Edith Cowan University

Research Online

Theses: Doctorates and Masters

Theses

2014

A preliminary investigation of the potential effects of the invasive Mozambique tilapia Oreochromis mossambicus on the native fish assemblages of Lake MacLeod, Western Australia

Hannah Cameron-Caluori
Edith Cowan University

Follow this and additional works at: https://ro.ecu.edu.au/theses

Part of the Environmental Indicators and Impact Assessment Commons, and the Environmental Monitoring Commons

Recommended Citation

Cameron-Caluori, H. (2014). *A preliminary investigation of the potential effects of the invasive Mozambique tilapia Oreochromis mossambicus on the native fish assemblages of Lake MacLeod, Western Australia*. https://ro.ecu.edu.au/theses/1568

This Thesis is posted at Research Online.
https://ro.ecu.edu.au/theses/1568

Edith Cowan University

Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study.

The University does not authorize you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following:

- Copyright owners are entitled to take legal action against persons who infringe their copyright.

- A reproduction of material that is protected by copyright may be a copyright infringement. Where the reproduction of such material is done without attribution of authorship, with false attribution of authorship or the authorship is treated in a derogatory manner, this may be a breach of the author's moral rights contained in Part IX of the Copyright Act 1968 (Cth).

- Courts have the power to impose a wide range of civil and criminal sanctions for infringement of copyright, infringement of moral rights and other offences under the Copyright Act 1968 (Cth). Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.

A preliminary investigation of the potential effects of the invasive Mozambique tilapia *Oreochromis mossambicus* on the native fish assemblages of Lake MacLeod, Western Australia

Thesis submitted in partial fulfillment of Master of Science (Biological Sciences)

July 2014

Hannah Cameron-Caluori

Supervisors: A. Prof. Glenn Hyndes and Prof. Pierre Horwitz

School of Natural Sciences
Edith Cowan University
270 Joondalup Drive,
Joondalup WA 6027

Abstract

As one of the major threats to biodiversity in aquatic systems, invasive species can alter the structure and function of a community, often through habitat and resource competition, and/or direct predation. This study aims to determine if invasive tilapia (*Oreochromis mossambicus*) is likely to have an effect on the native fish communities of Lake MacLeod, a unique and important inland, saline lake system in north-western Western Australia, through competition for key resources, namely habitat and food sources. Seven study sites were selected within the Northern Ponds of Lake MacLeod, from which fish abundances were assessed in three habitats (pneumatophore beds, nearshore and vents) using adapted fyke-nets and unbaited underwater videography. From three of the seven sites, dietary and stable isotope analyses were conducted.

The species richness of fish was low, with between 3-4 species caught at each site. *Amniataba caudavittata (*yellowtail grunters) and *Craterocephalus pauciradiatus* (hardyheads), the two most prevalent native fish species, were captured in all three habitats. Although observed in every habitat, *O. mossambicus* was only successfully captured from the vent habitats. The total length of *O. mossambicus* ranged from 48-385 mm but the cohort at one of the vents, 'Jack's vent', was considerably larger and more abundant than at all other sites. *C. pauciradiatus* remained consistent in its size structure across sites and habitats, whereas *A. caudavittata* tended to be slightly smaller in the pneumatophore habitat. Based on stomach contents analysis, *O. mossambicus* and *A. caudavittata* consumed many of the same food items, but often in different proportions. Little variation was seen among sites, except for Jack's, and the diets were more separated by species than by site. *A. caudavittata* consumed higher percentages of filamentous green algae, amphipods, and seagrasses than *O. mossambicus*, but *O. mossambicus* generally consumed a higher percentage of sand and/or sediment, foraminifera, and detritus. *A. caudavittata* consumed a wider variety of items.

From observations on recorded videos, and from the high percentage of sediment in the stomachs of *O. mossambicus* relative to *A. caudavittata*, it was concluded that the two species are utilizing different feeding strategies; the former selecting items from the benthos and the latter straight from the water column. *A. caudavittata* had a higher mean $\delta^{15}N$ than *O. mossambicus*, but not high enough to imply they occupy different trophic levels. The mixing models based on stable isotopes that incorporate food assimilated over longer periods than stomach content analysis, suggested a greater similarity in the diets between species within each site.

O. mossambicus is occupying some of the same habitats and using some of the same resources as the native fish, particularly *A. caudavittata*. However, based on behavioural differences, such as feeding strategies, and the observed differences in prey item proportions, along with the observations of high abundances of all species at many sites, it can be hypothesized that the fish species successfully co-exist with the present habitat and food resource availability. It would be prudent to examine other aspects of the ecology of *O. mossambicus* in this system to determine if they are impacting the system in ways other than its trophic and habitat interactions with other species.

Declaration

I certify that this thesis does not, to the best of my knowledge and belief:

i. *incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education;*

ii. *contain any material previously published or written by another person except where due reference is made in the text of this thesis; or*

iii. *contain any defamatory material*

Hannah Cameron-Caluori

Dec 2014

Acknowledgements

First and foremost, I would like to thank my research supervisors, Assoc. Prof. Glenn Hyndes and Prof. Pierre Horwitz. Without their assistance and dedication to every step throughout this process; from guidance through the proposal writing, to field technique expertise and sample collecting, to innumerable draft edits and suggestions, and everything in between, this thesis would have never been accomplished. I would like to thank you very much for your support and understanding over these past two and a half years.

Next, I would like to thank Dampier Salt Ltd (Rio Tinto) for funding this study, and their staff at Perth and Lake MacLeod offices, particularly Mrs Star Gianatti and Mr Brett Renton for their practical and administrative support. Without their permission and help, we would not have been able to access this unique and important study site. Thank you very much for allowing this research to take place. Mr Paul Richardson also gave permission to use tracks on the Gnaraloo pastoral station to access the lake, making research possible when other tracks to study sites were inaccessible.

I would also like to show my gratitude to my fellow postgraduate students who helped in various field data collection and laboratory analytical processes; namely Andrew Mackey for his help with the stable isotope analysis lab procedures, Natasha Dunham for her help with sample collection and field site acclimatization, Benjamin Jones for his unrivaled fishing abilities and invertebrate knowledge, Aldo Turco for his spearfishing expertise and perfect execution in the field. I especially thank Christopher Kavazos for his help in every aspect of this process, from field work assistance and sample collection, to carrying heavy equipment over kilometers of mud, to constant moral support and encouragement. This process would not have been the same without you. I would like to sincerely thank you all for your help and dedication.

None of this could have happened without my family and a few special friends. I would like to thank my whole family, especially my parents and grandparents Pat and Don, for their constant financial support and encouragement throughout this process. Thank you to all my incredibly supportive and encouraging friends in Perth, who made me feel at home on the other side of the world, especially Kristiina Bedford. A special thank you to my partner Jason Bond, for your encouragement and belief in me every time I doubted myself, and especially for your financial support over the past 2 years. This thesis would not have been possible without you all and stands as a testament to your support.

Table of Contents

Abstract ... ii

Declaration ... iv

Acknowledgements ... v

1. Introduction ... 1
- **1.1 Research Problem** ... 1
- **1.2 Background** ... 1
  - 1.2.1 Fish communities and invasive species ... 1
  - 1.2.2 *Oreochromis mossambicus* ... 3
  - 1.2.3 Lake MacLeod ... 5
  - 1.2.4 Significance ... 8
- **1.3 Research Aims** ... 8

2. Methods ... 10
- **2.1 Study Site** ... 10
- **2.2 Data Collection** ... 15
  - 2.2.1 Stage 1 –Fish community structures in Lake MacLeod ... 16
    - Fyke-netting ... 17
    - Stereo-videography ... 20
  - 2.2.2 Stage 2 – Dietary study of *A. caudavittata* and *O. mossambicus* ... 22
    - Processing of fish samples ... 25
    - Stomach contents analysis (SCA) ... 27
    - Stable isotope analysis (SIA) ... 29

3. Results ... 32
- **3.1 Fish Community Structures in Lake MacLeod** ... 32
  - 3.1.1 Water chemistry in fish habitats ... 32
  - 3.1.2 Fish community structures ... 33
- **3.2 Dietary Study of *O. mossambicus* and *A. caudavittata*** ... 40
  - 3.2.1 Sizes of fish used for dietary analysis and feeding observations ... 40
  - 3.2.2 Stomach content analysis (SCA) ... 41
  - 3.2.2 Stable isotope analysis (SIA) ... 48
    - Differences between months ... 48
    - Differences between species ... 49
    - Isotope ratios relative to potential dietary sources ... 51

4. Discussion ... 58
- **4.1 Fish community structures** ... 58
  - 4.1.1 *O. mossambicus* abundances and distribution ... 58
  - 4.1.2 Biodiversity and species interactions ... 64
- **4.2 Dietary study** ... 67
  - 4.2.1 Species differences ... 68
  - 4.2.2 Stable isotopes and mixing models ... 72
- **4.3 Conclusions** ... 74
  - 4.3.1 Summary and future studies ... 74

5. References ... 79

1. Introduction

1.1 Research Problem

Lake MacLeod is a unique and biologically significant ecosystem within Australia, as well as internationally (Phillips *et al.*, 2005). Unfortunately, it is facing a major potential threat due to an invasive fish species, the Mozambique tilapia *Oreochromis mossambicus*. This study focuses on quantifying the abundances of the invasive tilapia and native species in a range of habitats and to establish the potential effects of this invasive species on the native fish communities of Lake MacLeod, which has received limited research attention due to the location's inaccessibility and isolation.

1.2 Background

1.2.1 Fish communities and invasive species

Overlap in resource use is a common occurrence in all ecosystems; however, when a resource is limiting this overlap becomes competitive (Sale, 1974). In a stable ecosystem, this competition generally aids in controlling the sizes of the populations, allowing all species to co-exist (Sale, 1974). Interspecies competition commonly exists over a number of resources, such as desired habitats and food, which can be in limited supply (Sale, 1974; Molnar *et al*., 2008). Both of these resources are essential to the success of a species, and if two co-existing species require similar but limited habitats and food resources, one species is likely to be outcompeted (Sale, 1974).

One of the most common threats to a stable ecosystem is the establishment of non-native, or invasive, species (Bax *et al*., 2003; Garcia-Berthou, 2007; Molnar *et al*., 2008; Martin *et al*., 2010; Mangla *et al*., 2011). Invasive species have invaded ecosystems

worldwide, threatening native populations and leading to major conservation and management issues (Bax *et al*., 2003; Garcia-Berthou, 2007; Molnar *et al*., 2008; Martin *et al*., 2010; Mangla *et al*., 2011). The impacts of a successful invasion often include a significant altering of the community and food web structures, through habitat and resource competition, and the potential to displace native species (Molnar *et al*., 2008).

In marine and freshwater systems worldwide, plants and animals including mussels, fish, sea stars, urchins, and macrophytes can often enter new regions through the: ballast waters or attachment to chains and anchors of ships; attachment to drilling platforms; aquarium and aquaculture trades; or movement of fish products or seafood (Australian State of the Environmental Committee, 2001). One of the most widespread inland, aquatic invasions is the zebra mussel (*Dreissena polymorpha*) in the Great Lakes and the Saint Lawrence River in North America (MacIsaac, 1996). Their monotypic colonization encrusted onto hard surfaces damaged pipes, water treatment and power plants, ships and boats, and completely covered walls of waterways, harbours and historic shipwrecks (MacIsaac, 1996). The species also occupies ideal habitats for native species such as other mollusks and macrophytes, which in turns depletes food sources for higher-level consumers (MacIsaac, 1996). The impacts of invasive species on endemic fauna can be catastrophic; such as the reduction of biodiversity, or extinction of a native species all together (Molnar *et al*., 2008). Often it can be almost impossible to remove an invasive species once it has successfully established populations in a marine habitat (Thresher & Kurtis, 2004). Unfortunately, the rate at which invasive organisms are establishing themselves in coastal systems is increasing across the globe (Bax *et al*., 2003).

Various teleost species have established wild populations in Western Australia, such as the goldfish *Carassius auratus*, the carp *Cyprinus carpio*, the one-spot livebearers *Phalloceros caudimaculatus*, and the mosquitofish *Gambusia holbrooki* (see Morgan & Gill,

2001). These invasive species can often compete with native fish or other aquatic animals for food and/or habitat, exhibit aggressive behaviour towards them or exhibit direct predation, causing the native species to be displaced, or sometimes completely replaced, by the invasives (Morgan & Gill, 2001).

1.2.2 Oreochromis mossambicus

The Mozambique tilapia *Oreochromis mossambicus* (Peters 1852), a species of Tilapiine fishes of the Cichlidae family, is one of the most successful marine and freshwater invasive fish species worldwide (see Courtenay, 1997; Maddern *et al*., 2007; Martin *et al*., 2010; Russel *et al*., 2012). The grouping of fish generally referred to as "tilapia" include species in the genera *Tilapia*, *Sarotherodon* and *Oreochromis*, all within the Cichlidae family (Bradford *et al*., 2011). All genera are native to and generally very common in West Africa, with many species achieving invasive status worldwide (Bradford *et al.*, 2011), and are often considered the most widely distributed and most successful invasive fishes in the world (Doupé *et al.*, 2010). *Oreochromis mossambicus* has been listed as one of the top 100 worst invasive species across the globe (Global Invasive Species Database, 2004) and has successfully spread to over 90 countries or territories on 5 continents (Russel *et al*., 2012), forming wild populations in every country they have been introduced (De Silva *et al.*, 2004; Canonico *et al.*, 2005).

Historically, many species of tilapia were used worldwide for aquaculture because of their high fecundity, fast growth rates and an opportunistic generalist diet (Doupé *et al.*, 2010; Martin *et al*., 2010). They have the ability to withstand high fluctuations in salinity, temperature and dissolved oxygen levels, allowing them to survive in a wide variety of environments (see Scholfield *et al.* 2001; Canonico *et al.*, 2005; Martin *et al*., 2010). With their ability to adapt to many environments, the wide distribution of *O. mossambicus* through introductions for aquaculture, ornamental use, human consumption and weed control have

ultimately facilitated their success as invaders (Martin *et al*., 2010; Russel *et al*., 2012). The importation of *O. mossambicus* has been banned in many countries around the world, including Australia since 1963; however, its populations have continued to expand throughout Queensland and Western Australia (Russel *et al*., 2012).

O. mossambicus is known to be omnivorous but is generally herbivorous and/or detritivorous (Maitipe & De Silva, 1985; Arthington *et al.*, 1994). However, the species displays high dietary plasticity by feeding opportunistically on invertebrates, zooplankton, larval fish and eggs (Maitipe & De Silva, 1985; Arthington *et al.*, 1994). The potential for dietary competition between this species and native fish is highly likely, as well as the potential for the direct predation of small or juvenile fish. By invading a new system, *O. mossambicus* may disrupt the trophic processes causing changes that can propagate throughout the food web. Additionally, *O. mossambicus* have been documented consuming various algaes and macrophytes (see Maddern *et al.*, 2007). Macrophyte populations are integral in most aquatic systems, playing an important role in stabilizing the benthic substrate from erosion and disruption (Sand-Jensen, 1998). These communities also provide shelter and protection for many invertebrates as well as juvenile and adult fish (Sand-Jensen, 1998). Additionally, these macrophyte communities are host to a variety of periphyton, which form part of many aquatic species' diets as well as aiding in water filtration (Biggs & Close, 1989). Although tilapia may not specifically select for the periphyton (Doupé *et al*., 2010), by consuming the macrophytes there is a great chance that they will deplete the resource.

The *Oreochromis* genus is distinct from other tilapia genera in their breeding and maternal mouthbrooding behaviours (Bradford *et al.*, 2011). Males build and aggressively protect breeding areas (called leks) during the spawning season, while females mouthbrood the fertilised eggs (Maddern *et al.*, 2007; Doupé *et al.,* 2009a). This aggressive behaviour has the potential to affect the behaviour, movements, and activities of the native fish species

(Doupé *et al.,* 2009a). Other species in the presence of breeding *O. mossambicus* have shown a decline in egg production and fertilisation success, which may be due to the behavioural interactions and/or a possible chemical/hormonal release from the breeding males (Doupé *et al.,* 2009a). Additionally, the construction of these leks, which have been recorded to be up to 80cm in diameter (Maddern *et al.*, 2007), can affect the habitats and available food sources of the native fish communities by the removal of important macrophytes and periphyton, disruption of the sediment, and occupation of potentially key habitats of other fauna (Maddern *et al.*, 2007; Doupé *et al.,* 2009a).

Oreochromis mossambicus has the potential to cause drastic damage to an ecosystem in which it has become established (see Maddern *et al.*, 2007; Doupé & Burrows, 2008). However, there is little empirical or direct evidence to support these ideas (Maddern *et al.*, 2007; Doupé & Burrows, 2008). Since it was first sighted in Western Australia in 1978, *O. mossambicus* has made its way into the Gascoyne, Lyons, Minilya, Lyndon and Chapman rivers, where it is now often seen to be a dominant species (see Morgan *et al.*, 2004; Morgan and Gill, 2004; Maddern *et al*., 2007). Despite the continually increasing presence of invasive tilapia, and specifically *O. mossambicus*, in Australia, there has been little evidence of the ecological influences of this invasive species and quantifiable data of its effect on native fish communities is limited (Doupé & Burrows, 2008; Doupé *et al.,* 2009a).

1.2.3 Lake MacLeod

Lake MacLeod is an inland, saline lake in Western Australia, spanning approximately 120 km north to south and between 10-40 km east to west covering an area of almost 2000 km^2 between Carnarvon and Coral Bay (see Methods Figures 2.1 and 2.2). The majority of the area is a semi-dry lakebed, which is periodically filled by flooding events from the surrounding rivers, namely the Gascoyne, Lyndon and Minilya rivers (Phillips *et al.*, 2005).

In the northern area of the lake system are the Northern Ponds, a series of permanent, saline ponds spanning approximately 6000 ha (Phillips *et al.*, 2005). Water from the Indian Ocean travels underground, through karst to a series of vents, to continuously supply the ponds with seawater (Phillips *et al.*, 2005).

The Northern Ponds are known to be a biologically significant ecosystem and are a proposed Ramsar site as a Wetland of International Importance (Phillips *et al.*, 2005). Home to the largest inland population of the mangrove species *Avicennia marina* worldwide, Lake MacLeod is also host to unique and thriving communities of shorebirds and other waterbirds, invertebrates, and fish (Phillips *et al.*, 2005). The Northern Ponds have been classified as one of the most important stopovers for migrating birds in Australia, and are home to over 70 bird species (recorded in 2005), both migratory and residential (Phillips *et al.*, 2005). The invertebrate community consists of several rare species in addition to a number of Gondwanan relics (Phillips *et al.*, 2005), and communities unique to the area (McLure, 2011).

Although the native fish communities are abundant, the species richness is low (Shepherd, 1991; Streamtec 1998, 1999, 2001; Phillips *et al.*, 2005). Little quantitative work has been conducted on the fish communities of Lake MacLeod, but the native species that have been recorded are limited to yellowtail grunters *Amniataba caudavittata* (Richardson, 1845) and few-ray hardyheads *Craterocephalus pauciradiatus* (Gunther, 1861), along with occasional sightings of spangled perch *Leiopotherapon unicolor* (Gunther, 1859) and sea-mullet *Mugil cephalus* (Linnaeus, 1785) (Shepherd, 1991; Streamtec 2001; Phillips *et al.*, 2005). This preliminary work has suggested that the fish communities within Lake MacLeod are potentially one of the top consumers, while also possibly providing a food source for the large bird populations, although little quantitative evidence exists. The most widely recorded species (*A. caudavittata* and *C. pauciradiatus*) are both known to often inhabit estuaries and other euyhaline environments (see Prince *et al*., 1982; and Potter *et al.*, 1986; Wise *et al.*,

1994; Young *et al.*, 1997; Morgan and Gill, 2004; Allen *et al.*, 2005; Molony and Parry, 2006; Davis *et al*., 2012) which likely contributes to their success in the dynamic Lake MacLeod environment. *A. caudavittata* generally exhibits a fairly omnivorous diet (see Wise *et al.*, 1994; Young *et al.*, 1997; Molony and Parry, 2006; Davis *et al*., 2012) and atherinids such as *C. pauciradiatus* generally consume a range of small invertebrates and plankton (see Humphries and Potter, 1993; Allen *et al.*, 2005 for similar species), as well as being known detrivores.

Oreochromis mossambicus has been observed multiple times in the Northern Ponds for over a decade, but its presence has never been quantified (see Phillips *et al.*, 2005). During major flood events, most of these rivers can overflow into the Lake MacLeod basin, such as the flood event in the year 2000 that is hypothesised to have allowed the first populations of *O. mossambicus* to enter the lake (Phillips *et al.*, 2005).

The Northern Ponds system has been the focus of limited research attention due to logistical constraints and isolation. Current projects are underway, or have recently been completed on various aspects of the biology of Lake MacLeod ("Temporal & Spatial Variation in Aquatic Invertebrate Communities at Lake MacLeod, Northwestern Australia", N. McLure, 2011; "Influence of Hydrological and Environmental Conditions on Mangrove Vegetation at Coastal and Inland Semi-arid areas of the Gascoyne Region", N. Dunham, 2014; Untitled project investigating microbial communities, C. Kavazos, unpubl. data; and an ongoing untitled shorebird study, unpubl. data) in order to gain a better understanding of the ecological processes and interactions that are driving the system. As a highly abundant community within the ecosystem, understanding the fish community dynamics is essential to understanding the Lake MacLeod system as a whole. The presence of the invasive fish species makes it even more vital to understand and quantify the abundance, species composition and trophic structure of the fish communities. The lake system is currently not

being managed. As well as being applicable to other salinas across the globe, this research holds the potential to determine if management steps are indeed needed in order to preserve the native fauna at Lake MacLeod.

1.2.4 Significance

This study seeks to provide insight into the potential effects of the most widespread invasive fish group (Tilapia), and specifically one of the most successful invasive species worldwide (*Oreochromis mossambicus*), on native fish communities. The potential applications of such data are numerous and relevant to systems across the globe. The major concerns linked to the successful invasion of *O. mossambicus* into the Northern Ponds of Lake MacLeod, in regards to the native fish species in the ecosystem, are competition for habitat and food sources. Quantifying the habitat use and community structures of all fish species and investigating any dietary overlap will allow for informed management strategies of this highly unique ecosystem.

1.3 Research Aims

The overall aim of the study is to determine if the invasive *O. mossambicus* is likely to have an effect on the native fish communities of Lake MacLeod through competition for key resources. Specifically, this study aims to determine the abundances and size structures of *O. mossambicus* and native species (mainly *A. caudavittata* and *C. pauciradiatus*) in representative habitats of the Northern Ponds of Lake MacLeod to establish if there is overlap in the habitat distribution of these species and the potential competition with native species. Secondly, this study aims to determine if *O. mossambicus* might be affecting the most common native species through trophic processes, either through similar food resource use

and/or through direct predation. These aims will help to understand the potential effects of *O. mossambicus* on native fish, which will facilitate future management of the system.

2. Methods

2.1 Study Site

Lake MacLeod is situated along the north-western coast of Western Australia (WA) between the towns of Carnarvon and Coral Bay, lying parallel to the Indian Ocean (Figure 2.1). The majority of its expansive area, in most years, is a dry lakebed (Figure 2.2), with a depression of 3-4 m below sea level (Phillips *et al.*, 2005). However, there is a series of permanent, saline ponds within the lakebed, referred to as the 'Northern Ponds' (Figure 2.2b) (Phillips *et al.*, 2005) because they are in the northern part of the overall Lake MacLeod system.

The Northern Ponds consist of three main regions, the Cygnet and Ibis ponds which are situated south of the Sandy Bluff Sill, or the "panhandle", and the Chirrida Pond system to the north of the Sandy Bluff Sill (Figure 2.3) (Phillips *et al.*, 2005). These ponds are connected to the nearby Indian Ocean by an underground karst system, through which the seawater travels approximately 18-20 km inland and upwells through vents into the ponds (Phillips *et al.*, 2005). The combination of the below sea level elevation of the lakebed and the dry, windy climate with excessive evaporation creates a constant hydrostatic pressure resulting in discharge into the ponds, which can overflow into the lakebed (Phillips *et al.*, 2005). The upwelled and ponded water flows south and east from the vents, some of which ends up in the main lake (Phillips *et al.*, 2005). Small amounts of water spill into the surrounding mudflats and can be carried by strong winds throughout the lakebed (Phillips *et al.*, 2005). These varied dispersal processes of water cause the surrounding ponds to exist in a variety of formations, sizes and depths. When the water initially reaches the Northern Ponds, its salinity is similar to seawater, but can become hypersaline as it disperses away from the vents (Phillips *et al.*, 2005).

