resources available for the identification of fish larvae, which were used to identify them to family level.

Mean abundances of invertebrate larvae collected with the Neuston net was fairly low on the Madagascan shelf, with the highest recorded in the W-Ed zone (13.7 ind m^{-3}; Table 2). Crab larvae were the most abundant group on the shelf, while prawn larvae dominated the eddy. Phyllosoma larvae were found on both the west and east Madagascan shelf in low numbers but not in the eddy larvae dominated in the eddy. Mean invertebrate larval abundances collected with the Bongo net was much higher compared to the Neuston net both on the shelf and in the eddy (Table 2). Highest abundances were recorded on the east (41.5 ind m^{-3}) and west (55.6 ind m^{-3}) Madagascan shelf and in eddy zone W-Ed (39.3 ind m^{-3}). Shrimp larvae dominate the invertebrate meroplankton on the shelf and outside the eddy (E-Out), whereas prawn larvae dominated the eddy meroplankton. Phyllosoma larvae were found in low abundances in the west Madagascan shelf and in the outer eddy perimeter (W-Ed). Bivalve larvae were present in the eddy core and outside the eddy (E-Out), also in low numbers.

Low larval fish abundances was also recorded with the Neuston net on the Madagascan shelf, with the highest abundance in the eddy zone E-Out (6.9 ind m^{-3}; Table 3). The dominant families of fish larvae collected on the east Madagascan shelf with the Neuston net were Mullidae and Sphyraenidae on the Madagascan shelf, together, comprising > 60 % of total larval fish abundance. Outside of the eddy (E-Out) Centropomidae comprised 74.2 % of total larval fish abundance, and in the eddy periphery Mullidae comprised 26.3 % of total larval fish abundance. Mean larval fish abundances collected with the Bongo net (Table 3) was highest at the west shelf station (71.5 ind m^{-3}), and in the eddy abundance was greatest in the western periphery (W-Ed (17.9 ind m^{-3}). Dominant fish families include the Carangidae, Bregmacerotidae, Lutjanidae and Myctophidae, on both the Madagascan shelf and in the eddy. Collectively Bregmacerotidae, Lutjanidae and Myctophidae comprised 80 % of total larval fish abundance on the east Madagascan shelf, while Carangidae dominated on the west shelf and comprised 82.4 % of total larval fish abundance.

Moderate to low Neuston invertebrate and larval fish abundances on the Madagascan shelf (east and west) were associated with cool near-surface water (22-23 ˚C) and moderate near-surface chlorophyll a concentration (0.2-0.3 mg m^{-3}). In the eddy, moderate invertebrate and larval fish abundances outside of the eddy (E-Out) and on the eddy perimeter (W-Ed), was associated with a moderate near-surface chlorophyll a concentration in the eddy perimeter (0.15 mg m^{-3}), but a much lower concentration outside the eddy (0.08 mg m^{-3}). The highest near-surface chlorophyll a concentration in the core (0.35 mg m^{-3}) was associated with low neuston invertebrate and larval fish abundance (Table 4). Higher Bongo net invertebrate and larval fish abundances on the Madagascan shelf were associated with cool water (< 23˚C) and high chlorophyll a concentration in the upper 100 m, with higher chlorophyll a concentration on the west shelf (0.88 mg m^{-3}) compared to the east shelf (0.12 mg m^{-3}). In the eddy, moderate Bongo net invertebrate and larval fish abundances in the eddy perimeters, E-Ed and W-Ed, were associated with moderate chlorophyll a concentration in the W-Ed (0.11 mg m^{-3}) and higher chlorophyll a concentration in the E-Ed (0.28 mg m^{-3}). Lower Bongo net invertebrate and larval fish abundances in the eddy core were associated with high chlorophyll a concentration (0.30 mg m^{-3}) (Table 5).

Table 2 Mean (ind m^{-3}) and relative abundances (% of total) of invertebrate larvae of various taxonomic groups collected with the Neuston net (top) and Bongo net (bottom) on the Madagascan shelf and in the eddy zones.

Groups	East shelf no.m^{-3}	%	West shelf no.m^{-3}	%	E- Out no.m^{-3}	%	E-Ed no.m^{-3}	%	Core no.m^{-3}	%	W-Ed no.m^{-3}	%
Neuston												
Prawn larvae	0.88	33.33	-	-	3.10	50.00	0.66	60.0	2.65	92.31	5.97	43.55
Crab larvae	1.33	50.00	1.55	63.64	1.77	28.57	0.44	40.00	0.22	7.69	6.64	48.39
Shrimp larvae	0.22	8.33	-	-	1.33	21.43	-	-	-	-	1.11	8.06
Phyllosoma larvae	0.22	8.33	0.88	36.36	-	0.00	-	-	-	-	-	-
Total	**2.65**	**100**	**2.43**	**100**	**6.19**	**100**	**1.11**	**100**	**2.88**	**100**	**13.71**	**100**
Bongo												
Prawn larvae	13.85	33.33	11.26	20.24	1.07	33.33	4.98	45.45	1.60	50.00	26.79	68.18
Shrimp larvae	21.54	51.85	28.48	51.19	1.42	44.44	2.99	27.27	0.53	16.67	8.33	21.21
Crab larvae	6.15	14.81	14.57	26.19	0.36	11.11	2.99	27.27	0.53	16.67	2.98	7.58
Phyllosoma larvae	-	-	1.32	0.56	-	-	-	-	-	-	1.19	3.03
Bivalve larvae	-	-	-	-	0.36	11.11	-	-	0.53	16.67	-	-
Total	**41.54**	**100.00**	**55.63**	**100.00**	**3.20**	**100.00**	**10.95**	**100.00**	**3.19**	**100.00**	**39.29**	**100.00**

Table 3 Mean (ind m^{-3}) and relative abundances (% of total) of fish larvae of various families collected with the Neuston net (top) and Bongo net bottom) on the Madagascan shelf and in the eddy zones.

Families	East shelf no.m^{-3}	%	West shelf no.m^{-3}	%	E-Out no.m^{-3}	%	E-Ed no.m^{-3}	%	Core no.m^{-3}	%	W-Ed no.m^{-3}	%
Neuston												
Bothidae	-	-	-	-	0.22	3.23	-	-	-	-	0.44	10.53
Bregmacerotidae	-	-	-	-	-	-	-	-	-	-	0.22	5.26
Carangidae	-	-	-	-							0.22	5.26
Centropomidae	-	-	-	-	5.09	74.19	-	-	-	-	0.22	5.26
Clupeidae	0.22	6.67	-	-	0.22	3.23	-	-	-	-	0.44	10.53
Exocoetidae	0.22	6.67	-	-	-	-	-	-	-	-	-	-
Mullidae	0.88	26.67	-	-	-	-	-	-	-	-	1.11	26.32
Myctophidae	-	-	-	-	1.11	16.13	-	-	-	-	-	-
Scombridae	0.22	6.67	-	-	-	-	-	-	-	-	0.22	5.26
Scorpaenidae	-	-	-	-	-	-	-	-	-	-	0.22	5.26
Serranidae	0.22	6.67	-	-	-	-	-	-	-	-	0.44	10.53
Sphyraenidae	1.33	40.0	-	-	-	-	-	-	-	-	0.22	5.26
Synodontidae	-	-	-	-	0.22	3.23	-	-	-	-	-	-
Unidentified larvae	0.22	6.67	0.22	100.0	-	-	-	-	0.22	10.0	0.44	10.53
Total	**3.32**	**100**	**0.22**	**100**	**6.85**	**100**	**0.00**	**0.00**	**0.22**	**100**	**4.20**	**100**
Bongo												
Apogonidae	-	-	1.99	2.78	-	-	-	-	-	-	1.79	10.00
Blennidae	1.54	5.56	0.66	0.93	-	-	-	-	-	-	-	-
Bothidae	-	-	-	-	-	-	-	-	-	-	-	-
Bregmacerotidae	7.69	27.78	1.32	1.85	-	-	0.50	5.26	0.53	7.69	4.17	23.33
Carangidae	-	-	58.94	82.41	-	-	-	-	-	-	2.98	16.7
Carapidae	-	-	-	-	-	-	0.50	5.26	-	-	-	-
Centropomidae	-	-	-	-	1.07	21.43	-	-	-	-	-	-
Clupeidae	1.54	5.56	3.31	4.63	-	-	-	-	-	-	-	-
Exocoetidae	-	-	-	-	-	-	-	-	0.53	7.69	-	-
Gonostomatidae	1.54	5.56	-	-	-	-	0.50	5.26	-	-	1.19	6.67
Labridae	-	-	0.66	0.93	0.36	7.14	-	-	-	-	0.60	3.33
Lutjanidae	7.69	27.78	1.32	1.85	-	-	-	-	-	-	4.17	23.33
Mullidae	-	-	-	-	-	-	-	-	-	-	2.38	13.33
Myctophidae	7.69	27.78	-	-	2.85	57.14	5.97	63.16	5.85	84.62	2.98	16.67
Nomeidae	-	-	-	-	0.36	7.14	-	-	-	-	-	-
Scombridae	1.54	5.56	1.32	1.85	-	-	1.00	10.53	-	-	-	-
Scorpaenidae	-	-	-	-	-	-	-	-	-	-	-	-
Serranidae	-	-	0.66	0.93	-	-	-	-	-	-	1.79	10.00
Sphyraenidae	-	-	1.32	1.85	-	-	0.50	5.26	-	-	-	-
Synodontidae	1.54	5.56	-	0.00	-	-	0.50	5.26	-	-	-	-
Trichuridae	1.54	5.56	-	-	0.36	7.14	-	-	-	-	-	-
Unid larvae	3.08	11.11	1.32	1.85	-	-	-	-	-	-	-	-
Total	**27.69**	**100.00**	**71.52**	**100.00**	**4.98**	**100.00**	**9.45**	**100.00**	**6.91**	**100.00**	**17.86**	**100.00**

Table 4 Mean (± standard deviation) of Neuston net invertebrate and fish larval abundance (ind m^{-3}), near- surface (7 m) chlorophyll a concentration (mg m^{-3}), near-surface (7 m) temperature on the shelf on the west and east shelf and across the eddy zones.

Zones	Inverts (ind m^{-3})	Fish larvae (ind m^{-3})	Chlorophyll a (mg m^{-3})	Temperature (˚C)
East shelf	5.3 ± 1.95	0.26 ± 0.38	0.2 ± 0.04	22.86 ± 0.001
West shelf	0.97 ± 0.93	0.02 ± 0.06	0.28 ± 0.03	21.86 ± 0.001
E-Out	2.47 ± 2.10	0.53 ± 1.31	0.08 ± 0.02	22.87 ± 0.001
E-Ed	0.44 ± 0.42	0	0	22.49 ± 0.06
Core	1.15 ± 1.32	0.02 ± 0.06	0.35 ± 0.06	22.06 ± 0.001
W-Ed	5.48 ± 4.87	0.32 ± 0.27	0.15 ± 0.03	22.53 ± 0.003

Table 5 Mean (± standard deviation) of Bongo net invertebrate and fish larval abundance (ind m^{-3}), chlorophyll a concentration (mg m^{-3}) and temperature in the upper 100 m on the west and east shelf and across the eddy zones

Zones	Inverts (ind m^{-3})	Fish larvae (ind m^{-3})	Chlorophyll a (mg m^{-3})	Temperature (˚C)
East shelf	8.31 ± 9.33	1.32 ± 2.30	0.12 ± 0.10	21.98 ± 0.53
West shelf	11.31 ± 11.54	3.41 ± 12.76	0.88 ± 0.39	21.72 ± 0.06
E-Out	0.64 ± 0.58	0.24 ± 0.65	0.08 ± 0.07	22.33 ± 0.81
E-Ed	2.19 ± 2.16	0.45 ± 1.30	0.28 ± 0.15	22.33 ± 0.36
Core	0.64 ± 0.58	0.33 ± 1.28	0.30 ± 0.19	21.80 ± 0.52
W-Ed	7.86 ± 11.85	0.85 ± 1.30	0.11 ± 0.12	21.95 ± 0.49

4.4 DNA analysis

4.4.1 COI analysis

Traditional microscope identifications were difficult due to a lack of available reference material, and most of the decapod larvae were only identified to a high taxonomic level. However, with DNA barcoding analysis, more detailed identifications were possible. Due to budget constraints, randomly selected specimens both invertebrate and vertebrate larvae from each sampling station were used for DNA barcoding and the results include only the presence of taxa and not their abundance. A total of 51 barcodes were obtained for both invertebrate and fish larvae. Most sequences were identified to the generic level only, given the lack of online reference data. For the purpose of this thesis, divergence thresholds, which were first proposed by Hebert *et al.* (2004b), were used to identify unknown sequences. Past barcoding studies of marine fish (Ward *et al.* 2005) and decapods (da Silva *et al.* 2011) established ranges of sequences divergences that characterise individuals belonging to the same species. Hebert *et al.* (2003b) further demonstrated that a wide gap exists between the divergence values obtained in intraspecific and interspecific comparisons among congeners. These divergences increase with increasing taxonomic level (i.e., at generic, familial or ordinal level). For this study, sequence divergences were used for species identification, and to assess generic placement and family membership based on these divergence thresholds.

