Fish as mosquito control agents in mangroves

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Abstract

The saltwater mosquito, *Aedes vigilax* (Skuse), is a major vector of Ross River virus and Barmah Forest virus across sub-tropical and tropical Australia, and poses a significant human health risk, particularly in densely populated areas such as South East Queensland and Northern New South Wales regions of eastern Australia. The insect uses saltmarsh and mangrove basin forests as oviposition and larval habitats, and various mosquito control methods exist that target larval stages. Fish, acting as predators of larvae, have routinely been used as a component of mosquito control strategies worldwide. If managed appropriately biological control using fish can be effective, but if mismanaged, biological control can have serious environmental consequences. For example, the release of the Mosquitofish (*Gambusia holbrooki* (Girard)) for mosquito control in Australian waterways has caused significant ecological damage; *G. holbrooki* is now a major invasive pest. Nonetheless, mosquito control strategies that include the use of native fish populations in the strategy may be more effective for controlling mosquitoes in mangrove basins than those that do not include native fish. However, little is known of fish populations (both exotic and native) within mangrove basins, and the impact these fish do, and could have on mosquito populations. This research examines the relationship between resident fish and saltwater mosquitoes in eastern Australian mangrove basin forests. It focussed specifically on two mangrove basin sites in the South East Queensland/Northern New South Wales region, and evaluates the potential of resident mangrove basin fish as biological control agents in mangrove ecosystems.

This thesis includes five research aims: 1) identify the composition and abundance of resident fish populations in mangrove basin forests, 2) describe the dietary habits of resident fish populations, 3) examine predation by resident fish on larval *Ae. vigilax*, 4) examine oviposition deterrence of *Ae. vigilax* by presence of resident fish, and 5) quantify the impact of resident fish populations on mosquito production.

The research involves both field work and laboratory experiments. Field work was conducted in two mangrove basin forests in the South East Queensland and Northern New South Wales region and the laboratory experiments were conducted
in a controlled temperature facility at Griffith University. The research and findings are presented in nine chapters.

**Chapter one** presents a brief introduction to the study, outlining the key justifications of the research, stating the primary aims of the study, and providing an outline of the thesis.

**Chapter two** presents a review of the background literature relevant to this thesis. It began by examining mosquito control in mangroves, and proceeds to describe mosquito and fish populations in mangroves, as well as the factors that influence fish-mosquito interactions. This chapter identifies the three major knowledge gaps that were addressed in this study.

**Chapter three** presents a description of the two research sites in this study, and detailed methods that are used throughout.

**In Chapter four** the research describes resident fish populations that persist in eastern Australian mangrove basin forests during the critical period following tidal flooding, when interaction between fish and mosquitoes is most likely, thereby facilitating biological control. Resident fish populations in mangrove basin pools are sampled to describe the composition of species and abundance and distribution of fish, both spatially and temporally (across an entire year of sampling). Resident fish populations are dominated by a single species, the exotic *Gambusia holbrooki* (Girard), but also include three native species: *Pseudomugil signifer* (Kner), *Hypseleotris galii* (Ogilby) and *Pseudogobius* sp. (an unidentified species known locally as the Blue-Spot Goby). Fish were abundant during the warmer, wetter months, when *Ae. vigilax* is generally more active, which suggests that fish-mosquito interactions are likely. However fish populations were highly heterogeneous, both temporally and spatially, which may reduce the impact fish may have on mosquito populations overall.

**In Chapter five** dietary habits of resident fish populations examined in the laboratory are presented, with a focus on the extent to which fish diets incorporated mosquito larvae. Resident fish populations feed on a wide variety of prey. Diets range from small prey items such plant material and micro-crustaceans to larger invertebrates, including mosquito larvae, particularly in warmer months. However relatively little predation on mosquito larvae was documented in any species, even in fish present during peak mosquito season. This was most likely
due to the low *Ae. vigilax* populations throughout the study period (due to extreme weather events in the SEQ/NNSW region throughout 2011 and 2012), rather than a lack of interest in mosquito larvae as prey.

**In Chapter six** the predator-prey interaction between resident mangrove fish and *Ae. vigilax* larvae is described. A series of laboratory experiments compare predation rates of the four resident mangrove fish species on 2\textsuperscript{nd} and 4\textsuperscript{th} instar *Ae. vigilax*, and simulated day and night experiments. All four species show high predation rates of both 2\textsuperscript{nd} and 4\textsuperscript{th} instar larvae, and all show a similar pattern of larval consumption, gorging on larvae in the first hour of each experiment, before reducing to a constant background feeding rate. *Gambusia holbrooki* show the highest larval consumption rate, but is unsuitable as a mosquito control agent due to it being an exotic pest species. Of the three native species, *P. signifer* has the greatest potential as a mosquito control agent, having consumption rates comparable to *G. holbrooki*, and was the only species that does not show a significant reduction in larval consumption in the night experiments.

**In Chapter seven** the examination of the ability of the four resident mangrove fish species to deter *Ae. vigilax* oviposition is presented. A laboratory experiment compares *Ae. vigilax* oviposition in the presence of the four fish species, by presenting gravid mosquitoes a choice of three oviposition mediums: a clean pad, isolated from fish, a pad soaked with water that had contained fish but still isolated from fish, and a pad soaking within the tank and completely exposed to fish. *Ae. vigilax* adults were shown to overwhelmingly favour clean, isolated substrate for oviposition, which strongly suggests that *Ae. vigilax* can detect the presence of fish, either visually or chemically, and avoid areas where larvae would be at greater risk of predation. Oviposition is greatly influenced by all four species, but the effects are most acute in the presence of *Pseudogobius* sp. and *G. holbrooki*.

**In Chapter eight** the research describes whether resident fish populations have a significant, measureable impact on *Ae. vigilax* production in mangrove basin forests. A predation index is derived from the size, stability and composition of resident fish populations, which was is as a measure of the impacts of resident fish populations on mosquito eggshell density, a commonly used measure of mosquito production (and the only measure available in this study, given the absence of *Ae. vigilax* larvae). No relationship is found between the size, stability, composition or predator impact of resident fish and *Ae. vigilax* eggshell density. This result may be
caused by the high spatial and temporal heterogeneity of resident mangrove fish populations, but may also be due to the ineffectiveness of eggshell density to accurately observe the impacts of fish-mosquito interactions on mosquito production.

**Chapter nine** provides a synthesis and conclusion of the thesis, based on the findings of the research undertaken, answering the question: is biological control of *Ae. vigilax* using fish a viable option in eastern Australian mangroves?

Resident fish populations can have a major impact on *Ae. vigilax* populations, via predation on larvae and deterrence of oviposition. However, the observed patterns of fish distributions, both spatially and temporally, suggest that natural fish populations would not make significant contribution to mosquito control. However this may also be because environmental conditions throughout the study were unfavourable to mosquito production and were considered atypical of a normal mosquito season at the study sites. However, laboratory experimentation using resident fish identify that these fish are potential biological control agents, with predation by a native species being as efficient as the introduced *G. holbrooki*. Future research and management pathways that include biological control are discussed, including: examining fish-mosquito interactions in-situ when *Ae. vigilax* are abundant; improving mangroves as a fish habitat; and, improving native fish presence in mangrove forests by replacing the exotic *G. holbrooki* populations with native fish species that can effectively control mosquitoes, in particular *P. signifer*
Statement of originality

The work presented in this thesis has never been submitted as part of a previous degree or diploma in any university. To the best of my knowledge and belief this thesis contains no material previously published or written by another person, except where due reference is made within the thesis itself.

Lachlan Griffin

13 September 2013
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Chapter 1  General introduction

1.1  Problem statement

The saltwater mosquito, Aedes vigilax (Skuse), is voracious and persistent biter and is a major vector of Ross River virus and Barmah Forest virus across sub-tropical and tropical Australia, particularly in the South East Queensland (SEQ)/Northern New South Wales (NNSW) region in eastern Australia. This region has a rapidly growing population (Queensland Government 2012) with associated development pressures and an economy heavily reliant on outdoor tourism (Kelly-Hope et al 2002; Kelly-Hope et al 2004) which makes the problem of mosquito borne disease and thus control of mosquitoes critically important.

Ross River virus (RRV) and Barmah Forest virus (BFV) (Boyd and Kay 1999) are alphaviruses endemic to Australia and the Pacific (Aaskov et al 1981; Rosen et al 1981; Tesh et al 1981; Aldred et al 1990; Mackenzie et al 1994; Frances et al 2004). The viruses are transmitted to humans from reservoir mammals such as kangaroos (Frances et al 2004), possums (Boyd et al 2001) and horses (Kay et al 2007) while the female mosquito extracts a blood meal. Infection rates vary annually with 1500 to 3000 cases of RRV and 150 to 500 cases of BFV diagnosed in Queensland (Allaway et al 2012), however, because a similar number of cases go unreported, actual infection rates may be far higher (Russell 2002). While not fatal, RRV and BFV are polyarthritic diseases that can exhibit persistent and debilitating fatigue, joint and muscle pain (Flexman et al 1998) estimated to cost $3-6 million in healthcare and work absence each year (Woodruff et al 2006). In addition, traveller perception about these diseases has potential to cause major disruptions to tourism (Kelly-Hope et al 2002). Furthermore, there can be substantial costs associated with mosquito control, which is generally undertaken by local councils,
for example, Brisbane City Council pest control budget was ~$4 million in 2012, much of which is focussed on mosquito control and primarily the application of chemicals targeted at larval stages of the mosquito’s larval habitats (M. Muller pers. comm.).