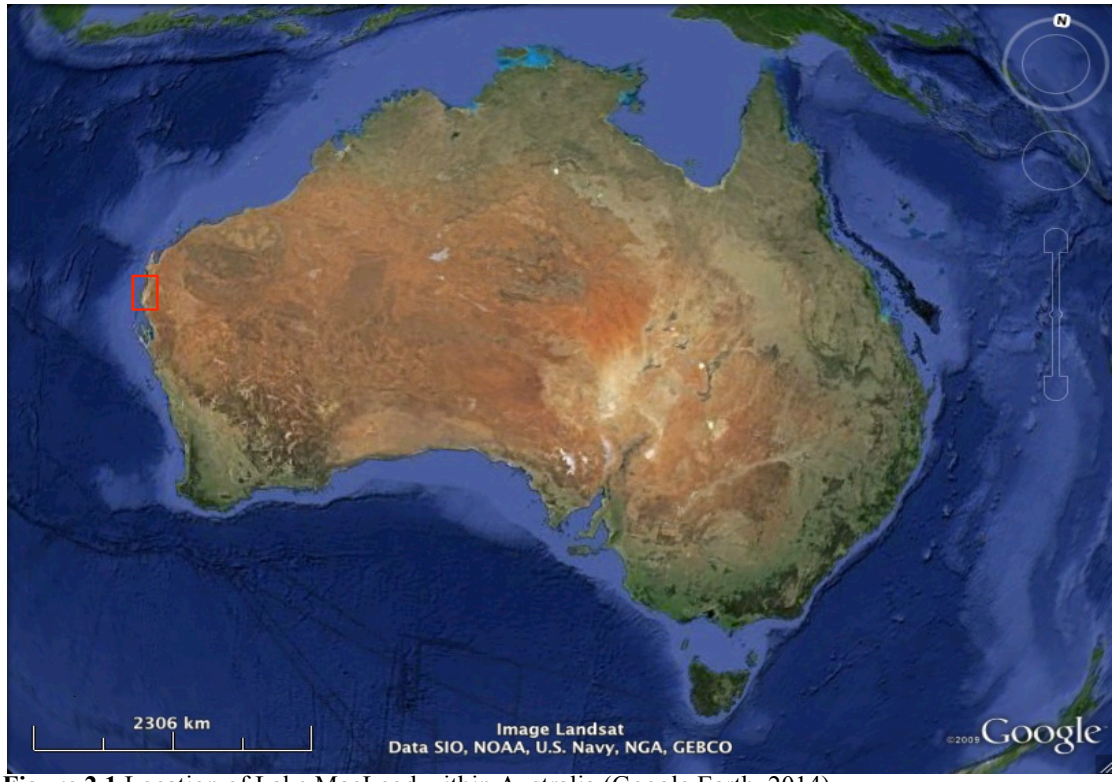

Figure 2.1 Location of Lake MacLeod within Australia (Google Earth, 2014).

a) **b)**

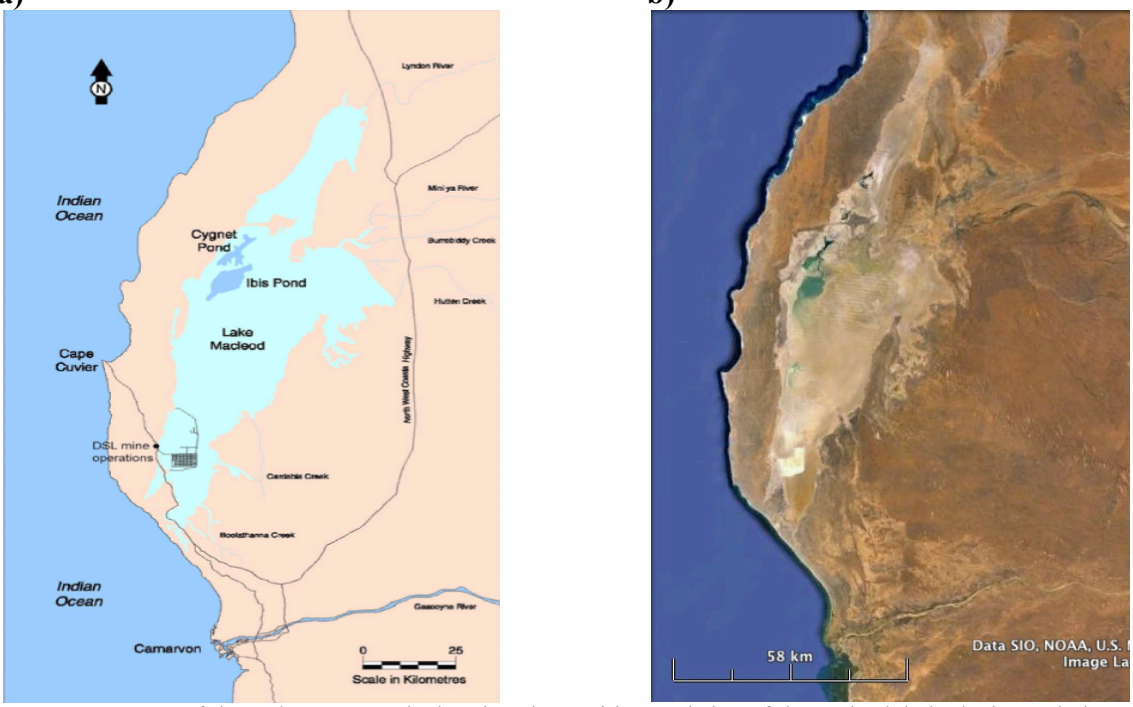

Figure 2.2. Map of the Lake MacLeod, showing the position and size of the entire lakebed, situated along north-western coast of WA, between Carnarvon and Coral Bay approximately 18 km inland from the Indian Ocean **(a)** (Phillips *et al*., 2005). Satellite image of the lakebed showing the dry lakebed and permanent ponds **(b)** (Google Earth, 2014).

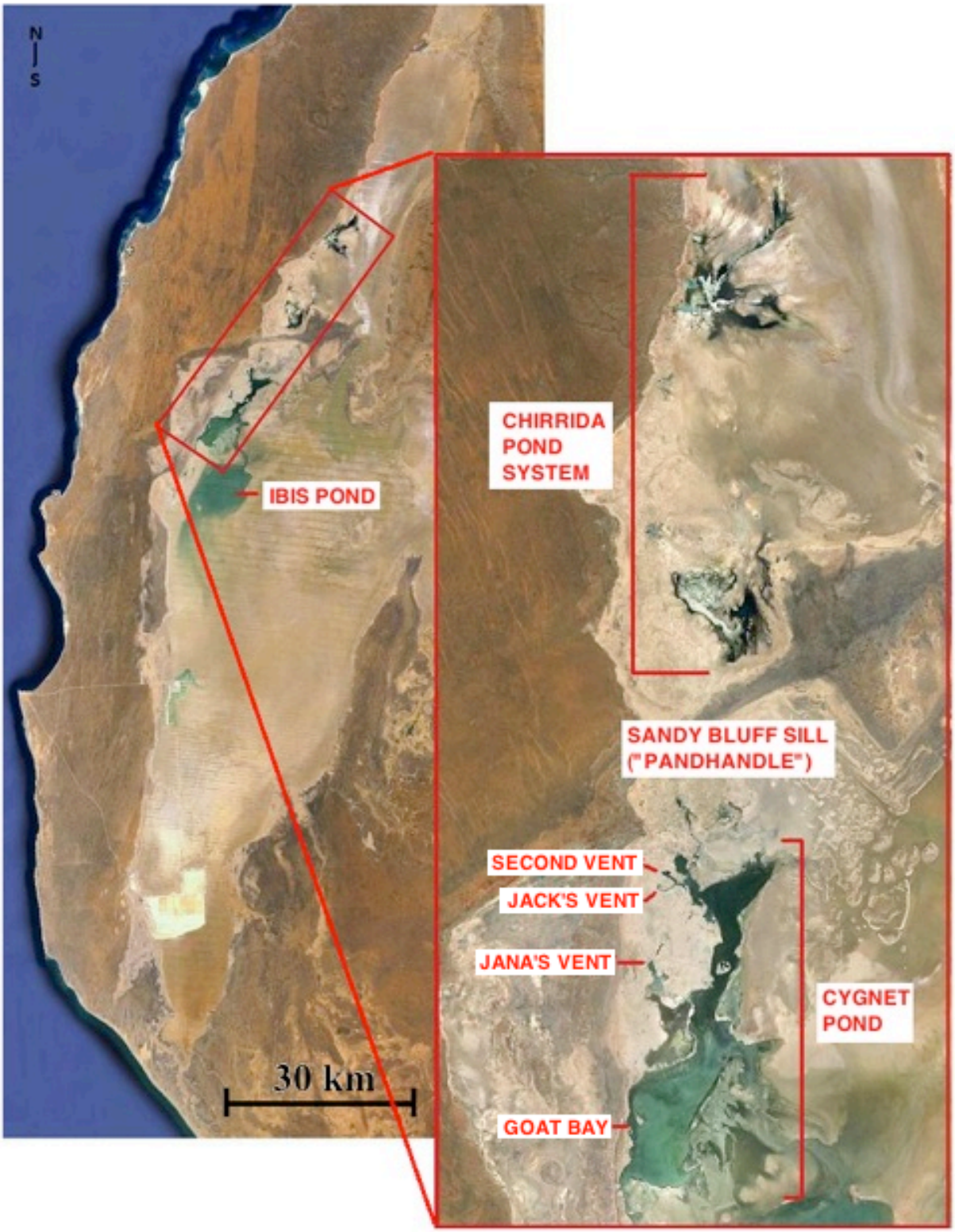

Figure 2.3 Satellite image of the Lake MacLeod lakebed, showing Ibis Pond and the Northern Ponds in more detail (see inset). Cygnet Pond and the Chirrida system of ponds are shown in more detail, with the major vents and ponds labeled (Images edited from Google Earth, 2014).

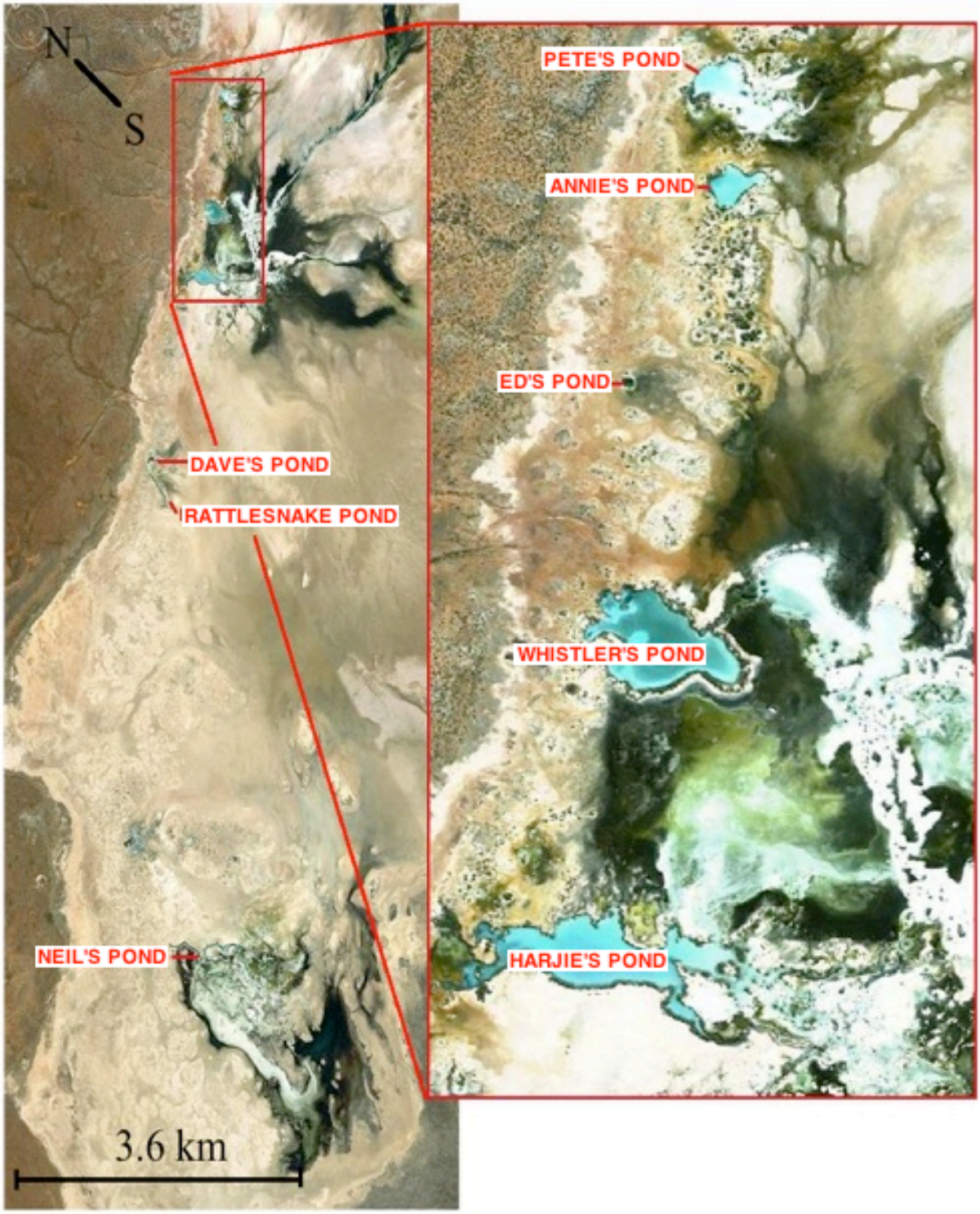

Figure 2.4 Satellite images of the Chirrida system of ponds, within the Northern Ponds of Lake MacLeod. The major permanent ponds and vents are labeled (Images edited from Google Earth, 2014).

Situated in the southern Carnarvon basin, this region experiences high temperatures year-round (with mean highs of 22.3-32.5°C over the past 69 years measured from the

Carnarvon Airport) (Bureau of Meteorology, 2014). This region also experiences a mean annual evaporation (2764 mm) that is over 10 times higher than the mean annual precipitation received (228.5 mm) (data from the Department of Agriculture WA and the Bureau of Meteorology respectively). These conditions create a very arid climate (Wyroll *et al.*, 2000). The region receives highly variable rains, with precipitation in the winter derived from cold frontal systems that cross the southwest of WA and occasionally are extensive enough to cross the mid-west or northwest of WA as well. In the summer, monsoonal thunderstorms and tropical lows or cyclones from the north occasionally cause massive flood events (Wyroll *et al.*, 2000). Such floods can cause runoff from the Gascoyne, Lyndon and Minilya rivers to discharge into the lakebed, potentially bringing with them invasive fauna (Phillips *et al.*, 2005). A major flood event that occurred in the year 2000, which was the largest flood in recorded history, is believed to have brought in the initial populations of the invasive tilapia *O. mossambicus* from established populations in the surrounding rivers (Phillips *et al.*, 2005). Another major flood event occurred in late December 2010 and January 2011, during which *O. mossambicus* were observed entering Lake MacLeod from distributaries of the Gascoyne River and from which individuals from Jana's Vent were identified as *O. mossambicus* by the Western Australian Museum (McLure, 2011).

In a pilot study conducted in July 2011 by researchers from Edith Cowan University, three main habitat types occupied by the fish communities within the Northern Ponds were identified: 1) pneumatophore beds; 2) nearshore habitats; and 3) open vents (Figure 2.5). The pneumatophore bed habitat is defined as a shallow water habitat (depth <0.5 m) containing dense pneumatophore roots (Figure 2.5a). This habitat extends from the beginning of the fringing vegetation along the shoreline to the edge of the pneumatophores in the pond, covering a width up to 10 m. The nearshore habitat is classified as the sloping littoral zones with soft sandy sediment containing few pneumatophores or the vertical drops around the

pond edges with over-hanging mangrove roots (Figure 2.5b). These nearshore habitats are typically between 0.5-1 m in depth. Lastly, the open vent habitat is classified as the deep-water vents (depth >2 m), where the seawater enters the ponds from the underground karst system. These vents typically have a high rugosity and rocky substrate (Figure 2.5c; see Appendix Figure 6.3).

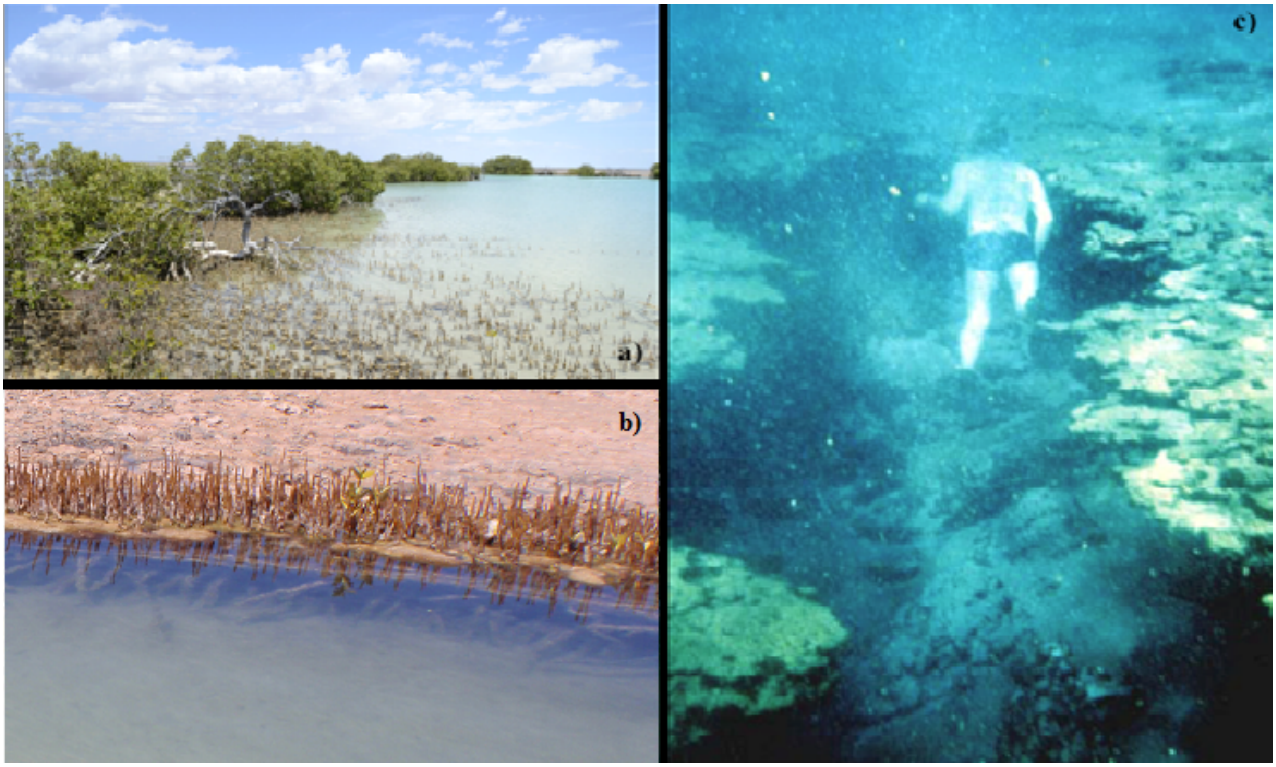

Figure 2.5. Examples of the three main fish habitats: pneumatophore bed **(a)**, nearshore **(b)** and open vent **(c)** habitats within the Lake MacLeod ponds.

2.2 Data Collection

This study consisted of two stages, firstly focusing on community structure then on a dietary analysis, to investigate the possible effects of *O. mossambicus* on the native fish communities in the lake. Table 2.1 shows a summary of the two stages and the techniques and analyses used.

Table 2.1. Summary table of the sampling design and analysis type, showing the parameters being measured, the various sampling techniques for each habitat type, the number of sites being sampled and a short description of the field or lab analysis. See text for abbreviations.

Stage of study	Aim	Habitat type	Sampling method	Number of sites sampled	Analysis summary (field or lab)
1	Community structure	Pneumatophore beds	Fyke-seine net	7	On-site count
		Nearshore habitats	Stereo RUV 5 min		EventMeasure
		Open vents	Stereo RUV 15 min		EventMeasure
2	Dietary analysis	Pneumatophore beds	Fyke-seine net	3 (4 with seasonal replicate)	SCA and SIA
		Nearshore habitats	Seine net, angling, traps		SCA and SIA
		Open vents	Angling, traps, nets		SCA and SIA

During both stages, water quality data were collected with a Thermo Fisher Scientific TPS 90FL from all sites that were used, before any fish sampling took place. Temperature, pH, dissolved oxygen, and electrical conductivity (which was then converted into salinity) levels were collected from the middle of the water column in shallow waters. In the vents and nearshore habitats where the water depth was greater than 0.5 m, measurements were made from the surface and bottom of the ponds. The water quality data were averaged where more than 1 sample was taken in a habitat. A degree of water clarity was given to each habitat at each site, based on direct observation, on a scale of 1-5, with 1 equating to near perfect clarity and visibility of at least 5 m, and 5 corresponding to a lack of clarity with visibility of less than 0.25 m. This technique was decided upon based on the observed levels of water clarity within the study sites, and adapted from the principle of the horizontal black disk technique for water clarity (as presented in Steel & Neuhausser, 2002), as opposed to the traditional vertical Secchi disk technique due to the shallow waters.

2.2.1 Stage 1 –Fish Community Structures in Lake MacLeod

To determine the abundances and size structures of *O. mossambicus* and the native species (*A. caudavittata* and *C. pauciradiatus*) in order to establish if there is overlap in the

habitat distribution of these species and the potential competition, seven sites were selected within Lake MacLeod. The original sampling design of this stage included eight sites within the Northern Ponds. Selection criteria for a sampling site were based on the site containing at least one pond with each of the three habitat types present and being realistically accessible from the main track for hauling heavy equipment. The ponds selected were: Pete's, Annie's, Whistler's, Harjie's, Jack's, Dave's, Neil's and Jana's (Figures 2.3 and 2.4). However, Annie's was excluded from the study, as the first round of sampling revealed it to contain very few fish, and showed that it had very similar characteristics, and was very close in proximity, to Pete's. At each site, the GPS coordinates were recorded using a Garmin Oregon 550 GPS. Each of the three main habitat types at each site was sampled separately using the appropriate technique for each habitat across all ponds (Table 2.1).

Fyke-netting

To sample the pneumatophore beds, a combination fyke- and seine-net was used. Fyke nets are commonly used to sample mangrove habitats with protruding pneumatophore roots (Clynick & Chapman, 2002). Most mangrove habitats are in tidal zones, thus the fyke-nets can be left in place and the fish are ushered into the net by the movement of water (Clynick & Chapman, 2002). In this case, however, Lake MacLeod has no tidal movement so modifications to this procedure were made. A custom made fyke-net was made comprising 4 mm mesh in two wings each 10m long with a 1 m drop, and a 2m long pocket with four round hoops. The net dimensions and mesh size allowed for the effective capture of the commonly observed species, with low probability of injuring the fish through gilling. There was no need for a breather hole to be cut for air breathing animals caught by accident, because the top of the net was open and there were very few observed animals in the sampling area other than fish and invertebrates. Detachable 10 m long extension wings with

the same 4 mm mesh and 1 m drop, were added onto the end of each wing (Figure 2.6). These extensions could be added or removed as required for the sampling area. The entire fyke-net was fitted with a float line and a lead line. Additionally, a 20 m long, small seine net made of 4 mm mesh and a 1 m drop was made, with a float line and a very light lead line.

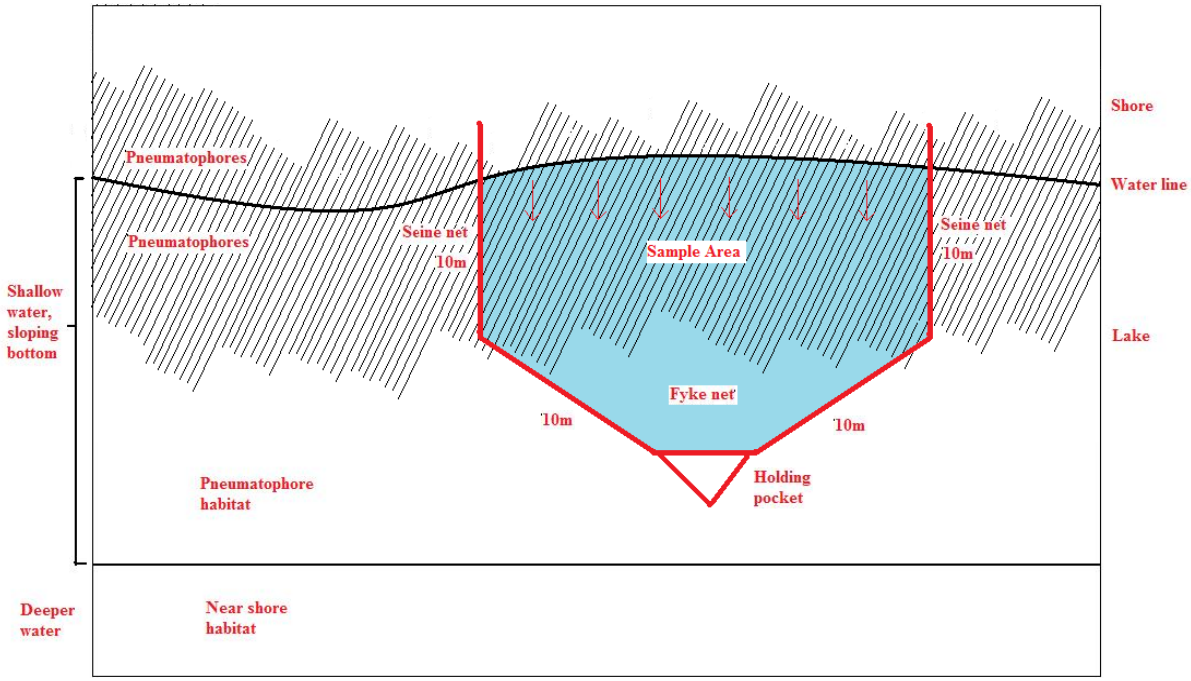

Figure 2.6. Diagram of sampling design of fyke-seine net system set up in pneumatophore bed habitats. The sample area, which will be measured for each sampling, is shaded blue. Arrows indicate the direction of fish corralling.

At one location at each site, the fyke-net was placed over the shallow pneumatophore area with the wings or wing extensions crossing the water line onto shore and the holding pocket in moderately deep water (around 1 m). The additional seine-net was then pushed through the pneumatophores by 2-4 people to corral the fish into the fyke-net. Occasionally, a larger and heavier seine net was required, depending on the robustness of the pneumatophores. While pushing the seine-net through the water, the lead line of the seine net was kept on, or as close to, the substrate as possible with every step. Loud noises and splashing were made as the net was pushed through the water in order to encourage the fish to swim towards the fyke-net pocket instead of back towards the seine-net. As the seine-net

was being moved forward, one or more persons, depending on the size and shape of the sampling area, began to circle the wings of the fyke-net in and behind the seine-net until the net was sealed. The trapped fish were then gently pushed through each ringed level of the fyke-net and into the holding pocket. Fish were emptied into a bin freshly-filled with pond water and brought back to shore. With the known size of the netting system at each site, the dimensions of the sampling areas could be determined. Under the assumption that the proportion of fish escaping was similar among sites, the netting areas (within the fyke- and seine-net set ups) were standardized (all to 187 m^2) and the abundance of fish per area measured (abundance per net area of 187 m^2) were calculated at each site to provide a relative measure of fish density for each site.

The fish were immediately processed. All individuals were identified to species level and counted. Up to the first 30 individuals of each species were measured for total length to the closest 1 mm using a measuring board. All fish were immediately placed into a recovery bin of clean pond water after being processed. Except for the invasive *O. mossambicus*, all healthy fish were released directly back into the pond. Any fish showing signs of distress were monitored in the recovery bin. If the fish did not improve after 10 minutes, they were immediately euthanased to avoid suffering. The majority of fish that were euthanased due to stress were *C. pauciradiatus*. All *O. mossambicus* caught were immediately euthanased due to their invasive status. All fish were euthanased using a 450 mg/L concentration of clove oil (Borski & Hodson, 2003; American Veterinary Medical Association (AVMA), 2013) in a bath of pond water. Prior to the field trip, the clove oil was dissolved in 95% ethanol at a 1:10 ratio, as per AVMA (2013). In the field, this clove oil mixture was mixed with pond water to produce a 450 mg/L concentration, with enough water for the fish to be fully submerged (Borski & Hodson, 2003; AVMA, 2013). Fish were left in the bath for 10 minutes after the

cessation of opercular movement (Borski & Hodson, 2003), which generally took 1-2 minutes. Any euthanased fish were placed in an ice slurry and frozen as soon as possible.

Stereo-videography

To sample the open vent and nearshore habitats, netting would have been ineffective due to the depth, sizes and shapes of the vents, and the lack of strong currents or tides. Videography was selected because it is efficient, non-disruptive or invasive and provides the quantitative abundance and size data that are needed for this type of study (Watson *et al.*, 2010). In order to accurately measure length from underwater video footage, a stereo-video system is needed (Watson *et al.*, 2010). Measurements are taken from both screens and an accurate length is calculated using computer software (Watson *et al.*, 2010). In this study, the SeaGIS software EventMeasure was used. The stereovideo set up in this study was custom ordered from SeaGIS and set up to give the most accurate measurements for the fish species previously known to occur at Lake MacLeod. The system comprised two Canon HFS21 video cameras secured inside two waterproof housings. The housings were secured onto a base bar 0.8m apart, angled slightly inwards. The system also included handholds and a light emitting diode. The whole system was weighted to be neutrally buoyant in full strength seawater.