For decapod larvae, two sequences were identified to species level: the shrimp *Rhynchocinetes durbanensis* and squat lobster *Allogalathea elegans* (Table 10). A further 18 sequences were identified to genus level. These included decapod larvae of crabs of the genera *Ashtoret, Calappa*, *Ebalia, Pilumnus, Portunus, Pseudoliomera* and *Xaiva,* prawns (*Bentheogennema, Marsupenaeus, Metapenaeopsis* and *Solenocera*), and shrimp (*Acanthephyra, Eualus, Periclimenes,* and *Plesionika*) (Table 6). These invertebrate larvae were found on both the Madagascan shelf and along the eddy transect. Fish larvae identified to species level included *Decapterus macrosoma*, *Parupeneus fraserorum*, *Pseudanthias squamipinnis* and *Sarda orientalis* (Table 7). There were two possible candidate species identified for genera *Gonostoma, Oplegnathus, Priacanthus* and *Scomber*, which may reflect misidentification of these species in the BOLD reference database. In this case, identification was made to genus level (Table 11). Those larvae that were identified to genus level include the genera *Bregmaceros*, *Callionymus*, *Chromis*, *Epinephelus*, *Gonostoma, Halichoeres*, *Lotella*, *Nealotus*, *Ostorhinchus*, *Oplegnathus*, *Polysteganus*, *Priacanthus*, *Pseudanthias*, *Pyramodon, Scomber*, *Symbolophorus, Taaningichthys* and *Vinciguerria*. Fish larvae

identified to family level included Ammodytidae, Bothidae, Gonostomatidae, Myctophidae and Notosudidae.

4.4.2 16S analysis

In cases of failed amplification with COI, amplification of 16S rDNA was used instead. Two unknown phyllosoma larvae and one fish larva were identified using 16S rDNA. These included phyllosoma larvae of the genus *Scyllarus* and *Panulirus* (Table 6), and a fish larva of the genus *Paralabrax* (Table 7).

Table 6 Unknown invertebrate larvae identified through DNA barcoding. Taxa in bold indicate sequences identified to species level.

Unknown specimen number	Family	Genus	Species	Gene	BOLD % similarity	GenBank % similarity
C1	Portunidae	*Xaiva*		COI	98.2	
C2	Portunidae	*Portunus*		COI	86.29	
C3	Leucosiidae	*Ebalia*		COI	86.29	
C7	Matutidae	*Ashtoret*		COI	87.71	
C8	Xanthidae	*Pseudoliomera*		COI	99.81	
C9	Pilumnidae	*Pilumnus*		COI	84.67	
C13	Calappidae	*Calappa*		COI	100	
P1.2	Penaeidae	*Metapenaeopsis*		COI	89.05	
P1.4	Benthesicymidae	*Bentheogennema*		COI	86.18	
P3	Solenoceridae	*Solenocera*		COI	89.38	
P5	Penaeidae	*Marsupenaeus*		COI	89.27	
S1	Acanthephyridae	*Acanthephyra*		COI	98.89	
S2	Palaemonidae	*Periclimenes*		COI	100	
S3	Hippolytidae	*Eualus*		COI	86.62	
S4	**Rhynchocinetidae**	***Rhynchocinetes***	***durbanensis***	COI	**100**	
S5	Pandalidae	*Plesionika*		COI	84.26	
C11	**Galatheidae**	***Allogalathea***	***elegans***	COI	**100**	
Ph1	Scyllaridae	*Chelarctus*		COI	82.36	
Ph2	Scyllaridae	*Scyllarus*		16S		81
Ph3	Palinuridae	*Panulirus*		16S		99

Table 7 Unknown fish larvae identified through DNA barcoding. Taxa in bold indicate sequences identified to species level.

Unknown specimen number	Family	Genus	Species	Gene	BOLD % similarity	Genbank % similarity
F2	Oplegnathidae	*Oplegnathus*	*peaolopesi/robinsoni*	COI	100	
F4	Bothidae			COI		89
F5	**Mullidae**	***Parupeneus***	***fraserorum***	COI	**100**	
F8	Notosudidae			COI	96.45	
F11	**Carangidae**	***Decapterus***	***macrosoma***	COI	100	
F12	Priacanthidae	*Priacanthus*	*aff. arenatus/ arenatus/ hamrur*	COI	99.11	
F13	Serranidae	*Epinephelus*		COI	99.65	
F14	Bregmacerotidae	*Bregmaceros*		COI	98.2	
F16	Scombridae	*Scomber*	*colias/japonicus*	COI	100	
F17	Myctophidae	*Symbolophorus*		COI	99.03	
F18	Myctophidae			COI	90.41	
F19	Gempylidae	*Nealotus*		COI		91
F20	Labridae	*Halichoeres*		COI	99.41	
F21	Carapidae	*Pyramodon*		COI	98.92	
F22	Apogonidae	*Ostorhinchus*		COI		82
F24	Pomacentridae	*Chromis*		COI	92.88	94
F25	Serranidae	*Pseudanthias*		COI	99.52	
F26	Callionymidae	*Callionymus*		COI	99.36	
F27	Moridae	*Lotella*		COI	91.59 *cf. tosaensis*	94 *rhacina*
F28	**Carangidae**	***Decapterus***	***macrosoma***	COI	100	
F29	**Mullidae**	***Parupeneus***	***fraserorum***	COI	**100**	
F30	**Scombridae**	***Sarda***	***orientalis***	COI	**100**	
F32	**Serranidae**	***Pseudanthias***	***squamipinnis***	COI	**100**	
F33	Serranidae	*Paralabrax*		16S		98
F34	Myctophidae	*Taaningichthys*		COI	98.28	
F35	Phosichthyidae	*Vinciguerria*		COI	98.21	
F36	Gonostomatidae			COI	99.38	
F37	Ammodytidae			COI	93.24	
F38	Sparidae	*Polysteganus*		COI	93.43	91 *coeruleopunctatus*
F39	Gonostomatidae	*Gonostoma*	*elongatum/ denudatum*	COI	100	
F40	**Mullidae**	***Parupeneus***	***fraserorum***	COI	**100**	

4.5 Phylogenetic Analysis

Sequences obtained in this study were combined with sequences downloaded from BOLD and GenBank (Table 12) to produce a Neighbour-joining (NJ) tree (Figure 13). The NJ tree was used to display a graphic representation of the divergence patterns and distance relationships between sequences. Due to a lack of online reference data and available sequences for the invertebrate larvae, the NJ tree was produced using only larval fish sequences. Tight clusters and short branch lengths among unknown larvae and downloaded data for representative species indicate very small genetic distances and, together with high bootstrap support, confirm highly accurate species identifications for these larvae. Sequence divergences were calculated using the K2P model (Kimura 1980) are presented in Appendices A and B. K2P distances among specimens in each of these clusters varied from 0% to 0.2%. These clusters represent *Decapterus macrosoma, Parupeneus fraserorum, Pseudanthias squamipinnis* and *Sarda orientalis*. Those unknown fish larvae which had two possible candidate species identifications (*Gonostoma elongatum, Oplegnathus robinsoni, Priacanthus* aff. *arenatus* and *Scomber colias* also showed tight clusters with downloaded fish species data and fell within the 2% threshold characterising conspecifics species. Certain unknown fish larvae also showed tight clusters with *Callionymus marleyi*, *Epinephelus rivulatus*, *Halichoeres zeylonicus* and *Pseudanthias connelli* respectively, but had higher genetic distances (from 3 to 8%), falling beyond the 2% threshold characterising congeneric species.

Some of the unknown larvae are clearly associated with representatives of some species and a particular genus in neat clusters, but with slightly longer branch lengths and, hence, larger genetic distances. This might suggest that the unknown larvae are congeneric, but belonging to a different species. These genetic distances between unknown larvae are congeneric, but belonging to a different species. These genetic distances between unknown larvae and downloaded data for species (*Callionymus marleyi, Chromis chrysura, Lotella* sp*., Nealotus tripes, Polysteganus baissaci, Pyramodon* sp. *Scopelosaurus lepidus, Symbolophorus rufinus, Taaningichthys minimus* and *Vinciguerria nimbaria* were between 1 and 10%.

Sequences separated by rather longer branches and K2P distances varying from 10 and 20% indicated that unknown larvae belong to a different genus, but to the same families as *Ammodytoides* sp*., Arnoglossus polyspilus, Cyclothone microdon* and *Diogenichthys laternatus*, for which data were downloaded.

Using both the K2P distances and NJ tree, DNA barcoding proved to be 100% successful at identifying unknown sequences to family level, 55% at genus level and 23% at species level.

Table 8 Fish COI sequences downloaded from BOLD or GenBank and used in conjunction with the present sequences to obtain a neighbour-joining tree. Sequences highlighted in blue are unpublished.

Species name	Location collected	BOLD Specimen number	Genbank Accession number
Oplegnathus robinsoni	South Africa, KwaZulu-Natal	TZMSC416-05	JF494026
Oplegnathus robinsoni	South Africa, KwaZulu-Natal	TZMSC575-06	JF494028
Arnoglossus polyspilus	China, Guangdong	FSCS116-06	EF607331
Arnoglossus polyspilus	China, Guangdong	FSCS117-06	EF607330
Parupeneus fraserorum	South Africa	DSFSF391-09	GU805064
Parupeneus fraserorum	South Africa	DSFSG628-11	KF489698
Scopelosaurus lepidus		MAECO527-09	
Scopelosaurus lepidus		MAECO528-09	
Decapterus macrosoma	Malaysia, Terengganu, South China Sea	DBMF279-10	JX261515
Decapterus macrosoma	Malaysia, Sabah, Sulawesi Sea	DBMF675-10	JX261514
Decapterus macrosoma	Malaysia, Sabah, South China Sea	DBMF754-10	JX261170
Decapterus macrosoma	South Africa, KwaZulu-Natal	TZMSB169-04	JF493342
Priacanthus aff. *arenatus*	South Africa, KwaZulu-Natal, Richards Bay	TZMSA195-04	DQ885041
Priacanthus aff. *arenatus*	South Africa, KwaZulu-Natal, Richards Bay	TZMSA209-04	DQ885042
Epinephelus rivulatus	South Africa, KwaZulu-Natal, Park Rynie	TZMSB198-04	DQ885003
Epinephelus rivulatus	South Africa, KwaZulu-Natal, Park Rynie	TZMSC151-05	DQ885006
Scomber colias	Portugal, Faro, Algarve	FCFPS118-06	JQ774713
Scomber colias	Portugal	FCFPW043-06	JQ775114
Symbolophorus rufinus			
Diogenichthys laternatus		ANGBF1270-12	HQ127668
Diogenichthys laternatus	Pacific Ocean	LIDMA1138-12	
Nealotus tripes	Taiwan, Northwestern, Taiwan	LFTW070-12	
Halichoeres zeylonicus	Mozambique	DSFSE680-08	JF493603
Pyramodon* sp. *W1	**Australia**	**FOAF724-07**	
Hemieleotris latifasciatus			AY722173.1
Chromis chrysura	Reunion, Hermitage	IPCOM365-10	JF458036
Chromis chrysura	Reunion, St Leu	IPCOM439-10	JF434873
Pseudanthias connelli	South Africa	DSFSF628-09	JF494282
Pseudanthias connelli	South Africa, KwaZulu-Natal, Durban	TZMSC542-06	JF494284
Callionymus marleyi	South Africa, KwaZulu-Natal	TZMSC053-05	JF493006
***Lotella* sp.**	**Australia**	**FOAF746-07**	
***Lotella* sp.**	**Australia**	**FOAF747-07**	
Sarda orientalis	China, Zhejiang, Zhejiang	GBGCA7703-15	KM055424
Sarda orientalis	South Africa, KwaZulu-Natal	TZMSC144-05	JF494396
Pseudanthias squamipinnis	South Africa	DSFSG568-11	KF489711
Pseudanthias squamipinnis	Sri Lanka, Western, Kochchikade	TZAIB904-07	FJ583946
Taaningichthys minimus		GBGCA11187-15	KJ555472
Taaningichthys minimus		GBGCA5879-13	AP012244
Vinciguerria nimbaria		GBGCA356-10	FJ918934
Cyclothone microdon	Atlantic Ocean	MAECO094-06	EU148135
Polysteganus baissaci	**Mauritius**		
Gonostoma elongatum		MAECO302-06	EU148180
Gonostoma elongatum		MAECO351-06	EU148179
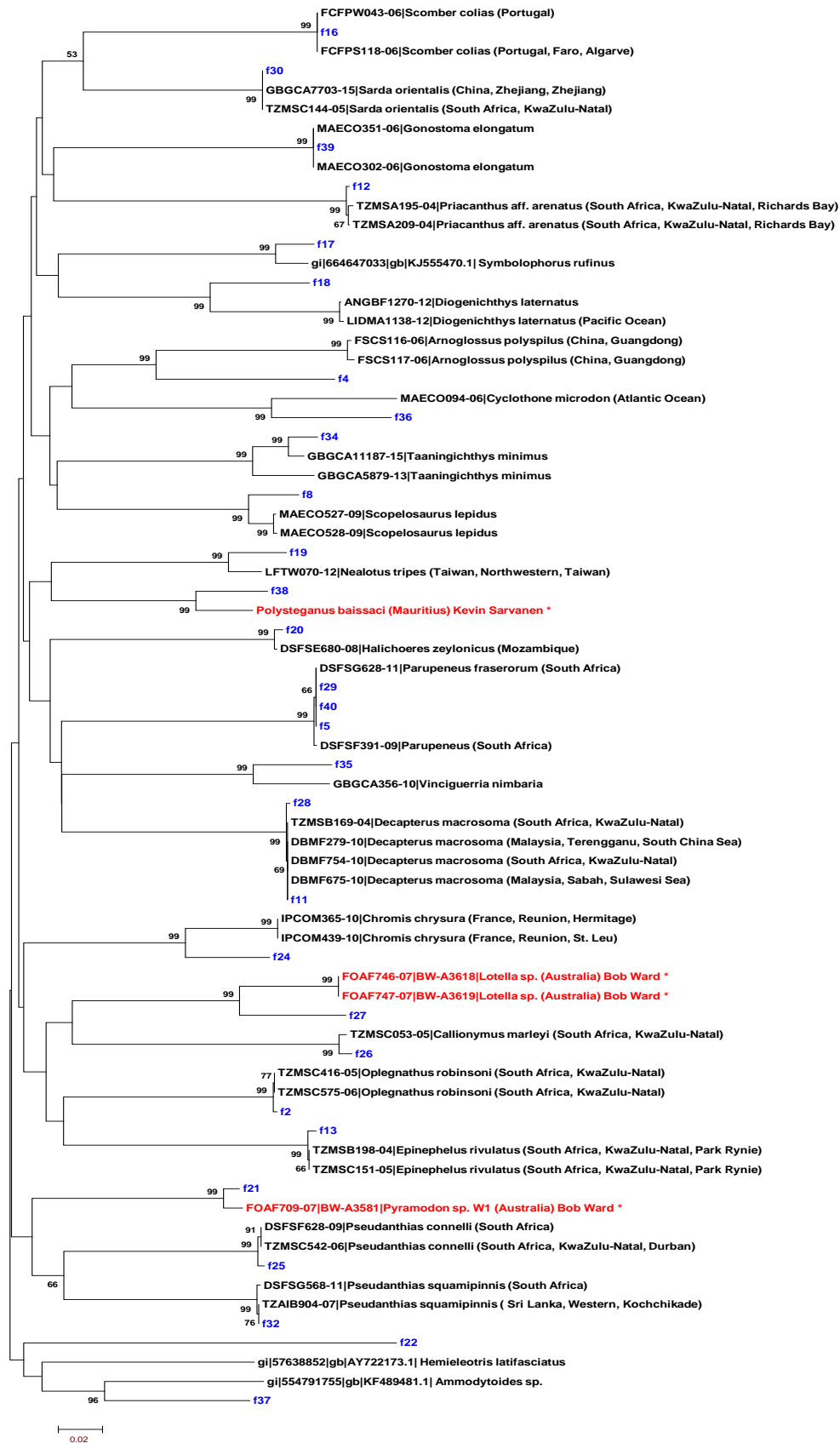
Figure 11 Neighbour-joining tree of COI sequences to display a graphic representation of the divergence patterns and distance relationships between sequences. The scale represents 2% K2P distance. Sequences in blue are unknown sequences, red represents unpublished data and black data are extracted from BOLD or GenBank. Numbers at nodes represent bootstrap values.