Saltwater mosquitoes use a range of coastal wetlands including saltmarsh (Dale et al 1986) and mangrove basin forests for oviposition and larval habitats (Knight 2011; Knight et al 2012). Mangroves in the region provide a habitat for a number of mosquito species, including *Culex sitiens* (Wiedmann), *Verralina funerea* (Theobald), *Aedes alternans* (Westwood) and *Ae. vigilax* (Skuse). Here the focus is on *Ae. vigilax* as it poses the greatest human health risk, but reference to *Ae. vigilax* larvae could equally apply to larvae of these other species, especially regarding predation of larvae by fish, as they are of similar size (with the exception of *Ae. alternans*, which is far larger) and found in the same mangrove pools.

The human health risks associated with mangrove mosquito populations can be mitigated by control of the vector species. A wide variety of methods exist for controlling mosquitoes, however the importance of mangroves needs to be taken into account. Mangroves have significant ecological, cultural and economic value. They provide a habitat for many organisms such as fish (Meynecke et al 2008; Wang et al 2009), birds (Ford 1982; Noske 1996) and invertebrates (Clay and Andersen 1996; Vance et al 1996; Nielsen 1997; Gwyther 2003; Sheridan and Hays 2003). They also provide essential ecosystem services including maintaining estuarine water quality (Mitsch and Gosselink 2000; Lin and Dushoff 2003), protecting coastlines and coastal communities from destructive weather (Badola and Hussain 2005; Kathiresan and Rajendran 2005), and provide economic value by supporting commercially important fish stocks (Faunce and Serafy 2006).

Considering these values mosquito control methods used in mangroves need to be environmentally sound. Fish, acting as predators of larvae, have routinely been used as a component of mosquito control strategies worldwide. If managed correctly biological control using fish can be effective and environmentally sustainable, especially if used as part of an integrated control strategy (Tomerini et al 2011). Successful integrated programs for mosquito control in saltmarshes are well established, and include a range of approaches including chemical and physical methods, such as runnelling (Hulsman et al 1989; Dale et al 2008). A complimentary effect of this is increased fish access to previously isolated larval
habitats (Connolly 2005), which may enable biological control using existing native fish populations to significantly contribute to an integrated mosquito management strategy.

However for mangrove systems there remains a critical lack of knowledge limiting the development of integrated approaches. Methods such as environmental modification and biological control using native fish have not been investigated or implemented. Research into the mosquito mangrove habitat has received recent attention that has led to new avenues of environmentally focused, minimal impact mosquito control methods using habitat modification that restore tidal processes and potentially increase fish-based biological control (Knight 2011; Knight et al 2012). However further research is required. To understand the impact these fish may have on disease vector mosquito populations, and thus contribute to mosquito control, we need to describe resident fish populations in eastern Australian mangrove basin forests, and investigate fish-mosquito interactions in these habitats.

1.2 Aims of study

The aims of this thesis were to investigate the interactions between fish and saltwater disease vector mosquitoes in eastern Australian mangrove basin forests, and to assess the potential that fish have as biological control agents in these ecosystems, both currently and in the future. The specific objectives of this thesis were to:

1. Identify the composition and abundance of resident fish populations living in mangrove basin pools between tidal connections (Chapter 4).
2. Determine the dietary habits of resident fish in mangrove basin pools (Chapter 5).
3. Examine predation by resident fish on larval *Ae. vigilax* (Chapter 6).
4. Examine oviposition deterrence of *Ae. vigilax* by presence of resident fish (Chapter 7).
5. Quantify the impact of resident fish populations on mosquito production (Chapter 8).

Investigating these five research objectives led to an improved understanding of fish in mangroves and their potential to control mosquito populations, and identified
significant future research and management pathways that may enhance biological control using fish in future, as shown in Fig 1.1.

Conducting this research required a broad, interdisciplinary approach, combining field sampling, laboratory experiments and statistical analyses to describe fish and mosquito populations in the mangrove basin, and directly observe fish-mosquito interactions. Study sites are described in Chapter 3, and field and laboratory methods are described in their relevant chapters.

1.3 Significance of research

Considering the important ecological and ecosystem service values of mangroves, mitigating mosquito borne disease in these ecosystems requires an environmentally sensitive approach. Biological control using existing fish populations may be an effective and environmentally sound method of mosquito control in mangrove environments that may be implemented as part of an integrated mosquito management approach. The findings of this research significantly contribute to this, as the improved understanding of the relationship between fish and mosquitoes may be able to inform biological control in eastern Australian region mangroves.

1.4 Outline of thesis

This thesis is presented in a series of chapters, beginning with an investigation of the relevant literature (Chapter 2) and a description of the study sites and of the general methods used (Chapter 3). Chapters 4-8 present five individual areas of research, and are presented as manuscript-style chapters, reviewing the relevant literature, describing the methodology used and presenting and interpreting results, particularly how they relate to the overall aim of the study. Chapter 9 synthesises the findings of the main research chapters to determine the potential of fish to act as biological control agents of mosquitoes. Figure 1.1 shows the general research space of the thesis and the relevant chapters. A brief summary of each chapter is presented below:
Chapter 1 - Has provided a brief introduction to the study, outlining the key justifications for the research, stating the primary aims of the study and providing an outline of the thesis.

Chapter 2 – Presents a review of background literature relevant to this thesis. The chapter begins by examining previous work on fish and mosquito populations in mangrove forests, and proceeds to identify factors that influence the degree to which fish may influence mosquito populations. It concludes by identifying three major knowledge gaps that need to be addressed to determine the biological control potential of mangrove basin fish, and these knowledge gaps will shape the research goals of the following chapters.

Chapter 3 – Describes the primary research sites of this study, and details some general methodologies to be used in multiple chapters of the study.

Chapter 4 – Presents research describing the composition and abundance of resident fish populations in SEQ/NNSW mangrove forest basins during the critical larval hatching and development period following tidal connectivity.

Chapter 5 – Presents research identifying the dietary habits of resident mangrove fish, with particular focus on the degree to which fish diets incorporate mosquito larvae.

Chapter 6 – Presents in a series of laboratory experiments examining the predator-prey interaction between resident mangrove fish and *Ae. vigilax* larvae.

Chapter 7 – Presents research examining whether resident mangrove basin fish can deter *Ae. vigilax* oviposition.

Chapter 8 – Presents research exploring whether the fish-mosquito interaction has a significant, measureable impact on immature *Ae. vigilax* production in SEQ/NNSW mangrove basin forests, that may indicate ongoing biological control.

Chapter 9 – Provides a synthesis and conclusion of the thesis, summarising the major findings and contributions to knowledge. This chapter discusses whether, based on the findings of this thesis, biological control of *Ae. vigilax* using fish is a viable option in SEQ/NNSW region mangrove basin forests. Furthermore, this chapter considers future research and management pathways that have arisen from the findings of the thesis.
Fig 1.1 Diagram of the general research space, showing the research objectives and outputs of this thesis.
Chapter 2  An overview of fish-mosquito interactions in mangrove basin forests


2.1 Introduction

Mangrove forests provide habitats for the saltwater mosquito *Ae. vigilax*, a significant vector of RRV and BFV in low-lying coastal regions of Australia. The human health risks and nuisance associated with mangrove mosquito populations can be mitigated by control of the vector mosquito species, however *Ae. vigilax* presents a difficult management issue. There is a great need to address the human health problems associated with this species, but also to preserve mangrove ecosystems and their vital ecological and ecosystem service roles. Considering this, mosquito control needs to be both effective at reducing mosquito population and be environmentally sound.

Control methods fall into three broad categories: chemical control (using chemical compounds to kill or disrupt mosquito life stages), source reduction (altering the morphology and/or hydrology of immature habitats to make them unsuitable for larval survival and oviposition) and biological control (using predators or pathogens to reduce mosquito populations) (Dale and Hulsman 1990). Successful mosquito control in coastal wetlands, including mangroves, uses examples of all three categories.

Application of various pesticides reduce mosquito populations directly via adult or larval mortality (Hurst et al 2007; Cilek 2008) or by impairing mosquito growth rates (Sullivan 1997; Lawler et al 1999; Butler et al 2006; Henrick 2007). Chemical control of larvae is common in Australia, particularly in the SEQ/NNSW region.
However chemical control can be ineffective and environmentally hazardous. Widespread chemical control throughout the 20th century has led to significant insecticide resistance in many mosquito species (Roberts and Andre 1994; Sharma et al 2007; Van Bortel et al 2008). Larvicides can also have negative effects on non-target invertebrates and fish, particularly in high concentrations (Ali 1980; Boyce et al 2007; Hurst et al 2007).

Source reduction (also known as habitat modification) is also a widely used method of mosquito control in coastal wetlands, and can vary in scale. Large scale construction projects such as impoundments in mangroves and saltmarshes in the United States, popular in the early to mid-20th Century, significantly altered tidal flushing patterns, and proved successful at reducing mosquito populations (Carlson and Vigliano 1985; Carlson and O'Bryan 1988). However many of these projects have had negative impacts on ecosystem health and non-target wetland organisms (Ruber et al 1994; Schmalzer 1995; Feller et al 2003). Smaller scale modification projects have proven to be effective without sacrificing environmental health. One example of this is runnelling in SEQ saltmarshes, where shallow channels connect larval pools to more frequent tidal inundation, providing a low maintenance method of disrupting immature mosquitoes with minimal impacts on water quality, stability and non-target organisms (Hulsman et al 1989; Breitfuss et al 2003; Dale 2007).

Biological control comes in many forms, often analogous to other forms of mosquito control. For example synthetic bacterial larval control agents such as *Bacillus thuringiensis israelensis* (Bti), are bio-rational methods (albeit synthetic), but in practice they are deployed and managed using similar methods to chemical larval control, by aerial or ground-based spraying of larval pools (Russell et al 2003; Russell and Kay 2008). Biological control can also take unique forms, such as the experimental infections of *Aedes aegypti* (L.) adult mosquitoes with specialised strains of the bacteria Wolbachia to disrupt Dengue transmission (Hoffmann et al 2011).