The physical limitations of the underwater video systems include low light conditions and highly turbid water. To reduce the chance of low light conditions, the camera was deployed during the middle of the day with plenty of sun exposure. Where possible, the camera system was not placed in a vent covered by shadows. However, after analysing the video footage, the picture quality proved to be very high and shadows or low light conditions did not affect the usability of footage. Water turbidity, however, was uncontrollable. All sites

contained one or more vents with suitably clear water, however, only three sites (Neil's, Whistler's and Pete's) contained nearshore habitats with sufficiently low turbidity.

Prior to use, the stereo-video cameras were calibrated (SeaGIS Pty Ltd, 2011) in a pool, using a calibration cube provided by SeaGIS, at 2m depth to simulate the approximate depth of the study sites. For detailed calibration procedures, see the SeaGIS "CAL" user manual (SeaGIS Pty Ltd, 2011). In the field, the camera system was fitted into an underwater, negatively buoyant, metal frame, effectively creating a stereo remote underwater video system (stereo-RUV). The unbaited stereo RUV was lowered into a vent at each of the seven study sites. Two ropes were attached to each side of the RUV frame, which was lowered into the vents from a boat or the shore near the vent. One snorkeler then entered the water to precisely position the RUV. The snorkeler then exited the water completely, and all persons left the surrounding area to allow the fish to resume natural behaviours. The RUV was left for at least 30 minutes in each vent, although the first 15 minutes were excluded as an acclimatization phase for the fish and to allow the sediment to settle. The following 10 minutes of each video were analysed from each vent.

To investigate the nearshore habitats, a similar technique was used. The RUV was deployed in three places along the side of the pond, positioned to film the fish swimming in and out of the pneumatophores. Again, the RUV was placed using the attached ropes and the precise location set by a snorkeler where necessary, who then exited the water and moved away, as for the vent. The RUV was left in each position for 10 minutes. The first 5 minutes of each video was excluded to allow the fish to acclimate. Originally, the stereo-videocamera system was going to be used as a diver-operated videocamera (DOV) to perform three 25 m transects along the sides of the pond to sample this nearshore habitat. However, after attempting transects in the nearshore area at Pete's, it became clear that the movement of the snorkeler and the camera was disruptive, causing all fish to hide.

All video data were imported from the Secure Digital (SD) memory cards onto a Dell PC laptop. The video files were spliced together using Windows Movie Maker. These files were then converted from Matroska files (.mkv) to Audio Video Interleaved files (.avi), which are compatible with the SeaGIS analysis software Event Measure (SeaGIS Pty Ltd, 2012). The video footage was used to: (i) determine the maximum number of fish observed in one frame over the duration of the video footage (MaxN); and (ii) measure the total lengths (TL) of each species present (Watson *et al.*, 2010; SeaGIS Pty Ltd, 2012). MaxN was calculated from one screen as opposed to two because in some instances the camera was angled too much to get an accurate count on both screens simultaneously. To measure the total lengths (TL) of each species, the frame in which the species had the highest number of individuals on both screens was used. Each individual was measured from the tip of the nose to the end of the caudal fin. This was done for each species observed at each site, and the mean TL determined.

Using SPSS v.22.0, two independent sample t-tests were conducted using species as factors (*A. caudavittata* and *O. mossambicus*) and sites as replicates: one on the abundance data from netting in the pneumatophores, and one on the MaxN data from the vents. Data from the pneumatophore habitat were normally distributed and the homogeneity of variances was accepted. The MaxN data were square root transformed to achieve normality. Two 2-way, independent sample Kolmogorov-Smirnov tests were conducted on the TL data from the pneumatophore habitats and the vents, to test for possible length differences between the species.

2.2.2 Stage 2 – Dietary Study of A. caudavittata and O. mossambicus

The second stage of the study aimed to investigate possible overlap of diets between *O. mossambicus* and the native fish species, and possible predation on native species by the invasive species. From the observations made and data collected from stage 1, *A.*

caudavittata was the most abundant species and was present at all sites. *A. caudavittata* can be omnivorous, and therefore, is likely to exhibit dietary overlap with *O. mossambicus*. *A. caudavittata* was also found to have similar habitat associations and similar size ranges to *O. mossambicus* (see Results section 3.1.2). Based on these results and observations, stage 2 of the study focused on the diets of *O. mossambicus* and *A. caudavittata*.

To investigate the diets of the two fish species, a combination of a stomach content analysis (SCA) and a stable isotope analysis (SIA) was used. Both of these techniques are commonly used to investigate diets of animals and the trophic structures of ecosystems (Beaudoin *et al.*, 1999; Davis *et al.*, 2012). Both techniques have shortcomings, and therefore, are often conducted simultaneously to increase the accuracy and resolution of studies (Beaudoin *et al.*, 1999; Davis *et al.*, 2012). SCA gives useful information on which resources are being consumed and in what proportions they are being consumed (Hyslop, 1980). While SCA allows for the identification of specific taxa and direct estimation of proportions or amounts of prey items consumed, this technique often requires large sample sizes, is limited to a very short time frame from ingestion (often only a few hours), and is highly dependent on the digestion rates of each species (Gearing, 1991; Polis & Strong, 1996; Lugendo *et al*., 2006). Digestion rates between consumer species can vary, which can affect species comparisons, and in this case, *O. mossambicus* is known to digest animal material rapidly, even showing evidence of rapid digestion of hard parts such as fish bones and crustacean exoskeletons (Doupé & Knott, 2010). Stomach contents may be highly digested, especially soft-bodied animal matter, and different dietary items may be digested at various rates, rendering some items unrecognisable and often under-represented in the measurement of gut content proportions (Hyslop, 1980; Burns *et al.*, 1998; Grey *et al.*, 2002).

Alternatively, stable isotope analyses (SIA) are utilised because they allow for temporal or spatial variation to be clearer, are based on the items that are assimilated into the

consumer's tissue over a longer time period (weeks to months), and provide information on the trophic structure and relationships within a system (Gearing, 1991; Pinnegar & Polunin, 2000; Lugendo *et al.*, 2006), however, they lack the fine detail provided by a SCA (Grey *et al.*, 2002). SIA used for dietary studies generally look at the stable isotopes of carbon ($^{13}C/^{12}C = \delta^{13}C$) and nitrogen ($^{15}N/^{14}N = \delta^{15}N$). The consistent enrichment of these isotopes as they are assimilated up the food web allows for this analysis to trace the major food sources from primary producers to consumers, and provides useful information on the trophic relationships of an ecosystem (Bouillon *et al.*, 2002; Pinnegar & Polunin, 2000; Peterson & Fry, 1987). As a general rule of thumb, the stable isotope ratios of animals reflect their diets up to $1.0^{0/}{}_{00}$ for $\delta^{13}C$ and approximately $3\text{-}3.5^{0/}{}_{00}$ enrichment for $\delta^{15}N$, based on both field and laboratory studies (e.g. DeNiro & Epstein, 1978; Fry & Sherr, 1984; Minagawa &Wada, 1984; Fry, 1988; Peterson & Fry, 1987). However, these discrimination factors can vary based on taxonomy, food source and quality, and a number of environmental variables (Vanderklift & Ponsard, 2003).

In November 2012, two sites within the Northern Ponds (Dave's and Pete's) were sampled to collect *O. mossambicus* and *A. caudavittata* samples for the dietary analyses. Also in November 2012, Neil's was sampled, however, only *A. caudavittata* was successfully collected. In April 2013, both species were collected from Jack's and Dave's. The capture techniques used varied between habitats, sites and species. The majority of *A. caudavittata* was caught using hook and line angling, with and without bait. Since this technique tended to favour the mid-to-large sized fish, *A. caudavittata* was also caught using baited opera house style traps and small seine nets (10 m in length with a 1 m and 4 mm mesh) to allow for a variety of sizes to be sampled. These techniques were successful for capturing *A. caudavittata* at all sites and seasons. To capture the *O. mossambicus*, a variety of netting techniques (fyke, seine and cast nets) were required (see Appendix Figure 6.1), as they were

not attracted to any type of bait on a hook. A limited number of *O. mossambicus* were caught using baited opera house style traps at Dave's. At Jack's, spear fishing was used to capture all *O. mossambicus* individuals.

Processing of fish samples

After the fish were caught, they were immediately euthanased, as in stage 1. During the first round of sampling (November 2012) and after being euthanased, the fish were placed directly into an ice bath to stop digestion and transported to the site house for processing that day/evening. Processing took place 2-5 hours after death. During the second round of sampling (April 2013), the processing took place on site, within minutes of death to ensure that the gut contents had not further digested.

Total length (TL) of each fish was measured to the nearest millimetre using a measuring board and the fish were dissected to determine the sex, remove the guts and take a tissue sample. All fish were cut from the vent using scissors, proceeding anteriorly to stop between the gills. The TL, sex and any distinct markings or colouring of each fish were recorded.

To remove the gut, the tissue attaching the intestine to the vent was cut, as well as the oesophagus anterior to the stomach. The entire gut (from above the stomach to the end of the small intestine) was then pulled out of the fish with forceps. Any attaching tissues were removed with scissors and the gut was placed into a clean, labeled sample jar containing 70% ethanol. The jars were then organized into bins and placed into the vehicles to ensure they stayed upright while being transported back to the ECU campus in Perth.

Dorsal muscle tissue samples were taken from 10 fish of each species at each site. To remove the tissue samples, a cleaned scalpel was used to cut through the skin and flesh tissue to the bone in a square or rectangular shape. Then the scalpel was used to separate the flesh

from the bones and the skin was completely removed. Enough flesh was taken to provide at least a 3 mg dried sample. The samples were placed into clean, labeled sample jars and put directly into an ice slurry. Once back at the site house, the samples were immediately frozen. All frozen samples were transported back to Perth in an ice-filled esky and stored in a freezer until processing began.

Samples of a wide variety of potential dietary sources were taken for the SIA. The potential dietary sources sampled (where present) included: filamentous green and red algae species, seagrasses, detritus, pneumatophores, mangrove leaves, samphire, biofilm, plankton, and invertebrates. For all algae, seagrass, and samphire samples, the plants were pulled off the substrate from five different areas per site, and placed directly into clean, labeled sample jars. Mangrove leaves and pneumatophores were cut with secateurs from live mangrove trees in five separate areas per site and placed directly into clean, labeled, sealed bags. Detrital material was collected in water from five different areas per site from the bottom of the pond using a clean, labeled sample jar as a scoop and then securing the lid. Benthic biofilm was collected in a similar way, where present, although extra care was taken to only sample the top layer of the substrate. Often the biofilm layer was easily removed without sampling the underlying sediment. The biofilm was also sampled from five different areas per site. Zooplankton and phytoplankton were each sampled using a different plankton net (mesh pore size 125um and 50um, respectively), and pulled through the water column until a large enough sample was collected. This varied between 1 and 20 pulls. For each, excess water was drained and the sample was stored in a clean, labeled sample jar. To collect the invertebrates, a sweep net was swept through shallow, algal or pneumatophore-dominated habitats. This was done once or twice per site. The invertebrate samples were placed in sealed plastic bags filled with pond water and kept cool. Once back at the site house, the samples picked in a sorting tray, and live representatives of main taxonomic groups placed into separate petri

dishes to avoid predation and kept alive in pond water overnight to purge their digestive tract. After at least 8 hours, they were drained and frozen in clean, labeled sample jars. All samples were frozen and transported back to Perth in an ice-filled esky and stored in a freezer until processing began. Table 2.2 shows a complete list of the dietary source samples collected at each site.

Table 2.2 Summary of the source samples collected at each site and season.

Source collected	**Dave's 11/12**	**Dave's 04/13**	**Jack's**	**Pete's**	**Neil's**
Cladophora sp.	✓	✓	✓	✓	✓
Ulva flexuosa			✓		
Boodlea sp.			✓		
Caulerpa chemnitzia					✓
Hypnea sp.			✓		
Pink benthic biolayer		✓	✓		
Detritus	✓	✓	✓	✓	✓
A. marina leaves	✓	✓	✓	✓	✓
A. marina pneumatophores	✓	✓	✓	✓	✓
Halodule univervis			✓		✓
Ruppia sp.	✓	✓		✓	
Acetabularia caliculus				✓	
Goat feces		✓	✓		
Tecticornia sp.		✓	✓		
Sediment		✓	✓		
Phytoplankton		✓	✓		
Zooplankton		✓	✓		
Amphipoda	✓	✓	✓	✓	✓
Gastropoda	✓	✓		✓	✓
Diptera larvae		✓			
Polychaeta		✓	✓		
Coleoptera	✓	✓	✓		
O. mossambicus tissue	✓	✓	✓	✓	
A. caudavittata tissue	✓	✓	✓	✓	✓
C. pauciradiatus tissue 07/12	✓		✓	✓	✓
E. hawaiensis tissue			✓		

Stomach contents analysis (SCA)

Once back at the lab, each fish gut was removed from its jar and unwound so it lay in a straight line. The total length of the gut was recorded and the first 1/3 of the gut was separated for SCA. The remaining 2/3 was placed back into the jar with ethanol for storage. This front section of the gut was used because of the differences between the anatomies of the two fish species; *A. caudavittata* has a distinct stomach, which then narrows into the intestine,

while *O. mossambicus* has no distinct stomach or narrowing, and therefore did not have a distinct section to analyse. Under a dissecting microscope, the first 1/3 of the gut was carefully cut down one side with a small pair of scissors. The gut contents, which was rinsed into a petri dish marked with 50 evenly spaced points and containing 70% ethanol, were then worked apart and spread out into a single layer. The 50-point system, an adaptation of the 100-point system, was used because the stomach contents of the study species, especially the *O. mossambicus*, consisted mainly of small prey items where more than 1 item lay on each point. Furthermore, a pilot study conducted on the stomach contents of 10 *O. mossambicus* and 10 *A. caudavittata* (Appendix Tables 6.2 and 6.3) indicated that the mean percent composition for each dietary item was very similar for both methods and therefore justified using the more efficient 50 point system. The dietary items and their proportions on each point were recorded.

The frequency of occurrence (FO), i.e. the proportion of all fish in a sample that had ingested a particular dietary item, was determined for each species at each site/season (see Maddern *et al*., 2007). Using the proportional stomach content data, the percent composition of the stomach contents of each fish was determined (also referred to as the percentage volumetric contribution of each dietary item) (Maddern *et al*., 2007). To compare the dietary composition between species and sites/seasons, a similarity matrix (based on the Bray-Curtis similarity measure) was created using PRIMER v6.0 with a square-root transformation on the individual stomach contents of all fish at each site. Multi-dimensional scaling (MDS) ordination was performed on the average composition data based on the similarity matrix in PRIMER v6.0 (PRIMER-E Ltd, Plymouth Marine Laboratory, 2013). To measure the differences in the dietary compositions between species, a two-way ANOSIM (site/season and species as factors) was run on the similarity matrix in PRIMER v6.0. The ANOSIM provided a measure (test statistic R) of discrimination (between 0 and 1; with 0 equating to no

differences) between the species groups. A SIMPER analysis was also conducted on the individual dietary composition data using PRIMER v6.0.

Stable isotope analysis (SIA)

All flesh and source samples were thawed and thoroughly cleaned using forceps, brushes and deionised water. Excess water was removed and the samples were placed into labelled, plastic epindorf vials in trays. Hardyheads (*C. pauciradiatus*), collected as by-catch from Pete's, Dave's, Jack's and Neil's in July 2012 were also processed for SIA. Muscle tissue samples were removed from fish specimens over 40mm in TL (Davis *et al*., 2012), while the heads, tails, viscera and skin/scales were removed and the whole bodies used for specimens under 40mm TL (as per Doucett *et al*., 1999 and Davis *et al*., 2012). Gastropods were placed into petri dishes under a dissecting microscope and the shells removed from the flesh with forceps. The flesh was then rinsed with deionised water and placed into a vial. A second sub-sample of sources with possible high calcium carbonate contents (e.g. detritus, sediment, pink biolayer, and benthic biofilm) were acid treated to remove the inorganic carbon (see Fry 1988, Cloern *et al.*, 2002; Jacob *et al.*, 2005). A 10% solution of 1M hydrochloric acid was used, one drop at a time until the effervescing ceased (Fry 1988, Cloern *et al.*, 2002; Jacob *et al.*, 2005). The samples were placed into a centrifuge for 1 hour or until all liquid was removed. All samples were then left in a 50-60°C oven for 48-72 hours, until completely dried. The vials were placed in a ball mill until the samples were ground into a fine powder. The samples were weighed and transferred into 8x5 mm tin capsules, which were then closed and sealed. Animal samples (fish and invertebrates) were weighed to 1-1.2 mg and plant samples were weighed to 3-3.2 mg. The samples were packaged into 96 well trays and sent to the LIENs lab at La Rochelle University in France for analysis in the Delta V Advantage Isotope-Ratio Mass Spectrometer (IRMS) (Thermo Scientific, Bremen,

Germany). Results were expressed as parts per mille (‰) of the difference between the sample and the standard (Peedee belemnite limestone for $\delta^{13}C$ and N_2 for $\delta^{15}N$). Standards were analysed two per every 20 samples in the IRMS with three blanks at the start of each run (LIENs, La Rochelle University). The overall precision from this machine is approximately $0.05^0/_{00}$ for C and $0.08^0/_{00}$ (LIENs, La Rochelle University).

The $\delta^{13}C$ data for all fish samples were reciprocally transformed to achieve normality and the $\delta^{15}N$ data for all fish samples were normally distributed without transformation. The $\delta^{13}C$ and $\delta^{15}N$ data for the source material were normally distributed without transformation. Using SPSS v22.0, a series of independent sample t-tests was run on the $\delta^{13}C$ and $\delta^{15}N$ data from both species and on the $\delta^{13}C$ and $\delta^{15}N$ data from the source material to test for differences between the two sample months at Dave's. Since the results suggested no differences between months, seasonal data were combined for further tests (see Results section 3.2.2). A two-way fixed factor ANOVA was run on the $\delta^{13}C$ and $\delta^{15}N$ data from both fish species to test for differences between the sites and species.

The data were then analysed using mixing models in the program MixSIR (Semmens & Moore, 2008) to determine the probabilities of each potential dietary source contributing to the diets of each species. Firstly, the fractionation (or discrimination) factors and standard deviations for $\delta^{13}C$ and $\delta^{15}N$ for both species of fish were determined from the literature. The fractionation values ($\delta^{13}C$ = 2.05‰, $\delta^{15}N$ = 1.74‰) and standard deviations ($\delta^{13}C$ = 1.03‰, $\delta^{15}N$ = 1.93‰) for *O. mossambicus* were calculated using an average of the values determined for *Oreochromis niloticus* (Nile tilapia) from the studies by Frocken (2001) and by Gaye-Siesseggar *et al.* (2003, 2004a and 2004b) (as presented in the meta-analysis by Post, 2002). The fractionation values ($\delta^{13}C$ = 1.73‰, $\delta^{15}N$ = 3.97‰) and standard deviations ($\delta^{13}C$ = 0.89‰, $\delta^{15}N$ = 2.19‰) for *A. caudavittata* were calculated by averaging the values calculated for *Fundulus heteroclitus* from various types of diets in the study by Eldson *et al.*

(2010). Although this is an unrelated species of fish, they are similar in their feeding behaviours, diets and sizes. Along with the appropriate fractionation value and standard deviation, the $\delta^{13}C$ and $\delta^{15}N$ values for each fish species at each site, and the average for $\delta^{13}C$ and $\delta^{15}N$ and standard deviation values for each likely food source were incorporated into the MixSIR analyses. The program was run at the appropriate number of iterations to satisfy the mixing model assumptions, this number varied from 10,000 to 10,000,000. For all sites but Dave's, there was no sample replication of invertebrates. However, each sample contained many individuals and therefore each sample effectively provided an average for several to many individuals. Therefore, the $\delta^{13}C$ and $\delta^{15}N$ values for the invertebrates were included in the mixing models. The standard deviation values for the invertebrates were calculated using the standard deviation values from Dave's in relation to the $\delta^{13}C$ and $\delta^{15}N$ values at each site. The mixing models were created for each species at each site. They were also run on the detritus $\delta^{13}C$ and $\delta^{15}N$ values with mangrove leaves, algae, seagrass and biofilm as possible sources, in order to determine the composition of the detritus.

3. Results

3.1 Fish community structures in Lake MacLeod

3.1.1 Water chemistry in fish habitats

In general, water temperature was higher at the vents and declined in the nearshore and pneumatophore habitats (Tables 3.1 and 3.2). At the vents, water temperature ranged from 25.1°C at Jack's to 28.3°C at Dave's. The dissolved oxygen percentage and concentration of the water were generally lower at the vents and increased in the nearshore habitats and pneumatophores areas. While varying slightly from site to site, pH (ranging from 6.28 to 7.95) and salinity (ranging from 32.5 to 38.4‰), remained relatively stable within each site. The water in the vents was generally clearer (less turbid) than the water in the nearby nearshore and pneumatophore habitats.

Table 3.1 Summary of water depth of each habitat and water quality parameters from each habitat at the study sites in July 2012, including salinity, temperature, pH and dissolved oxygen levels from all habitats at each study site.

Site	Habitat	Depth (m)	Time	Temperature (°C)	pH	DO%	DO (mg/L)	Salinity (‰)	Water clarity (1-5)
Pete's	Vent	1	16:15	26.95	6.99	52	-	32.6	2
	Nearshore	1	16:10	21.3	7.195	77.5	-	38.4	2
	Pneumatophore	0.3	16:20	23.6	7.27	104	-	35.7	2
Jana's	Vent	1.5	13:28	25.95	7.49	34	2.405	35.9	2
	Nearshore	0.6	13:35	25.85	7.475	45.5	3.61	35.9	5
	Pneumatophore	0.3	13:30	26	7.77	61	4.05	37.25	3
Neil's	Vent	1.8	9:24	26.7	6.36	21.5	1.75	36.3	1
	Nearshore	1.6	9:40	26.75	6.66	26.5	2.115	34.3	1
	Pneumatophore	0.5	12:05	25	7.37	80	6.27	36.1	2
Dave's	Vent	1.5	8:50	27.3	7	33	2.625	32.5	1
	Nearshore	1.2	10:23	22.9	7.385	61.5	5.045	36.4	5
	Pneumatophore	0.5	9:15	24.5	7.23	54	4.62	35	2
Harjie's	Vent	1.5	13:01	27.3	7.11	48.5	3.925	33	2
	Pneumatophore	0.1	14:00	25.1	7.85	225	17.9	35.1	2
Whistler's	Vent	2	11:00	25.9	6.28	-	-	33.4	3
	Nearshore	0.75	10:00	24.6	7.44	73	5.9	34.4	2
	Pneumatophore	0.4	13:30	24.7	7.37	31	2.69	34.4	3
Jack's	Vent	5	11:15	25.1	6.665	40	3.665	34.1	1
	Nearshore	1	11:30	24.9	7.405	49	4.195	34.1	5
	Pneumatophore	0.3	10:00	23.7	7.95	81	6.95	34.1	3

Table 3.2 Summary of water depth of each habitat and water quality parameters from each habitat at the study sites in November 2012 and April 2013, including salinity, temperature, pH and dissolved oxygen levels from all habitats at each study site.

Site	Habitat	Depth (m)	Time	Temperature (°C)	pH	DO%	DO (mg/L)	Salinity (ppt)
Pete's 11/12	Vent	1	11:00	26.7	7.02	52.5	3.99	32.5
	Nearshore	1	11:04	21.1	7.21	80.1	6.32	35.5
	Pneumatophore	0.25	11:05	22.7	7.54	82.4	6.73	36.1
Neil's 11/12	Vent	2	14:05	26.5	6.45	32.9	2.21	36.7
	Nearshore	1.5	14:10	26.3	6.99	53.5	4.42	35.2
	Pneumatophore	0.5	14:45	25.1	7.25	75.1	6.22	35.6
Dave's 11/12	Vent	1	10:30	27.1	6.90	33.9	2.60	34.5
	Nearshore	0.25	10:31	23.2	7.01	45.9	3.60	33.7
	Pneumatophore	0.30	11:40	23.2	6.91	41.5	3.03	33.9
Dave's 04/13	Vent	1	9:30	28.3	6.89	34.55	2.72	33.3
	Nearshore	0.25	9:35	28.9	6.99	74.4	5.77	33.1
	Pneumatophore	0.30	9:40	28.0	6.91	45.5	3.58	33.6
Jack's 04/13	Vent	1	13:15	25.1	7.31	33.25	2.23	35.7
	Nearshore	1	12:30	25.4	7.29	58.6	4.79	35.4

3.1.2 Fish community structures

Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852), yellowtail grunters *Amniataba caudavittata* (Richardson, 1845), few-ray hardyheads *Craterocephalus pauciradiatus* (Gunther, 1861), and giant herring *Elops hawaiensis* (Regan, 1909) were the four fish species recorded within the ponds sampled at Lake MacLeod (Table 3.3). *O. mossambicus*, *A. caudavittata*, and *C. pauciradiatus* were found in various habitats at most ponds. *E. hawaiensis* was only observed in one habitat at one site (vent habitat at Jack's).

Table 3.3 Summary of species caught during the study, with their common and family names, and the habitats in which they were caught (or observed only).

Species name	Common name	Family	Habitats
Oreochromis mossambicus (Peters, 1852)	Mozambique tilapia, Mozambique mouthbrooder	Cichlidae	Pneumatophores Vents (Nearshore)
Amniataba caudavittata (Richardson, 1845)	Yellowtail grunter, Yellowtail trumpeter	Terapontidae	Pneumatophores Nearshore Vents
Craterocephalus pauciradiatus (Gunther, 1861)	Few-ray hardyhead	Atherinidae	Pneumatophores Nearshore Vents
Elops hawaiensis (Regan, 1909)	Giant herring	Elopidae	Vent

Using the fyke net, only *A. caudavittata* and *C. pauciradiatus* were caught in the pneumatophore habitat, while *A. caudavittata*, *C. pauciradiatus*, *O. mossambicus*, and *E. hawaiensis* were observed using the RUV within the vent habitat but not all species were seen at each site (Table 3.4). Video footage of nearshore habitat was obtained from Pete's, Neil's and Whistler's, but not from the other four sites (Dave's, Jack's, Jana's, and Harjie's) as the water was too turbid to be analysed. Two species (*A. caudavittata* and *C. pauciradiatus)* were observed in the footage of nearshore habitats, however, both species were not observed at all three sites (Table 3.4).