CHAPTER 5: DISCUSSION

5.1 Zooplankton biovolume on the Madagascan shelf

In this study, a higher mean zooplankton biovolume was recorded with the Bongo net (500 μm) on the Madagascan shelf (0.63 mL m^{-3}) compared to the eddy (0.19 mL m^{-3}), whereas mean zooplankton biovolume collected with the Neuston net did not differ much between the Madagascan shelf (0.08 mL m^{-3}) and the eddy (0.06 mL m^{-3}). Knowledge of zooplankton biomass in the SWIO is scarce. Broad scale sampling was conducted during the International Indian Ocean Expedition (IIOE) between 1959-1965 and zooplankton biovolumes in the SWIO were lower compared to higher biovolumes found in the northern monsoon-driven part of the Indian ocean (Rao 1973). In the SWIO highest zooplankton biomass has been recorded mainly in coastal waters, for example, Huggett (2014) recorded a high mean mesozooplankton (>200 μm) biovolume (1.9 mL m^{-3}) near the coast, ~ 15°S south of Nacala, Mozambique, during a cruise in April/May 2010. Sampling of macrozooplankton during fisheries surveys between August 1977 to June 1978 off the Mozambique Coast yielded mean displacement volumes <0.5 mL m^{-3}, with higher values on the Sofala Bank, Mozambique (Saetre and de Paula e Silva 1979). Displacement volumes are more accurate compared to settled volume, due to the different shapes of organisms which may become evident while settling; with displacement volume this effect is minimised (Harris *et al.* 2000). Nehring *et al.* 1987 found higher zooplankton biomass on the Sofala Bank, between 20 and 40 mg m^{-3} over the outer shelf, whereas values up to 160 mg m^{-3} were found on the inner shelf. However, at Angoche which is farther north along the Mozambican shelf (~ 18°S), values reached 320 mg m^{-3}. A more detailed study by Leal et al. (2009) on the Sofala Bank (~ 18-20°S) of the Mozambican coast between 5-8 December 2007 revealed higher zooplankton biovolumes (settled biovolumes) nearshore (0.77-7.11 mL.l^{-1}) compared to a lower biovolume offshore (0.28 mL.l^{-1}). The Sofala Bank is a productive shelf region influenced by tidal currents and great hydrological variability determined by the Zambezi Delta discharges, as well as by mesoscale oceanographic features of the MC (Lutjeharms 2006b, Ternon *et al.* 2014). Malauene *et al.* (2014) found that elevated chlorophyll *a* concentrations north of Mozambique near Angoche (~ 16°S) are largely driven by north-easterly monsoon winds, but also through cyclonic/anticyclonic eddies interacting with the shelf. In the present study, elevated sea surface chlorophyll *a* concentration and low sea surface temperature on the southern Madagascan shelf were

associated with elevated zooplankton biovolume. These oceanographic conditions have been observed previously on the southern coast of Madagascar, where hydrographic data, and satellite and sea surface temperature imagery showed elevated chlorophyll *a* and cooler water, indicating upwelling, on the south-east coast of Madagascar (Di Marco *et al.* 2000, Machu *et al.* 2002). Similar conditions were also noted in a more recent study on the southern coast of Madagascar (26°S). Pripp *et al.* (2014) found elevated chlorophyll *a* and primary production on the south coast in response to upwelling as revealed by high sea surface salinity and low sea surface temperature.

5.2 Zooplankton biovolume in the eddy

In this study, highest mean zooplankton biovolumes were recorded in the eddy periphery, with lower zooplankton biovolumes in the eddy core. This is in contrast to chlorophyll *a* concentrations, which were highest in the eddy core (0.5-0.7 mg m^{-3}) and intermediate in the eddy periphery (0.3-0.5 mg m^{-3}). This pattern could be due to top-down control by zooplankton grazers as the main controlling primary producers on the eddy peripheries. Zooplankton biomass in the open ocean has previously been found to be relatively high in cyclonic eddies. In the MC, Huggett (2014) found higher mesozooplankton biovolume (0.3 mL m^{-3}) in cyclonic eddies compared to anti-cyclonic eddies, although the coarser sampling resolution, compared to this study, was not adequate to detect differences in biovolume between the core and periphery of the eddies. Landry *et al.* (2008) recorded mesozooplankton biomass in a cyclonic eddy to be 80% greater than the surrounding waters of the Hawaiian Islands. In the Western Bay of Bengal, mesozooplankton biomass was five times and density 18 times greater in cold-core eddies (Fernandes and Ramaiah 2009). In the Gulf of Mexico, acoustic backscatter intensity reflected from epipelagic zooplankton communities revealed elevated zooplankton and micronekton in cold-core eddies compared to warm-core eddies.

It is unclear in this study as to why zooplankton would accumulate in the eddy peripheries and not the core where more food is available. Interestingly studies conducted in the Sargasso Sea documented similar observations to this study with higher zooplankton biomass recorded in a cyclonic eddy's periphery compared to the core (Goldthwait and Steinberg 2008). Hernandez-LeonTorres *et al.* (2001) also found elevated biomass in the eddy periphery compared to the core of the eddy in the vicinity of the Canary Islands. In both

the above studies, the eddies were mature and it was suggested that higher zooplankton biovolume or biomass found at eddy peripheries could be due to an outward drift of zooplankton toward the eddy periphery during the eddy winding down, i.e. decaying or in the "spinning down" phase (Bakun 2006). In this study, however, the cyclonic eddy was sampled when it was about a month old, which was evident from the thermal structure of the eddy with a lack of doming in the temperature profile, indicating that the eddy was not fully "spun up". Furthermore, studies on micronekton and top predators in the MC also found higher abundance at the eddy peripheries (Weimerskirch *et al.* 2004, Sabarros *et al.* 2009, Tew-Kai and Marsac 2009, Sanchez-Velasco *et al.* 2013); these studies suggested that eddies tend to advect coastal waters at their periphery when they propagate along the coast and thereby distribute nutrient-rich waters in the open ocean. It is thus suggested that productive coastal waters on the southern Madagascan shelf become entrained into the eddy periphery as the eddy passes along the shelf and advects coastal rich waters into the open ocean.

5.3 Meroplankton distribution and species composition

5.3.1 Invertebrate larvae

No clear spatial patterns of abundance and species composition were observed for the invertebrate larvae collected with the Neuston and Bongo nets. Invertebrate larvae were evenly distributed on the Madagascan shelf and in the eddy (Table 9). Larvae of prawns, shrimps and crabs were the dominant decapods, with phyllosoma larvae also present but in lower numbers and mainly on the Madagascan shelf, with one only found in the eddy. Dominant prawn larvae represented two families, Penaeidae and Benthesicymidae. Penaeidae are normally found in shallow waters along the continental shelf and occur in large quantities (Chan 1998), while Benthesicymidae are either deep benthic dwellers or members of the meso- and bathypelagic fauna (Vazquez-Bader *et al.* 2004).

Shrimp larvae were dominated by the family Palaemonidae, genus *Periclimenes.* This genus is known to associate with anemones and is found throughout the world in warmer waters, with greatest diversity on tropical reefs (Bruce 2004). One larval shrimp identified to species level was the rhynchocinetid shrimp *Rhynchocinetes durbanensis.* This species, initially known only from South Africa (Gordon 1936) is widely distributed in the Indo-West Pacific and is associated with coral or rocky reefs in shallow waters (Gordon 1936).

Dominant crab larvae included portunids from the family Portunidae and were found on both the Madagascan shelf and in the eddy. Portunid crabs are commonly found in tropical and subtropical estuarine and nearshore habitats (Shields 1992).

Phyllosoma larvae represented two families, Palinuridae (spiny lobsters) and Scyllaridae (slipper lobsters). Spiny lobsters are inhabitants of reef and rocky shores in tropical and temperate seas (Diaz *et al.* 2002). The genus *Panulirus* is characteristic of shallow tropical waters and has a wide geographic distribution (Ptacek *et al.* 2001). Scyllaridae are widespread in shallow temperate and tropical waters, but are more abundant in tropical waters and have also been recorded in waters deeper than 300 m (Baisre 1994). Phyllosoma larvae identified from this study fall under the subfamily Scyllarinae. This subfamily consists of small lobsters which are found in tropical lagoons, on continental shelf and slopes, and on deep sea ridges and seamounts (Booth *et al.* 2005).

Lastly, a squat lobster larva was also identified to species level. *Allogalathea elegans,* widely distributed in the Indo-West Pacific region and sighted off northern Mozambique and Madagascar (Baba *et al.* 2008), is a shallow water species, but is sometimes found down to 146 m, and is associated with crinoids (Cabezas *et al.* 2011).

Table 9 Table of decapod larval groups recorded on the Madagascan shelf and in the eddy zones.

	W-Ed	E-Ed	Core	E-Out	West shelf	East shelf
Prawn larvae	Penaeidae	Penaeidae	Penaeidae	Penaeidae	Penaeidae	Penaeidae
	Benthesicymidae	Benthesicymidae	Benthesicymidae	Benthesicymidae	Benthesicymidae	Benthesicymidae
Shrimp larvae	Rhynchocinetidae	Rhynchocinetidae	Rhynchocinetidae	Rhynchocinetidae	Rhynchocinetidae	Rhynchocinetidae
	Palaemonidae	Palaemonidae	Palaemonidae	Palaemonidae	Palaemonidae	Palaemonidae
Crab larvae	Portunidae	Portunidae	Portunidae	Portunidae	Portunidae	Portunidae
Phyllosoma larvae	Palinuridae				Scyllaridae	Scyllaridae

5.3.2 Fish larvae

In contrast to the invertebrate larvae, clear spatial patterns of species composition were observed for the fish larvae, with more larvae recorded on the Madagascan shelf and in the western eddy periphery, and fewer larvae recorded in the eddy core (Table 10). Dominant fish larvae collected from the Neuston and Bongo nets belonged to coastal/reef, benthic, epipelagic and mesopelagic families. The coastal/reef fish families included the Centropomidae, Mullidae and Serranidae, epipelagic fish families included the Sphyraenidae and Carangidae, benthic fish families included the Bothidae and Bregmacerotidae, and mesopelagic fish families included the Myctophidae and Gonostomatidae. Carangids have been described as coastal or shelf families off eastern Australia (Keane and Neira 2008). The presence of Myctophidae is easily explained, being a diverse family of oceanic fishes which have been collected in many regions of the world and which represent a high proportion of the total larvae collected in oceanic plankton samples (Olivar and Beckley 1994). This also explains their high abundance in both nets. Myctophids also dominated eddy peripheries in the MC, with few caught in the eddy core, during a study on the influence of mesoscale features on micronekton and large pelagic fish communities in the MC (Potier *et al.* 2014). The genus *Symbolophorus* was one of the myctophids which prevailed in fish assemblages (Potier *et al.* 2014) and was also present in this study.