Predation is a more common form of biological control, using a wide variety of organisms to reduce mosquito populations (Fincke et al 1997; Brodman and Dorton 2006). Fish are an ideal predator for biological control, as aquatic larval populations are confined to pools, and are far less mobile than flying adults.
(Chapman et al 1999), and far more susceptible to external damage than hardened, conditioned eggs (Kumar and Hwang 2006).

Predation in biological control regularly involves introducing larval predators into mosquito habitats. When planned and managed correctly this can be effective and environmentally sound, especially in small, simple habitats (Brodman and Dorton 2006; Van Dam and Walton 2007; Chandra et al 2008). A biological project in this form already exists in the SEQ region, where small, larvivorous fish are introduced into ponds and water tanks to reduce backyard mosquito habitats (Moffat et al 2005; Allaway et al 2012).

However, stocking programs are far less effective in large, complex ecosystems. Stocking may not be able to introduce sufficient fish into the environment to provide comprehensive control, and ecological factors such as the presence of alternate prey and behavioural and reproductive characteristics of predator and prey may significantly reduce the capacity of fish to control mosquito populations (Davey et al 1974; Bence 1988; Hoddle 2004). For example, Blaustein (1992) found that the presence of alternate prey mitigated against the effectiveness of fish as controlling agents of mosquito populations in rice plantations, even at high levels of fish-stocking.

Stocking inappropriate fish to control mosquitoes can also have serious environmental consequences. A classic example of this is the Mosquitofish, Gambusia holbrooki (Girard), a species native to North America, which has been introduced into waterways worldwide as a biological control agent for pest mosquito species (Morton et al 1988; Angelon and Petranka 2002; Chandra et al 2008). Gambusia holbrooki was released in Australia in 1925 as part of a worldwide effort to eradicate disease vector mosquitoes (Wilson 1960; Walton et al 2012). Not only has G. holbrooki failed to have a significant impact on mosquitoes, but it has become a major exotic pest in many waterways across Australia. Its adaptability and wide environmental tolerances allow G. holbrooki to rapidly colonise and dominate freshwater and brackish ecosystems (Arthington et al 1983; Keane and Neira 2004; Pyke 2005), and has a drastic impact on many native aquatic vertebrates, including frogs (Webb and Joss 1997) and fish, particularly small fish with similar diets and habitats (Howe et al 1997; Warburton and Madden 2003; Macdonald et al 2012). For this reason it is directly linked to significant reductions
in native populations, both in Australia (Arthington and Marshall 1999) and worldwide (Kumar and Hwang 2006; Laha and Mattingly 2007).

In order for biological control to be successful and environmentally sound, species selected for mosquito control need to be compatible with their environment (Van Dam and Walton 2007). Many native Australian fishes may be just as, if not more, effective at reducing mosquito populations than *G. holbrooki* (Hurst et al 2004; Willems et al 2005). However considering the ecological importance of mangroves, artificially stocking fish to control *Ae. vigilax*, would likely be expensive, require constant restocking and maintenance, and ultimately may not be feasible.

Biological control may be most easily achieved using existing fish populations residing within mangrove basins. Increasing access of resident fish populations to mosquito larval habitats may be an effective and environmentally sound method of controlling immature mosquito populations. Fish-mosquito interactions, and thus mosquito control, may be possible through an integrated management approach, which incorporates aspects of chemical, biological and source reduction methods. Successful integrated management has been used in saltmarshes, for example runnelling, where shallow channels directly disrupt the mosquito life cycle, but also have a secondary impact on mosquitoes by allowing existing fish populations better access to larval pools (Dale and Hulsman 1990; Connolly 2005). This may increase predator-prey interactions between resident fish and immature mosquito life stages, thereby naturally increasing biological control.

While runnelling-type methods are currently confined to saltmarshes, an improved understanding of the hydrological processes in mangroves may allow physical modification based on similar principles (Knight 2008). This may allow a similar expansion of resident fish populations throughout the mangrove basin, and thus potentially allow biological control of *Ae. vigilax*. In the rest of this chapter an overview of the literature relating to mangrove *Ae. vigilax* and fish populations is presented, and the factors that influence fish-mosquito interactions, and thus allow biological control, are described. Several key knowledge gaps are also identified.
2.2 Life cycle and habitat requirements of *Ae. vigilax*

Addressing the risks of mosquito borne disease associated with mangrove forests requires a thorough understanding of the life cycle and habitat requirements of *Ae. vigilax* in sub-tropical mangroves. The life cycle of the species is reasonably well understood as it has been studied in detail over the last four decades. Examining similar salt-water wetland mosquitoes gives further insight into *Ae. vigilax*. *Aedes taeniorhynchus* (Wiedemann) is one such species, an American mosquito that shares similar behavioural, morphological and ecological characteristics with *Ae. vigilax* (Ritchie 1993). Figure 2.1 summarises the *Ae. vigilax* life cycle:

![Figure 2.1 Summary of *Ae. vigilax* life cycle. (Life stage images Source: NSW Arbovirus Surveillance & Vector Monitoring Program, 2002)](image-url)
2.2.1 Oviposition

Eggs are deposited on moist soil surrounding wetland pools (Kerridge 1971). Eggs, once laid, continue to develop over 1-2 weeks. During this time eggs are vulnerable to drowning if completely submerged by tidal or rainfall flooding, and desiccation if not kept moist. Suitable soil moisture for egg survival is dependent on local elevation, where eggs are located close enough to the water’s edge to prevent desiccation, but high enough to prevent submersion (Dale et al 1986; Ritchie and Jennings 1994). Shading from ground cover and/or mangrove tree canopy further reduces desiccation (Kerridge 1971; Kay and Jorgensen 1986; Gislason and Russell 1997; Dale et al 2008). Once fully developed eggs harden and become relatively resistant to desiccation and submersion, and can lie dormant in a state of diapause until conditions are suitable for hatching (Kerridge 1971).

2.2.2 Hatching and larval development

Hatching is triggered by decreased dissolved oxygen (DO) following tidal inundation, where increased microbial activity within the soil lowers dissolved oxygen (Borg and Horsfall 1953; Horsfall et al 1958; Horsfall 1965; Kerridge 1971). The nature of the tidal regime is therefore an important factor in the suitability of an area for immature *Ae. vigilax* habitat. Tidal inundation needs to be frequent enough to flood the substrate and trigger egg hatching, while still being infrequent enough to allow oviposition and full egg development (Knight et al 2008).

Larvae are vulnerable to predation (Jenkins 1964; Morton et al 1988). Therefore optimal larval survival depends on environments that provide pools that are free from potential predators and isolated from regular tidal flooding that can wash larvae into less favourable habitats (Dale et al 1986). Pools need to be isolated from predators until larvae develop into pupae and emerge into adults, which can take as few as 5-6 days, or as many as 9-13 days, depending on temperature (Jeffery et al 2005). After emergence adults begin flying after a few hours, and reach reproductive maturity within several days (Clements 1992; Jeffery et al 2005).
2.2.3 Other mangrove mosquito species in mangroves

In addition to *Ae. vigilax*, mangrove basin pools provide a habitat for several other mosquito species, including *Ve. funerea*, *Ae. alternans* and *Cx. sitiens*. *Verralina funerea* and *Ae. alternans* share many life cycle and habitat requirements with *Ae. vigilax*, the one significant exception being that *Ae. alternans* is significantly larger, and larvae of the species can prey upon *Ae. vigilax* larvae in coastal wetland pools (Kerridge 1971). However the third species often found in mangroves, *Cx. sitiens*, has noticeably different in life cycle and habitat requirements. Unlike other mangrove mosquito species *Cx. sitiens* oviposits in rafts of eggs on the water’s surface. Therefore this species does not require submersion for hatching stimulus, and can therefore complete its entire life cycle largely independent of tidal or rainfall. For this reason it can establish in large numbers in pools isolated from regular tidal flushing, and, if not directly treated, can persist for long periods (Prummongkol et al 2012). *Culex sitiens* larvae are highly vulnerable to fluctuations in water quality, particularly salinity, and are generally believed to be unable to withstand salinities approaching that of seawater (Mottram et al 1994; Roberts 1996).

*Cx. sitiens* is perhaps the most abundant mosquito species (after *Ae. vigilax*) in Australian coastal wetlands (Webb and Russell 1999; Jeffery et al 2002). However this species generally receives far less attention than *Ae. vigilax*, most likely due to it being far less of a human health risk, as it is not a vector of BFV (Boyd and Kay 2000), and is a relatively ineffective RRV vector (Ritchie et al 1997). However it is a vector of Japanese Encephalitis in tropical regions such as northern Australia and South East Asia (Van Den Hurk et al 2006; Prummongkol et al 2012).

2.3 Eastern Australian mangrove forests as immature *Ae. vigilax* habitat

While the life cycle and ecology of mosquitoes such as *Ae. vigilax* are well understood, much of what we know of this species stems from studies conducted within saltmarsh environments. Until recently little attention had been paid to mangrove forests as significant mosquito habitats. Mosquito activity was noted in the past, for example Kerridge (1971) found *Ae. vigilax* larvae in pools along the mangrove/saltmarsh fringe. However mangroves were for the most part, considered
a far less suitable habitat, especially compared to saltmarshes. Mangrove forests were thought to lack suitable exposed substrate for oviposition (Kay and Jorgensen 1986) and hydrology was thought to be uniform across mangrove forests, making them unsuitable for larval survival (Hamlyn-Harris 1933).

This lack of attention to mangrove basins as an *Ae. vigilax* habitat stemmed from a lack of understanding of topography and hydrology of sub-tropical mangrove forests in Australia. These particular forests are relatively species poor compared to other mangrove systems, which makes traditional forms of species based classification difficult (Watson 1928). South East Queensland/Northern New South Wales region mangrove forests typically comprise only a few species and are dominated by stands of *Avicennia marina* (Forsk) var *Australasica* (Walp.), interspersed with small communities of *Aegiceras corniculatum* (L.) Blanco, *Bruguiera gymnorrhiza* (L.) Savigny, *Rhizophora stylosa* Griff., *Excoecaria agallocha* L., *Ceriops australis* (C.T.White) Ballment, T.J.Sm. & J.A Stoddart and saltmarsh vegetation, including *Sporobolus virginicus* ((L.) Kunth) and *Sarcocornia quinqueflora* (Bunge ex (Ung.-Stern)). The landscape is also relatively flat, with little noticeable variation in elevation.