In the pneumatophore habitats, both *A. caudavittata* and *C. pauciradiatus* were netted at every site (Figure 3.1). Based on a single sample from each site, *C. pauciradiatus* generally had higher abundances (ranging from 118-514) (abundance per net area of 187m^2) than *A. caudavittata* (ranging from 3-236) (Table 3.4 and Figure 3.1). However, at Jana's, *A. caudavittata* was observed at its highest abundance of any pneumatophore habitat and had a higher abundance than *C. pauciradiatus*, which was observed at its lowest abundance of any pneumatophore site (236 and 118, respectively) (Table 3.4 and Figure 3.1). In the pneumatophore habitat at Jack's, *C. pauciradiatus* was observed at its highest abundance of any site and *A. caudavittata* was observed at its lowest abundance (514 and 3, respectively) (Figure 3.1). Over all sites, *C. pauciradiatus* had a significantly higher abundance than *A. caudavittata* ($F=7.021$, $p=0.021$, $df=1$).

In the vent habitat, *A. caudavittata* was observed at every site and often at the highest abundance of any species. For this species, the MaxN ranged from 11-46 depending on the site, while *O. mossambicus* generally had a lower MaxN than *A. caudavittata*, excluding Jack's, and ranged from 3 to 101 (Table 3.4 and Figure 3.2a). However, in the vent habitats across all sites, there were no significant differences between the MaxN for *A. caudavittata* and the MaxN for *O. mossambicus* ($F=2.184$, $p=0.165$, $df=1$). *O. mossambicus* was observed

at four sites, whereas *C. pauciradiatus* was only observed at two sites in the vent habitats and at very low abundances (6-10) (Table 3.4 and Figure 3.2a). Similarly, *E. hawaiensis* was only observed at Jack's vent with a MaxN of 1.

More limited RUV sampling was available for the nearshore habitat due to the turbity of the water; *A. caudavittata* was observed at each of the three sites at abundances ranging from a MaxN of 5-114 (Table 3.4 and Figure 3.2b). *C. pauciradiatus* was only observed at one nearshore site with a MaxN of 27 (Table 3.4 and Figure 3.2b). No other recordings are available from the RUV in this habitat, although *O. mossambicus* was observed whilst snorkelling around the nearshore habitat of some sites.

Table 3.4 Summary of the abundances or MaxN, average total lengths, and total length ranges for each species observed in each habitat in July 2012.

Site	Species	Technique	Habitat	Abundance or MaxN (from1 screen)	Average TL (mm)	TL range (mm)
Pete's	*A. caudavittata*	Netting	Pneumatophores	32	66.37	42-104
	C. pauciradiatus			188	24.87	20-41
Dave's	*A. caudavittata*			94	81.38	39-126
	C. pauciradiatus			131	26.67	20-53
Harjie's	*A. caudavittata*			182	71.83	39-97
	C. pauciradiatus			230	33.67	23-45
Jack's	*A. caudavittata*			3	45.00	43-46
	C. pauciradiatus			514	30.30	22-45
Jana's	*A. caudavittata*			236	94.90	76-135
	C. pauciradiatus			118	20.67	17-25
Neil's	*A. caudavittata*			19	87.25	54-158
	C. pauciradiatus			290	36.34	26-58
Whistler's	*A. caudavittata*			84	68.77	32-185
	C. pauciradiatus			311	38.87	25-60
Pete's	*A. caudavittata*	Videography	Vents	34	98.11	59-130
	O. mossambicus			10	126.88	85-174
Dave's	*A. caudavittata*			20	55.63	24-89
	O. mossambicus			15	57.40	48-69
	C. pauciradiatus			10	22.00	20-25
Harjie's	*A. caudavittata*			28	60.35	45-77
Jack's	*A. caudavittata*			44	105.75	98-121
	O. mossambicus			101	367.96	341-385
	E. hawaiensis			1	553	-
Jana's	*A. caudavittata*			46	75.44	54-95
	O. mossambicus			7	138.67	129-145
Neil's	*A. caudavittata*			14	106.75	99-121
	O. mossambicus			4	71.75	61-78
	C. pauciradiatus			6	-	-
Whistler's	*A. caudavittata*			11	113.33	105-122
Pete's	*A. caudavittata*		Nearshore	5	120.33	118-124
	C. pauciradiatus			27	21.18	19-24
Neil's	*A. caudavittata*			114	112.17	87-141
Whistler's	*A. caudavittata*			22	81.44	54-110

Table 3.5 Kolmogov-Smirnov results for differences in the total lengths of the fish from the pneumatophore and vent habitats, sampled in July 2012.

Test groups	N	Z value	Sig
A. caudavittata and *C. pauciradiatus* Netting - Pneumatophores	400	9.101	< 0.001
A. caudavittata and *O. mossambicus* Videos - Vents	110	3.643	< 0.001

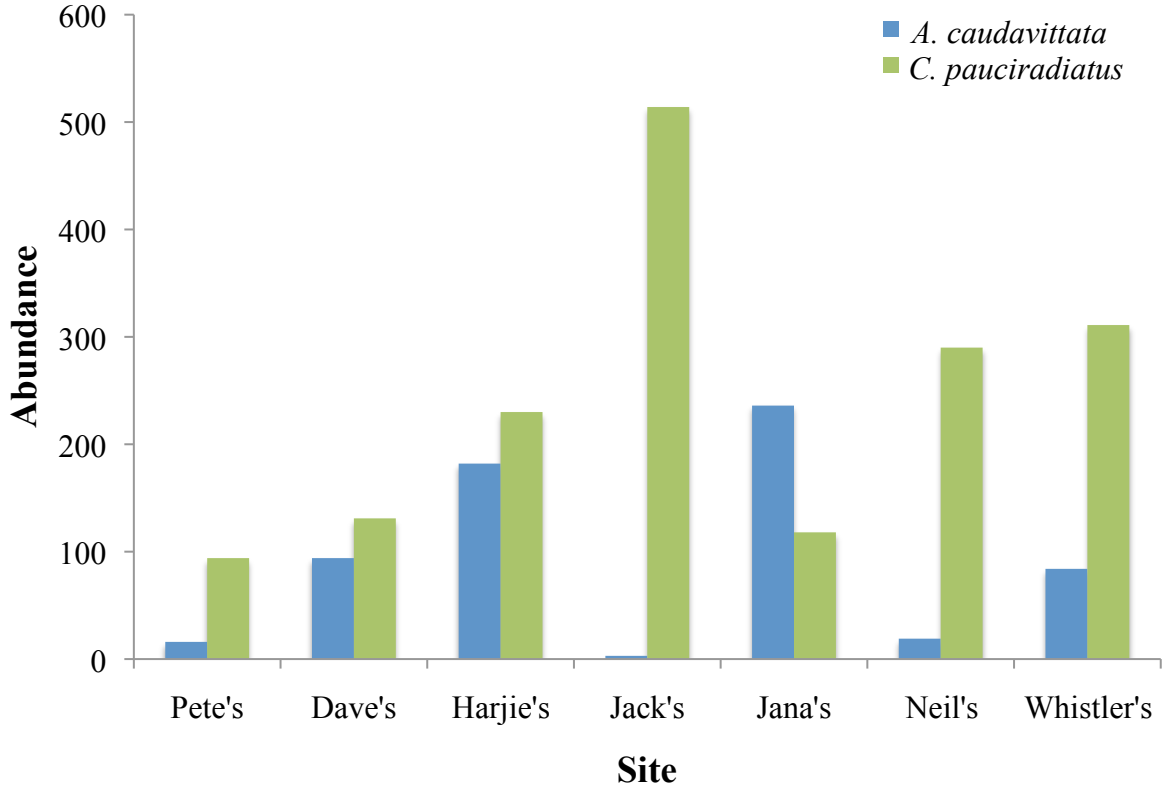

Figure 3.1 Number of fish of each species caught in one sweep of the fyke net (abundance per net area of 187m^2) in the pneumatophore habitats of the 7 sample sites.

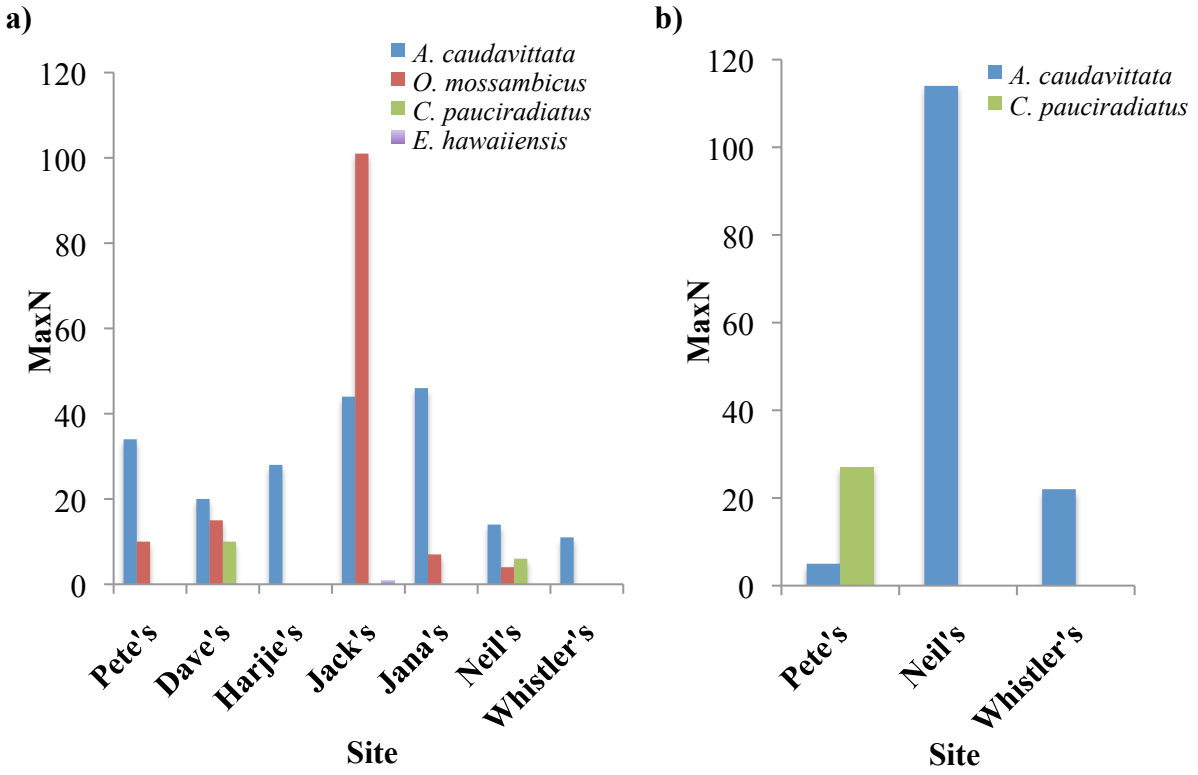

Figure 3.2 The maximum number of fish (MaxN) of each species appearing on one screen of the RUV footage at one for: the vents of the 7 study sites (**a**), and the nearshore habitats of 3 study sites (**b**).

Whenever they were caught together, *A. caudavittata* had a larger average total length (TL) than *C. pauciradiatus* (Table 3.4; see also the length frequency distributions in Figure 3.3), significantly so in the pneumatophore habitat at all sites ($Z=9.101$, $p<0.001$) (Table 3.5). The average TLs and the length frequency distributions of *A. caudavittata* in the vents were not uniform among sites (Table 3.4, Figure 3.3). Comparisons between *O. mossambicus* and *A. caudavittata* showed that the former species had a higher average TL than *A. caudavittata* at most vents (Table 3.4), however, the length frequency distributions varied between sites (Figure 3.3). *O. mossambicus* was larger in Jack's vent than any other site or habitat sampled (Table 3.4, Figure 3.3). The only species with a higher TL than *O. mossambicus* was *E. hawaiensis*, (observed on the video footage in only one vent at a very low density) (Table 3.4). Over all the vents, *O. mossambicus* was significantly larger than *A. caudavittata* ($Z=3.643$, $p<0.001$) (Table 3.5; Figure 3.4).

In summary, *O. mossambicus* was the largest of the three main fish species known to occur at more than one study site and had an overall total length range of 48-385 mm (Table 3.4). *O. mossambicus* could be put into three different size cohorts, the cohort at Jack's being conspicuously larger than at all other sites (Table 3.4; Figures 3.3). *A. caudavittata* was seen to be most consistently present among all habitats and all sites (Table 3.4). *A. caudavittata* was, on average, smaller than *O. mossambicus*, with an overall total length range of 23-185 mm, and had less distinct size cohorts, but was often smaller in the pneumatophore habitat (Table 3.4; Figure 3.4). *C. pauciradiatus*, which was mainly observed in the pneumatophores, was by far the smallest of the three main fish species, with a size range from 19-58 mm and no distinct size cohorts or distinct variation between habitats (Table 3.4; Figure 3.4).

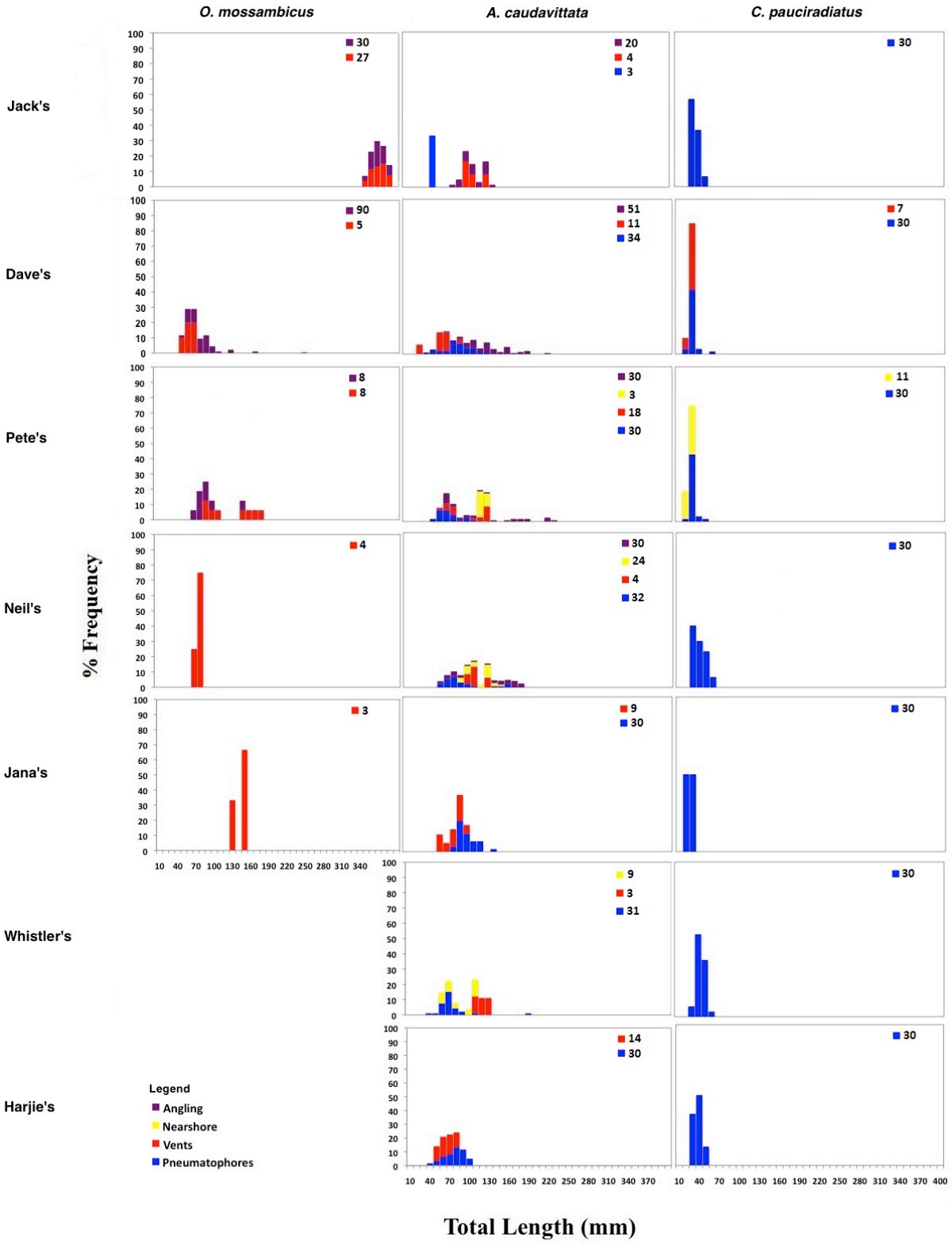

Figure 3.3 Percentage frequencies of total lengths for each species caught in each habitat/capture technique: *Oreochromis mossambicus*, *Amniataba caudavittata* and *Craterocephalus pauciradiatus* in 5mm size classes at all seven sites. Different habitats (pneumatophores, vents and nearshores) or caught for the dietary study (in traps or on fishing line) are labelled by various colours with the number of samples per method (N) displayed within each graph.

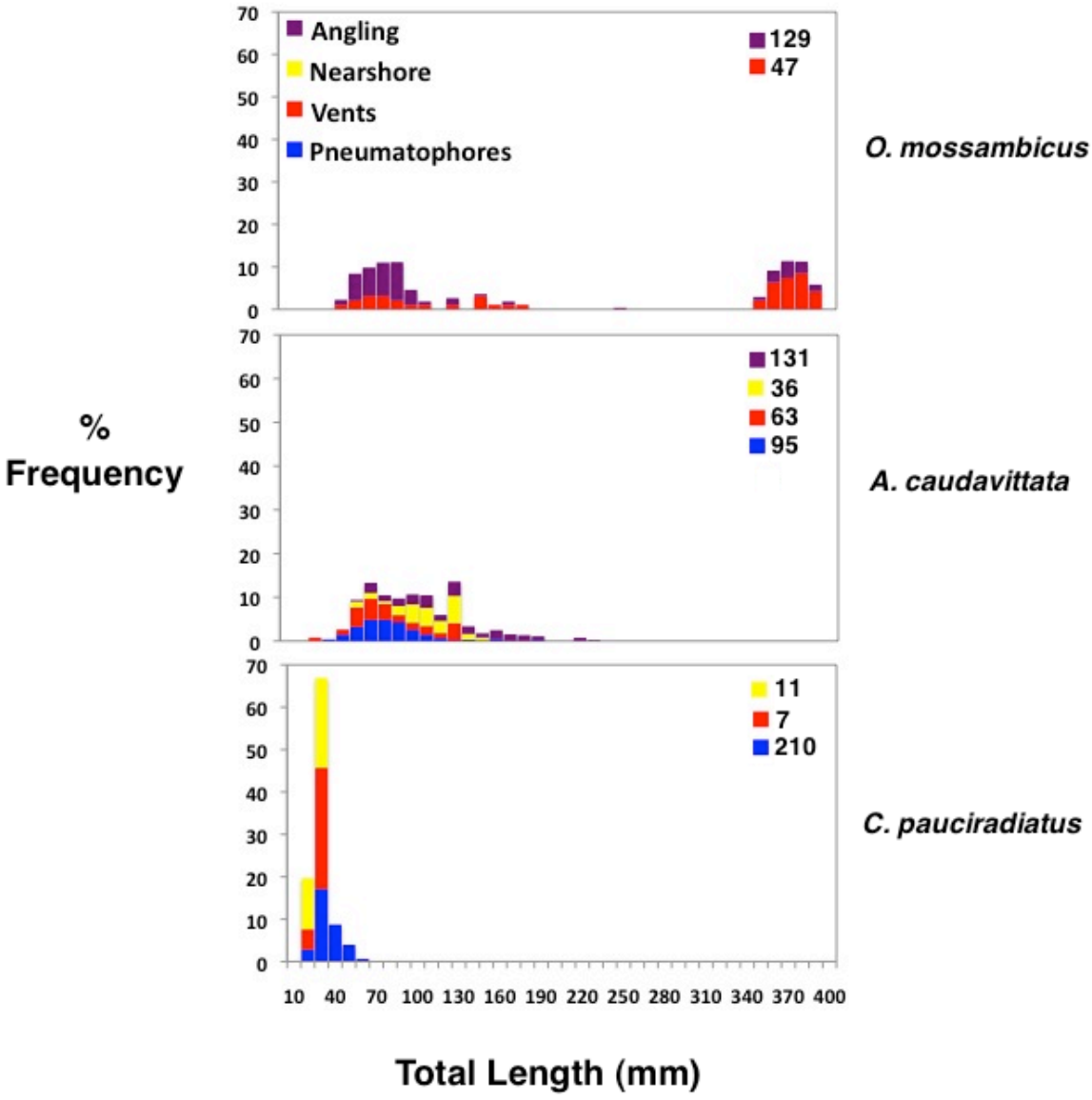

Figure 3.4 Percentage frequencies of total lengths for each species caught in each habitat:/capture technique: *Oreochromis mossambicus*, *Amniataba caudavittata* and *Craterocephalus pauciradiatus* in 5mm size classes at all seven sites combined. Different habitats (pneumatophores, vents and nearshores) or caught for the dietary study (in traps or on fishing line) are labeled by various colours with the number of samples per method (N) displayed within each graph.

3.2 Dietary study of *O. mossambicus* and *A. caudavittata*

3.2.1 Sizes of fish used for dietary analysis and feeding observations

The *A. caudavittata* individuals used for dietary studies varied in TL (ranging from 53-212 mm), although the average TL was fairly consistent (between 107-133 mm) (Table 3.6). For individuals of *O. mossambicus*, excluding Jack's where a very different size

category was observed, the TL varied more than *A. caudavittata* (45-247 mm) but the average TL was fairly consistent (71-96 mm) (Table 3.6). Jack's showed a very different size structure for *O. mossambicus* with minimal variation in TL (from 343-388 mm) and a significantly higher average TL (367.7 mm) ($Z=4.447$, $p<0.001$).

Table 3.6 Summary of the sample size and TLs of *A. caudavittata* and *O. mossambicus* caught for the dietary analysis at each site and time.

Site/Month	Species	N	Average TL (mm)	TL range (mm)
Pete's	*A. caudavittata*	30	124.5	61-229
	O. mossambicus	8	89.5	68-150
Neil's	*A. caudavittata*	30	121.0	53-179
Dave's 11/12	*A. caudavittata*	30	127.2	63-212
	O. mossambicus	33	96.0	60-247
Dave's 04/13	*A. caudavittata*	21	133.6	89-183
	O. mossambicus	58	70.7	45-122
Jack's	*A. caudavittata*	20	106.8	74-131
	O. mossambicus	30	367.7	343-388

Observations on the video footage taken in stage 1 showed that *O. mossambicus* was often selecting food items from the benthic substrate, whereas *A. caudavittata* was more often seen consuming food straight from water column. *C. pauciradiatus* was seen selecting food from the epiphytic algae on the pneumatophores and from disturbed sediment and macrophytes.

3.2.2 Stomach content analysis (SCA)

The percent composition of the stomach contents shows the percentage of the overall stomach contents made up by each item, which in turn provides evidence for which items are being consumed in what proportions (Table 3.7 and Figure 3.5). In comparison, the frequency of occurrence shows how many individuals (converted into a frequency out of 100) of fish consumed each item, which gives evidence for the variety or lack of variety in dietary items that the species are consuming (Table 3.8 and Figure 3.6).

The stomach contents of *O. mossambicus* from all sites over 2 times of the year generally showed sand/sediment at the highest percentage contribution, closely followed by diatoms (Figure 3.5a). Moderate amounts of filamentous green algae, Foraminifera, amphipods, and detritus were also recorded, along with the occasional rocks and various invertebrates. In comparison, the stomach contents for *A. caudavittata* were generally dominated by filamentous green algae with the highest percentage contribution, followed by diatoms and amphipods (Figure 3.5b). Moderate proportions of seagrass, Foraminifera, ostracods and smaller amounts of sand/sediment were recorded, and occasionally seeds and various invertebrates were noted.

Based on the data for percentage contributions, both species of fish consumed some of the same dietary items, however, most often the quantities and proportions differed between the species (Figure 3.5). *O. mossambicus* always showed a higher percentage of sand/sediment in its guts, while *A. caudavittata* consumed more filamentous green algae (Table 3.5; Figure 3.5). Both species consumed relatively moderate amounts of diatoms, while *A. caudavittata* consumed higher percentages of amphipods and seagrass, but less foraminifera and detritus than *O. mossambicus*. Both species occasionally consumed bivalves, ostracods, and gastropods (Table 3.5; Figure 3.5).

There was little variation in the percentage compositions of diets from site to site, for both species (Figure 3.5). The exception to this was the proportion of the dietary items in the guts of both species from Jack's, which varied from the other sites, although the dietary items themselves were similar. The stomach contents of *O. mossambicus* at Jack's contained slightly more filamentous green algae, amphipods, and sand/sediment and less Foraminifera than at the other sites (Table 3.5; Figure 3.5a). *A. caudavittata* from Jack's contained less filamentous green algae, seagrass and Foraminifera but more amphipods than those from the other sites (Table 3.5; Figure 3.5b).

Between the two times of year at Dave's, *O. mossambicus* showed less differences in percent composition of stomach contents; the only difference of note was a very small amount of seagrass for November 2012 samples, whereas no seagrass was recorded from April 2013 (Table 3.7; Figure 3.5a). *A. caudavittata* showed only slight variations in the proportions of the percent composition of their stomach contents between the two months sampled at Dave's (Table 3.7; Figure 3.5b). The samples from November 2012 showed a slightly higher percentage of seagrass, diatoms and Foraminifera, and a lower percentage of filamentous green algae compared to April 2013 (Table 3.7; Figure 3.5b).

The frequency of occurrence (FO) of some dietary items differed substantially between the two species of fish. *O. mossambicus* generally consumed items at a higher FO than *A. caudavittata* (Figure 3.6). However, *A. caudavittata* consumed more items overall, although often at a lower FO (Figure 3.6). Over all sites and months, almost all individuals of both species consumed filamentous green algae and diatoms (Figure 3.6). In addition, almost all *O. mossambicus* consumed amphipods, Foraminifera, detritus and sand/sediment, while approximately 30-40% ingested rocks and ostracods and only approximately 1-3% ingested seagrasses, bivalves and gastropods (Figure 3.6). *A. caudavittata* consumed amphipods and seagrasses at relatively high frequencies (72.6% and 57.3% respectively), while around/between 20 and 40% each of consumed ostracods, Foraminifera, detritus, sand/sediment, and only 2-5% of all individuals ingested bivalves, gastropods, polychaetes, dipteran larvae, and seeds (Figure 3.6).

O. mossambicus consistently consumed filamentous green algae, diatoms, amphipods, Foraminifera, detritus and sand/sediment, irrespective of site (Table 3.8). The FO of dietary items differed the most at Jacks, where almost all items that were consumed were ingested by every individual (Table 3.8). At Jack's, no fish consumed seagrass, whereas at all other sites individuals that had ingested seagrass ranged from 10 to 100% of those sampled (Table 3.8).

Rocks were observed in every individual at Jack's compared to the 0-12.5% occurrence at the other sites (Table 3.8). Similarly, gastropods were only ingested at Jack's, and not observed as a dietary item in any individuals for this species elsewhere (Table 3.8).

A. caudavittata showed a lot more variation in the FO of dietary items among sites. At most sites, excluding Jack's, this species showed high FO of filamentous green algae (93-100%) and diatoms (100%) across sites (Table 3.8). Considerably fewer individuals ingested filamentous green algae (55%) and diatoms (75%) at Jack's (Table 3.8). The FO for the other dietary items varied from site to site, but suggested that *A. caudavittata* had more variety in its diet across sites compared to *O. mossambicus*.