Fish larvae identified to species level include the scad mackerel *Decapterus macrosoma*, the serranid *Pseudanthias squamipinnis* (sea goldie), the goat fish *Parupeneus fraserorum* and the striped bonito *Sarda orientalis. D. macrosoma* is reef-associated, found in depth ranges between 20 to 214 m and widely distributed in the tropical Indo-West Pacific (Paxton et al. 1989). *P. squamipinnis* is widely distributed in the Indo-West Pacific and is a reef-associated fish, occurring in shallow waters down to 55 m (Randall *et al.* 1997). The goat fish *Parupeneus fraserorum* is a newly described mullid species from the coast of KwaZulu-Natal, South Africa, in a depth range of 39-57 m but has also been recorded off the southeastern coast of Madagascar from a depth range of 27-87 m (Randall and King 2009). According to Randall and King (2009), *P. fraserorum* is known only from KZN and the southeastern coast of Madagascar. *S. orientalis* is a coastal species occurring at a depth range of 1-167 m and has a wide distribution in the Indo-Pacific region, from the east coast of Africa to the west coast of America in the Eastern Pacific region (Collete and Nauen 1983).

Other fish larvae identified in this study but not found in high abundance include families of reef-associated fishes. The Priacanthidae are distributed in tropical and subtropical waters of

the Atlantic, Indian and Pacific Oceans, and are generally associated with rock formations or coral reefs (Starnes 1988). The genus *Pseudanthias* (subfamily Anthiinae, family Serranidae), are small colourful, coral-reef fishes mainly from the Indo-Pacific (Randall 2011). The Labridae inhabit tropical marine and temperate waters around the world, and are mainly found in shallow-water habitats, such as coral reefs, rocky reefs, sand, grass and algae (Westneat and Alfaro 2005). The Apogonidae, also known as cardinal fishes, are nocturnal, active inhabitants of tropical, subtropical and warm temperate reefs (Fraser and Allen 2010). The Pomacentridae (damsel fishes) occur in all tropical oceans, mainly the Indo-Pacific (Allen 1991). Lastly, the Sparidae are widely distributed in tropical and temperate waters and occur in rocky and sandy areas nearshore or on offshore reefs to 450 m (Heemstra and Heemstra 2004).It was evident that reef associated fish dominated the dominated the Madagascan shelf as well as the eddy zone (W-Ed).

5.3.3 Larval entrainment in eddies

Several studies portrayed the entrainment and advection of planktonic larvae by mesoscale eddies in other oligotrophic waters. Graber and Limouzy-Paris (1997) found that fish larval assemblages within spin-off eddies in the Straits of Florida consisted predominantly of reef fishes, indicating that fish larvae were entrained from the shelf. In the Gulf of California, the highest plankton larval abundance was concentrated at the edge of an eddy, offshore, consisting mainly of coastal pelagic and demersal species. This suggests that the edge of the eddy "captured" larvae close to the coast and transported them around the eddy (Sanchez-Velasco *et al.* 2013). In the Leeuwin Current, larval fish assemblages collected within the eddy were different from the surrounding oceanic waters (Holliday *et al.* 2011). The eddy larval assemblages consisted mainly of oceanic meso-pelagic fishes, but neritic taxa were also present. Holliday *et al.* (2011) therefore confirmed that the occurrence of neritic taxa in the eddy was due to larvae being incorporated within the eddy as it developed in proximity to the shelf.

The majority of the fish larvae found in this study were reef associated and were present on the Madagascan shelf, but also in the eddy periphery (W-Ed) in the Mozambique Basin. The occurrence of coastal/reef fish larvae on the Madagascan shelf is to be expected, with the majority being coastal/reef associated. However, the presence of coastal/reef fish families in the open ocean is less easily explained. Coastal/reef- associated fish present in the eddy periphery (W-Ed) in the Mozambique Basin suggests that fish larvae were wrapped around the edges of the cyclonic eddy, which originated on the southern Madagascan shelf, and

were transported into the MC. Higher zooplankton biovolumes, larval abundances and reef-associated larval assemblages found in the periphery of the cyclonic eddy, compared to the core in this study provide support for the suitcase hypothesis that planktonic organisms are entrained within eddies as they propagate south-westwards of the Madagascan shelf.

Table 10 Table of fish larval species composition recorded on the Madagascan shelf and in the eddy zones. Colour codes indicate habitats of larval fish.

W-Ed	Core	E-Ed	E-Out	West shelf	East Shelf
Priacanthus (Bigeyes)	Diogenichthys (lanternfish)	Bregmaceros (Codlets)	Arnoglossus (Flounders)	Ostorhinchus (Cardinal fish)	Bregmaceros (Codlets)
Halichoeres (Wrasses)	Symbolophorus (lanternfish)	Lotella (Morid cods)	Nealotus (Snake mackerel)	Chromis (Damselfish)	Nealotus (Snake mackerel)
Ostorhinchus (Cardinal fish)	Lotella (Morid cods)	Pyramodon (Pearlfishes)	Symbolophorus (Lanternfish)	Polysteganus (Seabream)	Cyclothone (Bristlemouths)
Chromis (Damselfish)		Diogenichthys (Lanternfish)	Halichoeres (Wrasses)	Halichoeres (Wrasses)	Symbolophorus (Lanternfish)
Pseudanthias squamipinnis (Sea goldie)		Gonostoma (Bristlemouth)		Bregmaceros (Codlets)	Pseudanthias (Sea goldie)
Polysteganus (Seabream)		Symbolophorus (Lanternfish)		Callionymus (Dragonets)	
Parupeneus fraserorum (goatfish)		Polysteganus (Seabream)		Bleekeria (Sand lances)	
				Epinephelus (Groupers)	
				Scomber (Mackerels) Sarda orientalis (Striped bonito)	
				Decapterus (Kingfish)	

Reef

Epipelagic

Mesopelagic

Benthic

5.4 Connectivity between the Madagascar and South Africa?

The overall aim of this thesis was to determine whether eddies off southern Madagascar create a potential pathway for gene flow across the MC to southern Africa. The goatfish *P. fraserorum*, a newly described mullid which is found off the coasts of both Madagascar and KZN, was identified and its larvae were present on the Madagascan shelf and in the eddy. Invertebrate and fish larvae identified in this study were either coastal or reef-associated and found on the Madagascan shelf and in the eddy. Based on these findings, it is suggested that cyclonic eddies do entrain planktonic larvae from the Madagascan shelf and transport them into the open ocean. It has also been observed through numerical models and altimetry data (Quartly and Srokosz 2004, Halo *et al.* 2014b) that cyclonic eddies off the southwest region of Madagascar have strong vorticity, and are most energetic, living longer and travelling longer distances compared to eddies from the southeast region, suggesting that they are most likely to trap material in their cores and to transport them over long distances.

Assuming that eddies can entrain larvae from the Madagascan shelf, can they transport larvae across the MC in time before metamorphosis? The core of a cyclonic eddy is clearly a favourable niche for larvae; however, the propagation speed of cyclonic eddies recorded crossing the Mozambique Channel from south-west Madagascar is 6-9 km.day^{-1}, which would take 5-7 months (Marsac *et al.* 2014). This would not be feasible for the survival of most marine organisms, especially benthic species. For example, some benthic organisms have a pelagic larval phase of 80 days (Shulman and Bermingham 1995). Another possible route for biota to cross the MC in a suitable time-frame for larval survival is via the regions between contra-rotating eddies (Marsac *et al.* 2014). Hancke *et al.* (2014) recorded a (westward) horizontal velocity of 52-170 km day-1 in contra-rotating eddies. In this case, it would take less than 50 days to cross the MC, favouring larval survival for most benthic and pelagic species.

5.5 Efficacy of DNA barcoding of plankton

DNA barcoding of meroplankton has been revealed to be an effective tool for identification in this study, although taxonomic identification could mostly be done only to genus level. This indicates the limited reference information available for invertebrate and fish species in this region. On a global scale, DNA barcoding has been successful as an identification tool, identifying basic groups of zooplankton (Webb *et al.* 2006, Machida *et al.* 2009, Bucklin *et al.* 2010, Heimeier *et al.* 2010, Cheng *et al.* 2014) and fish larvae (Pegg *et al.* 2006, Valdez-Moreno *et al.* 2010, Baldwin *et al.* 2011). DNA barcoding has also confirmed the accurate identification and discrimination within various groups of holozooplankton, for example, copepods (Bucklin *et al.* 2003, Bucklin and Frost 2009, Blanco-Bercial *et al.* 2014), cnidarians (Ortman 2008, Ortman *et al.* 2010), euphausiids (Bucklin *et al.* 2007), ostracods (Angel *et al.* 2008), pteropods (Jennings *et al.* 2010a) and chaetognaths (Jennings *et al.* 2010b).

This was the first study in the SWIO, using DNA barcoding as an identification tool, to identify meroplankton. Plankton taxonomic analysis using morphological identification is time-consuming and difficult, especially for meroplanktonic organisms, but becomes even worse when dealing with fragile and damaged organisms (Bucklin *et al.* 2011). This study has shown the efficacy of DNA barcoding to identify meroplankton where only broad identifications were possible using microscope analysis. This study has also brought new knowledge on meroplankton composition and abundance in the SWIO.

CHAPTER 6: CONCLUSIONS

This study was conducted to investigate whether mesoscale eddies formed off southern Madagascar can create a pathway for gene flow between KZN and Madagascar, helping to explain the similarity of marine fauna found along both of these coasts. The highest plankton biovolumes were recorded on the Madagascan shelf and in the outer region of an eddy in the Mozambique Channel, with lower plankton biovolumes found in the core and inner region of the eddy. It thus seems evident that biological material gets wrapped in the outer region of eddies along the southern Madagascan shelf as they move away from the shelf in a south-westerly direction. Shelf-originating decapod larvae (*Allogalathea elegans* and *Rhynchocinetes durbanensis*) and fish larvae (*Pseudanthias squamipinnis* and *Parupeneus fraserorum*) were found in the Mozambique Basin, and this clearly indicates that they were transported via the cyclonic eddy into the Mozambique Basin. The goatfish *P. fraserorum*, which is known from both the KZN and southern Madagascan coasts, was also recorded in the eddy, suggesting that more species that are known to occur on both these coasts could be revealed with further survey work and more comprehensive analysis. However, more detailed studies would be required to determine whether eddy-entrained larvae reach the KZN coast in time to settle. Mesoscale eddies that form off southern Madagascar take up to several (5 to 7) months to cross the MC (Marsac *et al.* 2014), thus only organisms with a long larval phase (e.g. > 7 months) would be able to survive such a journey. This is highly unlikely since most pelagic larval stages for benthic organisms can last for only 80 days before settlement (Shulman and Bermingham 1995). Frontal zones (between contra-rotating eddies) could provide a faster, effective route for planktonic larvae to cross the Mozambique Channel, due to fast-flowing currents that exist in these zones. Studies have shown that the journey across the Mozambique Channel in the frontal zone would take less than 50 days (Hancke *et al.* 2014). Further studies are therefore required, focusing mainly on these frontal zones to confirm whether planktonic larvae do indeed use this route to cross the Mozambique Channel and settle along the KZN coast.

This was the first study in the SWIO to use DNA barcoding as an identification tool to identify meroplanktonic larvae. Most taxa were successfully identified to genus level, and a few individuals were identified to species level. DNA barcoding has thus proved to be an accurate tool, even when dealing with fragile, damaged and minute planktonic larvae. This method is therefore recommended to be used in conjunction with the morphological identification of plankton for accurate results, especially when dealing with species that are morphologically similar.

This study confirms that cyclonic eddies that form off southern Madagascar can entrain planktonic larvae as they move offshore, but further studies are required to determine whether planktonic larvae are able to cross the Mozambique Channel and reach the KZN coast in time to settle.

REFERENCES

Angel, M.V., Nigro, L., Bucklin, A,. 2008. DNA barcoding of oceanic planktonic ostracoda: species recognition and discovery (abstract). World Conference on Marine Biodiversity, Valencia, Spain, 11-15 November 2008.

Allen, G.R., 1991. *Damselfishes of the world*. Melle, Germany: Mergus Publishers.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J., 1990. Basic local alignment search tool. *Journal of molecular biology*, 215, 403–10.

Atwood, E., Duffy-Anderson, J.T., Horne, J.K., and Ladd, C., 2010. Influence of mesoscale eddies on ichthyoplankton assemblages in the Gulf of Alaska. *Fisheries Oceanography*, 19, 493–507.

Avise, J.C., 1994. *Molecular Markers, Natural History, and Evolution*. New York: Chapman & Hall.