The key to understanding how sub-tropical eastern Australian mangrove systems function is the relationship between hydrology and topography, at both landscape and substrate scales. This relationship is well documented in sub-tropical mangrove systems in other parts of the world. In one early study Lugo and Snedaker (1974) identified significant variation in Florida mangrove forests, which was dependent on broad hydrological and physical processes across the landscape. Forest structure was influenced by the position within the landscape, as different areas of the forest are subjected to different hydrological and physical processes, and by position in relation to other forest types.

The same hydro-physical relationships accurately describe the *A. marina* dominated mangroves of the SEQ/NNSW region. Knight et al (2008) incorporated measurements of tidal hydrology and hydrodynamics into landscape-scale definitions of mangrove forest types, and highlighted the critical role tidal hydrology plays within these ecosystems. Tidal flooding and topography interact within the mangroves, so that the lower internal areas of mangrove forests (described by Lugo and Snedaker as basins) are isolated from the adjacent estuary by higher areas along the fringe of mangroves (referred to as berms). These basin forests make ideal
habitat for immature mosquitoes such as *Ae. vigilax* as they comprise a mosaic of exposed substrate and pneumatophores (for oviposition) and isolated pools (for larvae) (Knight et al 2012), as shown in Fig 2.2.

Fig 2.2 A typical SEQ, *A. marina* mangrove forest basin. The substrate/pool mosaic, formed by dense pneumatophore structure and isolated pools, creates an ideal habitat for *Ae. vigilax* oviposition and larval development.

Whether a mangrove basin is suitable for oviposition depends on a complex relationship between hydrology and topography. Because berms block tidal flow into the basin, the height of the berm determines the frequency of tidal inundation of the basin. Basins behind low berms experience more frequent tidal inundation, and basins behind high berms experience less frequent inundation. This has a profound effect on the distribution of suitable immature *Ae. vigilax* habitat; Knight (2008) identified areas of optimal immature *Ae. vigilax* habitat where the adjacent berm created a tidal flooding regime that was frequent enough to trigger egg hatching but infrequent enough to allow egg and larval development. These findings mirror those incorporating broad scale hydrology and topography to mangrove mosquito populations in the United States, where immature populations
of *Ae. taeniorhynchus* were concentrated behind berms of a specific height (Ritchie and Addison 1992; Lang 2003).

The relationship between topography and tidal flow is not limited to the fringe of the mangrove forest; differences in surface morphology within basins create significant variation in tidal flow. Minor changes in elevation across mangrove basins have significant impacts on tidal inundation frequency and duration (Cohen et al. 2004; Lara and Cohen 2006). For example, Knight et al. (2009) used high resolution topographic data to describe internal mangrove microtopography and hydrology, and found that slight elevation differences result in different tidal flooding frequency and duration, and that internal ridges can impede tidal flooding into adjacent lower areas.

Internal basin topography also has a significant impact on immature mosquito distribution in mangroves. Griffin et al. (2010) found suitable immature *Ae. vigilax* habitat in slightly raised (as little as 5 cm higher) areas within a mangrove basin. Higher areas contained more exposed substrate for oviposition, slightly less frequent tidal inundation to allow egg and larval development, and had less hydrological connectivity thereby minimising larval exposure to predators.

### 2.4 Mangrove forests as a fish habitat

Mangroves are known as an important habitat for many species of fish (Vidy et al. 2004; Wang et al. 2009), some of which may be predators of mosquitoes, and thus potential biological control agents (Kumar and Hwang 2006). Many species of fish are attracted to mangrove forests (Laegdsgaard and Johnson 1995), and dwell within mangroves their entire lives (Nagelkerken et al. 2000; Faunce and Serafy 2006). Others move into the mangroves for short periods, often during specific life stages (Sheridan and Hays 2003).

Many studies link fish to mangroves indirectly, by examining the densities and species richness of fish in mangrove-lined estuaries (Laegdsgaard and Johnson 1995; Nagelkerken et al. 2001; Hajisamae and Chou 2003; Aburto-Oropeza et al. 2008) or along creeks adjacent to mangrove stands (Beumer 1978; Bell et al. 1984; Robertson and Duke 1987). Others have focussed directly on fish entering and leaving mangroves, by sampling along the mangrove fringe (Morton 1990;
Robertson and Duke 1990b; Laroche et al 1997; Nagelkerken et al 2000; Hindell and Jenkins 2004). Whether indirectly or directly, all of these studies have established a strong correlation between presence of mangrove forests and the size and diversity of fish communities. Studies such as these highlight the importance of mangroves for commercial fisheries; for example, Hajisamae and Chou (2003) and Aburto-Oropeza et al (2008) identified a strong association between robust commercially important fish species and mangroves in estuaries in South East Asia and Central America respectively.

These studies have identified a number of key factors that make mangrove forests ideal fish habitats, and have determined which fish use them. Our knowledge of fish may also be extended by examining fish in other habitats, especially those with similar features such as saltmarshes and freshwater wetlands. However, drawing conclusions from non-mangrove habitats needs to be done with caution, as the factors influencing fish habitat can vary dramatically between species, given the wide, prolific dispersal of fish into almost all aquatic and marine habitats (Lowe-McConnell 1977).

2.4.1 Structural complexity of mangroves

Mangrove forests are structurally complex environments. The physical complexity of the extensive root and pneumatophore system of the mangrove plants provides a refuge from predation for small species and juveniles of larger species (Thollot et al 1999; Faunce et al 2004; Nagelkerken et al 2008; Nagelkerken and Faunce 2008). In the presence of predators, small species of fish actively seek the protection of structurally complex pneumatophores in Avicennia forests (Laegdsgaard and Johnson 2001). This protection is critical for many fish species, so much so that if small mangrove fish are isolated from mangroves they seek out other structurally complex environments that provide similar protection (Johnston and Sheaves 2007).

Structural complexity also acts as a base for mangrove food webs. The protruding roots and pneumatophores provide a large surface area for algal and fungal growth (Potts 1980; Hyde 1990; Aikanathan and Sasekumar 1994; Swe et al 2009), providing a source of food for herbivorous fish and species of invertebrates (Proches et al 2001; Proches and Marshall 2002; Proches 2004), which are fed upon by
predatory fish (Verweij et al 2006; Nagelkerken et al 2008). The mangrove roots systems are also eaten by a number of fish species (Robertson 1991). For many species, food source is as important as refuge for attracting fish to mangroves; in laboratory trials Laegdsgaard and Johnson (2001) found that clean simulated mangrove roots attracted only few fish compared to open areas; however, when the ‘roots’ were covered with algae and cyanobacteria fish presence increased dramatically.

2.4.2 Water quality in mangroves

Turbidity

Turbidity influences the distribution of fish both within mangrove and non-mangrove habitats (Robertson and Duke 1987; Abrahams and Kattenfeld 1997; Thollot et al 1999; Johnston et al 2007; Tse et al 2008; Payne and Gillanders 2009). As much fish predation is based on sight (Mathavan et al 1980), the dense vegetation of mangrove forests and sediment trapped by the mangrove roots creates shaded, turbid water and provides protection for small and juvenile fish (Blaber and Blaber 1980; Cyrus and Blaber 1992; de la Moriniere et al 2004).

Water chemistry

Water chemistry is another factor important for fish in mangroves. While not necessarily attracting fish, it plays a critical role in determining the types of fish that can live within mangroves. Factors such as pH, temperature salinity and DO significantly affect fish growth, metabolism and behaviour (Howells et al 1983; Leung et al 1999; Buentello et al 2000; Frick and Wright 2002; Teien et al 2004; Augley et al 2008; Brandt et al 2009), and extremes in these factors can significantly increase fish mortality (Alabaster et al 1957; Weatherley 1972; Alabaster et al 1979; Cech et al 1985; Peterson 1990; Dunson and Dunson 1999). In mangroves these water chemistry factors can fluctuate wildly, influenced by cycles of evaporation and replenishment of pools, which is highly dependent on frequency of tidal connectivity and rainfall (Gardner and Gorman 1984; Orozoco Storni et al 1984; Buffoni and Cappelletti 1999). Pools in areas isolated from
frequent tidal or rainfall flushing generally experience poorer water chemistry, creating less suitable fish habitat (Dunson and Dunson 1999; Nagelkerken et al 2000).

DO and pH are particularly important in SEQ/NNSW region mangroves, which are commonly associated with iron sulfite rich acid sulfate soils (Saffigna and Dale 1999). These soils, if exposed to air, whether by physical manipulation of the substrate or evaporation lowering water levels, oxidise to produce sulphuric acid, which, if released into the water column by tidal or rainfall flooding can significantly impact estuarine ecosystems (Ukpong 1995; Saffigner and Hey 2001). Monosulfitic black ooze (MBO), a substance that forms on the bottom of pools in acid sulfate areas, can cause similar problems when churned up by water movement; its release into the water column causes rapid deoxygenation of pools, resulting in major disruptions to aquatic organisms (Bush et al 2004a; Bush et al 2004b). Acid sulfate soils and MBOs result in very low DO concentrations in mangrove basin pools for long periods. Knight et al (2013) showed that NNSW region mangrove basin pools were consistently hypoxic between tides, and while DO concentrations increased during tidal flooding, when DO rich water entered the system, on the ebb of the tide DO concentrations rapidly lowered to zero.