For *O. mossambicus*, the only item to show a considerable difference in FO between the two times of year (November and April) at Dave's was seagrass (10% and 97%) (Table 3.8). Similarly, most dietary items were consumed at relatively similar FO by *A. caudavittata* between these times (Table 3.8). The only items that showed considerable differences in the frequencies of ingestion between the two months were amphipods (70% and 5%), Foraminifera (73% and 25%) and sand/sediment (13% and 85%) (Table 3.8).

Table 3.7 Summary of the percent composition of the stomach contents (the percentage volumetric contribution of each dietary item) for *O. mossambicus* and *A. caudavittata* from all sites/seasons.

	Percent Composition								
	O. mossambicus				*A. caudavittata*				
Dietary Item	Pete's	Dave's 11/12	Dave's 04/13	Jack's	Pete's	Neil's	Dave's 11/12	Dave's 04/13	Jack's
Algae	6.08	10.26	9.54	16.65	38.45	33.98	33.28	51.42	13.38
Seagrasses	0	0.28	0	0	6.30	2.92	8.88	5.37	0.14
Diatoms	24.70	29.11	24.22	18.98	7.05	13.51	21.72	17.10	13.52
Rocks	0.13	0.03	0	1.11	0	0	0	0	0
Ostracoda	0.44	0.11	0.03	0.71	0	3.44	1.04	0.08	1.01
Amphipoda	5.15	5.44	6.80	13.76	20.11	13.72	12.31	13.46	25.49
Foraminifera	12.10	11.20	11.83	0.69	7.12	2.30	4.28	1.62	0.25
Detritus	8.69	5.79	4.01	5.48	0	2.08	1.12	0.05	0.58
Sand/Sediment	32.63	36.22	31.55	42.96	0.03	3.51	3.51	4.63	0.25
Bivalvia	0	0	0.31	0	0.63	0.38	0.65	1.56	0.25
Gastropoda	0	0	0	0.40	1.96	0.06	0.15	0	0
Diptera (Larvae)	0	0	0	0	1.17	1.10	0.07	0.81	0.93
Seeds	0	0	0	0	2.43	0.07	0.10	0	0.14
Unknown item 1	0	0	0	0	0.10	0.03	0.36	0	0
Polychaeta	0	0	0	0	0	0	0.64	0	3.44
Unidentifiable	0	0	0	0	0.23	0.37	0.65	0	0

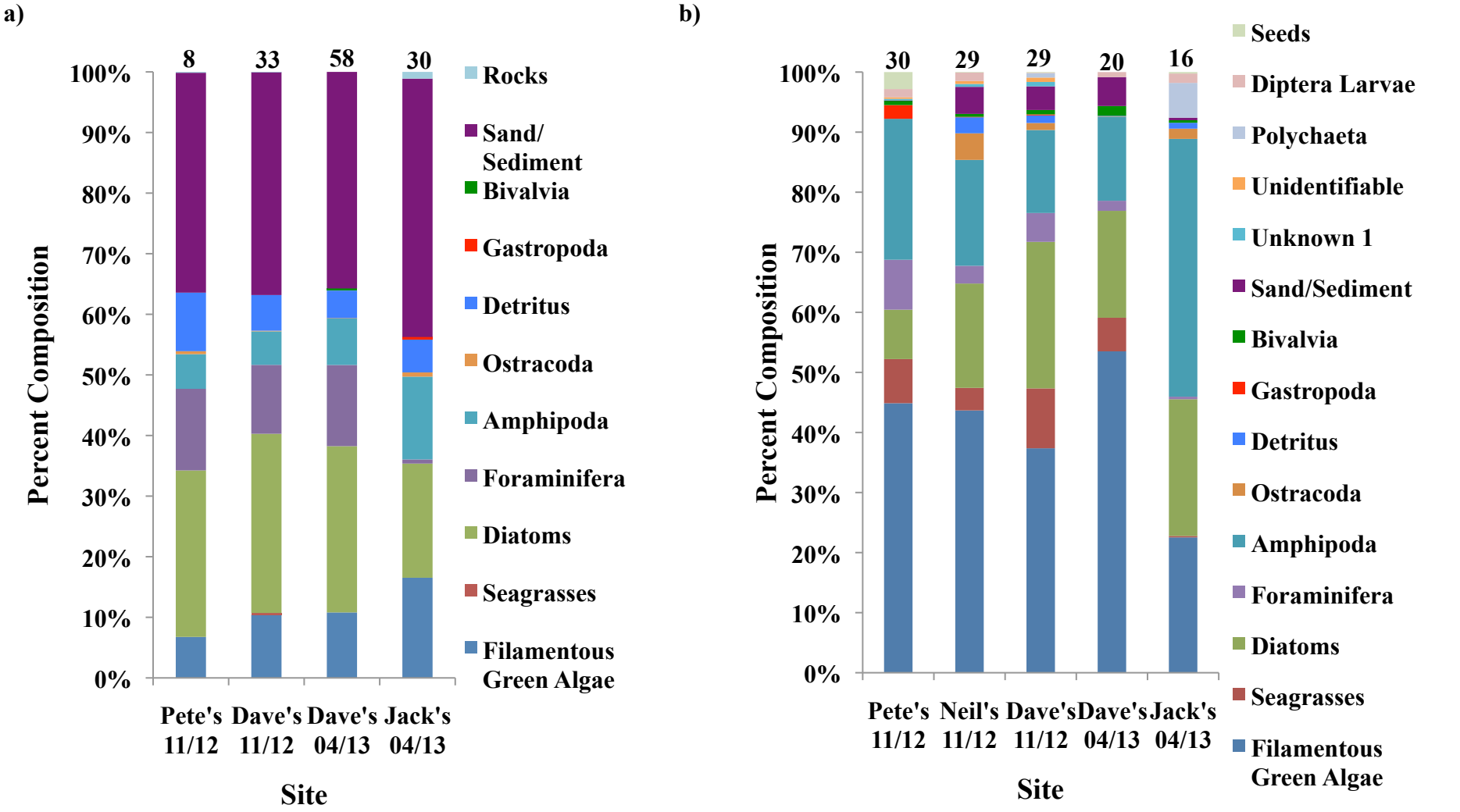

Figure 3.5 The percent composition of the stomach contents for *O. mossambicus* **(a)** and *A. caudavittata* **(b)** from a variety of sites and seasons, with sample size (N) displayed above each site.

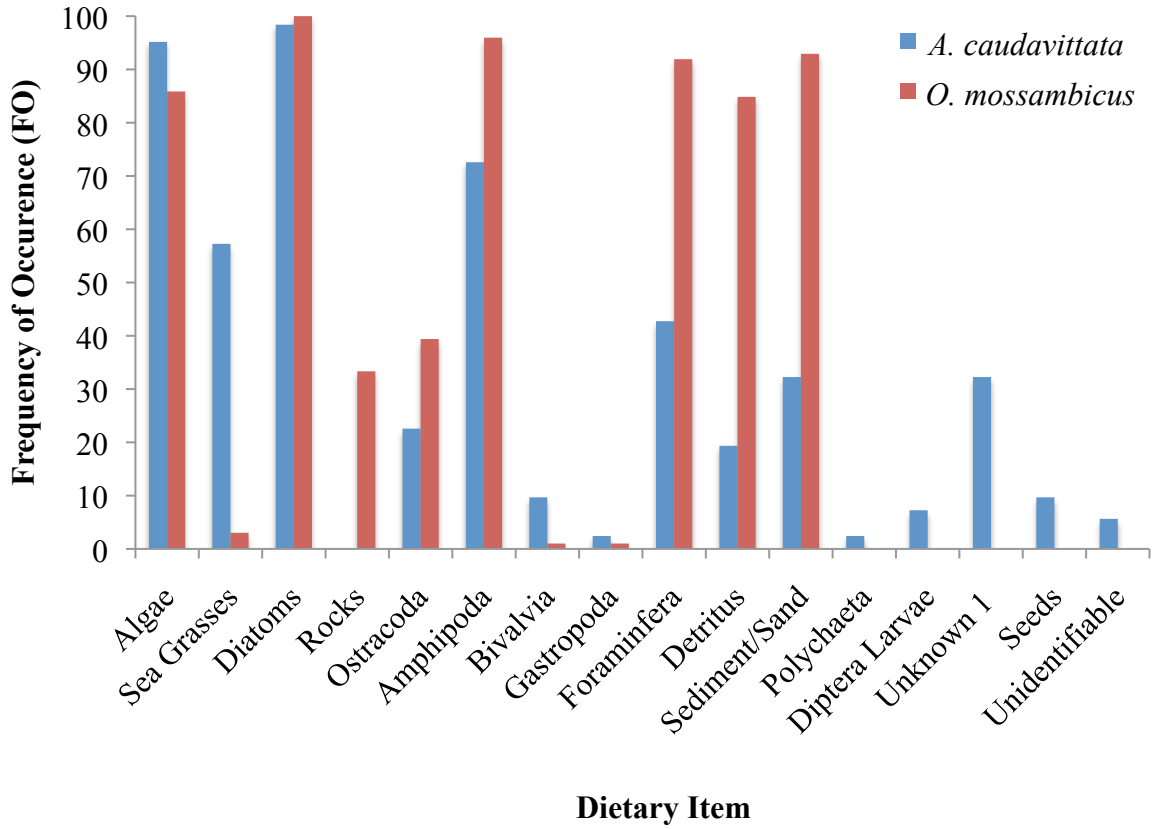

Figure 3.6 The percent frequency of occurrence of each dietary item observed in the stomach contents of *O. mossambicus* and *A. caudavittata.*

Table 3.8 Summary of the frequency of occurrence (the proportion of all fish in a sample that had ingested a particular dietary item) for *O. mossambicus* and *A. caudavittata* from all sites/seasons.

	Frequency of Occurrence								
	O. mossambicus				*A. caudavittata*				
Dietary Item	Pete's	Dave's 11/12	Dave's 04/13	Jack's	Pete's	Neil's	Dave's 11/12	Dave's 04/13	Jack's
Algae	75	67	93.5	100	93	97	93	100	55
Seagrasses	100	10	97	0	63	73	63	80	0
Diatoms	100	100	100	100	100	100	100	100	75
Rocks	12.5	3	0	100	0	0	0	0	0
Ostracoda	75	20	7	100	0	3	4	0	10
Amphipoda	100	100	93.5	100	100	20	70	5	70
Foraminifera	97.5	87	87	100	17	17	73	25	65
Detritus	75	83	71	100	37	40	37	10	5
Sand/Sediment	75	97	87	100	1	57	13	85	5
Bivalvia	0	0	3	0	7	10	10	10	5
Gastropoda	0	0	0	3	7	3	3	0	0
Diptera Larvae	0	0	0	0	0	10	3	5	10
Seeds	0	0	0	0	10	3	3	0	5
Unknown item 1	0	0	0	0	0	97	87	80	10
Polychaeta	0	0	0	0	3	0	7	0	5
Unidentifiable	0	0	0	0	0	3	3	0	5

The Multi-dimensional scaling (MDS) plot, based on the average composition within each site, showed a clear separation in the dietary composition between *O. mossambicus* and *A. caudavittata* (Figure 3.7). The ANOSIM, based on replicate data within each site, confirmed that the samples were more closely grouped according to species (R=0.724, p=0.001) than sites/seasons (R=0.316, p=0.001) (Figure 3.7). The SIMPER analysis showed a few diagnostic dietary items (Table 3.9). Most notably, when the two species were compared, sand/sediment and Foraminifera were diagnostic for the diets of *O. mossambicus,* whereas filamentous algae was diagnostic for *A. caudavittata* (Table 3.9a).

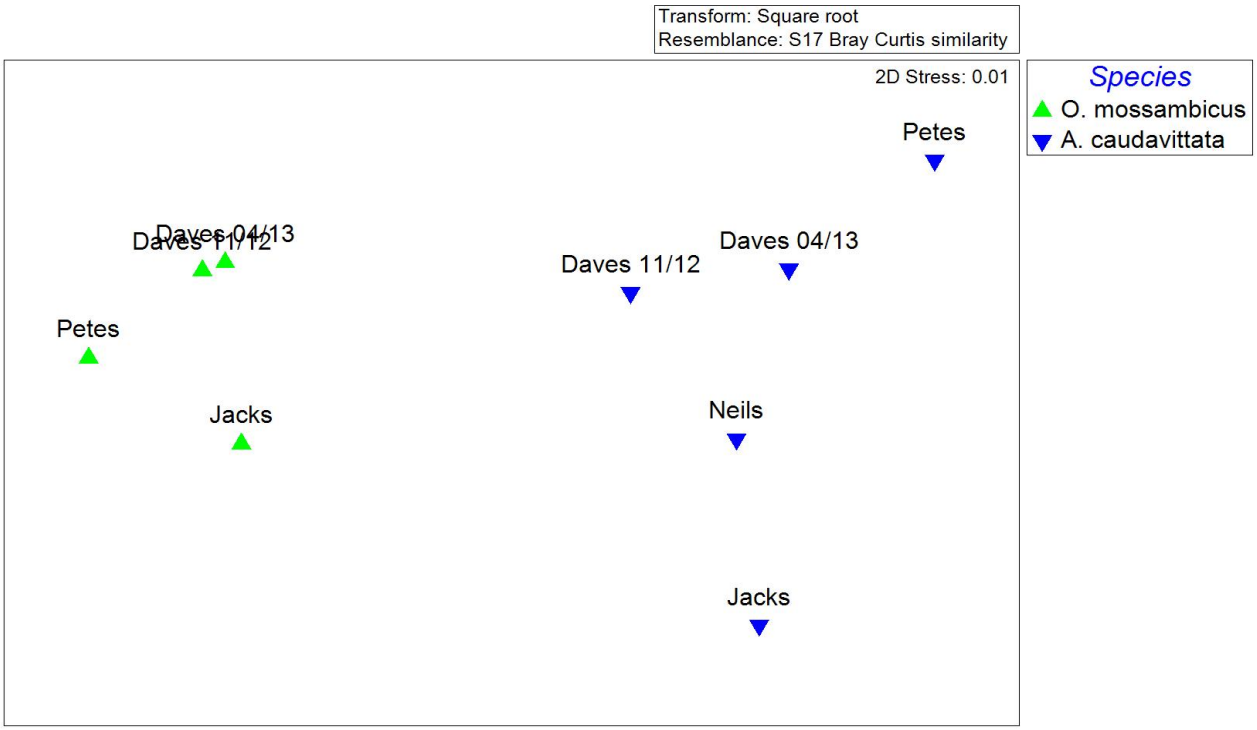

Figure 3.7 Multidimensional scaling (MDS) based on the Bray-Curtis matrix of similarity, of the percent composition of the stomach contents of each species of fish at each site, labelled by species. Samples were more closely grouped by species (R=0.724, p=0.001) than the site/season (R=0.316, p=0.001).

Table 3.9 Diagnostic species based on ratio values (Similarity/Std Dev) determined by a SIMPER analysis using the percent composition of items in the stomach contents between the two species **(a)** and the fish collected at each site **(b)**, with individual fish as replicates. Dietary items with ratio values greater than or equal to 1.5 are presented. Dietary items for each pair-wise comparison in the matrix are diagnostic of the site/season or species labelled at the top of each column.

(a)

	O. mossambicus		*A. caudavittata*	
	Dietary Item	Sim/SD	Dietary Item	Sim/SD
O. mossambicus			Filamentous Algae	1.6
A. caudavittata	Sand/Sediment	2.2		
	Foraminifera	1.5		

(b)

	Neil's		Dave's 11/12		Dave's 04/13		Jack's	
	Dietary Item	Sim/SD	Dietary Item	Sim/SD	Dietary Item	Sim/SD	Dietary Item	Sim/SD
Pete's	Diatoms (elliptical)	1.6			Diatoms (elliptical)	1.5	Diatoms (elliptical)	1.5
Neil's					Sand/ Sediment	1.9		
Dave's 11/12								
Dave's 04/13							Rocks	1.6
Jack's			Foraminifera	1.82	Foraminifera	1.8		
			Diatoms (round)	2.25	Diatoms (round)	1.9		

3.2.2 Stable Isotope Analysis

The stable isotope analysis (SIA) was conducted on the same samples as the stomach contents analysis. Firstly, the differences in stable isotope signatures between the two months sampled at Dave's were compared, then the different sites were investigated, followed by a comparison between species. A mixing model analysis was conducted on the SIA results, also comparing sites and species.

Differences between months

For both species, no significant differences were seen, neither for the $\delta^{13}C$ values ($F=0.760$, $p=0.389$, $df=35$) nor for the $\delta^{15}N$ values ($F=0.161$, $p=0.691$, $df=35$) between the two months at Dave's (Table 3.10). Of the sources that were sampled at Dave's at each time of year, only one (detritus) showed a statistically significant difference in isotopic signature

between months, and this difference was only $\delta^{13}C$ values (F=36.281, p=0.002, Table 3.11). The difference between the mean $\delta^{13}C$ values of the detritus was very low (0.53‰), and this, combined with the overall lack of seasonal differences for both $\delta^{13}C$ and $\delta^{15}N$, has been interpreted as evidence for the homogeneity of data for the fish and the sources of food across months. Accordingly, seasonal variation in the data was determined to be minimal and for the remainder of the study, data from both months at Dave's were pooled.

Table 3.10 Results of the independent samples t-test run on the $\delta^{13}C$ and reciprocally transformed $\delta^{15}N$ values from both species of fish comparing the two seasons at Dave's (November 2012 and April 2013).

Season	Isotope	N	Mean	Std deviation	Std Error	d.f.	F	Sig.
Dave's 11/2012	$\delta^{13}C$	20	-21.10	1.102	0.25	35	0.760	0.389
Dave's 04/2013		17	-21.06	0.902	0.22			
Dave's 11/2012	$\delta^{15}N$	20	11.22	1.039	0.002	35	0.161	0.691
Dave's 04/2013		17	11.39	1.263	0.002			

Table 3.11 Summary of descriptive statistics and results of independent sample t-tests for the $\delta^{15}N$ and $\delta^{13}C$ signatures of source material from Dave's 11/12 and Dave's 04/13.

Source material	Variable	Season	Mean	Std. error	F	Sig.
Cladophora sp.	$\delta^{15}N$	11/12 04/13	5.72 4.48	0.37 0.73	2.329	0.165
	$\delta^{13}C$	11/12 04/13	-22.51 -25.91	1.73 2.91	1.008	0.345
A. marina leaves	$\delta^{15}N$	11/12 04/13	4.72 6.07	0.72 1.20	0.925	0.364
	$\delta^{13}C$	11/12 04/13	-26.54 -27.37	0.69 1.16	1.92	0.203
A. marina pneumatophores	$\delta^{15}N$	11/12 04/13	2.77 3.42	0.82 1.36	0.168	0.693
	$\delta^{13}C$	11/12 04/13	-25.41 -24.53	0.33 0.66	1.422	0.267
Detritus	$\delta^{15}N$	11/12 04/13	4.90 4.61	0.71 0.23	0.294	0.611
	$\delta^{13}C$	11/12 04/13	-25.41 -25.94	0.06 0.05	36.281	0.002
Ruppia sp.	$\delta^{15}N$	11/12 04/13	3.44 2.67	0.37 0.69	0.956	0.357
	$\delta^{13}C$	11/12 04/13	-14.89 -14.55	0.63 2.01	0.433	0.529

Differences between species

At each site, the mean $\delta^{15}N$ was significantly higher for *A. caudavittata* compared to *O. mossambicus* (F=376.192, p<0.001) (Figure 3.8 and Table 3.12), while the mean $\delta^{13}C$ was

not significantly different between the two species (F=1.252, p=0.266) (Figure 3.8 and Table 3.12). For both species, the $\delta^{15}N$ (F=67.404, p<0.001) and $\delta^{13}C$ (F=293.803, p<0.001) signatures of the fish showed a significant difference between sites (Table 3.12). All sites were significantly different in their $\delta^{13}C$ signatures, with the lowest $\delta^{13}C$ values at Jack's being obvious (Figure 3.8). The $\delta^{15}N$ signatures only differed significantly between some sites (Table 3.13 and Figure 3.8). Dave's and all other sites showed significantly different $\delta^{15}N$ signatures, and a significant difference was also seen between Neil's and Pete's (Table 3.13).

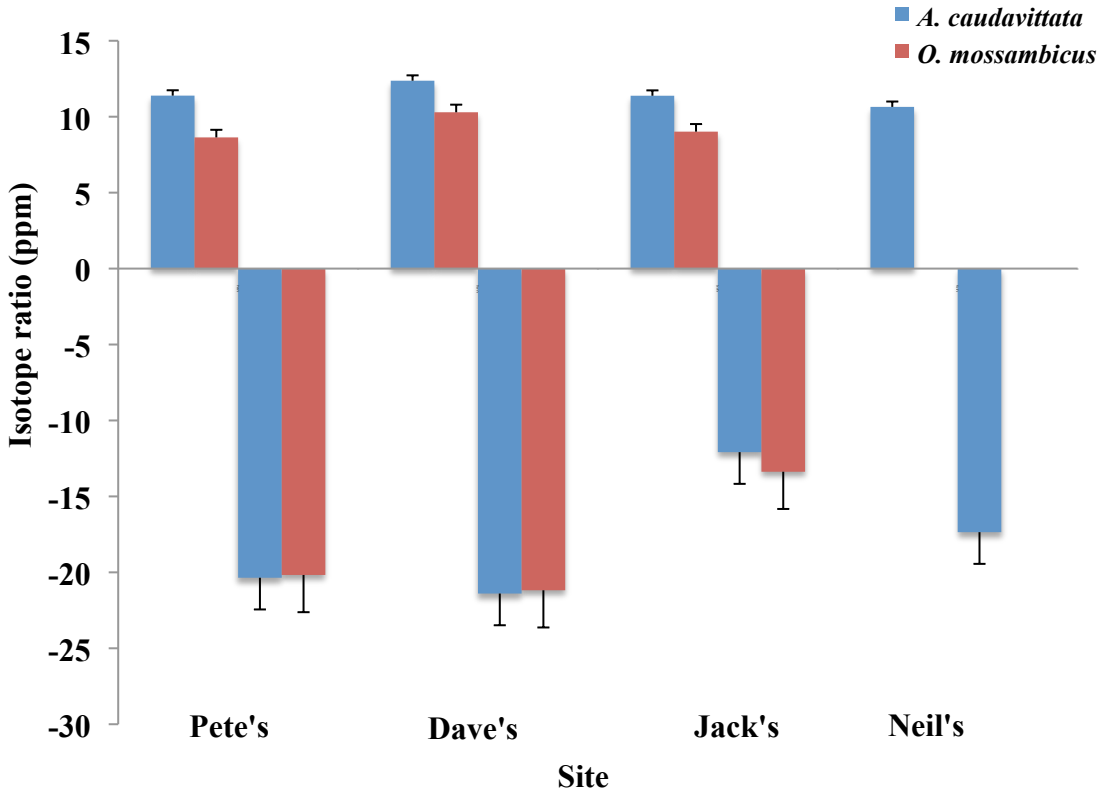

Figure 3.8 The average (±SE) isotopic $\delta^{15}N$ (above the horizontal) and $\delta^{13}C$ (below the horizontal) signatures of *A. caudavittata* and *O. mossambicus* at each site.

Table 3.12 Results of 2-way fixed factor ANOVA for the δC and square root transformed $\delta^{15}N$ values for fish.

	Isotope	d.f.	F	Sig
Species	$\delta^{13}C$	1	1.252	0.266
Site		3	293.803	<0.001
Species	$\delta^{15}N$	1	376.192	<0.001
Site		3	67.404	<0.001

Table 3.13 Tukey's post hoc results of the 2-way fixed factor ANOVA for the $\delta^{15}N$ and $\delta^{13}C$ values for both fish species between all sites.

Site		$\delta^{13}C$		$\delta^{15}N$	
		Std. Error	Sig.	Std. Error	Sig.
Dave's	Jack's	0.29683	0.000	0.03622	0.000
	Neil's	0.39612	0.000	0.04833	0.000
	Pete's	0.31757	0.011	0.03953	0.000
Jack's	Dave's	0.29683	0.000	0.03622	0.000
	Neil's	0.42835	0.000	0.05226	0.080
	Pete's	0.35696	0.000	0.04425	0.920
Neil's	Dave's	0.39612	0.000	0.04833	0.000
	Jack's	0.42835	0.000	0.05226	0.080
	Pete's	0.44297	0.000	0.05461	0.029
Pete's	Dave's	0.31757	0.011	0.03953	0.000
	Jack's	0.35696	0.000	0.04425	0.920
	Neil's	0.44297	0.000	0.05461	0.029

Isotope ratios relative to potential dietary sources

At Dave's, Jack's and Neil's, both fish species showed higher mean $\delta^{15}N$ values than any source collected (Figure 3.9). For Pete's, the epiphytic alga, *Acetabularia caliculus*, showed an average $\delta^{15}N$ signature almost identical to *O. mossambicus* (Figure 3.9).

At Pete's and Dave's, the mean $\delta^{13}C$ values of both *A. caudavittata* and *O. mossambicus* were relatively mid-way in the range of the $\delta^{13}C$ signatures of the potential source material, implying that the sources collected are likely to have contributed to the diets of the fish (Figures 3.9). In comparison, the mean $\delta^{13}C$ values of the fish at Neil's and, even more so at Jack's, were skewed to the right, showing mean $\delta^{13}C$ values higher than most or all of the potential source material (Figures 3.9). *Cladophora* sp. showed signatures also skewed in the same direction at these sites, and at Jack's, both the seagrass *Halodule univervis* and amphipods were skewed in the same direction (Figure 3.9).