Baba, K., Macpherson, E., Poore, G.C.B., Ahyong, S.T., Bermudez, A., Cabezas, P., Lin, C.W., Nizinski, M., Rodrigues, C., and Schnabel, K.E., 2008. Catalogue of squat lobsters of the world (Crustacea: Decapoda: Anomura - Families Chirostylidae, Galatheidae and Kiwaidae). *Zootaxa*, 1905, 1–220.

Bailey, K.M., Picquelle, S.J., and Spring, S.M., 1996. Mortality of larval walleye pollock *Theragra chalcogramma* in the western Gulf of Alaska, 1988–91. *Fisheries Oceanography*, 5, 124–136.

Baisre, J.A., 1994. Phyllosoma larvae and the phylogeny of Palinuroidea (Crustacea: Decapoda): a review. *Marine and Freshwater Research*, 45, 925–944.

Bakun, A., 2006. Fronts and eddies as key structures in the habitat of marine fish larvae: opportunity, adaptive response and competitive advantage. *Scientia Marina*, 70S2, 105–122.

Baldwin, C.C., Brito, B.J., Smith, D.G., Weigt, L.A., and Escobar-Briones, E., 2011. Identification of early life-history stages of Caribbean *Apogon* (Perciformes: Apogonidae) through DNA Barcoding. *Zootaxa*, 36, 1–36.

Barlow, R., Lamont, T., Morris, T., Sessions, H., and van den Berg, M., 2014. Adaptation of phytoplankton communities to mesoscale eddies in the Mozambique Channel. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 106–118.

Batten, S.D. and Crawford, W.R., 2005. The influence of coastal origin eddies on oceanic plankton distributions in the eastern Gulf of Alaska. *Deep Sea Research Part II: Topical Studies in Oceanography*, 52, 991–1009.

Berry, P.F., 1974. Palinurid and Scyllarid lobster larvae of the Natal coast, South Africa. *South African Association for Marine Biological Research, Investigational Report*, 34, 1–44.

Berry, P.F., 1978. Reproduction, growth and production in the mussel, *Perna perna* (Linnaeus), on the east coast of South Africa. *South African Association for Marine Biological Research, Investigational Report*, 48, 1–28.

Biastoch, A. and Krauss, W., 1999. The role of mesoscale eddies in the source regions of the Agulhas Current. *Journal of Physical Oceanography*, 29, 2303–2317.

Blanco-Bercial, L., Cornils, A., Copley, N., and Bucklin, A., 2014. DNA barcoding of marine copepods: assessment of analytical approaches to species identification. *PLoS Currents*, 6, 1–24.

Booth, J.D. and Phillips, B.F., 1994. Early life history of spiny lobster. *Crustaceana*, 66, 271–294.

Booth, J.D., Webber, W.R., Sekiguchi, H., and Coutures, E., 2005. Diverse larval recruitment strategies within the Scyllaridae. *New Zealand Journal of Marine and Freshwater Research*, 39, 581–592.

Braby, L., 2014. Dynamics, interactions and ecosystem implications of mesoscale eddies formed in the southern region of Madagascar. MSc. Dissertation, University of Cape Town.

Brownell, C.L., 1979. Stages in the early development of 40 marine fish species with pelagic eggs from the Cape of Good Hope. *Ichthyological Bulletin of the J.L.B. Smith Institute of Icthyology*, 40, 1–84.

Bruce, A., 2004. A partial revision of the genus *Periclimenes* Costa, 1884 (Crustacea : Decapoda : Palaemonidae). *Zootaxa*, 26, 1–26.

Bryden, H.L.H., Beal, L.M.L., and Duncan, L.M.L., 2005. Structure and transport of the Agulhas Current and its temporal variability. *Journal of Oceanography*, 61, 479–492.

Bucklin, A. and Frost, B., 2009. Morphological and molecular phylogenetic analysis of evolutionary lineages within *Clausocalanus* (Copepoda: Calanoida). *Journal of Crustacean Biology*, 29, 111–120.

Bucklin, A., Frost, B., Bradford-Grieve, J., Allen, L., and Copley, N., 2003. Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. *Marine Biology*, 142, 333–343.

Bucklin, A., Hopcroft, R.R., Kosobokova, K.N., Nigro, L.M., Ortman, B.D., Jennings, R.M., and Sweetman, C.J., 2010. DNA barcoding of Arctic Ocean holozooplankton for species identification and recognition. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 57, 40–48.

Bucklin, A., Steinke, D., and Blanco-Bercial, L., 2011. DNA barcoding of marine metazoa. *Annual Review of Marine Science*, 3, 471–508.

Bucklin, A., Wiebe, P.H., Smolenack, S.B., Copley, N.J., Beaudet, J.G., Bonner, K.G., Färber-Lorda, J., and Pierson, J.J., 2007. DNA barcodes for species identification of euphausiids (Euphausiacea, Crustacea). *Journal of Plankton Research*, 29, 483–493.

Burgess, S.C., Kingsford, M.J., and Black, K.P., 2007. Influence of tidal eddies and wind on the distribution of presettlement fishes around One Tree Island, Great Barrier Reef. *Marine Ecology Progress Series*, 341, 233–242.

Cabezas, P., Macpherson, E., and Machordom, A., 2011. *Allogalathea* (Decapoda: Galatheidae): a monospecific genus of squat lobster? *Zoological Journal of the Linnean Society*, 162, 245–270.

Canino, M., Bailey, K., and Incze, L., 1991. Temporal and geographic differences in feeding and nutritional condition of walleye pollock larvae *Theragra chalcogramma* in Shelikof Strait, Gulf of Alaska. *Marine Ecology Progress Series*, 79, 27–35.

Caterino, M.S. and Tishechkin, A.K., 2006. DNA identification and morphological description of the first confirmed larvae of Hetaeriinae (Coleoptera: Histeridae). *Systematic Entomology*, 31, 405–418.

Chan, T.Y., 1998. Shrimps and prawns. *FAO species identification guide for fishery purposes. The Living Marine Resources of the Western Central Pacific*, 2, 851–791.

Chapman, P., Di Marco, S.F., Davis, R.E., and Coward, A.C., 2003. Flow at intermediate depths around Madagascar based on ALACE float trajectories. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 50, 1957–1986.

Charbonnier, D. and Crosnier, A., 1961. Quelques données sur la pêche des langoustes à Madagascar. *La Pêche Maritime*, 1–3.

Cheng, F., Wang, M., Sun, S., Li, C., and Zhang, Y., 2014. DNA barcoding of Antarctic marine zooplankton for species identification and recognition. *Advances in Polar Science*, 24, 119–127.

Collete, B.B. and Nauen, C.E., 1983. Scombridae of the world, an annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. Species Catalogue 2, FAO 139 pp.

Condie, S. and Condie, R., 2016. Retention of plankton within ocean eddies. *Global Ecology and Biogeography*, 1264–1277.

Conway, D., White, R., Huges-Dit-Ciles, J., Gallienne, C.P., and Robins, D., 2003. *Guide to the coastal and surface zooplankton of the south-western Indian Ocean.* Occasional Publication of the Marine Biological Association of the United Kingdom. Plymouth, UK.

Crochelet, E. and Lagabrielle, E., 2012. *Fish larvae dispersal in the Western Indian Ocean and implications for marine spatial planning.* [http://www.asclme.org (August 2013)].

da Silva, J.M., Creer, S., dos Santos, A., Costa, A.C., Cunha, M.R., Costa, F.O., and Carvalho, G.R., 2011. Systematic and evolutionary insights derived from mtDNA COI Barcode diversity in the Decapoda (crustacea: Malacostraca). *PLoS ONE*, 6, e19449. doi:10.1371/journal.pone.0019449.

de Ruijter, W.P.M., Van Aken, H.M., Beier, E.J., Lutjeharms, J.R.E., Matano, R.P., and Schouten, M.W., 2004. Eddies and dipoles around South Madagascar: Formation, pathways and large-scale impact. *Deep-Sea Research Part I: Oceanographic Research Papers*, 51, 383–400.

Diaz, D., Mari, M., Abello, P., and Demestre, M., 2002. Settlement and juvenile habitat of the European spiny lobster *Palinurus elephas* (Crustacea: Decapoda: Palinuridae) in the western Mediterranean Sea (Erratum). *Scientia Marina*, 66, 93–94.

Di Marco, S.F., Chapman, P., and Nowlin, W.D., 2000. Satellite observations of upwelling on the continental shelf south of Madagascar. *Geophysical Research Letters*, 27, 3965–3968.

Falkowski, P.G., Ziemann, D., Kolber, Z., Bienfang, P.K., Ziemann, D., and Bienfang, P.K., 1991. Role of eddy pumping in enhancing primary production in the ocean. *Nature*, 352, 55–58.

Felsenstein, J., 1985. Confidence limits on phylogenies : an approach using the bootstrap. *Evolution*, 39, 783–791.

Fernandes, V. and Ramaiah, N., 2009. Mesozooplankton community in the Bay of Bengal (India): spatial variability during the summer monsoon. *Aquatic Ecology*, 43, 951–963.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Fraser, T.H. and Allen, G.R., 2010. Cardinalfish of the genus *Apogonichthyoides* Smith, 1949 (Apogonidae) with a description of a new species from the West-Pacific region. *Zootaxa*, 1949, 40–56.

Garland, E.D. and Zimmer, C.A., 2002. Techniques for the identification of bivalve larvae. *Marine Ecology Progress Series*, 225, 299–310.

Godø, O.R., Samuelsen, A., Macaulay, G.J., Patel, R., Hjøllo, S.S., Horne, J., Kaartvedt, S., and Johannessen, J. a., 2012. Mesoscale eddies are oases for higher trophic marine life. *PLoS ONE*, 7, 1–9.

Goldthwait, S.A. and Steinberg, D.K., 2008. Elevated biomass of mesozooplankton and enhanced fecal pellet flux in cyclonic and mode-water eddies in the Sargasso Sea. *Deep Sea Research Part II*, 55, 1360–1377.

Gopal, K., Tolley, K.A., Groeneveld, J.C., and Matthee, C.A., 2006. Mitochondrial DNA variation in spiny lobster *Palinurus delagoae* suggests genetically structured populations in the southwestern Indian Ocean. *Marine Ecology Progress Series*, 319, 191–198.

Gordon, I., 1936. On the macruran genus *Rhynchocinetes*, with the description of a new species. *Proceedings of the Zoological Society of London*, 1936, 75–88.

Govoni, J.J., 2005. Fisheries oceanography and the ecology of early life histories of fishes: a perspective over fifty years. *Scientia Marina*, 69, 125–137.

Govoni, J.J., Hare, J.A., Davenport, E.D., Chen, M.H., and Marancik, K.E., 2010. Mesoscale, cyclonic eddies as larval fish habitat along the southeast United States shelf: A Lagrangian description of the zooplankton community. *ICES Journal of Marine Science*, 67, 403–411.

Graber, H.C. and Limouzy-Paris, C.B., 1997. Transport patterns of tropical reef fish larvae by spin-off eddies in the Straits of Florida. *Oceanography*, 10, 68–71.

Gründlingh, M.L., 1985. Occurrence of Red Sea water in the southwestern Indian Ocean, 1981. *Journal of physical oceanography*, 15, 207–212.

Gründlingh, M.L., 1987. Cyclogenesis in the mozambique ridge current. *Deep Sea Research Part A. Oceanographic Research Papers*, 34, 89–103.

Gründlingh, M.L., 1993. On the winter flow in the southern Mozambique Channel. *Deep Sea Research Part I: Oceanographic Research Papers*, 40, 409–418.

Gründlingh, M.L., 1995. Tracking eddies in the southeast Atlantic and southwest Indian oceans with TOPEX/POSEIDON. *Journal of Geophysical Research: Oceans (1978–2012)*, 100, 24977–24986.

Gurney, R., 1942. Larvae of Decapod Crustacea. *British Journal of Community Nursing*, 16, 305.

Halo, I., Backeberg, B., Penven, P., Ansorge, I., Reason, C., and Ullgren, J.E., 2014a. Eddy properties in the Mozambique Channel: A comparison between observations and two numerical ocean circulation models. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 38–53.

Halo, I., Penven, P., Backeberg, B., Ansorge, I., Shillington, F., and Roman, R., 2014b. Mesoscale eddy variability in the southern extension of the East Madagascar Current: Seasonal cycle, energy conversion terms, and eddy mean properties. *Journal of Geophysical Research: Oceans*, 119, 7324–7356.

Hamner, W.M.W. and Hauri, I.R., 1981. Effects of island mass: Water flow and plankton pattern around a reef in the Great Barrier Reef lagoon Australia. *Limnology and Oceanography*, 26, 1084–1102.

Hancke, L., Roberts, M.J., and Ternon, J.F., 2014. Surface drifter trajectories highlight flow pathways in the Mozambique Channel. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 27–37.

Hare, J.A., Churchill, J.H., Cowen, R.K., Berger, T.J., Cornillon, P.C., Dragos, P., Glenn, S.M., Govoni, J.J., and Lee, T.N., 2002. Routes and rates of larval fish transport from the southeast to the northeast United States continental shelf. *Limnology and Oceanography*, 47, 1774–1789.

Hare, M., Palumbi, S., and Butman, C., 2000. Single-step species identification of bivalve larvae using multiplex polymerase chain reaction. *Marine Biology*, 137, 953–961.