These low pH and DO conditions create significant issues for aquatic life, both throughout estuaries and within mangroves. For example Easton (1989) documented exposed acid sulphate soils (from coastal wetland drained to create pastoral land) forming a highly acidic terrestrial rainfall runoff, which, upon entering a NNSW region estuary, rapidly lowered pH and DO concentrations, causing major fish and crustacean kills. The low pH and hypoxic conditions in mangrove basin pools are also likely to limit fish population diversity and reduce survivability of fish in these pools (Moss and Scott 1961; Dunson and Dunson 1999).

However some fish species have behavioural or physiological adaptations to cope with extremes in water quality, including DO and pH. Many fish are able to detect changes in DO (Loesch 1960; Pihl 1994; Wannamaker and Rice 2000) and can alter their behaviour to avoid being exposed to lethal hypoxic or anoxic conditions; some fish move into waters richer in DO or congregate at the air-water interface and breathe air to a limited degree (Peterson 1990; Eby and Crowder 2002; Seymour et al 2007). Others can reduce swimming speed to reduce their metabolic rate.
(Herbert and Steffensen 2005) or increase opercular beat rate to increase their oxygen intake (Maxime et al 2000). Some fish species also have wide environmental tolerances that allow them able to endure extreme hypoxia, for example Peterson (1990) found that two mangrove fish species can endure DO concentrations as low as 1.6ppm. Therefore, given the wide adaptability of fish, the low pH and DO concentrations in SEQ/NNSW region mangrove basins may not completely exclude them as fish habitats, but may serve to severely limit the species of fish that can survive within them. This may even increase the refuge function of mangrove forest basins, as it restricts survival in the mangroves to a select group of fish.

2.4.3 Tidal connectivity

Tidal hydrology may have several significant impacts on fish populations. Firstly, as discussed above, tidal flooding frequency has been linked strongly to environmental conditions within the mangrove basin. As discussed in the previous section, mangrove forests experience significant fluctuations in water availability and water quality, especially in areas that are isolated from regular tidal connectivity for long periods (Dunson and Dunson 1999; Nagelkerken et al 2000).

Tidal connectivity is also critical for allowing fish to colonise mangrove pools. In environments where suitable habitat is located in isolated patches, the distribution of organisms using these habitats depends on factors that enable them to reach these patches. These factors are examined in metapopulation theory, where populations in isolated patches persist due to the relationship between extinction factors, which determine the survivability of populations in small patches, and colonisation factors, which determine levels of immigration into patches (Hanski 1998). The influence these factors have largely depends on environment type, as different environments have different conditions that influence patch populations and different means by which organisms can move between patches (Hanski 1998; Freckleton and Watkinson 2002).

The distribution of fish in isolated pool habitats appears to follow metapopulation theory. In areas with little immigration, population size and persistence are driven by extinction factors. For example Magnuson et al (1998) examined isolated fish populations within temperate freshwater lakes in Finland and the United States. Fish populations within these lakes were far more influenced by extinction factors,
such as water quality, than by colonisation factors, as immigration between lakes was rare. However in environments with more frequent flooding, such as intertidal habitats, colonisation factors are considered to be more critical (Sheaves 2009). Sheaves and Johnston (2008) examined tidal and freshwater flooding in a sub-tropical Queensland estuary floodplain and found that variation in flooding frequency affected hydrological connectivity, which in turn influenced the distribution of fish in isolated pools. More frequently flooded areas, which were regularly connected to the main estuary, had larger and more varied fish populations, whereas less frequently flooded areas, which were rarely connected to the estuary, had smaller and less varied fish populations.

Given the significance of tidal variability within mangrove forest basins, where pools exist as isolated patches of potential fish habitat, mangrove fish populations may also function consistently with metapopulation theory. Pools in frequently flushed areas may provide more suitable habitat for fish than those in less frequently flushed areas.

2.4.4 Limitations of knowledge of SEQ/NNSW region mangrove basin fish populations

Despite the strong links between fish and mangroves, there are two important, largely unexplored areas of mangrove fish biology relevant to this study: the lack of direct, mangrove-based studies of fish populations, and a concomitant lack of understanding of fish communities specifically within intermittently flooded A. marina dominated mangrove basin forests.

Lack of direct mangrove-based studies

Although the preference by certain species for mangrove habitat is reasonably well established, this link has been made largely by studying areas around mangroves, rather than within the forests themselves. Much of the literature examines the effect of mangroves on fish populations in nearby creeks and estuaries, where the benefit of mangrove forests is demonstrated by the relatively high abundance of fish in adjacent habitats (Beumer 1978; Bell et al 1984; Robertson and Duke 1987).
Thus, while a strong link has been made between fish and mangrove forests, relatively few studies directly examine fish populations within mangroves, and therefore the mechanisms by which fish use mangroves are poorly understood.

The lack of direct mangrove fish studies may be linked to the overall utilitarian goals of much research on this topic. Faunce and Serafy (2006) noted that a primary motive for understanding and protecting mangroves was to protect commercially important species, in particular fish. Many studies relating to this topic do indeed focus their findings on commercially important species, either directly (Morton et al 1988; Laegdsgaard and Johnson 1995; Laroche et al 1997; Hajisamae and Chou 2003; Aburto-Oropeza et al 2008) or indirectly by emphasising the importance of some mangrove fish as prey for commercially important species (Robertson and Duke 1990a).

Lack of research on resident fish in *A. marina* mangrove basins

Some studies sample around the edges of mangrove forests, demonstrating that estuarine fish species dwell within these habitats (see Morton 1990; Laroche et al 1997; Nagelkerken et al 2000; Hindell and Jenkins 2004; Huxham et al 2004). However, these studies limit themselves to the mangrove fringe and predominantly focus on interactions within fringing mangrove tree species such as *Rhizophora* and *Ceriops*, which are morphologically dissimilar to *A. marina*, the dominant species in SEQ/NNSW mangroves. The former species have thick prop roots and buttresses and the latter (*A. marina*) has a dense pneumatophore root system, which forms a complex environment of dense pneumatophore stands and isolated pools (Amarasinghe and Balasubramaniam 1992).

This issue is compounded by the morphological variation between fringing and basins areas of *A. marina* dominated mangroves. Studies conducted around the edges of *A. marina* basins have identified significant fish populations (Morton 1990; Hindell and Jenkins 2004; Smith and Hindell 2005), however few studies have penetrated into mangrove basin forests. Morton (1990) and Hindell and Jenkins (2004) believed the internal basins of SEQ region *A. marina* mangrove forests, with their dense pneumatophore system, were too complex to study using conventional fish netting methods and therefore limited their study to the fringe of the mangrove.
Smith and Hindell (2005) examined only a narrow, 10-20 m band of mangroves, which lacked an internal basin.

Several studies have been able to penetrate significantly into mangroves, albeit not into A. marina basins forests, identifying a relationship between distance from the mangrove fringe and fish and crustacean species diversity and density, which decreased as distance increased (Vance et al 1996; Huxham et al 2008). However these studies only used distance from the estuary as a factor, and did not investigate the unique features of internal mangrove basins, such as structural complexity and tidal hydrology, which, as demonstrated in the previous sections, play important roles in the mangrove ecosystem.

The lack of studies specifically examining how fish use A. marina mangrove basin forests presents a significant gap in the knowledge that needs to be addressed. This knowledge gap also presents a major obstacle to our understanding of how mangrove fish may interact with immature Ae. vigilax populations, which only live in basin forest areas. Addressing this knowledge gap will be a major focus of this study.

2.5 Interactions between fish and mosquitoes in mangroves

Considering the adaptability of fish it is likely that fish populations exist within mangrove basins where immature mosquitoes are found. However evidence that these fish influence mosquito populations is required if these fish are to be considered as potential biological control agents. Fish interact with mosquito populations in several ways, but due to the lack of direct mangrove studies these interactions are poorly understood in mangrove systems. Fish-mosquito interactions are far better understood in other habitats however, and incorporating these may allow us to better understand mangrove fish-mosquito interactions.

2.5.1 Predation of larvae

There is a long standing assumption that predation by fish, particularly of larval stages of mosquitoes, has a significant impact on the size and distribution of mosquito populations (Chandra et al 2008). Studies document fish feeding on
mosquito larvae across many mosquito species, occupying completely different habitats, ranging from freshwater (Hurst et al 2004; Becker et al 2005) to brackish (Taylor et al 1992) to saline habitats (Hess and Tarzwell 1942; Morton et al 1988), and from habitats as small as discarded household containers (Connor 1922) to large habitats such as flooded agricultural land (Davey et al 1974; Blaustein 1992) and riverine floodplains (Louca et al 2009).

Predation of mosquito larvae by fish can vary significantly. Fish, despite being present in areas typically associated with mosquitoes, may show little or no interest in larvae as a food source (Morton et al 1987; Hollingsworth and Connolly 2006), or may eat little else but larvae (Morton et al 1988). Several factors known to influence the degree to which fish feed on mosquitoes are discussed below.

**Resident versus transient fish species**

One significant factor influencing fish predation of mosquitoes is their association with mangroves. Different fish species use mangroves in different ways; some (known as transient species) enter only for short periods (Nagelkerken et al 2000; Faunce and Serafy 2006), whereas others (known as resident species) remain in mangroves their entire lives (Sheridan and Hays 2003).

Studies conducted in other periodically connected habitats demonstrate little species or feeding overlap between transient and resident fish populations. For example in two studies Morton et al described completely different resident (Morton et al 1988) and transient (Morton et al 1987) fish population in a SEQ saltmarsh. Transient species, as they frequently move between mangroves and the adjacent estuary, are not as reliant on mangrove-produced food or refuge. This is reflected in their diets, which are often more selective or more focussed on estuarine prey items (Nemerson and Able 2004; Valinas et al 2010; Mazumder et al 2011). For example, Vaslet et al (2012) found that the diets of resident fish in mangroves in Florida and Belize mainly comprised mangrove-sourced food, whereas transient species fed predominantly from adjacent seagrass beds. Transient species are therefore far less likely to be mosquito larval predators, and studies of transient diets in significant mosquito habitats reflect this (Morton et al 1987; Hollingsworth and Connolly 2006); Platell and Freewater (2009). For example, Hollingsworth and Connolly (2006) found very little evidence of predation of *Ae. vigilax* larvae by
transient fish, despite strong evidence that they were feeding while visiting inundated SEQ region saltmarshes, which are significant *Ae. vigilax* habitats.