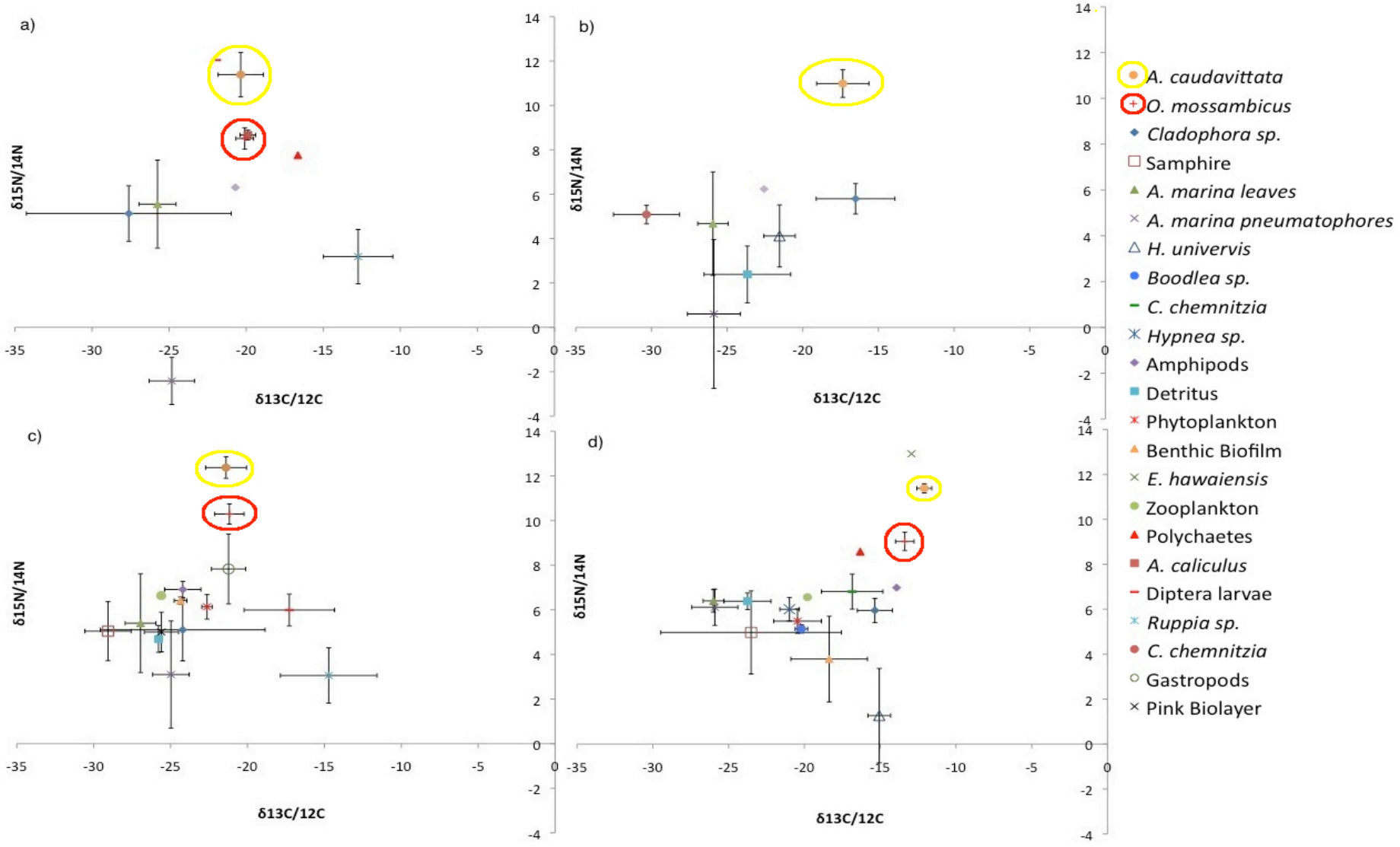

Figure 3.9 Scatterplot showing mean $\delta^{15}N$ vs mean $\delta^{13}C$ with standard deviation for fish and source material from Pete's **(a)**, Neil's **(b)**, Dave's **(c)** and Jack's **(d)**. Lack of replicates available is indicated where standard deviation bars are absent.

Based on mixing model outputs, both *A. caudavittata* and *O. mossambicus* at Dave's appeared to have similar dietary compositions, although both species had a high degree of uncertainty (Figure 3.10). Both species showed a probability (albeit highly uncertain) that amphipods and, to a slightly lesser extent, gastropods contribute a large proportion (median = ~0.5-0.6) to their diets, while they had a high probability of *Cladophora* sp., *Ruppia* sp., *A. marina* leaves, biofilm, and dipteran larvae contributing almost nothing to their diets (median <0.1) (Figure 3.10). Greater uncertainty in the predicted contributions of sources in the diets of fish was present at Dave's.

At Jack's, both *A. caudavittata* and *O. mossambicus* showed a high probability, with a high level of certainty, that amphipods contributed a moderate to large proportion (Median = ~0.80 and 0.55, respectively) to their diets (Figure 3.11). *Hypnea* sp., *A. marina* leaves, *H. univervis,* and biofilm contributed very little to the diets of both species (median <0.1) (Figure 3.11), while *Cladophora* sp. and *U. flexuosa* made greater contributions albeit with low levels of certainty to the diets of *O. mossambicus*.

The diets of *A. caudavittata* and *O. mossambicus* at Pete's contained fairly high levels of uncertainty, but again were fairly similar, although all sources collected appeared to contribute little to their diets (Figure 3.12). *Cladophora* sp. and dipteran larvae likely contributed small proportions to the diets of *A. caudavittata* (median = ~0.2-0.3) compared to *O. mossambicus*, while *A. caliculus*, *A. marina* leaves and amphipods likely contributed similar proportions to the diets of the latter species (Figure 3.12). All the other sources collected showed fairly low levels of certainty with extremely low possible contributions to either species' diet. Overall, both species at Pete's showed the lowest likely contribution of amphipods to their diets at any site.

At Neil's, there was a high probability, with a high degree of certainty, that *C. chemnitzia* (a green alga only collected at Neil's) contributed a high proportion (0.7) to the

diet of *A. caudavittata* (Figure 3.13). There was a high probability that *Cladophora* sp., *A. marina* leaves and *H. univervis* made very small contributions to the diet of this species (<0.1) (Figure 3.13).

The detritus samples from Dave's, Neil's and Jack's showed, with high levels of certainty, that the *A. marina* leaves contributed the highest proportion of the sources that make-up the detritus (Figure 3.14). Although there was no detritus sample taken from Pete's, the consistency of these results at the other sites suggests the make-up of the detritus would not differ much at other sites.

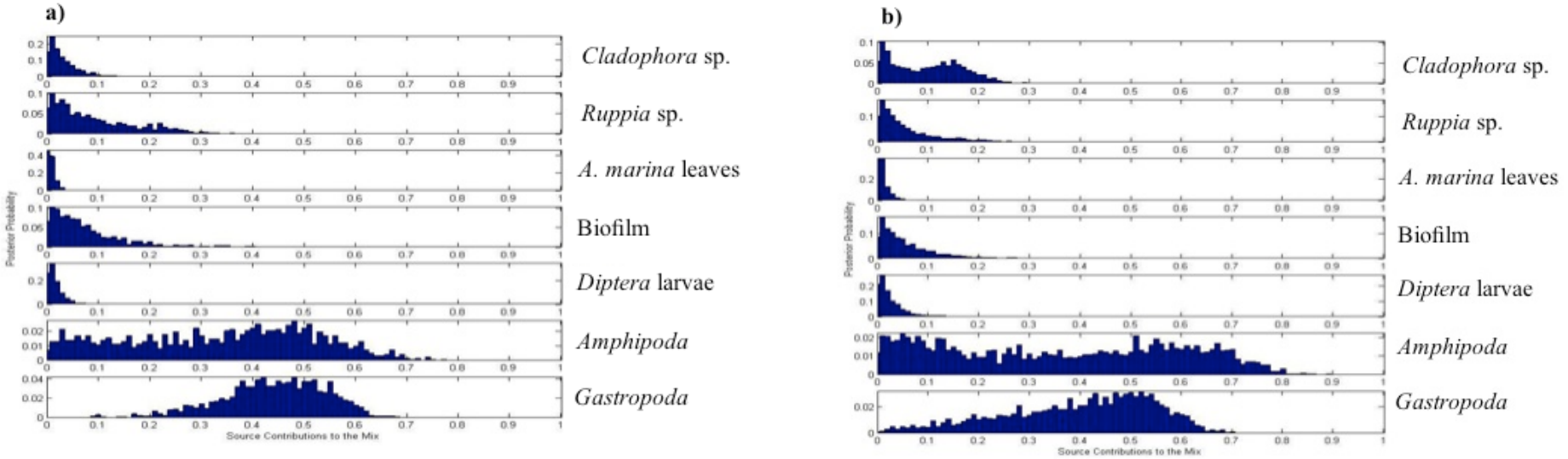

Figure 3.10 Probability distributions of the contributions of sources, based on mixing model (MixSIR) results, to the diets of **(a)** *O. mossambicus* and **(b)** *A. caudavittata* from Dave's (11/12 and 04/13 combined).

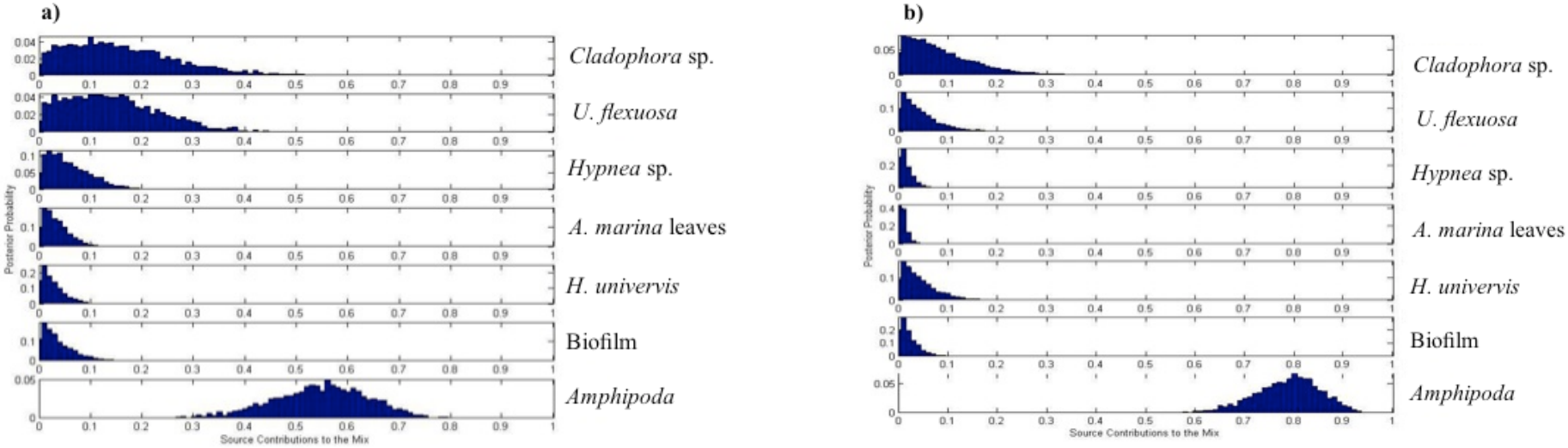

Figure 3.11 Probability distributions of the contributions of sources, based on mixing model (MixSIR) results, to the diets of **(a)** *O. mossambicus* and **(b)** *A. caudavittata* from Jack's 04/13.

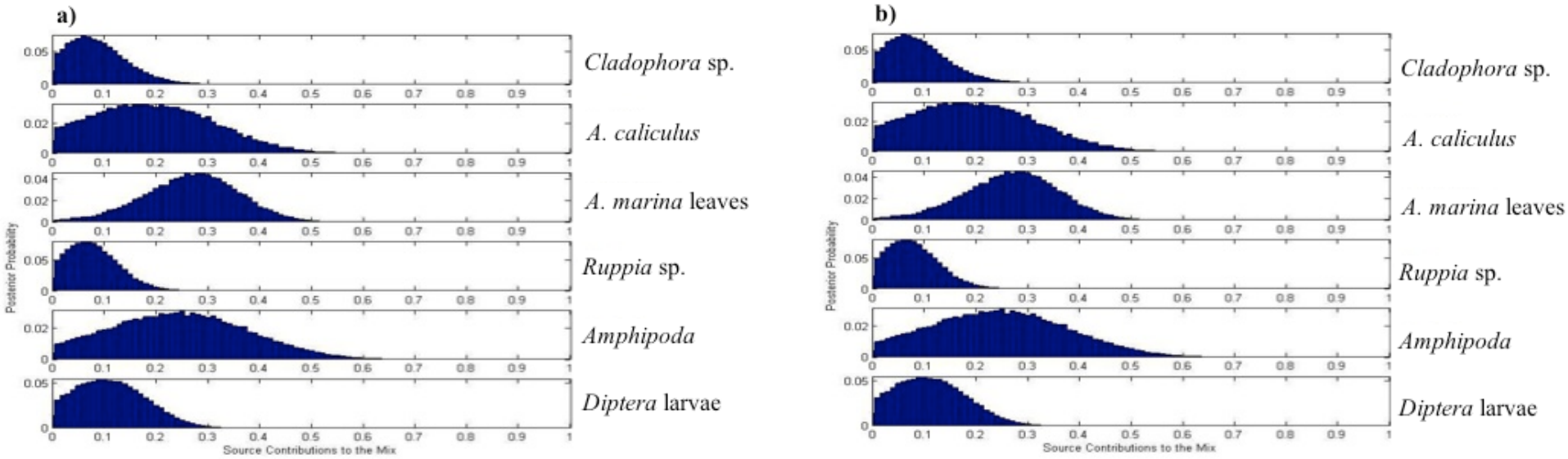

Figure 3.12 Probability distributions of the contributions of sources, based on mixing model (MixSIR) results, to the diets of **(a)** *O. mossambicus* and **(b)** *A. caudavittata* from Pete's 11/12.

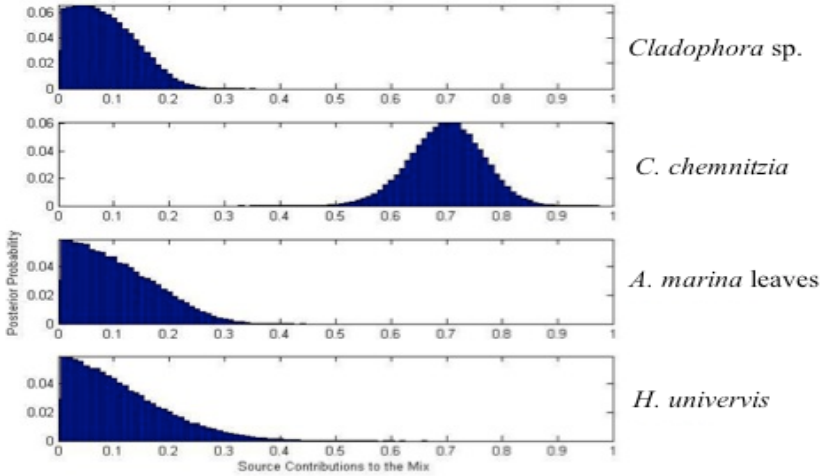

Figure 3.13 Probability distributions of the contributions of sources, based on mixing model (MixSIR) results, to the diets of *A. caudavittata* from Neil's 11/12.

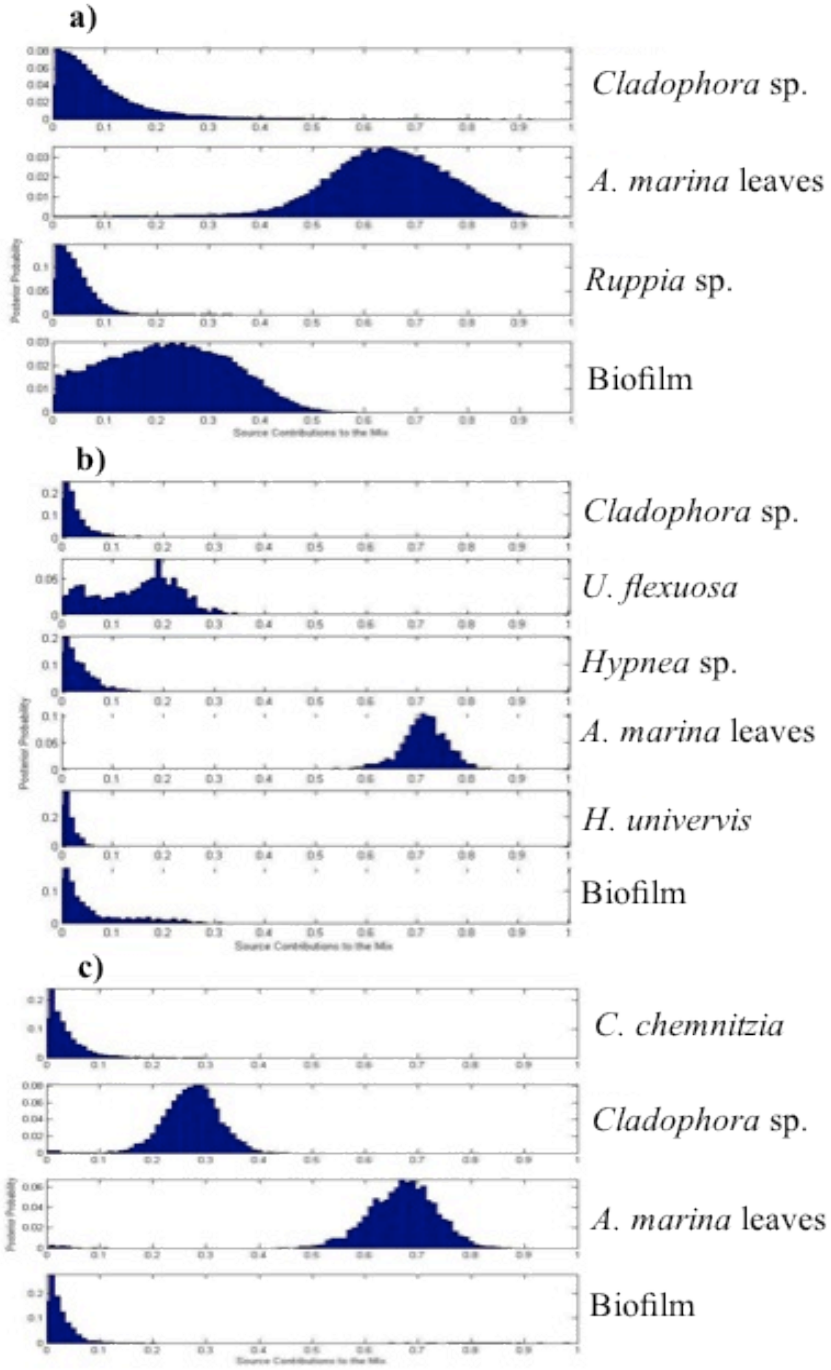

Figure 3.14 Probability distributions of the contributions of sources, based on mixing model (MixSIR) results, to the composition of detritus from Dave's **(a)**, Jack's **(b)** and Neil's **(c).**

4. Discussion

This is the first study to quantify the fish communities of Lake MacLeod, an important inland salt-lake system in north-western Australia. These fish community estimates and descriptions are essential to the understanding and conservation of the ecosystem, and are especially important to determine the potential threats posed by the invasive *O. mossambicus*. Although the negative effects of *O. mossambicus* have been hypothesized, there is little actual evidence of its impact on native fishes in Australia or worldwide (see Maddern *et al.*, 2007). This study aimed to provide evidence to either support or refute this hypothesis. The findings suggest some overlap between *O. mossambicus* and the native fish species *A. caudavittata* in habitat use and prey selection. Although this study does not necessarily provide evidence of *O. mossambicus* as currently having an impact on the native fish, it does show the potential for a negative impact to the fish and the ecosystem, by suggesting that if resources were limited, competition could occur.

4.1 Fish community structures

4.1.1 O. mossambicus *abundances and distribution*

The tilapia *O. mossambicus* was recorded in high abundance in the vent habitat at many of the study sites (5 out of 7 ponds), suggesting its wide distribution and high abundance throughout the ponds of Lake MacLeod. In addition, *O. mossambicus* individuals were often visually observed within almost all study sites, often swimming

within and between all habitats. It is likely that they are widespread throughout many, if not all, of the ponds in the system and utilize all habitats. It is well established that this species can withstand a variety of environments, including conditions that are too harsh for other species (e.g. Doupé *et al.*, 2010; Martin *et al*., 2010), which is likely a contributing factor to their success at Lake MacLeod. They are known to: reproduce and survive in conditions from freshwater to hypersaline (Trewavas, 1983), reach sexual maturity at stunted sizes (Lamboj, 2004), show dietary plasticity (Trewavas, 1983), tolerate low levels of dissolved oxygen and even utilize atmospheric oxygen (de Moor and Bruton, 1988), and survive in water temperatures from 8-42°C (Phillippart and Ruwet, 1982). *O. mossambicus* is most commonly found in coastal lakes and protected estuaries, with calm, standing water (Blaber, 1997), often with plenty of vegetation (Allen *et al.*, 2002). While the species was not recorded in the vents at Neil's and Harjie's, the vent habitats at both these sites were located in a small area at the end of a fairly long channel. By contrast the vents where they were observed were either larger and more open or connected to a larger part of the pond through a very short and wide channel (such as in Dave's). Although *O. mossambicus* can survive in a variety of habitats, the smaller, more closed-in vents may be less desirable for them and not allow as much movement in and out of the vents.

Within the five ponds in which they were able to be quantified and not simply observed, *O. mossambicus* were only recorded in the vent habitats by the stereo RUV. Although *O. mossambicus* was observed in the pneumatophores and nearshore habitats, they were unable to be caught and thus quantified or measured. The use of all three habitats by *O. mossambicus* is not surprising based on their general habitat use patterns.

They are generally known to remain in shallow areas, close to shore while they are young, and slowly move towards the deeper, open water as they mature (Fryer and Iles, 1972). Large, adult *O. mossambicus* often inhabit both the deeper, open water habitats and the shallower, near shore areas (Fryer and Iles, 1972). Nearshore, shallow areas are often utilized for the construction of leks (Fryer and Iles, 1972). At many of the study sites, leks were observed in the nearshore habitats (see Appendix 6.1), suggesting that *O. mossambicus* have, at least, attempted to mate in these shallow habitats. This was supported by an observation at Pete's, where a female *O. mossambicus* spat out the fry from its mouth during an attempt to capture it. No active mating was observed. This concurs with other studies which have shown that *O. mossambicus*, as well as most other tilapia species, utilize relatively shallow, soft sandy sediment dominated habitats for reproduction (Doupé *et al.,* 2009a; Maddern *et al.*, 2007). These habitat-use patterns have been shown to remain consistent even in very small bodies of water, especially when multiple habitats are present within one site (Pet & Piet, 1993). It is highly possible that small, especially small juvenile *O. mossambicus* were missed by the fyke net in the pneumatophore habitat. While high abundances of small *C. pauciradiatus* were captured in all the pneumatophore habitats, members of its family are typically easy to catch (see Valesini *et al.*, 1997; Younge *et al.,* 1997), whereas individuals of *O. mossambicus* have proved to be very quick and hard to catch in this study. The results from this study suggest that *O. mossambicus* at Lake MacLeod generally prefer to utilize the more open vents, however, it appears they utilize the nearshore habitats when mating and as juveniles. The preferred use of the more open vents may be due to a number of factors including the colder water temperatures, deeper and larger spaces that allow for large

schools, increased protection from predators due to the depth and rugosity, or the possible abundance of prey in the substrate.

The population structure of *O. mossambicus* was different at Jack's compared to the sites within the Chirrida Pond system to the north. At Jack's, the species had significantly larger total lengths (341-385 mm at Jack's vs. 45-174 mm at all other sites) and appeared to be more abundant. Although Jack's had high densities of *O. mossambicus* and at large sizes, high abundances of *C. pauciradiatus* and *A. caudavittata* (relative to other sites) were also recorded (see Results Figures 3.1 and 3.2a). There are a number of possible explanations for the difference in community structure at Jack's. The mechanisms that may cause such a difference in fish community structures between Jack's and the other sites include water chemistry and physical characteristics of the ponds, the accessibility and connectivity of the pond, the locations of the ponds with respect to the Gascoyne River, possible predator load, and the sizes of the ponds themselves.

The ability of *O. mossambicus* to withstand fluctuations in salinity, temperature, and dissolved oxygen levels allows *O. mossambicus* to survive in a wide variety of environments (Blaber, 1997; Allen *et al.*, 2002). There were some water chemistry differences between Jack's and the other study sites, mainly the lower water temperatures, which may contribute to the different fish communities. The vent at Jack's is much deeper, wider and colder than at any other study site (see Appendix Figures 6.2 and 6.3), and the size of the overall pond was the largest of all sites. These open spaces and deeper water may provide a more favourable and spacious habitat for larger sized and greater abundance of fish.

Lowe-McConnell (1982) showed that the only factors controlling community structures of tilapia in multi-species cichlid populations in Africa were major physico-chemical events, such as droughts or floods, and predation. This theory may be applicable to the Lake MacLeod. Cygnet Pond (including Jack's) is located closer to the Gascoyne River and has a higher degree of connectivity between ponds through large channels, whereas the ponds in the Chirrida system (north of the Sandy Bluff Sill) are mainly connected by water movement through strong winds (see Methods Figure 2.3) (Logan, 1987; Shepherd, 1991). The Chirrida system is also partially separated from the Cygnet and Ibis Ponds by the Sandy Bluff Sill, sits at a slightly higher elevation, and tends to slope southwards (Logan, 1987; Shepherd, 1991), which suggest less flood waters would successfully reach this part of the lake and/or a higher magnitude flood event would be required (McLure, 2011). The size, depth and high level of connectivity to surrounding ponds, may be factors that contribute to the larger and more abundant fish within Jack's. It can also be hypothesized that the Ibis and Cygnet Ponds were subject to heavier invasion pressures, compared to the more northern Chirrida Pond system, because of its closer proximity to the Gascoyne River during major flood events, from which the majority of the invasion is thought to have originated (Phillips *et al.*, 2005). If the species is not reproducing at Jack's, it is possible that members in the school(s) that originally entered the pond were similar sizes and ages, or have grown to the maximum optimal size for this habitat. Although there was evidence of lek construction at Jack's, no active mating or juveniles were observed at this site. Only one observation of active mouthbrooding was made, and it was observed at the other end of the system at Pete's.

Additionally, the predation levels surrounding Jack's are likely to be higher than the other study sites. The bird population around Jack's is much higher in abundance than at any other study site (see Appendix Figure 6.4) (Phillips *et al.*, 2005). This includes many piscivorous water birds including cormorants, pelicans, darters, ospreys, egrets and herons (Phillips *et al.*, 2005), which could provide a higher level of predation on smaller fish. These birds may be selecting the smaller sized *O. mossambicus* (and for that matter also *E. hawaiensis,* see below), which are likely to be more easily consumed, leaving behind the large sized fish that were observed. Although no direct predation from birds was observed, partly consumed mid-sized *O. mossambicus* carcasses were seen on the shore around many of the study sites, including Jack's (see Appendix Figure 6.5).

Another explanation for vast differences in sizes of the *O. mossambicus* at Jack's compared to the other sites may be stunting of size. Stunting, where sexually mature fish have reduced maximum size, is very common in multi-species assemblages of tilapia and a wide variety of freshwater fishes (Lorenzen, 2000). Although the causes of stunting are unclear, it is most often linked to small bodies of water with isolated populations and low fish biodiversity (Lorenzen, 2000). Size of the pond has been shown to affect growth of tilapia, even when fish density is constant (Chen & Prowse, 1964). While all the study sites at Lake MacLeod meet these criteria of relatively small bodies of water with isolated populations and low biodiversity, Jack's is by far the largest site with slightly less isolation through increased connectivity to other ponds and Lake McLeod. Therefore, having significantly larger *O. mossambicus* (and a population of large *E. hawaiensis*) than fish in other smaller ponds may possibly be attributed to stunting. Further study is needed to determine the mechanism behind such a distinct community at Jack's.