Harris, R., Wiebe, P., Lenz, J., Skoldal, H., and Huntley, M., eds., 2000. *ICES Zooplankton Methodology Manual*. New York: Academic Press.

Hebert, P., Penton, E., Burns, J., Janzen, D., and Hallwachs, W., 2004a. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 14812–14817.

Hebert, P.D., Stoeckle, M.Y., Zemlak, T.S., and Francis, C.M., 2004b. Identification of birds through DNA barcodes. *PLoS Biology*, 2, 1657–1663.

Hebert, P.D.N., Cywinska, A., Ball, S.L., and DeWaard, J.R., 2003a. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B,Biological Sciences*, 270, 313–321.

Hebert, P.D.N., Ratnasingham, S., and DeWaard, J.R., 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B, Biological Sciences*, 270, 96–99.

Heemstra, P.C. and Heemstra, E., 2004. *Coastal fishes of southern Africa*. Grahamstown, South Africa: NISC-SAIAB.

Heimeier, D., Lavery, S., and Sewell, M.A., 2010. Using DNA barcoding and phylogenetics to identify Antarctic invertebrate larvae: Lessons from a large scale study. *Marine genomics*, 3, 165–77.

Hermann, A.J., Hinckley, S., Megrey, B.A., and Stabeno, P.J., 1996. Interannual variability of the early life history of walleye pollock near Shelikof Strait as inferred from a spatially explicit, individual-based model. *Fisheries Oceanography*, 5, 39–57.

Hernandez-LeonTorres, S., Almeida, C., Gomez, M., Torres, S., Montero, I., and Portillo-Hahnefeld, A., 2001. Zooplankton biomass and indices of feeding and metabolism in island-generated eddies around Gran Canaria. *Journal of Marine Systems*, 20, 51–66.

Hicks, D.W. and Tunnell, J.W., 1995. Ecological notes and patterns of dispersal in the recently introduced mussel, *Perna perna* (Linneaus, 1758), in the Gulf of Mexico. *American Malacological Bulletin*, 11, 203–206.

Holliday, D., Beckley, L.E., and Olivar, M.P., 2011. Incorporation of larval fishes into a developing anti-cyclonic eddy of the Leeuwin Current off south-western Australia. *Journal of Plankton Research*, 33, 1696–1708.

Huggett, J.A., 2014. Mesoscale distribution and community composition of zooplankton in the Mozambique Channel. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 119–135.

Ivanova, N. V., Zemlak, T.S., Hanner, R.H., and Hebert, P.D.N., 2007. Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 7, 544–548.

Jackson, L.F., Smale, M.J., and Berry, P.F., 1991. Ghost Crabs of the Genus *Ocypode* (Decapoda, Brachyura, Ocypodidae) of the East Coast of South Africa. *Crustaceana*, 61, 280–286.

Jennings, R., Bucklin, A., Ossenbrügger, H., and Hopcroft, R., 2010a. Analysis of genetic diversity of planktonic gastropods from several ocean regions using DNA barcodes. *Deep-Sea Research II*, 57, 2199–2210.

Jennings, R., Bucklin, A., and Pierrot-Bults, A., 2010b. Barcoding of arrow worms (Phylum Chaetognatha) from three oceans: genetic diversity and evolution within an enigmatic phylum. *PLoS One*, 5, e9949. doi:10.1371/journal.pone.0009949.

Keane, J.P. and Neira, F.J., 2008. Larval fish assemblages along the south-eastern Australian shelf: Linking mesoscale non-depth-discriminate structure and water masses. *Fisheries Oceanography*, 17, 263–280.

Kendall, A.W., Schumacher, J.D., and Kim, S., 1996. Walleye pollock recruitment in Shelikof Strait: applied fisheries oceanography. *Fisheries Oceanography*, 5, 4–18.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.

Kinlan, B.P. and Gaines, S.D., 2003. Propagule dispersal in marine and terrestrial environments: A community perspective. *Ecology*, 84, 2007–2020.

Lalli, C.M. and Parson, T.R., 1993. *Biological Oceanograpghy: An Introduction*. Oxford: Butterworth-Heinemann.

Lamont, T., Barlow, R.G., Morris, T., and van den Berg, M.A., 2014. Characterisation of mesoscale features and phytoplankton variability in the Mozambique Channel. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 94–105.

Landry, M.R., Decima, M., Simmons, M.P., Hannides, C.C.S., and Daniels, E., 2008. Mesozooplankton biomass and grazing responses to Cyclone Opal, a subtropical mesoscale eddy. *Deep Sea Research Part II*, 55, 1378–1388.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., Mcgettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., and Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948.

Leal, M.C., Sá, C., Nordez, S., Brotas, V., and Paula, J., 2009. Distribution and vertical dynamics of planktonic communities at Sofala Bank, Mozambique. *Estuarine, Coastal and Shelf Science*, 84, 605–616.

Lebourges-Dhaussy, A., Huggett, J.A., Ockhuis, S., Roudaut, G., Josse, E., and Verheye, H., 2014. Zooplankton size and distribution within mesoscale structures in the Mozambique Channel: A comparative approach using the TAPS acoustic profiler, a multiple net sampler and ZooScan image analysis. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 136–152.

Ledoyer, M., 1982. Faune de Madagascar. *Crustacés Amphipodes Gammariens*, 598 pp.

Lee, T.T.N., Clarke, M.E., Williams, E., Szmant, A.A.F., and Berger, T., 1994. Evolution of the Tortugas Gyre and its influence on recruitment in the Florida Keys. *Bulletin of Marine Science*, 54, 621–646.

Leis, J.M., 1991. The pelagic phase of coral reef fishes: larval biology of coral reef fishes. *In*: P. Sale, ed. *The ecology of fishes on coral reefs*. San Diego: Academic Press, 183–230.

Leis, J.M. and Carson-Ewart, B.M., 1997. In situ swimming speeds of the late pelagic larvae of some Indo-Pacific coral-reef fishes. *Marine Ecology Progress Series*, 159, 165–174.

Limouzy-Paris, C.B., Graber, H.C., Jones, D.L., Röpke, A.W., and Richards, W.J., 1997. Translocation of larval coral reef fishes via sub-mesoscale spin-off eddies from the Florida current. *Bulletin of Marine Science*, 60, 966–983.

Logerwell, E.A. and Smith, P.E., 2001. Mesoscale eddies and survival of late stage Pacific sardine *(Sardinops sagax)* larvae. *Fisheries Oceanography*, 10, 13–25.

Lutjeharms, J.R.E., 1988a. Remote sensing corroboration of retroflection of the East Madagascar Current. *Deep Sea Research Part A. Oceanographic Research Papers*, 35, 2045–2050.

Lutjeharms, J.R.E., 1988b. On the role of the East Madagascar Current as a source of the Agulhas Current. *South African Journal of Science*, 84, 236–238.

Lutjeharms, J.R.E., 2006a. Three decades of research on the greater Agulhas Current. *Ocean Science Discussions*, 3, 939–995.

Lutjeharms, J.R.E., 2006b. The coastal ocean of south-eastern Africa. *In*: A.R. Robinson and K. Brink, eds. *The Sea*. Harvard University, Cambridge, MA, 783–834.

Lutjeharms, J.R.E., Bang, N.D., and Duncan, C.P., 1981. Characteristics of the currents east and south of Madagascar. *Deep Sea Research Part A, Oceanographic Research Papers*, 28, 879–899.

Lutjeharms, J.R.E. and Machu, E., 2000. An upwelling cell inshore of the East Madagascar Current. *Deep Sea Research Part I: Oceanographic Research Papers*, 47, 2405–2411.

Machida, R.J., Hashiguchi, Y., Nishida, M., and Nishida, S., 2009. Zooplankton diversity analysis through single-gene sequencing of a community sample. *BMC genomics*, 10, 438 doi:10.1186/1471-2164-10-438.

Machu, E., Lutjeharms, J.R.E., Webb, A.M., and Van Aken, H.M., 2002. First hydrographic evidence of the southeast Madagascar upwelling cell. *Geophysical Research Letters*, 29, 1–5.

Mackas, D.L. and Galbraith, M.D., 2002. Zooplankton distribution and dynamics in a North Pacific Eddy of coastal origin: I. Transport and Loss of continental margin species. *Journal of Oceanography*, 58, 725–738.

Mackas, D.L., Tsurumi, M., Galbraith, M.D., and Yelland, D.R., 2005. Zooplankton distribution and dynamics in a North Pacific Eddy of coastal origin: II. Mechanisms of eddy colonization by and retention of offshore species. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 52, 1011–1035.

MacNae, W., 1969. A general account of the fauna and flora of mangrove swamps and forests in the Indo-West-Pacific region. *Advances in Marine Biology*, 6, 73–270.

Malauene, B.S., Shillington, F.A., Roberts, M.J., and Moloney, C.L., 2014. Cool, elevated chlorophyll-a waters off northern Mozambique. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 68–78.

Marsac, F., Barlow, R., Ternon, J.F., Ménard, F., and Roberts, M., 2014. Ecosystem functioning in the Mozambique Channel: Synthesis and future research. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 212–220.

McGillicuddy, D.J. and Robinson, A.R., 1997. Eddy-induced nutrient supply and new production in the Sargasso Sea. *Deep Sea Research Part I: Oceanographic Research Papers*, 44, 1427–1450.

McGillicuddy, D.J., Robinson, A.R., Siegel, D.A., Jannasch, H.W., Johnson, R., Dickey, T.D., McNeil, J., Michaels, A.F., and Knap, A.H., 1998. Influence of mesoscale eddies on new production in the Sargasso Sea. *Nature*, 394, 263–266.

McLoughlin, S., 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany*, 49, 271–300.

Ménard, F., Benivary, H.D., Bodin, N., Coffineau, N., Le Loc'h, F., Mison, T., Richard, P., and Potier, M., 2014. Stable isotope patterns in micronekton from the Mozambique Channel. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 153–163.

Meyer, C.P., 2003. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society*, 79, 401–459.

Morris, T., Lamon, T., and Roberts, M., 2013. Effects of deep-sea eddies on the northern KwaZulu-Natal shelf, South Africa. *343-350*, 35, 343–350.

Morrow, R., Birol, F., Griffin, D., and Sudre, J., 2004. Divergent pathways of cylonic and anti-cyclonic ocean eddies. *Geophysical Research Letters*, 31, 1–5.

Muhling, B.A., Beckley, L.E., and Olivar, M.P., 2007. Ichthyoplankton assemblage structure in two meso-scale Leeuwin Current eddies, eastern Indian Ocean. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 54, 1113–1128.

Mullaney, T.J. and Suthers, I.M., 2013. Entrainment and retention of the coastal larval fish assemblage by a short-lived, submesoscale, frontal eddy of the East Australian Current. *Limnology and Oceanography*, 58, 1546–1556.

Muths, D., Gouws, G., and Mwale, M., 2012. Genetic connectivity of the reef fish *Lutjanus kasmira* at the scale of the western Indian Ocean. *Canadian Journal of Fisheries and Aquatic Sciences*, 69, 842–853.

Muths, D., Tessier, E., and Bourjea, J., 2015. Genetic structure of the reef grouper *Epinephelus merra* in the West Indian Ocean appears congruent with biogeographic and oceanographic boundaries. *Marine Ecology*, 36, 447–461.

Nakata, H., Kimura, S., Okazaki, Y., and Kasai, A., 2000. Implications of meso-scale eddies caused by frontal disturbances of the Kuroshio Current for anchovy recruitment. *Journal of Marine Science*, 57, 143–152.

Nauw, J.J., van Aken, H.M., Webb, A., Lutjeharms, J.R.E., and de Ruijter, W.P.M., 2008. Observations of the southern East Madagascar Current and undercurrent and countercurrent system. *Journal of Geophysical Research: Oceans*, 113, 1–15.

Nehring, D., Hagen, E., da Silva, A.J., Schemainda, R., Wolf, G., Mechelchen, N., Kaiser, W., Postel, L., Gosselck, F., and Brenning, U., 1987. Results of oceanological studies in the Mozambique Channel in February-March 1980. *Beiträge zur Meereskunde*, 56, 51–63.

Nurdiani, R. and Zeng, C., 2007. Effects of temperature and salinity on the survival and development of mud crab, *Scylla serrata* (Forsskål), larvae. *Aquaculture Research*, 38, 1529–1538.

Olivar, M. and Beckley, L., 1994. Influence of the Agulhas Current on the distribution of lanternfish larvae off the southeast coast of Africa. *Journal of Plankton Research*, 16, 1759–1780.

Ortman, B., 2008. DNA barcoding the medusozoa and ctenophora. Ph.D. Dissertation, University of Connecticut.

Ortman, B., Bucklin, A., Pages, F., and Youngbluth, M., 2010. DNA barcoding the Medusozoa using mtCOI. *Deep Sea Research Part II*, 57, 2148–2156.

Palastanga, V., van Leeuwen, P.J., Schouten, M.W., and de Ruijter, W.P.M., 2007. Flow structure and variability in the subtropical Indian Ocean: Instability of the South Indian Ocean countercurrent. *Journal of Geophysical Research: Oceans*, 112, 1–11.

Palero, F. and Abello, P., 2007. The first phyllosoma stage of *Palinurus mauritanicus* (Crustacea: Decapoda: Palinuridae). *Zootaxa*, 1508, 49–59.