As resident species remain in mangroves they rely on mangroves as a significant source of food (Vaslet et al 2012) and therefore are far more likely to be larval mosquito predators. Studies conducted on resident fish in mosquito habitats appear to confirm this, demonstrating significant mosquito predation (Hess and Tarzwell 1942; Morton et al 1988; Becker and Laurenson 2007). For example Morton et al (1988) identified significant predation of *Ae. vigilax* larvae (as high as 90% of diet of some species) by resident fish in a SEQ saltmarsh. Therefore, if resident fish populations exist within mangrove forest basins they are far more likely to be biological control agents than transient species.

**Seasonality of prey items**

Another critical factor influencing predation on mosquito populations is the ability of fish to cope with the seasonal nature of mosquito populations. Mosquito populations are low (or virtually non-existent) in winter months and very high in summer months, and in order to effectively utilise so variable a food source fish need to be able to shift their diet to other food sources when mosquito larval populations diminish. Therefore, fish species that exhibit generalist feeding behaviour are the most likely to feed on mosquito larvae (Harrington and Harrington 1961; Murdoch 1969; Pen and Potter 1991; Schleuter and Eckmann 2008; Laufer et al 2009). Resident coastal wetland species are known to exhibit such behaviour, albeit not in mangroves (due to the lack of mangrove-based studies). Morton et al (1988) found that several saltmarsh fish species that fed exclusively on mosquito larvae during peak mosquito season were able to switch to a generalist behaviour when mosquitoes became scarce, whereas non-generalist species tended not to feed on mosquitoes at all.

**Preference for alternate prey**

Another significant factor determining the rate of predation of mosquitoes by fish is the presence of alternate prey, in particular prey items that are preferable to mosquito larvae. A number of studies have shown that when available, fish favour
alternative food sources, despite there being an abundance of mosquito larvae (Bence 1988; Knight et al 2004; Manna et al 2008). For example, Manna et al (2008) found that while Culex mosquito larvae were eaten in large quantities by the fish Poecilia reticulata (Peters), rates of predation was reduced in the presence of chironomid (midge) larvae and worms.

The preference for alternate prey can affect both the predator-prey relationship and the mosquito population. If another prey species is preferred to mosquito larvae this can lead to a significant increase in mosquito populations, especially if fish predation concentrates on competitors or predators of mosquito larvae (Chesson 1989; Walton 2007). This may partly explain failed biological control using fish, where populations of predators and competitors of mosquitoes are preyed upon or outcompeted by other fish species (such as G. holbrooki) which are less efficient at reducing mosquito populations species (Bence 1988; Arthington and Marshall 1999; Kumar and Hwang 2006).

The reasons why fish select alternate prey over mosquitoes are unclear. Selection of prey in many organisms, including fish, is largely driven by the need to maximise energy gained while minimising energy expended, by consuming the largest, easiest to capture prey available, and therefore predators would be expected to focus their attention on the largest prey they can fit into their mouths (Hurlbert et al 1972; Eggers 1977; Hambright 1991; Luczkovich et al 1995). For example, the Bluegill Sunfish (Leopnis macrochirus (Rafinesque)) not only specifically targets the largest prey, but also the closest, which, from the fish’s perspective, appears to be the largest (Obrien et al 1976). Preference for mosquito larvae may therefore be reduced by the availability of larger, less agile prey; however, this needs further research.

**Fish morphology**

Fish morphology may also be a significant factor influencing predation of mosquitoes. Mosquito larvae are relatively small (Shinkarenko et al 1986), and are only a worthwhile food source for smaller fish, either small species or juveniles of larger species, often no larger than 5cm (Chandra et al 2008). There is a clear, well established relationship between fish size and larval predation. Studies have found that younger (smaller) individuals of a species eat the earlier instar mosquito larvae, whereas older (larger) individuals feed primarily on older instars and pupae.
Harrington and Harrington (1961) examined this in detail, looking at *Ae. taeniorhynchus* predation by a number of larvivorous fish in Florida saltmarshes. There was a strong correlation between the size of the fish and the stage of development of larvae consumed; smaller fish predominantly ate 1–3 instar larvae and larger fish ate 4\textsuperscript{th} instar larvae. In another example Taylor et al (1992) found that the size of *Ae. taeniorhynchus* and *Culex quinquefasciatus* (Say) larvae consumed by fish were directly proportional to the size of the fish.

One critical component of the predator and prey size relationship is gape size. As fish instinctively feed upon the largest prey they can fit into their mouths (Hambright 1991; Luczkovich et al 1995), differences in gape size between different fish may influence their capacity to feed upon mosquitoes. Gape size may explain differences in larvae size preference between different sized individuals within a species, such as identified by Taylor et al (1992), who found a correlation between gape size and ability to prey on different mosquito larval instars. It may also explain differences in larval predation between species, as those with larger gape sizes may be more efficient larval predators. Booth et al (1985) found that *Pseudomugil signifier* (Kner) fed on a wide variety of insects, however predation was limited to those individual prey items smaller than its gape size. Fish with a larger gape size may be able to feed on a wider variety of prey, including mosquito larvae. This was suggested by Hurst et al (2004) as a significant factor influencing predation of *Culex annulirostris* (Skuse) by fish, as species with larger gape sizes were more effective mosquito predators.

Position of the mouth may also be another important characteristic of fish that may influence their diet. The mouthparts can be located on the top, bottom or front of a fish’s head, and their location gives an indication of the predominant feeding habits of the species, which affects the type of prey typically consumed (Hurst et al 2004; Chandra et al 2008). Chandra et al (2008) reviewed the major larvivorous fish species in India, and found that surface feeders and column feeders that feed within the upper water column were more significant larval predators. As mosquito larvae spend much of their time at the surface, fish species with upturned mouths - surface feeders - are far more likely to be larvivorous species. Column feeders may also feed on mosquito larvae, but to a lesser extent than surface feeders.

The literature above suggests that small, generalist, resident fish species are potentially significant predators of mosquito larvae, and therefore may contribute
to biological control in mangrove basins. However none of this understanding is based on direct observations in mangrove basin forests or of species known to inhabit SEQ/NNSW region mangroves. A high degree of mosquito predation is likely, given the strong association fish and mosquitoes have to mangrove forests, and while studies in other habitats suggest that significant predation is at least possible, the fish-mosquito interaction between fish and mosquitoes needs to be directly examined. This will form a significant portion of this study.

2.5.2 Oviposition deterrence

The presence of fish may also disrupt mosquito oviposition. Studies have identified an ability of mosquitoes to detect larval predators and adjust their oviposition behaviour to avoid areas where hatching larvae would be at greater risk of predation. This behaviour has been documented in the presence of many types of predators, including invertebrates (Chesson 1989; Spencer et al 2002; Silverbush and Blaustein 2008; Silverbush et al 2010), tadpoles (Mokany and Shine 2003; Hagman and Shine 2007) and fish (Petranka and Fakhoury 1991; Ritchie and Laidlawbell 1994; Angelon and Petranka 2002; Walton et al 2009; Hurst et al 2010).

Visual vs. chemical oviposition deterrence

Exactly if and how predators are detected by mosquitoes is not clear. Detection may be sight based, as suggested by Chesson (1989), who documented a reduction of mosquito oviposition in the presence of predatory invertebrates. However detection may also be chemical based. Mosquitoes have the ability to detect chemical cues in the environment (Davis 1976; Bentley and Day 1989). These chemical cues allow adults to detect other organisms, such as blood hosts (Willems et al 2005) and competitors (Kesavaraju and Juliano 2004). Mosquitoes may also be able to sense chemical cues exuded by predatory organisms. This was first suggested by Petranka and Fakhoury (1991), who found that artificial pools containing fish and tadpoles were not oviposited on by *Anopheles* mosquitoes, despite fish being caged and not visible to the gravid mosquitoes. This suggested that factors other than visible detection were responsible for alteration in
oviposition behaviour, and the authors concluded that mosquitoes possessed an ability to detect chemical compounds exuded by fish, a trait that maximised larval survival by minimising exposure to predation. Many studies since have documented similar behaviour, attributing oviposition deterrence to chemical, rather than sight based cues (Angelon and Petranka 2002; Van Dam and Walton 2008).

Species specific oviposition deterrence

Whether visual or chemically based, the ability to detect potential predators is species specific. Some mosquitoes appear to be capable of detecting fish, whereas others are not (Van Dam and Walton 2008; Louca et al 2009; Hurst et al 2010). In one example, Zurharah and Lester (2010) found no evidence of an oviposition response in *Culex pervigilans* (Bergroth), even when exposed to a wide variety of predators, including fish. However, in another example some species avoided oviposition in areas long after larval predators were gone, which further suggests that factors beyond visible detection (most likely chemical cues) are responsible (Angelon and Petranka 2002).

Not all fish species appear to deter oviposition to the same degree. Pamplona et al (2009) compared the oviposition deterrence of two American fish species (*Betta splendens* (Regan) and *P. reticulata* (Peters)), and found that *Culex* mosquitoes responded to *B. splendens*, but not to *P. reticulata*. Mosquitoes may be able to respond differently to different species of fish, because, as the authors suggested, different fish may exude different chemicals, some of which may be more potent or more easily detectable than others.