4.1.2 Biodiversity and species interactions

The ponds of Lake MacLeod have a very low fish biodiversity. Only four species were observed in the ponds in this survey and only three of these species were observed in the Chirrida system ponds. Since diversity of fish is often influenced by pond or lake size and connectivity (e.g. Scheffer *et al.,* 2006; Matuszek & Beggs, 1988), the low diversity shown here is not an unexpected finding for this type of ecosystem; which comprises small, relatively shallow ponds with limited connectivity. In addition to the partial isolation of the lake and the ponds within the lake, the dynamic environment of Lake MacLeod may explain the low fish species richness. The three most common fish species (*O. mossambicus*, *A. caudavittata*, and *C. pauciradiatus*) observed at Lake MacLeod are obviously able to withstand the fairly harsh conditions of the ponds. This low biodiversity appears to be stable, based on the data collected in this study. Some previous studies (Shepherd, 1991; Streamtec 1999; Phillips *et al.*, 2005) observed one or two additional species, or sightings of additional species. Streamtec studies from 1999 recorded dead and partially decomposed sea-mullet (*Mugil cephalus*) in the macroinvertebrate samples, a species also observed by Shepherd (1991). Streamtec also reported limited sightings of spangled perch (*Leiopotheron unicolour*) from 2000 and 2001. Neither of these two species was recorded by netting, cameras, or observed during this study making it unlikely that they are currently present at any of the study sites. Other than one additional species at Jack's, the low species richness of three remained relatively consistent. Low species diversity can be caused by a variety of factors relevant

to this study, including isolation and limited ability for recruitment, spatial variation and changing salinities within one environment and occasional influxes of freshwater, high predation pressure and competition, limited habitat diversity and complexity, water temperature, and flow regime (see Rahel, 1986; Oberdorff *et al.*, 1995).

Both *A. caudavittata* and *C. pauciradiatus* are commonly found in estuaries around Australia, as well as in marine waters (see Wise *et al.*, 1994; Potter & Hyndes, 1999; Morgan and Gill, 2004; Molony and Parry, 2006; Davis *et al*., 2012, for *A. caudavittata* and Prince *et al*., 1982, Potter *et al.*, 1986, Potter & Hyndes, 1999 for similar species to *C. pauciradiatus*), which provides evidence that both species are euryhaline. The wide distribution of both species in the ponds at Lake MacLeod is most likely attributed to their ability to withstand a variety of changing salinities, which therefore provides them with the ability for recruitment into this system. The harsh environment surrounding the ponds makes it hard for most species to successfully establish themselves across the system, however both of these native species have done so.

There is no evidence from the present study that *O. mossambicus* is excluding native species from available habitats. While the vents were the only habitat where both *O. mossambicus* and *A. caudavittata* were caught together, there appears to be no discernable pattern between the presence of *O. mossambicus* and the abundance of *A. caudavittata*. Neither did the average sizes of *A. caudavittata* show a significant pattern associated with the presence or sizes of *O. mossambicus*. At some vents, where both species were present, they had similar average sizes, and at some vents *A. caudavittata* even had larger average sizes. Excluding Jack's, there was considerable overlap in the

size frequency distributions of these two species. Both species are widespread throughout the system and overlap in their overall distribution. However, based on the size and abundance data, there is no evidence available to suggest that the presence of *O. mossambicus* is negatively affecting the abundances or sizes of *A. caudavittata*.

The pneumatophore habitats appeared to have a distinct fish assemblage, comprising mostly *C. pauciradiatus* and juvenile *A. caudavittata*. Various factors may cause the fish assemblages to be different in this habitat compared to the vent and nearshore habitats, such as shallower water which may be too shallow for aquatic predators and the presence of highly dense pneumatophores that are useful for the protection of small fish and juveniles in addition to the possible epiphytic food sources present (Laegdsgaard & Johnson, 2001). Tropical and temperate mangrove habitats are known to provide shelter and protection for juvenile fishes, worldwide and within Australia (Laegdsgaard & Johnson, 1987 and 2001). Juvenile and small fishes tend to inhabit mangrove habitats more often than adjacent open seagrass beds (Robertston & Duke, 1987; Thayer *et al.*, 1987; Laegdsgaard & Johnson, 1995). In Australian estuarine systems, there have been between 4-32 times more fish recorded in mangroves and prop root environments than in surrounding seagrass beds (Robertston & Duke, 1987; Thayer *et al.*, 1987; Laegdsgaard & Johnson, 1995). Additionally, *C. pauciradiatus* in Lake MacLeod was observed feeding on the epiphytes of the pneumatophore roots especially when disturbed and on disrupted benthic biofilm, so it is also likely that this habitat provides a food source for this species, in addition to their normal diet of plankton. The pneumatophores are, therefore, likely to provide a food source for the small fish in

addition to shelter and protection from potential water bird predators, compared to the more open vent and nearshore habitats.

Jack's was the only site with a recorded fish species richness of four (See Results Figures 3.1 and 3.2), containing one additional species: the elopid *E. hawaiensis.* Although only one individual was seen at a time on the video footage, many more *E. hawaiensis* were observed at Jack's. All the individuals observed appeared similar in size to that recorded on the video (553 mm, see Results Table 3.4). This species was larger than *O. mossambicus* at Jack's, but again a limited size range was estimated. This limited, but large size range is likely too large to be predated on by the piscivorous birds present. *E. hawaiensis* is bentho-pelagic euryhaline species that often occupies lagoons, bays and estuaries containing high densities of mangroves (Morgan and Gill, 2004; Mundy, 2005). They are active swimmers and generally reside in schools (Morgan and Gill, 2004; Mundy, 2005). As previously stated, Jack's is larger, deeper pond that has a higher level of connectivity to the surrounding ponds than the other study sites, which provide a more optimal environment for this large, mobile schooling species, such as *E. hawaiensis,* to occupy.

4.2 Dietary study

O. mossambicus is known to be opportunistic and highly plastic in terms of its diet (see De Silva *et al.*, 1984; Maitipe & De Silva, 1985). Maitipe & De Silva (1985) have shown *O. mossambicus* in reservoirs in Sri Lanka to change their diets from exclusively feeding on one food source to another. Furthermore, De Silva *et al.* (1984) have shown that a range of diets was sufficient for adequate growth in the fish. Therefore,

it has been hypothesized that specific food source availability is not a major controlling factor in the structures of *O. mossambicus* populations (De Silva, 1985). The present study conforms to this conclusion, showing that *O. mossambicus* in Lake MacLeod had a highly broad diet.

Similarly, when compared to previous studies, both *O. mossambicus* and *A. caudavittata* consumed a variety of items, mainly invertebrates and plant matter (see Maddern *et al.*, 2007). Interestingly, there were some differences in diets observed between the species' diets at Lake MacLeod when compared to the diets of the same species in nearby rivers (the Chapman and the Gascoyne) based on the stomach contents analysis (see Maddern *et al.*, 2007). Neither *O. mossambicus* nor *A. caudavittata* were observed with gastropods or amphipods in their stomach contents in these rivers (Maddern, *et al.*, 2007), whereas both of these invertebrate groups were widely ingested at Lake MacLeod, especially Amphipoda. Other major differences between the diets between the studies included that *A. caudavittata* had evidence of teleost fish but no algae in their stomach contents when collected from Chapman River (see Maddern *et al.*, 2007). A likely explanation for this is the resource availability differences between Lake MacLeod and these rivers. Additionally, although located in WA, Chapman River is not actually in the Pilbara region near the location of the Lake, therefore differences in various characteristics (eg. climate, resources, competition, etc.) most likely exist.

4.2.1 Species differences

The wide variety of ingested food items, ranging from algal material to invertebrates indicates both *O. mossambicus* and *A. caudavittata* at Lake MacLeod are omnivorous, which supports the findings of other studies (see Maddern *et al*., 2007 for *O.*

mossambicus and Wise *et al.*, 1994 for *A. caudavittata*). Furthermore, there was considerable overlap in the diets between species, however items were consumed at vastly different ratios and frequencies resulting in different dietary compositions. *A. caudavittata* consumed more items at a lower frequency across individuals, which implies that they may be less selective than *O. mossambicus.* In contrast, *O. mossambicus* was more consistent with its diets from individual to individual, but consumed fewer items overall.

Whether this difference is due to competitive pressure of *O. mossambicus* on *A. caudavittata* or not is unclear. Based on observations from video footage in this study, the two species have different feeding strategies. *O. mossambicus* was constantly seen selecting food items from the benthic substrate, whereas *A. caudavittata* appeared to be selecting its food more often out of the water column. This observation was supported by the presence of more sediment in the stomachs of *O. mossambicus*. Also, *A. caudavittata* was caught very easily with a baited hook, whereas not a single *O. mossambicus* was successfully caught this way, suggesting that the former species has a greater attraction to animal tissue. Studies have described *A. caudavittata* as a benthic feeder (e.g. Wise *et al.*, 1994), and at Lake MacLeod, they were mainly observed selecting food items suspended in the water column. The invasive *O. mossambicus* and the native *A. caudavittata* at Lake MacLeod, therefore, appear to co-exist with separate feeding strategies under limited competition for food.

Although *O. mossambicus* shows high levels of dietary plasticity and omnivory, the species is often thought to be mainly herbivorous (de Moor and Bruton, 1988). The results of the dietary study concur with the omnivorous nature of this species. They

consumed a variety of prey, both flora (such as filamentous green algae, detritus and diatoms) and fauna (such as various invertebrates, especially amphipods), but there was no evidence of them predating on juvenile or small fish.

The diet of *A. caudavittata* in this study was similar to that in eastern Australia found by Davis *et al.* (2011). In that study the proportions of items consumed were slightly different, with dipteran larvae contributing the largest portion of the diets (Davis *et al.*, 2011). Items such as filamentous algae, macrophytes and various invertebrates, including bivalves and ostracods, made up similar proportions of the diets at both locations (Davis *et al.*, 2011, present study). A study in the Swan River estuary, approximately 1000 km south of Lake MacLeod, showed the species consumed algae and small crustaceans as juveniles and mainly polychaetes as adults (Wise *et al.*, 1994). The difference between these studies may be due to food source availability or to different feeding strategies.

Based on the stomach contents analysis, *O. mossambicus* and *A. caudavittata* exhibited very similar diets among sites, excluding Jack's. Both species showed some differences in prey item proportions at Jack's compared to the other sites (see Results Figure 3.5), suggesting some dietary plasticity among sites. Resource availability and the size structures of the fish could both play a role in the differences of the diets at Jack's. *A. caudavittata* consumed more amphipods, while consuming less filamentous algae and seagrasses, suggesting a shift towards a more carnivorous diet at Jack's (see Results Figure 3.5). *O. mossambicus* consumed more amphipods and filamentous algae at Jack's compared to the other sites, but the ratio of plant to animal material was relatively constant among sites because they also consumed fewer diatoms (see Results Figure 3.5).

The fact that both species altered their diets at Jack's, compared to the other sites, implies this shift in diets between sites may be dependent on specific resource availabilities at each site. Availability of various food sources is a common limiting factor in a species' diet; low availability with high value can cause competition between or within a species and/or a depletion of the resource (e.g. Pouilly *et al.*, 2003; Sternberg *et al.*, 2008). The abundances of food sources may be different at Jack's compared to the other sites, causing the fish to preferentially select items at different proportions.

Body size is also known to affect many aspects of a species' ecology, including diet (Werner & Gilliam, 1984), and the body sizes of *O. mossambicus* at Jack's were significantly larger than at any other site. Differences in body sizes can affect the energetic requirements of a species and change various physiological characteristics of the fish, such as intestinal length and physiology and mouth gape, all of which can alter the types and proportions of prey items required (see Werner & Gilliam, 1984; Schafer *et al.*, 2002). Based on previous studies, *O. mossambicus* juveniles tend to be more carnivorous than adults (de Moor and Bruton, 1988). Adults generally consume mostly algae and phytoplankton, but there is evidence of them feeding on invertebrates, larvae, and zooplankton (de Moor and Bruton, 1988; Maddern *et al.*, 2007) and on small or juvenile fishes (Trewavas, 1983). These ontogenetic dietary shifts do not completely correlate with the shifts in diets from this study, where the larger fish at Jack's were shown to consume more amphipods than the smaller fish at other sites, however, they were also seen to consume more algae. Although determining if ontogenetic shifts in diets occurred within a site was outside the scope of this study, there was a notable difference between the larger fish at Jack's and the smaller fish at the other sites.

Additionally, seasonal differences, specifically over long time periods and during major physio-chemical events, such as floods, likely cause changes in resource availability, although this was also outside of the scope of this study. With the available data it cannot be determined whether the differences in diets at Jack's are caused mainly by site differences and resource availability or by ontogenetic shifts. It is likely that both mechanisms play a role in the dietary shift of both species at Jack's.

4.2.2 Stable isotopes and mixing models

The mixing models analysis of the stable isotope signatures, which represent longer-term assimilation of diets, showed some of the same trends as the SCA, which is more representative of the short-term, ingested items. Mixing model results suggest that the two species' diets do not appear to differ drastically, but do show differences in the predicted amounts that each item is likely to contribute. Compared to SCA, the mixing models showed more similarities in the predicted diets between species within a site, and more differences in the diets among sites. Mixing models predicted a greater presence of gastropods in the diets of both species at Dave's, which was not observed in the diets based on SCA, and algae was shown to contribute little to the diets of both species at Pete's using mixing models, when it comprised a large proportion of the stomach contents at this site. However, these differences may be explained by the propensity of animal material to be assimilated more easily than plant material (see Gannes *et al.*, 1997). Additionally, *O. mossambicus* digests food, particularly animal material, rapidly (Doupé *et al*., 2009b; Doupé & Knott, 2010). It could, therefore, be possible that this species or possibly both species are consuming higher abundances of invertebrates than

were seen through SCA, but this was not observed due to their rapid digestion rates. Differences in the results from SCA and SIA are often due to differences in the time frames of the two types of analyses. SCA gives useful information of items very recently ingested, whereas, the SIA shows the long-term, assimilated trends (France, 1994 & 1996; Michener & Schell, 1994; Fry, 1983). This is why both techniques are often used simultaneously (see Grey *et al.*, 2002; Lugendo *et al*., 2006).

When mixing models are run, there is a limited number of possible sources that can be applied to the mix, and in this study, some different source materials were available for each site. Although the study aimed to collect consistent sources across each site, it was not always possible. The inclusion of different sources in some mixing models, and not others, would possibly change the outcome of the models. It may be that some or all sites are missing potential sources. Diatoms and Foraminifera, which were shown to contribute large proportions of the diets of both species through the SCA, could not be isolated in sufficient biomass for the SIA. Attempts were made to extract them from the sediment samples but because the sediment at the Northern Ponds is high in calcium carbonate, the samples were too small to analyse after the acid treatment to remove inorganic carbon. These important missing sources may have changed the output of the mixing models.

There was a significant difference between the average $\delta^{15}N$ signatures between the two species, however, the average $\delta^{13}C$ did not differ between species. The differences between the average $\delta^{15}N$ values of the two species at each site were between 2.1 and 2.9‰, which is slightly lower than the between 3-4‰ values often used to determine the separation of trophic levels (eg. Cocheret de la Moriniere *et al.,* 2003). A

fractionation value of 1.74‰ for $\delta^{15}N$ was used in this study for the *O. mossambicus* (based on a laboratory calculation of a species in the same genus by Frocken (2001) and Gaye-Siesseggar *et al.* (2003, 2004a and 2004b)), and 3.97‰ for *A. caudavittata* (based on a similar species (Eldson *et al.*, 2001)). Since a higher fractionation value was used for *A. caudavittata*, these two species appear to be occupying the same trophic level (Cocheret de la Moriniere *et al.,* 2003). However, the ease of capture of *A. caudavittata* with animal tissue bait (prawn, worm and fish tissue), suggests this species has a higher natural propensity for fauna than *O. mossambicus*.

At Neil's, where no *O. mossambicus* was collected, *A. caudavittata* showed a lower average $\delta^{15}N$ value of 10.6‰ than at other sites (which range from 11.4-12.4‰), while the source material collected at Neil's did not show notable differences in $\delta^{15}N$ values than at the other sites (Figure 3.9). One hypothesis therefore might be that in the absence of *O. mossambicus*, *A. caudavittata* may feed more predominantly on sources at lower trophic levels. However, since the stomach contents of both species from Neil's did not show any major differences from other sites, and a few *O. mossambicus* were observed throughout the site although not captured, the cause of this low $\delta^{15}N$ is still unknown. The successful capture of *O. mossambicus* at this site to determine whether its $\delta^{15}N$ is also lower than other sites would help to clarify this question.

4.3 Conclusions

4.3.1 Summary and future studies

O. mossambicus is abundant within many of the Northern Ponds of Lake MacLeod, where it occurs mainly in the vent habitats. They have been visually observed

in all habitats and at all sites, although none was successfully caught using the fyke nets in the pneumatophore habitats or recorded on camera in the nearshore habitats. They were successfully filmed in the vent habitats of 5 out of 7 study sites within the Cygnet and Chirrida Pond systems. At many sites, most notably Pete's and Jack's, partially consumed *O. mossambicus* carcasses have been seen along the shoreline. The evidence of lek building in the softer sediments of nearshore areas, suggests that at least courtship for mating, if not mating itself, occurs in that habitat.

Although highly abundant, evidence does not clearly suggest a direct, negative effect of tilapia on the native fishes. The abundance and sizes of *A. caudavittata* and *C. pauciradiatus* do not change significantly when *O. mossambicus* is present, and although *O. mossambicus* and *A. caudavittata* consume many of the same items, they may occupy different niches based on different feeding strategies and proportions of prey items in their stomachs. Thus, these two species do not appear to be directly competing for limited resources. However, these conclusions are based on short-term data, which may not accurately describe any previous effects of *O. mossambicus* on the native fish.

In terms of sites, Jack's Vent is the clear outlier. These differences in the fish community are not only seen in the size, abundances, and species richness of fish, but also in their diets. Based on the differences observed at Jack's in relation to the other sites, it would be beneficial to complete surveys at additional vents and channels immediately east and south of Jack's in order to determine if the population at Jack's is unique or if it is a pattern that occurs elsewhere in the Cygnet Lake system. These comparisons would be important in aiding to determine why Jack's Vent exhibited differences in the fish communities compared to the other sites. It would determine if other sites in the Cygnet

Lake system exhibited similar trends as Jack's, and therefore suggest whether the characteristics unique to the Cygnet ponds and vents (such as size, depth, water temperature, proximity to the Gascoyne River, waterbird predator load, etc.) are, or are not, causing the differences in the fish communities seen at Jack's compared to the Chirrida system ponds. Additionally, determining the exact mechanism responsible for such a distinct cohort at Jack's would be beneficial. This could be done through determining the age structure of the fish, determining whether successful reproduction is occurring, determining whether the waterbirds are predating on the small *O. mossambicus* and at what frequency, and whether the same observed size distribution pattern for *E. hawaiensis* can contribute more to our understanding of these questions. A more extensive study of the waterbird populations, comparing predatory bird load and shelter availability or cover at different sites, would be highly beneficial in determining their potential effect on keeping the *O. mossmabicus* populations under control. A dietary study of *E. hawaiensis* may be useful to acquire a more comprehensive understanding of the trophic structure of the fish communities of Lake MacLeod, however, it is unlikely that this species is interacting with other species through its diets as it generally occupies different habitats (pelagic vs benthic) (Mundy, 2005).

To fully understand the trophic structure of the fish communities, more work needs to be invested into the SIA. The successful acidification, and therefore analysis, of sources such as Foraminifera and diatoms would be greatly beneficial and provide a higher level of completeness to the SIA. Additionally, determining if *O. mossambicus* is changing the trophic structure of the ponds, through a long-term study and investigating the diets of piscivorous waterbirds (for example pelicans and cormorants, both of which

are abundant in the system), would be useful. Currently we have no data to determine whether the bird population is aiding in keeping *O. mossambicus* under control or whether the abundances of *O. mossambicus* are contributing to increased bird populations. Providing an abundant food source for the water bird populations may be a positive effect of *O. mossambicus* on the Lake MacLeod system. The relationship between *O. mossambicus* and the water bird populations needs to be quantified.

This study did not take into account the effects of *O. mossambicus* on aspects of the system other than the native fish, such as the invertebrate and microbial communities. It is clear that *O. mossambicus* is consuming some level of invertebrate prey and microphytobenthos. Although this study quantified the diet of the species, it is still unknown whether this feeding pressure is having a long-term effect on the abundance or biodiversity of these invertebrates, microbes and microphytobenthos itself. As many unique invertebrates and communities with rare combinations of marine and saline-tolerant species inhabit this system (McLure, 2011), it is important to know if they are under threat. It is now clear that *O. mossambicus* feeds directly on the benthos, and consumes a large amount of sediment in the process of feeding. It is also known that they are creating leks in the nearshore areas, as was observed in many of the study sites. Lek building can cause the depletion of vital resources such as seagrass beds and macrophytes and disrupt benthic composition (Maddern *et al.*, 2007). This behaviour may be disruptive to the invertebrates, microbes, and microphytobenthos. Along with quantifying these communities and determining their importance to the system, a study quantifying the possible damage from this type of behaviour is having on these communities would provide a more thorough ecosystem-level assessment of the impact of *O. mossambicus*.

Aggressive behaviour by male *O. mossambicus* during breeding season has been shown to negatively affect native fish species (see Maddern *et al.*, 2007; Doupé *et al.,* 2009a), causing them to avoid the breeding areas (Doupé *et al.,* 2009a). Doupé *et al.* (2009a) hypothesized that it is likely that there is also a chemical or hormonal release from breeding male *O. mossambicus*, which can affect the other fish species. Native fish in the presence of breeding *O. mossambicus* show a decline in egg production and fertilization success, which may be due to the behavioural and/or chemical interactions of the invasive tilapia (Doupé *et al.,* 2009a). Although the construction of leks was observed in many of the study sites (see Appendix Table 6.1), only one direct observation of juvenile fry was observed. The extent and success of their breeding within the system is still unknown. This is especially curious at Jack's, where leks were observed but only large fish were seen. Determining if active breeding is occurring, and if it is, whether the aggressive behaviours of the male fish and/or a hormonal release is negatively affecting the native fish, are important in understanding the possible threats to the fish communities of Lake MacLeod.

Oreochromis mossambicus within Lake MacLeod is widespread and abundant. This study aimed to quantify and describe the distribution and habitat use of the invasive *O. mossambicus* and native fish species within and across the ponds of Lake MacLeod, and determine if the invasive species is likely to be competing for the same food resources and/or predating on native fish species. However, these impacts appear unlikely. The complexity of this system demands further study to fully understand the effects of *O. mossambicus*, not only on the native fish, but on all aspects of the unique and important Lake MacLeod ecosystem.

5. References

Allen, GR, SH Midgley and M Allen (2002). Field guide to the freshwater fishes of Australia. pp. 394 (Western Australian Museum, Perth, WA).

Allen MG, DL Morgan, and HS Gill (2005). Distribution, zoogeography and biology of the Murchison River hardyhead (*Craterocephalus cuneiceps* Whitley, 1944), an atherinid endemic to the Indian Ocean (Pilbara) Drainage Division of Western Australia. *Ecology of Freshwater Fish*, **14:** 209-224.

American Veterinary Medical Association (2013). AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.

Arthington, AH, DR Bludorn, ML Kennard (1994). Food resource partitioning by the introduced cichlid, *Oreochromis mossambicus*, and two native fishes in a sub-tropical Australian impoundment. *Third Asian Fisheries Forum*, 425-428.

Australian Bureau of Meterology (2014). Climate Data Online. Retrieved on 15/01/2014. http://www.bom.gov.au/climate/data

Australian State of the Environment Committee (2001). Coasts and Oceans, pp. 60 (CSIRO Publishing on behalf of the Department of the Environment and Heritage, Collingwood, Australia).

Bax, N, A Williamson, M Aguero, E Gonzalez and W Geeves (2003). Marine invasive alien species: A threat to global biodiversity. *Marine Policy,* **27:** 313-323.

Beaudoin, CP, WM Tonn, EE Prepas and LI Wassenaar (1999). Individual specialization and trophic adaptability of northern pike (*Esox lucius*): an isotope and dietary analysis. *Oecologia*, **120:** 386-396.

Biggs, BJF and ME Close (1989). Periphytic biomass dynamics in gravel bed rivers: The relative effects of flows and nutrients. *Freshwater Biology*¸ **22:** 209-231.

Blaber, SJM (1997). Fish and fisheries of tropical estuaries. In: Fish and Fisheries Ser. 22, pp. 367 (Chapman and Hall, London, UK).

Borski, RJ and RG Hodson (2003). Fish Research and the Institutional Animal Care and Use Committee. *Institute for Laboratory Animal Research Journal*, **44(4):** 286-294.

Bouillon, S, AV Raman, P Dauby and F Dehairs (2002). Carbon and nitrogen stable isotope ratios of subtidal benthic invertebrates in an estuarine mangrove ecosystem (Andhra Pradest, India). *Estuarine, Coastal and Shelf Science,* **54(5):** 901-913.

Bradford, M, FJ Kroon and DJ Russell (2011). The biology and management of *Tilapia mariae* (Pisces: Cichlidae) as a native and invasive species: A review. *Marine and Freshwater Research,* **62(8):** 902-917.

Burns, JM, SJ Trumble, MA Castellini and JW Testa (1998). The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. *Polar Biology,* **19:** 272-282.

Canonico, GC, A Arthington, JK McCrary and ML Thieme (2005). The effects of introduced tilapias on native biodiversity. *Aquatic Conservation: Marine & Freshwater Ecosystems*, **15:** 463-483.

Chen, FY and GA Prowse (1964). The effect of living space on the growth rate of fishes. *Ichthyologica*, **3:** 11-20.

Cloern, JE, EA Canuel and D Harris (2002). Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnology & Oceanography*, **47:** 713-729.

Clynick, B and MG Chapman (2002). Assemblages of small fish in patchy mangrove forests in Sydney Harbour. *Marine and Freshwater Research,* **53(3):** 669-677.

Cocheret de la Moriniere, E, BJA Pollux, I Nagelkerken, MA Hemminga, AHL Huiskes and G van der Velde (2003). Ontogenetic dietary changes of coral reef fishes in the mangrove-seagrass-reef continuum: Stable isotopes and gut-content analysis. *Marine Ecology Progress Series,* **246:** 279-289.

Courtenay, WR (1997). Tilapias as non-indigenous species in the Americas: environmental, regulatory and legal issues. In: Tilapia Aquaculture in the Americas Vol. I. (Eds BA Costa-Pierce and JE Rakocy), pp. 18 (World Aquaculture Society: Los Angeles, CA).

Davis, AM, RG Pearson, BJ Pusey, C Perna, DL Morgan and D Burrows (2011). Trophic ecology of northern Australia's terapontids: ontogenetic dietary shifts and feeding classification. *Journal of Fish Biology,* **78:** 265-286.

Davis, AM, ML Blanchette, BJ Pusey, TD Jardine and RG Pearson (2012). Gut content and stable isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river. *Freshwater Biology,* **57:** 2156-2172.

Davis, AM, PJ Unmack, BJ Pusey, RG Pearson and DL Morgan (2013). Ontogenetic development of intestinal length and relationships to diet in an Australasian fish family (Terapontidae). *BMC Evolutionary Biology,* **13:** 53-68.