Palumbi, S.R., 1996. Nucleic acids II: the polymerase chain reaction. *Molecular systematics*, 2, 205–247.

Pardo, L.M., Ampuero, D., and Véliz, D., 2009. Using morphological and molecular tools to identify megalopae larvae collected in the field: the case of sympatric *Cancer* crabs. *Journal of the Marine Biological Association of the United Kingdom*, 89, 481–490.

Paxton, J.R., Hoese, D.F., Allen, G.R., and Hanley, J.E., 1989. Zoological catalogue of Australia. Vol. 7. Pisces. Petromyzontidae to Carangidae. Australian Government Publishing Service, Cranberra.

Pegg, G.G., Sinclair, B., Briskey, L., and Aspden, W.J., 2006. MtDNA barcode identification of fish larvae in the southern Great Barrier Reef , Australia. *Scientia Marina*, 70S2, 7–12.

Penven, P., Lutjeharms, J.R.E., and Florenchie, P., 2006. Madagascar: A pacemaker for the Agulhas Current system? *Geophysical Research Letters*, 33, L17609, doi:10.1029/2006GL026854.

Petrocci, C. and Lipton, D., 1994. The Warmwater Crab Fishery in Asia: Implications for the Chesapeake Bay Blue Crab Industry. *Virginia Sea Grant Marine Resource Advisory*, 49, 45.

Potier, M., Bach, P., Ménard, F., and Marsac, F., 2014. Influence of mesoscale features on micronekton and large pelagic fish communities in the Mozambique Channel. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 184–199.

Pripp, T., Gammelsrd, T., and Krakstad, J.O., 2014. Physical influence on biological production along the western shelf of Madagascar. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 174–183.

Ptacek, M.B., Sarver, S.K., Childress, M.J., and Herrnkind, W.F., 2001. Molecular phylogeny of the spiny lobster genus *panulirus* (Decapoda: Palinuridae). *Marine and Freshwater Research*, 52, 1037–1047.

Quartly, G.D., Buck, J.J.H., Srokosz, M. A., and Coward, A. C., 2006b. Eddies around Madagascar - The retroflection re-considered. *Journal of Marine Systems*, 63, 115–129.

Quartly, G.D. and Srokosz, M. A., 2004. Eddies in the southern Mozambique Channel. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 51, 69–83.

Randall, J.E., 2011. Two New Serranid Fishes of the Genus *Pseudanthias* from the Western Indian Ocean. *Smithiana Bulletin*, 13, 79–87.

Randall, J.E., Allen, G.R., and Steene, R.C., 1997. *Fishes of the Great Barrier reef and Coral Sea*. Honolulu, HI: University of Hawaii Press.

Randall, J.E. and King, D.R., 2009. *Parupeneus fraserorum*, a new species of goatfish (Perciformes: Mullidae) from South Africa and Madagascar. *Smithiana Bulletin*, 10, 31–35.

Rao, T., 1973. Zooplankton Studies in the Indian Ocean. *In:* Zeitschel, B. (Ed.). *The Biology of the Indian Ocean.* Springer Berlin Heidelberg.

Ratnasingham, S. and Hebert, P.D.., 2007. Barcoding BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes*, 7, 355–364.

Reddy, M., Macdonald, A., and Groeneveld, J.C., 2014. Phylogeography of the scalloped spiny-lobster *Panulirus homarus* rubellus in the Southwest Indian Ocean. *Journal of Crustacean Biology*, 36, 773–781.

Rhines, P.B., 2001. Mesoscale Eddies. *In*: J. Steele, K. Turekian, and S. Thorpe, eds. *Encyclopedia of Ocean Sciences*. London, UK: Academic Press, 1717–1729.

Richards, W.J., 2005. *Early stages of Atlantic fishes: an identification guide for the western central north Atlantic.* Taylor & Francis, Boca Raton, Florida.

Ridderinkhof, H., Van Der Werf, P.M., Ullgren, J.E., Van Aken, H.M., Van Leeuwen, P.J., and de Ruijter, W.P.M., 2010. Seasonal and interannual variability in the Mozambique Channel from moored current observations. *Journal of Geophysical Research: Oceans*, 115, C06010, doi:10.1029/2009JC005619.

Ridderinkhof, W., Le Bars, D., Heydt, A.S., and de Ruijter, W.P.M., 2013. Dipoles of the South East Madagascar Current. *Geophysical Research Letters*, 40, 558–562.

Riginos, C., Douglas, K.E., Jin, Y., Shanahan, D.F., and Treml, E. a., 2011. Effects of geography and life history traits on genetic differentiation in benthic marine fishes. *Ecography*, 34, 566–575.

Riginos, C. and Victor, B.C., 2001. Larval spatial distributions and other early life-history characteristics predict genetic differentiation in eastern Pacific blennioid fishes. *Proceedings of the Royal Society B, Biological Sciences*, 268, 1931–1936.

Robinson, A.R., 1983. Eddies in marine science. *In*: *Eddies in marine science*. New York: Springer-Verlag, 609.

Rodrıguez, J.M., Hernández-León, S., and Barton, E.D., 1999. Mesoscale distribution of fish larvae in relation to an upwelling filament off Northwest Africa. *Deep Sea Research Part I: Oceanographic Research Papers*, 46 (11), 1969–1984.

Sabarros, P.S., Ménard, F., Lévénez, J.J., Tew-Kai, E., and Ternon, J.F., 2009. Mesoscale eddies influence distribution and aggregation patterns of micronekton in the Mozambique Channel. *Marine Ecology Progress Series*, 395, 101–107.

Saetre, R. and de Paula e Silva, R., 1979. The marine fish resources of Mozambique. <www.fao.org/WAIRDOCS/FNS/FN130E>.

Saitou, N and Nei, M., 1987. The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees'. *Molecular Biology and Evolution*, 4, 406–425.

Sale, P.F., 1970. Distribution of larval Acanthuridae off Hawaii. *Copeia*, 1970, 765–766.

Sanchez-Velasco, L., Lavin, M.F., Jimenez-Rosenberg, S.P.A., Godinez, V.M., Santamaria-del-Angel, E., and Hernandez-Becerril, D.U., 2013. Three-dimensional distribution of fish larvae in a cyclonic eddy in the Gulf of California during the summer. *Deep-Sea Research Part I: Oceanographic Research Papers*, 75, 39–51.

Savolainen, V., Cowan, R.S., Vogler, A.P., Roderick, G.K., and Lane, R., 2005. Towards writing the encyclopedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 360, 1805–1811.

Schouten, M.W., de Ruijter, W.P.., van Leeuwen, P.J., and Ridderinkhof, H., 2003. Eddies and variability in the Mozambique Channel. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 50, 1987–2003.

Schouten, M.W.W., de Ruijter, W.P.M., and van Leeuwen, P.J., 2002. Upstream control of Agulhas Ring shedding. *Journal of Geophysical Research: Oceans (1978–2012)*, 107, 21–23.

Shanks, A.L., 2009. Pelagic larval duration and dispersal distance revisited. *Biological Bulletin*, 216, 373–385.

Shanks, A.L., Grantham, B.A., and Carr, M.H., 2003. Propagule dispersal distance and the size and spacing of Marine Reserves. *Ecological Applications*, 13, S159–S169.

Shields, J., 1992. Parasites and Symbionts of the crab *Portunus pelagicus* from Moreton Bay, eastern Australia. *Journal of Crustacean Biology*, 12, 94–100.

Shulman, M.J.M. and Bermingham, E., 1995. Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. *Evolution*, 49, 897–910.

Siedler, G., Rouault, M., Biastoch, A., Backeberg, B., Reason, C.J.C., and Lutjeharms, J.R.E., 2009. Modes of the southern extension of the East Madagascar current. *Journal of Geophysical Research: Oceans*, 114, 1–15.

Siedler, G., Rouault, M., and Lutjeharms, J.R.E., 2006. Structure and origin of the subtropical South Indian Ocean Countercurrent. *Geophysical Research Letters*, 33, 1–5.

Starnes, W.C., 1988. Revision, phylogeny and biogeographic comments on the circumtropical marine percoid fish family Priacanthidae. *Bulletin of Marine Science*, 43, 117–203.

Stobutzki, I.C. and Bellwood, D.R., 1994. An analysis of the sustained swimming abilities of presettlement and postsettlement coral-reef fishes. *Journal of Experimental Marine Biology and Ecology*, 175, 275–286.

Stobutzki, I.C. and Bellwood, D.R., 1997. Sustained swimming abilities of the late pelagic stages of coral reef fishes. *Marine Ecology Progress Series*, 149, 35–41.

Stoeckle, M., 2003. Taxonomy, DNA, and the Bar Code of Life. *BioScience*, 53, 796.

Stramma, L. and Lutjeharms, J.R.E., 1997. The flow field of the subtropical gyre of the South Indian Ocean. *Journal of Geophysical Research: Oceans*, 102, 5513–5530.

Sunnucks, P. and Hales, D.F., 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, 13, 510–524.

Swallow, J., Fieux, M., and Schott, F., 1988. The boundary currents east and north of Madagascar: 1. Geostrophic currents and transports. *Journal of Geophysical Research: Oceans (1978–2012)*, 93, 4951–4962.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S., 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.

Ternon, J.F., Bach, P., Barlow, R., Huggett, J., Jaquemet, S., Marsac, F., Ménard, F., Penven, P., Potier, M., and Roberts, M.J., 2014a. The Mozambique Channel: From physics to upper trophic levels. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 1–9.

Ternon, J.F., Roberts, M.J., Morris, T., Hancke, L., and Backeberg, B., 2014b. In situ measured current structures of the eddy field in the Mozambique Channel. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 100, 10–26.

Teske, P., Von Der Heyden, S., McQuaid, C.D., and Barker, N.P., 2011. A review of marine phylogeography in southern Africa Coastal phylogeography, 107, 1–11.

Teske, P.R., Papadopoulos, I., Zardi, G.I., McQuaid, C.D., Edkins, M.T., Griffiths, C.L., and Barker, N.P., 2007. Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: Planktonic, abbreviated and direct development. *Marine Biology*, 152 (3), 697–711.

Tew-Kai, E. and Marsac, F., 2009. Patterns of variability of sea surface chlorophyll in the Mozambique Channel: A quantitative approach. *Journal of Marine Systems*, 77 (1–2), 77–88.

Valdez-Moreno, M., Vasquez-Yeomans, L., Elias-Gutierrez, M., Ivanova, N., and Hebert, P., 2010. Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management. *Marine and Freshwater Research*, 61, 665–671.

Vazquez-Bader, A.R., Carrero, J.C., Garcia-Varela, M., Gracia, A., and Laclette, J.P., 2004. Molecular phylogeny of superfamily Penaeoidea Rafinesque-Schmaltz, 1815, based on mitochondrial 16S partial sequence analysis. *Journal of Shellfish Research*, 23, 911–918.

Visram, S., Yang, M.C., Pillay, R.M., Said, S., Henriksson, O., Grahn, M., and Chen, C.A., 2010. Genetic connectivity and historical demography of the blue barred parrotfish *(Scarus ghobban)* in the western Indian Ocean. *Marine Biology*, 157, 1475–1487.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., and Hebert, P.D.N., 2005. DNA barcoding Australia's fish species. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 360, 1847–1857.

Webb, K.E., Barnes, D.K.A.A., Clark, M.S., and Bowden, D.A., 2006. DNA barcoding: a molecular tool to identify Antarctic marine larvae. *Deep Sea Research Part II: Topical Studies in Oceanography*, 53, 1053–1060.

Weersing, K. and Toonen, R.J., 2009. Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series*, 393, 1–12.

Weimerskirch, H., Le Corre, M., Jaquemet, S., Potier, M., and Marsac, F., 2004. Foraging strategy of a top predator in tropical waters: Great frigatebirds in the Mozambique Channel. *Marine Ecology Progress Series*, 275, 297–308.

Werle, E., Schneider, C., Renner, M., Volker, M., and Fiehn, W., 1994. Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic Acids Research*, 22, 4354–4355.

Werner, F.E. and Quinlan, J. a, 2002. Fluctuations in marine fishes populations: physical processess and numerical modelling. *ICES Journal of Marine Science*, 215, 264–278.

Westneat, M.W. and Alfaro, M.E., 2005. Phylogenetic relationships and evolutionary history of the reef fish family Labridae. *Molecular Phylogenetics and Evolution*, 36, 370–390.

Yentsch, C.S. and Phinney, D.A., 1985. Rotary motions and convection as a means of regulating primary production in warm core rings. *Journal of Geophysical Research: Oceans (1978–2012)*, 90, 3237–3248.

Yeung, C., Jones, D.L., Criales, M.M., Jackson, T.L., and Richards, W.J., 2001. Influence of coastal eddies and counter-currents on the influx of spiny lobster, *Panulirus argus*, postlarvae into Florida Bay. *Marine and freshwater research*, 52, 1217–1232.