Whether visually or chemically based, oviposition deterrence by fish may play a significant role in mangrove mosquito population dynamics, such as *Ae. vigilax*. A single study exists in a similar habitat that suggests that oviposition deterrence by fish may be possible; Ritchie and Laidlawbell (1994) found that there was significantly less oviposition by *Ae. taeniorhynchus* in saltmarsh pools containing fish compared to those without fish. *Ae. taeniorhynchus* also lives in mangrove basin forests and is behaviourally and morphologically similar to *Ae. vigilax* (Ritchie 1993), and therefore similar behaviour in *Ae. vigilax* is possible.
There are no studies that document an oviposition response to fish by *Ae. vigilax*, and, while the role of fish as mosquito oviposition deterrent in mangroves has been suggested (Ritchie and Addison 1992; Ritchie and Montague 1995), it has never been directly examined. Instead, oviposition deterrence has been assumed, based on the behaviour of similar species in similar habitats. While we can speculate based on the behaviour of other species, ultimately a predator-sensory ability in *Ae. vigilax* needs to be directly studied before any definitive conclusions can be made, and this will be a major component of this study.

2.5.3 *Fish-mosquito interactions within mangrove basin forests*

The literature above indicates that a fish-mosquito interaction in mangroves, by predation of larvae or deterrence of oviposition, is likely, and may have a significant impact on mosquito populations. This is an assumption often used in studies of mosquito populations in coastal wetlands. Many studies incorporate fish populations into models of immature mosquito populations in mangroves. For example, Ritchie and Montague (1995) incorporated fish into a model of *Ae. taeniorhynchus* populations in Florida mangroves. Fish were estimated to contribute significantly to mosquito population declines; a single fish introduced into a mangrove was believed to reduce mosquito populations by approximately 500 larvae per week.

The influence of predators on a mosquito population has also been documented from a mosquito control perspective (albeit not in mangroves), where a larval population is monitored for changes following the introduction of predatory organisms, including fish (Bence 1988; Kumar & Hwang 2006; Walton 2007; Chandra et al 2008). These studies indicate that fish predation may have a significant influence on mosquito populations at a population level. For example, Van Dam and Walton (2007) found a significant reduction of mosquito populations following the introduction of a mosquito predator into freshwater pools. However these studies deal with the introduction of an exotic predator into a far less complex ecosystem than the mangroves, and therefore may not reflect predator-prey relationships in natural complex ecosystems.

To my knowledge only one study has attributed a change in a mangrove mosquito population to the presence of fish. This study, conducted by Ritchie (1984),
documented an unusually low adult population of *Ae. taeniorhynchus* in Florida, which was attributed to heavy winter rainfall sustaining large larvivorous fish populations in saltmarsh and mangroves. While this study may suggest fish predation as the cause, the predator-prey relationship was not directly studied, and conclusions based on changes in localised adult mosquito populations need to be considered carefully given the wide dispersal of many adult mosquitoes; adult *Ae. vigilax* for example, can travel 60km from its site of emergence (Chapman et al 1999).

A fish-mosquito interaction is also often incorporated into studies of mosquito ecology. To varying degrees, studies incorporate a need for refuge from predation, usually by fish, into the understanding of the ecology of coastal wetland mosquito species such as *Ae. vigilax* in Australia and *Ae. taeniorhynchus* in the United States (Dale et al 1986; Ritchie and Montague 1995). The extent to which fish are believed to influence mangrove basin mosquito populations varies. Ritchie and Addison (1992) believed that predation and oviposition deterrence by fish was a primary factor excluding certain areas of mangrove forests as mosquito habitats, however in another study Knight (2008) acknowledged that fish may be important, but placed far more emphasis on tidal dynamics determining the suitability of areas as larval habitat.

Patterns of fish distribution in other habitats, applied to mangrove forests, have also been used to support the idea of a strong fish presence influencing mosquito populations. Areas of higher hydrological connectivity are believed to have larger fish populations than less tidally connected areas in saltmarshes and tidal flats (Hess and Tarzwell 1942; Sheaves and Johnston 2008), and it is believed that similar fish distribution in mangroves may correspond with lower mosquito populations in more frequently flushed areas (Knight et al 2009; Griffin et al 2010). However, a limitation of this approach is that models can appear conclusive or complete when neither may be the case. Populations of immature mosquitoes are influenced by multiple factors, and disruption of mosquito life stages by unsuitable hydrological condition may be just as, or even more important than predation at influencing the distribution of mosquito populations. Although fish-mosquito interactions have been documented in other coastal wetland habitats, neither the distribution of fish, nor extent of fish-mosquito interactions have been directly investigated in mangrove basins, and therefore the relative impact fish may have on mosquito populations is not known. In order to address this, fish populations, and
their interactions with mangrove mosquitoes such as *Ae. vigilax*, need to be directly studied, and this will form a major component of this study.

### 2.6 Conclusions

This chapter has presented a review of the literature surrounding fish populations in mangrove basins and their interactions with mosquitoes. Three significant knowledge gaps were identified, which be addressed by the five research aims of this study (see section 1.2):

1. **The nature of fish populations within mangrove forest basins**

Mangroves are thought to provide a critical habitat for fish, and their presence within mangrove basins may enable biological control of disease vector mosquitoes such as *Ae. vigilax* in the SEQ/NNSW region. However little is known about fish activity within mangrove basins, as few studies penetrate beyond the mangrove fringe. We do not know what fish populations are found in mangrove basins, or whether control of mosquitoes by fish is possible. Mangrove basin fish populations therefore need to be directly examined, surveys of fish in this habitat needs to be undertaken and the extent to which they feed on mosquitoes needs to be established. This forms the first two research aims of this study and will be addressed in Chapters 4 and 5.

2. **Interaction between fish and mosquitoes in mangrove basins**

Fish can significantly influence immature mosquito populations. Small, generalist, resident fish species can consume large numbers of larvae and deter oviposition in other habitats, and may do the same in mangrove basins. However the interaction between fish and mosquitoes is highly dependent on species-specific characteristics of predator and prey, and the interaction between fish and *Ae. vigilax* has not yet been described. This is directly tied to the potential of mangrove fish to act as biological control agents, and therefore the fish-mosquito interaction between mangrove fish and *Ae. vigilax* needs to be directly investigated. This knowledge gap forms the third and fourth research aims of this study, and will be addressed Chapters 6 and 7.
3. *Influence of fish populations on immature mosquito production*

That fish populations influence mosquito populations is a long standing assumption in mangrove forest basin studies. However, due to the knowledge gaps surrounding the nature of mangrove basin fish and the interaction between fish and mosquitoes in mangrove basins this assumption has not been properly tested. Therefore it is unclear whether fish can influence the distribution of immature mosquito populations, which severely limits our understanding of the biological control potential of disease vector *Ae. vigilax* in SEQ. This knowledge gap forms the fifth research aim of this study, and will be addressed in Chapter 8.
Chapter 3 Study site description and related data collection

3.1 Study location

Field work in this study was conducted in two mangrove forests in the SEQ/NNSW region (Fig 3.1b), one of the most rapidly developing areas in Australia (Queensland Government 2012). The two sites were chosen because they are part of different tidal systems, but are ecologically, structurally and hydrologically similar, containing berms and ridges that influence tidal penetration into the mangrove basin, and both sites support significant immature mosquito populations.
Fig 3.1 Location of study sites in (a) Australia, (b) in the SEQ/NNSW region, and in the landscape ((c) Coombabah Lake – Study site 2 and (d) Terranora Broadwater – Study site 2 (d))
3.2 Site 1 – Terranora

Site 1 was located along the southern edge of Terranora Broadwater in New South Wales (28°13.5’S, 153°30.3’E), an inland tidal lake along Terranora Creek. The site comprises approximately 20 ha of mangroves, dominated by *A. marina*, but with *A. corniculatum*, *B. gymnorrhiza* and *R. stylosa* intermixed with *A. marina* along the lake edge and patches of saltmarsh (mainly *S. Virginicus*) and mangrove fern (*Acrostichum speciosum* Willd.) at the landward edge. It is bordered on the western and eastern sides by artificial ditches, and a narrow track and steep forest/grassland slope runs along the southern edge of the site. The areas surrounding the site comprised bushland, pastoral land and extensively ditched mangrove forest and saltmarsh.

3.2.1 Mosquito activity at site

The site is a significant immature mosquito habitat, supporting large populations of *Ae. vigilax*, *Cx. sitiens* and periodically *Ve. funerea* (C. Easton and B. Falkner, pers. comm.). As a consequence, it is monitored and treated regularly during warmer months to reduce mosquito production by Tweed Shire Council mosquito control. The Terranora site is also the location of a habitat modification project that aims to increase tidal hydrology throughout the mangrove basin. The berm is breached by an ~1m wide channel along the northern edge (directly in front of the connected front basin pool TW08 in Fig 3.2), and the pools immediately behind the breach are connected by a series of channels and pipes, extending into the back basin. This has reduced the tidal height necessary to flood the connected areas of the basin (see Fig 3.3), which therefore increased the frequency of tidal flushing into these areas.
Fig 3.2 Terranora study site (a) aerial photo, showing study sites, (b) transposed LiDAR derived topography with major features labelled (Image source: Google Earth 2011). Contours are measured as metres above Australian Height Datum (AHD).
3.2.2 Internal structure

The site (Fig 3.2) has an internal structure typical of subtropical, eastern Australian *A. marina* dominated mangrove forests, with high berms running along the northern, eastern and western edges of the site and basin forest within the internal areas of the site. An internal ridgeline runs approximately northeast to southwest through the middle of the site, effectively cutting the site into a front and back basin, which both comprise typical mangrove basin substrate mosaic of pools, substrate and pneumatophores. The two basin types and hydrological modification project divides the basin into four distinct areas. Fig 3.3 shows typical flooding patterns within the basin, recorded of 14/12/2012, which correspond to the four basin areas.