De Moor, IJ and MN Bruton (1988). Atlas of alien and translocated indigenous aquatic

animals in southern. In: South African Scientific Programmes Report No. 144, pp. 310 (National Scientific Programmes Unit: CSIR, Port Elizabeth, South Africa).

De Niro, MJ and S Epstein (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta,* **42:** 495-506.

Department of Agriculture Western Australia (2014). Evaporation Data for Western Australia. Retrieved on 15/01/2014. https://www.agric.wa.gov.au/

De Silva, SS, MK Perera and P Maitipe (1984). The composition, nutritional status and digestibility of the diets of *Sarotherodon mossambicus* from nine man-made lakes in Sri Lanka. *Environmental Biological Fisheries*, **11:** 205-219.

De Silva, SS (1985). Status of the introduced cichlid *Sarotherodon mossambicus* (Peters) in the reservoir fishery of Sri Lanka: a management strategy and ecological implications. *Aquaculture and Fisheries Management,* **1:** 91-102.

De Silva, SS, RP Subasinghe, DM Bailey and A Lowther (2004). Tilapias as alien aquatics in Asia and the Pacific: A Review. *FAO Technical Paper No. **453***, Rome.

Doucett, RR, RK Booth, G Power and RS McKinley (1999). Effects of the spawning migration on the nutritional status of anadromous Atlantic salmon (*Salmo salar*): insights from stable-isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, **56(11):** 2172-2180.

Doupé, RG and DW Burrows (2008). Thirty years later, should we be more concerned for the ongoing invasion of Mozambique Tilapia in Australia? *Pacific Conservation Biology*, **14(4):** 235-238.

Doupé, RG, J Schaffer, MJ Knott and DW Burrows (2009a). How might an exotic fish disrupt spawning success in a sympatric native species? *Marine and Freshwater Research*, **60(5):** 379-383.

Doupé, RG, MJ Knott, J Schaffer and DW Burrows (2009b). Investigational piscivory of some juvenile Australian freshwater fishes by the introduced Mozambique tilapia *Oreochromis mossambicus*. *Journal of Fish Biology*, **74(10):** 2386-2400.

Doupé, RG, MJ Knott, J Schaffer, DW Burrows and AJ Lymbery (2010). Experimental herbivory of native Australian macrophytes by the introduced Mozambique tilapia *Oreochromis mossambicus*. *Australian Ecology*, **35(1):** 24-30.

Doupé, RG and MJ Knott (2010). Rapid digestion of fish prey by the highly invasive 'detritivore' *Oreochromis mossambicus*. *Journal of Fish Biology,* **76(4):** 1019-1024.

Eldson, TS, S Ayvazian, KW McMahon, SP Thorrold (2010). Experimental evaluation of stable isotope fractionation in fish muscle and otoliths. *Marine Ecology Progress Series,* **408:** 195:205.

Focken, U (2001). Stable isotopes in animal ecology: The effect of ration size on the trophic shift of C and N isotopes between feed and carcass. *Isotopes in Environmental and Health Studies,* **37:** 199-211.

France, RL (1994). Nitrogen isotopic composition of marine and freshwater invertebrates. *Marine Ecological Progress Series*, **115:** 205-207.

France, RL (1996). Absence or masking of metabolic fractionations of ^{13}C in a freshwater benthic food web. *Freshwater Biology,* **36(1):** 1-6.

Fry, B (1983). A $^{13}C/^{12}C$ comparison of food webs in Caribbean seagrass meadows and coral reefs. *Aquatic Botany*, **14:** 389-398.

Fry, B and EB Sherr (1984). $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Sciences,* **27:** 13-47.

Fry, B (1988). Food web structure on Georges Bank from stable C, N, and S isotopic compositions. *Limnology & Oceanography,* **33:** 1182-1190.

Fryer, G and TD Iles (1972). The cichlid fishes of the Great Lakes of Africa. (Oliver and Boyd, Edinburgh, UK).

Gannes, LZ, DM O'Brien and C Martinez del Rio (1997). Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Journal of Ecology,* **78(4):** 1271-1276.

Garcia-Berthou, E (2007). The characteristics of invasive fishes: What has been learned so far? *Journal of Fish Biology*, **71:** 33-55.

Gaye-Siessegger, J, U Focken, H-J Abel and K Becker (2003). Feeding level and diet quality influence trophic shift of C and N isotopes in Nile tilapia (*Oreochromis niloticus*). *Isotopes in Environmental and Health Studies,* **39(2):** 125-134.

Gaye-Siessegger, J, U Focken, H-J Abel and K Becker (2004a). Inividual protein balance strongly influences $\delta^{15}N$ and $\delta^{13}C$ values in Nile tilapia, *Oreochromis niloticus*. *Naturwissenschaften,* **91:** 90-93.

Gaye-Siessegger, J, U Focken, H-J Abel and K Becker (2004b). Dietary lipid content influences the activity of lipogenic enzymes in the liver and on whole body $\delta^{13}C$ values of Nile tilapia, *Oreochromis niloticus*. *Isotopes in Environmental and Health Studies,* **40(3):** 181-190.

Gearing, JN (1991). The study of diet and trophic relationships through natural abundance ^{13}C. In: *Carbon Isotope Techniques* (Eds DC Coleman & B Fry), pp. 201-218 (Academic Inc., San Diego, USA).

Global Invasive Species Database (2004). 100 of the World's Worst Invasive Alien Species. Retrieved on 20/04/2012.
http://www.issg.org/database/species/reference_files/100English.pdf

Grey, J, SJ Thackeray, RI Jones and A Shine (2002). Ferox Trout (*Salmo trutta*) as 'Russian dolls': complementary gut content and stable isotope analyses of the Loch Ness foodweb. *Freshwater Biology,* **47(7):** 12-35-1243.

Hoeksema, SD and IC Potter (2006). Diel, seasonal, regional and annual variations in the characteristics of the ichthyofauna of the upper reaches of a large Australian microtidal estuary. *Estuarine, Coastal and Shelf Science,* **67:** 503-520.

Humphries, P and IC Potter (1993). Relationship between the habitat and diet of three species of atherinids and three species of gobies in a temperate Australian estuary. *Marine Biology,* **116(2):** 193-204.

Hyslop, EJ (1980). Stomach contents analysis- a review of methods and their application. *Journal of Fish Biology,* **17:** 411-429.

Jacob, U, K Mintenback, T Brey, R Knust and K Beyer (2005). Stable isotope food web studies: A case for standardized sample treatment. *Marine Ecological Progress Series,* **287:** 251-253.

Laegdsgaard, P and CR Johnson (1995). Mangrove habitats as nurseries: Unique assemblages of juveniles fish in the subtropical mangroves in eastern Australia. *Marine Ecology Progress Series*, **126:** 67-81.

Laegdsgaard, P and C Johnson (2001). Why do juvenile fish utilize mangrove habitats? *Journal of Experimental Marine Biology and Ecology,* **257:** 229-253.

Lamboj, A (2004). The Cichlid Fishes of Western Africa, pp. 255 (Birgit Schmettkamp Verlag, Bornheim, Germany).

Logan, BW (1987). The MacLeod Evaporite Basin Western Australia Holocene environments, sediments and geological evolution. (The American Association of Petroleum Geologists, Tulsa, USA).

Lorenzen, K (2000). Population Dynamics and Management. In: Tilapias: Biology and Exploitation, pp. 163-225. (Kluwer Academic Publishers, Dordrecht, Netherlands).

Lowe-McConnell, RH (1982). Tilapias in fish communities. In: *The Biology and Culture of Tilapias* (Eds RSV Pullin and RH Lowe-McConnell), pp. 83-113 (ICLARM, Manila, Philippines).

Lugendo, BR, I Nagelkerken, G van der Velde and YD Mgaya (2006). The importance of mangroves, mud and sand flats, and seagrass beds as feeding areas for juvenile fishes in Chwaka Bay, Zanzibar: gut content and stable isotope analyses. *Journal of Fish Biology,* **69(6):** 1639-1661.

MacIssac, HJ (1996). Potential abiotic and biotic impacts of Zebra Mussels on the inland waters of North America. *American Zoology*, **36:** 287-299.

Maddern, MG, DL Morgan and HS Gill (2007). Distribution, diet and potential ecological impacts of the introduced Mozambique mouthbrooder *Oreochromis mossambicus* Peters (Pisces: Cichlidae) in Western Australia. *Journal of the Royal Society of Western Australia*, **90:** 203-214.

Maitipe, P and SS De Silva (1985). Switches between zoophagy, phytophagy and detritivory of *Sarotherodon mossambicus* (Peters) populations in twelve man-made Sri Lankan lakes. *Journal of Fish Biology*, **26:** 49-61.

Mangla, S, RL Sheley, JJ James and SR Radosevich (2011). Intra and interspecific competition among invasive and native species during early stages of plant growth. *Plant Ecology,* **212:** 531-542.

Matuszek, JE and GL Beggs (1988). Fish species richness in relation to lake area, pH, and other abiotic factors in Ontario lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **45(11):** 1931-1941.

Martin, CW, MM Valentine, JF Valentine (2010) Competitive interactions between invasive Nile tilapia and native fish: The potential for altered trophic exchange and modification of food webs. *PLos One,* **5(12):** 1-6.

McLure, N (2011). Temporal & Spatial Variation in Aquatic Invertebrate Communities at Lake MacLeod, Northwestern Australia. Unpublished Honours Thesis, Edith Cowan University.

Michener, RH and DM Schell (1994). Stable isotope ratios as tracers in marine and aquatic food webs. In: *Stable isotopes in ecology and environmental science* (Eds K Lajtha and RH Michener) pp. 138-157 (Blackwell Scientific Publications, Oxford, UK).

Minagawa, M and E Wada (1984). Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta,* **48(5):** 1135-1140.

Molnar, JL, RL Gamboa, C Revenga and MD Spalding (2008). Assessing the global threat of invasive species to marine biodiversity. *Front Ecological Environment*, **6(9):** 485-492.

Morgan, DL, HS Gill, MG Maddern and SJ Beatty (2004). Distribution and impacts of introduced freshwater fishes in Western Australia. *New Zealand Journal of Marine and Freshwater Research*, **38(3):** 511-523.

Morgan, DL and HS Gill (2001). The green swordtail *Xiphophorus helleri* Heckel (Poeciliidae): another aquarium fish established in the wild in Western Australia. *Record of the Western Australian Museum,* **20:** 349-352.

Morgan DL and HS Gill (2004). Fish fauna in inland waters of the Pilbara (Indian Ocean) Drainage Division of Western Australia – evidence for three subprovinces. *Zootaxa,* **636:** 1-43.

Mundy, BC (2005). Checklist of the fishes of the Hawaiian Archipelago. *Bishop Museum Bulletins in Zoology,* **6:** 1-704.

Molony, BW and GO Parry (2006). Predicting and managing the effects of hypersalinity on the fish community in solar salt fields in north-western Australia. *Journal of Applied Ichthyology,* **22:** 109-118.

Oberdoff, T, J-F Guégan and B Hugueny (1995). Global scale patterns of fish species richness in rivers. *Ecography,* **18(4):** 345-352.

Pet, JS and GJ Piet (1993). The consequences of habitat occupation and habitat overlap of the introduced tilapia *Oreochromis mossambicus* and indigenous fish species for fishery management in a Sri Lankan reservoir. *Journal of Fish Biology,* **43:** 193-208.

Peterson, BJ and B Fry (1987). Stable isotopes in ecosystem studies. *Annual Reviews in Ecological Systems,* **18:** 293-320.

Philippart, J-C and J-C Ruwet (1982). Ecology and distribution of tilapias. In: The biology and culture of tilapias, pp. 15. (Proceedings of the International Conference on the Biology and Culture of Tilapias, Bellagio, Italy).

Phillips, B, R Butcher, J Hale and M Coote (2005). Ecological character of the Lake MacLeod wetland of international importance. *Australian Government Department of the Environment and Heritage, Natural Heritage Trust*.

Pinnegar, JK and NVC Polunin (2000). Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia*, **122:** 399-409.

Plymouth Marine Laboratory (2013). PRIMERv6.0 User Manual (PRIMER-E Ltd, Plymouth, UK).

Polis, GA and DR Strong (1996). Food web complexity and community dynamics. *American Naturalist,* **147:** 813-846.

Post, DM (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Journal of Ecology,* **83(3):** 703-718.

Potter, IC, Ivantsoff, W, Cameron R and J Minnard (1986). Life cycles and distribution of atherinids in the marine and estuarine waters of southern Australia. *Hydrobiologia,* **139:** 23-40.

Potter, IC and GA Hyndes (1999). Characteristics of ichthyofaunas of south-western Australian estuaries, including comparisons with holarctic estuaries and estuaries elsewhere in temperate Australia. *Australian Journal of Ecology,* **24:** 395-421.

Pouilly, M, F Lino, J-G Bretenoux, C Rosales (2003). Dietary-morphological relationships in a fish assemblage of the Bolivian Amazonian floodplain. *Journal of Fish Biology,* **62(5):** 1137-1158.

Prince, JD, IC Potter, RCJ Lenanton and NR Loneragan (1982). Segregation and feeding of atherinid species (Teleostei) in south-western Australian estuaries. *Australian Journal of Marine and Freshwater Research,* **33(5):** 865-880.

Rahel, FJ (1986). Biogeographic Influences on Fish Species Composition of Northern Wisconsin Lakes with Applications for Lake Acidification Studies. *Canadian Journal of Fisheries and Aquatic Sciences,* **43(1):** 124-134.

Robertston, AI and NC Duke (1987). Mangroves as nursery sites: Comparisons of the abundance and species composition of fish and crustaceans in mangroves and other nearshore habitats in tropical Australia. *Marine Biology,* **96:** 193-205.

Russel, DJ, PA Thuesen and FE Thomson (2012). A review of the biology, ecology, distribution and control of Mozambique tilapia, *Oreochromis mossambicus* (Peters 1852) (Pisces: Chiclidae) with particular emphasis on invasive Australian populations. *Reviews in Fish Biology and Fisheries*, **22(3):** 533-554.

Sale, PF (1974). Overlap in resource use, and interspecific competition. *Oecologia,* **17:** 245-256.

Sand-Jensen, K (1998). Influence if submerged macrophytes on sediment composition and near-bed flow in inland streams. *Freshwater Biology*, **39:** 663-679.

Schafer, LN, ME Platell, FJ Valesini, IC Potter (2002). Comparisons between the influence of habitat type, season and body size on the dietary compositions of fish species in nearshore marine waters. *Journal of Experimental Marine Biology and Ecology,* **278(1):** 67-92.

Scheffer, M, GJ Van Geest, K Zimmer, E Jeppesen, M Sondergaard, MG Butler, MA Hanson, S Declerck and L De Meester (2006). Small habitat size and isolation can promote species richness: Second-order effects on biodiversity in shallow lakes and ponds. *Oikos,* **112(1):** 227-231.

Scholfield, PJ, MS Peterson, MR Lowe, NJ Brown-Peterson and WT Slack (2001). Survival, growth and reproduction of non-indigenous Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758). I. Physiological capabilities in various temperatures and salinities. *Marine and Freshwater Research,* **62:** 439-449.

SeaGIS Pty Ltd (2011). CAL User Guide v2.00.

SeaGIS Pty Ltd (2012). EventMeasure User Guide v3.32.

Semmens, BX and JW Moore (2008). MixSIR: A Bayesian stable isotope mixing model, Version 1.0. Retrieved on 15/01/2014. http://www.ecologybox.org

Shepherd, MJ (1991). Hydrological environments and sedimentation, Cygnet seepage face, Lake MacLeod, Western Australia.PhD Thesis, University of Western Australia.

Steel, EA and S Neuhausser (2002). Comparison of methods for measuring visual water clarity. *Journal of the North American Benthological Society,* **21(2):** 326-335.

Sternberg, D, S Balcombe, J Marshall and J Lobegeiger (2008). Food resource variability in an Australian dryland river: evidence from the diet of two generalist native species. *Marine and Freshwater Research,* **59(2):** 137-144.

Streamtec Ecological Consultants (1999). Lake MacLeod Northern Ponds Aquatic Biomonitoring Programme 1999. *Report to Dampier Salt Limited, Report ST17/00.*

Streamtec Ecological Consultants (2000). Lake MacLeod Northern Ponds Baseline Aquatic Survey 2000. *Report to Dampier Salt Limited, Report ST01/01.*

Streamtec Ecological Consultants (2001). Lake MacLeod Northern Ponds Baseline Aquatic Survey 2001. *Report to Dampier Salt Limited, Report ST05/01.*

Thayer, GW, DR Colby and WF Hettler Jr (1987). Utilisation of the red mangrove prop habitat by fisheries in south Florida. *Marine Ecological Progress Series*, **35:** 25-38.

Thresher, RE and AM Kurtis (2004). Options for managing invasive marine species. *Biological Invasions,* **6:** 295-300.

Trewavas, E (1983). Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia, pp. 583 (British Museum of Natural History, London, UK).

Ute, J, K Mintenbeck, T Brey, R Knust and K Beyer (2005). Stable isotope food web studies: a case for standardized sample treatment. *Marine Ecological Progress Series*, **287:** 251-253.

Valesini, FJ, IC Potter, ME Platell, GA Hyndes (1997). Ichthyofaunas of a temperate estuary and adjacent marine embayment: implications regarding choice of nursery area and influence of environmental changes. *Marine Biology,* **128:** 317-328.

Vanderklift, MA and S Ponsard (2003). Sources of variation in consumer-diet of ^{15}N enrichment: A meta-analysis. *Oecologia*, **136:** 169-182.

Watson, DL, ES Harvey, BM Fitzpatrick, TJ Langlois and G Shedrawi (2010). Assessing reef fish assemblage structure: how do different stereo-video techniques compare? *Marine Biology*, **157(6):** 1237-1250.

Werner, EE and JF Gilliam (1984). The ontogenetic niche and species interactions in size-structured populations. *Annual Reviews of Ecological Systems,* **15:** 393-425.

Wise, BS, IC Potter and JH Wallace (1994). Growth, movements and diet of the terapontid *Amniataba caudavittata* in an Australian estuary. *Journal of Fish Biology,* **45(6):** 917-931.

Wyroll, KH, J Courtney and P Sandercock (2000). The climatic environment of the Carnarvon Basin, Western Australia. *Records of the Western Australia Museum* Supplement, **61:** 13-27.

Young, GC, Potter, IC, Hyndes, GA and S de Lestang (1997). The ichthyofauna of an intermittently open estuary: implications of the bar breaching and low salinities on faunal composition. *Estuarine, Coastal and Shelf Science*, **45:** 53-68.

6. Appendix

Table 6.1 Description of the locations and sizes of the lek's at sites where they were observed.

Site	Leks observed	Diameter of leks (cm)	Notes
Jana's	Yes	64, 50, 66, 51, 64, 85, 74, 73, 84, 86, 49, 54, 61, 44, 74	All leks < 20cm deep, located in nearshore habitats
Neil's	Yes	71, 52, 77, 63, 85, 83, 58, 86, 59, 46, 51, 60, 62, 66, 60, 88	Most leks between 20-30cm deep, located mainly in nearshore habitats, some on the verge of pneumatophores
Pete's	Yes	Not measured	Located mainly in nearshore habitats, some on the verge of pneumatophores
Jack's	Yes	Not measured	Located in nearshore habitats
Whistler's	Yes	Not measured	Located in nearshore habitats
Harjie's	No	N/A	
Dave's	No	N/A	

Table 6.2 Dietary composition of 10 *A. caudavittata* individuals showing little variation between the standard 100 point system and the adapted 50 point system.

Dietary Item	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**
Filamentous Aglae	0.46	0.45	0.37	0.38	0.36	0.34	0.39	0.35	0.42	0.43
Seagrass	0.20	0.21	0.31	0.35	0.38	0.39	0.30	0.33	0.26	0.27
Round Diatoms	0.11	0.14	0.07	0.10	0.06	0.08	0.09	0.07	0.09	0.10
Foraminifera	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.01
Amphipoda	0.07	0.07	0.11	0.14	0.08	0.08	0.10	0.13	0.10	0.10
Ostracoda	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.00
Black Round (unknown)	0.01	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.01
Unidentified	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.01
Sand/Silt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dietary Item	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**
Filamentous Aglae	0.50	0.52	0.47	0.44	0.47	0.51	0.46	0.45	0.35	0.37
Seagrass	0.27	0.25	0.32	0.39	0.26	0.22	0.29	0.33	0.38	0.39
Round Diatoms	0.07	0.06	0.07	0.05	0.09	0.11	0.11	0.11	0.12	0.10
Foraminifera	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01
Amphipoda	0.07	0.09	0.07	0.07	0.10	0.08	0.09	0.04	0.08	0.06
Ostracoda	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02
Black Round (unknown)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01
Unidentified	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.02	0.01	0.01
Sand/Silt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 6.3 Dietary composition of 10 *O. mossambicus* individuals showing little variation between the standard 100 point system and the adapted 50 point system.

Dietary Item	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**
Unidentified	10.64	11.24	11.52	11.70	9.94	10.24	8.60	8.50	10.60	10.40
Sediment	20.75	20.40	17.10	17.30	18.25	17.90	17.00	17.90	21.00	20.90
Filamentous algae	5.15	5.40	4.90	4.50	6.50	5.90	7.75	7.10	7.15	6.90
Round diatom	0.40	0.48	0.33	0.34	0.68	0.74	0.58	0.70	0.59	0.65
Elliptical diatom	8.75	8.64	9.53	9.26	9.53	8.98	8.30	8.62	9.30	9.12
Rocks	0.33	0.36	0.29	0.32	0.18	0.16	0.00	0.00	0.00	0.00
Ostracoda	0.14	0.16	0.17	0.18	0.03	0.05	0.07	0.10	0.09	0.10
Amphipoda	0.11	0.10	0.15	0.14	0.08	0.08	0.05	0.08	0.05	0.07
Detritus	0.20	0.17	0.11	0.09	0.07	0.08	0.21	0.18	0.11	0.18
Dietary Item	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**	**100pt**	**50pt**
Unidentified	8.81	8.64	12.87	12.00	6.77	7.50	11.95	11.60	11.00	11.40
Sediment	15.90	15.00	15.30	16.00	17.42	16.70	21.75	21.00	22.70	22.55
Filamentous algae	2.00	1.80	4.25	5.30	5.15	5.90	4.60	4.10	4.10	4.00
Round diatom	0.22	0.20	0.39	0.32	0.40	0.34	0.80	0.72	0.20	0.42
Elliptical diatom	9.10	10.04	10.58	11.10	8.79	8.44	9.49	10.16	10.45	10.30
Rocks	0.17	0.15	0.19	0.15	0.15	0.22	0.16	0.14	0.20	0.25
Ostracoda	0.18	0.19	0.09	0.12	0.00	0.00	0.11	0.13	0.09	0.12
Amphipoda	0.11	0.14	0.13	0.15	0.03	0.02	0.22	0.22	0.10	0.15
Detritus	0.04	0.06	0.40	0.40	0.10	0.16	0.24	0.26	0.45	0.39

Figure 6.1 Fyke and seine netting of Dave's pond to acquire dietary samples of *O. mossambicus*.

Figure 6.2 Vent at Pete's pond.

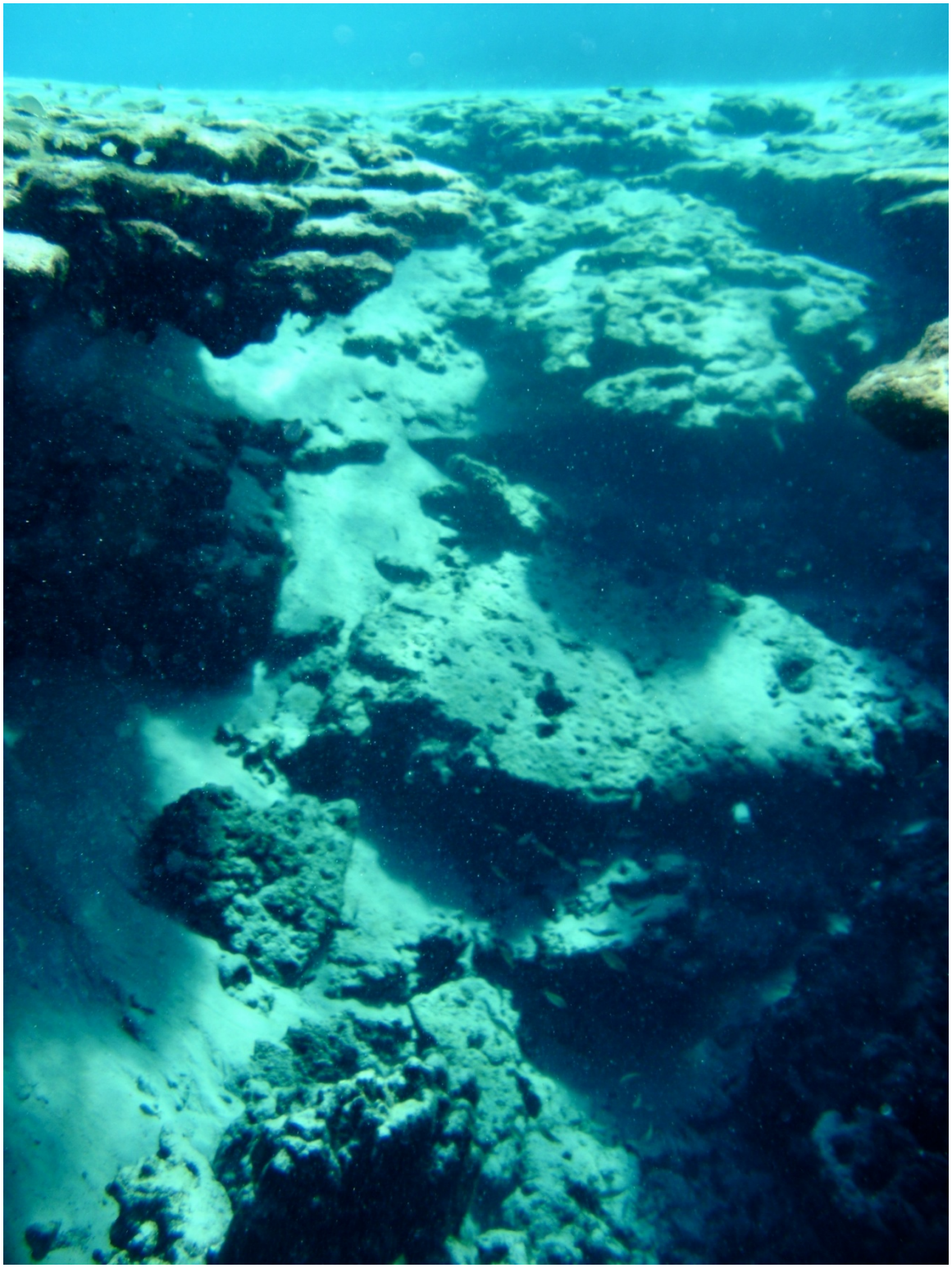

Figure 6.3 Photo taken from within Jack's vent, showing rugosity and habitat complexity.

Figure 6.4 Jack's pond in July 2012 with water birds flying above.

Figure 6.5 *O. mossambicus* carcass found around Jack's pond.