Appendix A 1 Kimura 2-parameter distances among unknown and downloaded sequences, where identifications to genus level were determined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
1) *Scomber colias*																																															
2) *Scomber colias*	0.000																																														
3) *F16 (Scomber)*	0.000	0.000																																													
4) *F34 (Taaningichthys)*	0.246	0.246	0.246																																												
5) *Taaningichthys minimus*	0.246	0.246	0.246	0.020																																											
6) *Taaningichthys minimus*	0.260	0.260	0.260	0.060	0.058																																										
7) *Taaningichthys minimus*	0.274	0.274	0.274	0.224	0.212	0.218																																									
8) *F17 (Symbolophorus)*	0.244	0.244	0.244	0.244	0.235	0.252	0.266																																								
9) *Symbolophorus rufinus*	0.222	0.222	0.222	0.220	0.211	0.227	0.247	0.032																																							
10) *Oplegnathus robinsoni*	0.233	0.233	0.233	0.256	0.260	0.256	0.292	0.250	0.241																																						
11) *Oplegnathus robinsoni*	0.233	0.233	0.233	0.256	0.260	0.256	0.292	0.250	0.241	0.000																																					
12) *F2 (Oplegnathus)*	0.230	0.230	0.230	0.253	0.257	0.259	0.296	0.253	0.244	0.002	0.002																																				
13) *Epinephelus rivulatus*	0.293	0.293	0.293	0.257	0.261	0.251	0.303	0.252	0.252	0.217	0.217	0.220																																			
14) *Epinephelus rivulatus*	0.293	0.293	0.293	0.257	0.261	0.251	0.303	0.252	0.252	0.217	0.217	0.220	0.000																																		
15) *F13 (Epinephelus)*	0.293	0.293	0.293	0.257	0.261	0.251	0.303	0.252	0.252	0.217	0.217	0.220	0.002	0.002																																	
16) *Pseudanthias connelli*	0.251	0.251	0.251	0.271	0.265	0.264	0.273	0.264	0.261	0.256	0.256	0.259	0.227	0.227	0.227																																
17) *Pseudanthias connelli*	0.251	0.251	0.251	0.271	0.265	0.264	0.273	0.264	0.261	0.256	0.256	0.259	0.227	0.227	0.227	0.000																															
18) *F25 (Pseudanthias)*	0.255	0.255	0.255	0.268	0.261	0.261	0.270	0.267	0.264	0.259	0.259	0.263	0.233	0.233	0.233	0.004	0.004																														
19) *F21 (Pyramodon)*	0.260	0.260	0.260	0.240	0.228	0.256	0.255	0.241	0.238	0.247	0.247	0.250	0.239	0.239	0.242	0.198	0.198	0.201																													
20) *Pyramodon sp.*	0.266	0.266	0.266	0.237	0.224	0.253	0.262	0.247	0.244	0.246	0.246	0.250	0.245	0.245	0.248	0.201	0.201	0.198	0.016																												
21) *Callionymus marleyi*	0.293	0.293	0.293	0.249	0.243	0.249	0.307	0.335	0.313	0.266	0.266	0.269	0.246	0.246	0.246	0.268	0.268	0.271	0.246	0.243																											
22) *F26 (Callionymus)*	0.300	0.300	0.300	0.252	0.246	0.246	0.310	0.335	0.320	0.269	0.269	0.272	0.243	0.243	0.243	0.264	0.264	0.268	0.253	0.249	0.009																										
23) *Lotella sp.*	0.290	0.290	0.290	0.284	0.281	0.291	0.308	0.300	0.300	0.265	0.265	0.268	0.224	0.224	0.224	0.282	0.282	0.286	0.248	0.251	0.261	0.268																									
24) *Lotella sp.*	0.290	0.290	0.290	0.284	0.281	0.291	0.308	0.300	0.300	0.265	0.265	0.268	0.224	0.224	0.224	0.282	0.282	0.286	0.248	0.251	0.261	0.268	0.000																								
25) *F27 (Lotella)*	0.295	0.295	0.295	0.287	0.281	0.301	0.305	0.298	0.288	0.256	0.256	0.252	0.250	0.250	0.250	0.283	0.283	0.286	0.281	0.278	0.271	0.271	0.098	0.098																							
26) *Chromis chrysura*	0.277	0.277	0.277	0.257	0.247	0.247	0.287	0.247	0.235	0.219	0.219	0.222	0.239	0.239	0.239	0.240	0.240	0.247	0.222	0.231	0.269	0.276	0.263	0.263	0.250																						
27) *Chromis chrysura*	0.277	0.277	0.277	0.257	0.247	0.247	0.287	0.247	0.235	0.219	0.219	0.222	0.239	0.239	0.239	0.240	0.240	0.247	0.222	0.231	0.269	0.276	0.263	0.263	0.250	0.000																					
28) *F24 (Chromis)*	0.264	0.264	0.264	0.263	0.260	0.263	0.298	0.262	0.260	0.213	0.213	0.216	0.249	0.249	0.249	0.235	0.235	0.241	0.216	0.226	0.287	0.273	0.260	0.260	0.254	0.082	0.082																				
29) *Gonostoma elongatum*	0.264	0.264	0.264	0.281	0.282	0.289	0.315	0.261	0.248	0.260	0.260	0.257	0.287	0.287	0.287	0.259	0.259	0.263	0.274	0.278	0.299	0.299	0.272	0.272	0.273	0.243	0.243	0.256																			
30) *Gonostoma elongatum*	0.264	0.264	0.264	0.281	0.282	0.289	0.315	0.261	0.248	0.260	0.260	0.257	0.287	0.287	0.287	0.259	0.259	0.263	0.274	0.278	0.299	0.299	0.272	0.272	0.273	0.243	0.243	0.256	0.000																		
31) *F39 (Gonostoma)*	0.264	0.264	0.264	0.281	0.282	0.289	0.315	0.261	0.248	0.260	0.260	0.257	0.287	0.287	0.287	0.259	0.259	0.263	0.274	0.278	0.299	0.299	0.272	0.272	0.273	0.243	0.243	0.256	0.000	0.000																	
32) *Polysteganus coeruleopunctatus*	0.235	0.235	0.235	0.242	0.239	0.249	0.245	0.235	0.232	0.245	0.245	0.249	0.256	0.256	0.253	0.237	0.237	0.234	0.231	0.228	0.292	0.303	0.269	0.269	0.265	0.210	0.210	0.228	0.257	0.257	0.257																
33) *Polysteganus coeruleopunctatus*	0.241	0.241	0.241	0.236	0.233	0.243	0.242	0.235	0.232	0.245	0.245	0.248	0.259	0.259	0.256	0.237	0.237	0.234	0.237	0.234	0.288	0.299	0.276	0.276	0.284	0.207	0.207	0.224	0.256	0.256	0.256	0.004															
34) *Polysteganus coeruleopunctatus*	0.244	0.244	0.244	0.239	0.236	0.246	0.242	0.235	0.232	0.245	0.245	0.248	0.259	0.259	0.256	0.237	0.237	0.234	0.237	0.234	0.292	0.302	0.276	0.276	0.284	0.210	0.210	0.228	0.256	0.256	0.256	0.007	0.002														
35) *Polysteganus coeruleopunctatus*	0.241	0.241	0.241	0.236	0.233	0.243	0.242	0.235	0.232	0.245	0.245	0.248	0.259	0.259	0.256	0.237	0.237	0.234	0.237	0.234	0.292	0.299	0.276	0.276	0.284	0.207	0.207	0.224	0.256	0.256	0.256	0.004	0.000	0.002												
36) *Polysteganus coeruleopunctatus*	0.241	0.241	0.241	0.233	0.230	0.240	0.239	0.235	0.232	0.245	0.245	0.249	0.256	0.256	0.253	0.237	0.237	0.234	0.235	0.231	0.285	0.295	0.279	0.279	0.288	0.207	0.207	0.224	0.259	0.259	0.259	0.007	0.002	0.004	0.002											
37) *Polysteganus baissaci*	0.228	0.228	0.228	0.227	0.224	0.228	0.227	0.245	0.236	0.235	0.235	0.238	0.252	0.252	0.249	0.215	0.215	0.218	0.222	0.218	0.272	0.282	0.275	0.275	0.288	0.195	0.195	0.215	0.233	0.233	0.233	0.078	0.073	0.075	0.073	0.075										
38) *F38 (Polysteganus)*	0.250	0.250	0.250	0.253	0.250	0.244	0.252	0.249	0.242	0.239	0.239	0.235	0.259	0.259	0.256	0.234	0.234	0.230	0.241	0.231	0.295	0.299	0.286	0.286	0.278	0.210	0.210	0.212	0.227	0.227	0.227	0.088	0.083	0.086	0.083	0.085	0.060									
39) *F19 (Nealotus)*	0.259	0.259	0.259	0.273	0.270	0.286	0.305	0.273	0.260	0.235	0.235	0.232	0.244	0.244	0.244	0.247	0.247	0.244	0.232	0.222	0.260	0.267	0.262	0.262	0.265	0.278	0.278	0.253	0.236	0.236	0.236	0.238	0.238	0.238	0.238	0.235	0.204	0.192								
40) *Nealotus*	0.239	0.239	0.239	0.260	0.251	0.260	0.267	0.259	0.247	0.220	0.220	0.223	0.254	0.254	0.254	0.222	0.222	0.219	0.213	0.204	0.270	0.270	0.242	0.242	0.259	0.261	0.261	0.230	0.248	0.248	0.248	0.222	0.222	0.222	0.222	0.219	0.195	0.200	0.043							
41) *F20 (Halichoeres)*	0.287	0.287	0.287	0.237	0.240	0.253	0.276	0.261	0.254	0.239	0.239	0.236	0.262	0.262	0.266	0.244	0.244	0.244	0.243	0.243	0.281	0.281	0.292	0.292	0.286	0.254	0.254	0.232	0.270	0.270	0.270	0.207	0.207	0.207	0.207	0.204	0.201	0.219	0.222	0.225							
42) *Halichoeres zeylonicus*	0.287	0.287	0.287	0.231	0.234	0.246	0.273	0.257	0.251	0.239	0.239	0.236	0.256	0.256	0.259	0.247	0.247	0.247	0.240	0.240	0.275	0.275	0.289	0.289	0.283	0.248	0.248	0.226	0.266	0.266	0.266	0.204	0.204	0.207	0.204	0.201	0.219	0.222	0.225	0.210	0.004						
43) *F35 (Vinciguerria)*	0.317	0.317	0.317	0.279	0.279	0.263	0.280	0.276	0.278	0.269	0.269	0.272	0.269	0.269	0.269	0.266	0.266	0.269	0.242	0.246	0.271	0.278	0.253	0.253	0.271	0.235	0.235	0.255	0.320	0.320	0.320	0.246	0.252	0.252	0.252	0.253	0.268	0.269	0.250	0.259	0.233	0.233					
44) *Vinciguerria nimbaria*	0.310	0.310	0.310	0.272	0.273	0.270	0.270	0.278	0.278	0.268	0.268	0.271	0.279	0.279	0.279	0.249	0.249	0.252	0.239	0.236	0.263	0.263	0.270	0.270	0.271	0.264	0.264	0.282	0.306	0.306	0.306	0.263	0.269	0.269	0.269	0.269	0.269	0.253	0.262	0.248	0.245	0.245	0.067				
45) *Priacanthus aff. arenatus*	0.256	0.256	0.256	0.288	0.288	0.288	0.305	0.278	0.258	0.251	0.251	0.248	0.260	0.260	0.263	0.283	0.283	0.283	0.293	0.279	0.283	0.286	0.286	0.286	0.272	0.272	0.272	0.276	0.249	0.249	0.249	0.247	0.247	0.243	0.247	0.244	0.266	0.260	0.264	0.291	0.260	0.257	0.257	0.271			
46) *Priacanthus aff. arenatus*	0.256	0.256	0.256	0.288	0.288	0.288	0.305	0.278	0.258	0.251	0.251	0.248	0.260	0.260	0.263	0.283	0.283	0.283	0.293	0.279	0.283	0.286	0.286	0.286	0.272	0.272	0.272	0.276	0.249	0.249	0.249	0.247	0.247	0.243	0.247	0.244	0.266	0.260	0.264	0.291	0.260	0.257	0.257	0.271	0.000		
47) *F12 (Priacanthus)*	0.256	0.256	0.256	0.288	0.288	0.288	0.305	0.278	0.258	0.251	0.251	0.248	0.260	0.260	0.263	0.283	0.283	0.283	0.293	0.279	0.283	0.286	0.286	0.286	0.272	0.272	0.272	0.276	0.249	0.249	0.249	0.247	0.247	0.243	0.247	0.244	0.266	0.260	0.264	0.291	0.260	0.257	0.257	0.271	0.000	0.000	

Appendix A 2 Kimura 2-parameter distances among unknown and downloaded sequences, where identification to family level were determined.

	1	2	3	4	5	6	7	8	9	10
1) *Arnoglossus polyspilus*										
2) *Arnoglossus polyspilus*	0.004									
3) F4 (Bothidae)	0.172	0.172								
4) *Ammodytoides* sp.	0.266	0.260	0.244							
5) F37 (Ammodytidae)	0.262	0.262	0.271	0.140						
6) *Diogenichthys laternatus*	0.276	0.276	0.300	0.277	0.225					
7) *Diogenichthys laternatus*	0.276	0.276	0.304	0.277	0.225	0.002				
8) F18 (Myctophidae)	0.263	0.269	0.267	0.253	0.206	0.107	0.104			
9) *Cyclothone microdon*	0.285	0.285	0.260	0.299	0.299	0.288	0.288	0.284		
10) F36 (Gonostomatidae)	0.281	0.288	0.264	0.319	0.337	0.304	0.304	0.275	0.114	