![Fig 3.3 Comparison of tidal flooding at the four areas of the Terranora site – 14/12/2012.](image)

(Source: Knight, unpublished data)

1. The connected front basin, which floods earliest and most frequently, being closest to the tidal source (~50m), lying in front of the central ridgeline and being directly connected to the estuary by the modification project.
2. The unconnected front basin, which floods soon after (~15 minutes) after the connected front basin, due to its position in front of the central ridgeline, but therefore less frequently than the connected front basin, being further from the tidal source (~100-150m) and not directly connected by the modification project.
3. The connected back basin, which floods later (~35 minutes after the
connected front basin) and less frequently due to it being further from the tidal source (~100-150m) and lying behind the central ridge line, but more frequently than the rest of the back basin due to its connection to the modification project.

4. The unconnected back basin, which is last to flood (~55 minutes after the connected front basin) due to being far from the tidal source (~150-200m), lying behind the central ridgeline and being unconnected to the modification project.

Data was collected in and around 12 pools across the Terranora study site. The pools chosen were all completely isolated from nearby pools by dense pneumatophore stands spread across the basin so as to lie within the four basin areas. These areas were of different sizes, so pools could not be evenly spread between the treatments. Table 3.1 summarises the area and depth the sample pools.

<table>
<thead>
<tr>
<th>Pool ID.</th>
<th>Basin Area</th>
<th>Surface Area (m²)</th>
<th>Mean standing water depth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TW01</td>
<td>Unconnected back</td>
<td>90</td>
<td>20</td>
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<tr>
<td>TW02</td>
<td>Unconnected back</td>
<td>40</td>
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</tr>
<tr>
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<td>22</td>
</tr>
</tbody>
</table>
3.2.3 Climatic conditions

Fig 3.4 shows climatic data in the Terranora site region.

![Climatic Data Graph](image)

During the study period the Terranora site experienced average rainfall throughout Winter and Spring, but was marked by above average rainfall in January 2012 (600mm – approximately three times the average rainfall for the area). This rainfall resulted in significant flooding of the study site at the end of January 2012. See Appendix 1 for detailed climate data.

3.3 Site 2 – Coombabah

Site 2 (Fig 3.5) was located on the eastern edge of Coombabah Lake, an inland tidal lake in Queensland (27° 54’ 18.4"S, 153° 21’ 56.3E”). The site lies within the Coombabah Lake Nature Reserve, a protected area of bushland administered by the Gold Coast City Council, and is immediately adjacent to the Coombabah Lake Marine National Park Zone, a designated protected fish habitat within Moreton Bay Marine Park.

The site comprises approximately 18 ha of *A. marina* dominated mangrove forest
situated along a tidal creek branching from the lake. The site was dominated by saltmarsh vegetation as late as the 1940s, however mangroves have gradually spread across much of the site over the last century. This change is thought to be caused by extensive ditching to attempt to drain the saltmarsh to improve pasture (Knight 2008). Ditching probably allowed mangrove propagules to colonise further inland, which allowed mangroves to spread across much of the area (Breitfuss 2003; in Knight 2008). This transition to mangrove may also be driven by climate change, as sea level rise and increased rainfall has been linked to mangrove encroachment into saltmarsh ecosystems (Eslami-Andargoli et al 2009).

The site is part of a larger system of mangroves that surround the lake. Mangroves are bordered on the landward side by a 20-50m strip of saltmarsh, behind which lies dense eucalypt forest. The site is also in close proximity to considerable industrial and residential development - Coombabah Lake has been gradually surrounded by urban development with the growth of the SEQ/NNSW region, with development spreading virtually up to the lake’s western edge. An airfield and water treatment plant lie directly south of the study site, and suburbia has encroached to within 1km of the study site.

3.3.1 Mosquito activity at site

Coombabah Lake is a significant mosquito habitat, supporting large immature populations of *Ae. vigilax*, *Ae. alternans*, *Cx. sitiens* and *Ve. funerea*. *Ae. vigilax* is particularly prevalent at the site; Knight (2008) measured eggshell densities as high as 26 eggshells/cm³ (0.05 eggshells/cm³ is been considered sufficient to indicate a significant mosquito problem (Addison et al 1992)).
Fig 3.5 Coombabah study site (a) aerial photo showing study sites, (b) transposed LiDAR derived topography with major features labelled (Image source: Google Earth 2013). Contours are measured as metres above Australian Height Datum (AHD).
3.3.2 Internal structure

The Coombabah site comprises a similar internal structure to that of the Terranora site, with a basin lying behind a berm along the creek edge. LiDAR derived microtopography shows that the system has no significant internal ridges or berms that impede tidal flow. Instead an area of low dieback runs through the middle of the site and extends to the south, comprising large area of very warm shallow water (in situ measurements of daytime water temperatures rose above 40°C in summer), with little or no exposed substrate or vegetation (with the exception of dead tree stumps and fallen branches). The lack of stabilising pneumatophore structure makes the substrate in this area extremely boggy and virtually impassable.

The berm area runs between the creek and the area of dieback, comprising dense pneumatophores and exposed substrate, with few pools and occasional patches of shallow (1-3cm) standing water. This higher area extends to the west of the dieback to the landward edge of mangroves, effectively cutting the site off from the lower area of mangroves running along the mouth of the creek and the lake edge. The rear of the site comprises a large, deep water (~40-60cm) area. This area appears to be unique within the site, being relatively open, with relatively sparse vegetation, with little exposed substrate or pneumatophore structure and is largely covered by a single expanse of standing water.

The dieback and rear flooded area divides the basin into three distinct areas. Tidal data during the study period was not available for this site, however hydrology of the study site was examined in detail by Knight et al (2008), which shows variation between the three basin areas in terms of the highest non-flooding tide (the maximum tide height above which tides flood the basin), the high tide percentage exceedance (the proportion of high tides that are high enough to flood into the basin) and the duration of tidal flooding, as described in table 3.2.
Table 3.2  Tidal hydrology data for the three basin types (source: Knight et al (2008)). The missing value in the Front basin (site 7) denotes a flooding duration exceeding the period between floods.

<table>
<thead>
<tr>
<th>Area</th>
<th>Corresponding site (Knight et al 2008)</th>
<th>Highest non-flooding tide (m)</th>
<th>High tide exceedance (%)</th>
<th>Duration of flooding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Front</td>
<td>Site 7</td>
<td>1.564</td>
<td>14.92%</td>
<td>-</td>
</tr>
<tr>
<td>Dieback</td>
<td>Site 12</td>
<td>1.642</td>
<td>8.29%</td>
<td>10-12hrs</td>
</tr>
<tr>
<td>Back</td>
<td>Site 11</td>
<td>1.642</td>
<td>8.29%</td>
<td>9-10hrs</td>
</tr>
</tbody>
</table>

1. **Front basin area** - Located in the west of the study site, between the dieback and the higher western edge of the site (outlined in red in Fig 3.5). This area lies closer (50-150m) to the tidal source (the creek) than the other basin areas. Knight et al (2008) measured tidal flooding within this basin area and determined that flooding of this area in front of the dieback is triggered by a slightly lower tide (7.8cm lower than the rear areas of the site), and therefore floods the basin more frequently (by 14% of all tides) and remains flooded for a longer period.

2. **Dieback basin area** - The wide band of mangroves along the eastern edge of the site (outlined in yellow in Fig 3.5), running almost north to south along the edge of the central dieback area and the landward edge of the mangroves, further from the tidal source than the front basin area (200-275m). Knight et al (2008) determined that flooding in this area is triggered by a higher tide (1.642m) and floods less frequently (by 8.29% of tides) than in the front basin area, and for a shorter period (9-10hrs).

3. **Deep basin area** - The basin area running along the southern edge of the deep standing water area at the northern area of the site (outlined in blue in Fig 3.5). This area was the furthest distance from the tidal source (~400m). Knight et al (2008) determined that the deep area exhibits similar flooding characteristics to the dieback area, however it has distinctly different morphological characteristics to the dieback area, being deeper (40-50cm) and more open, but still retaining the basin pool/pneumatophore/soil mosaic.

Data was collected in and around 12 pools across the Coombabah study site. As with the Terranora site, the pools were spread across the basin so as to lie within the four basin areas. These areas were of different sizes, so pools could not be
evenly spread between the treatments. Table 3.3 summarises the area and depth of the sample pools.

<table>
<thead>
<tr>
<th>Pool ID</th>
<th>Basin Area</th>
<th>Surface Area (m²)</th>
<th>Mean standing water depth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM01</td>
<td>Front</td>
<td>250</td>
<td>32</td>
</tr>
<tr>
<td>CM02</td>
<td>Front</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>CM03</td>
<td>Front</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>CM04</td>
<td>Back</td>
<td>160</td>
<td>35</td>
</tr>
<tr>
<td>CM05</td>
<td>Back</td>
<td>12</td>
<td>41</td>
</tr>
<tr>
<td>CM06</td>
<td>Back</td>
<td>45</td>
<td>37</td>
</tr>
<tr>
<td>CM07</td>
<td>Back</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>CM08</td>
<td>Dieback</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>CM09</td>
<td>Dieback</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>CM10</td>
<td>Dieback</td>
<td>85</td>
<td>20</td>
</tr>
<tr>
<td>CM11</td>
<td>Dieback</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>CM12</td>
<td>Dieback</td>
<td>90</td>
<td>25</td>
</tr>
</tbody>
</table>
3.3.3 Climatic conditions

Fig 3.6 summarises climatic conditions for the Coombabah site.

Fig 3.6 Coombabah temperature and rainfall over the study period (Coombabah wastewater treatment plant). The green line represents mean monthly temperature and the blue and red columns represent total monthly rainfall over the study period and mean historical (1992-present) total monthly rainfall respectively (Source: Bureau of Meteorology).

Climatic conditions followed a similar pattern to that of Terranora: low winter and spring rainfall followed by above average rainfall in January 2012 (506.8mm – three times the average January rainfall). As with Terranora, this rainfall caused the site to be completely flooded in late January 2012. See Appendix 1 for detailed climate data.

3.4 Data collection regime

Sites were sampled for fish from April 2011-April 2012 (refer to Appendix 2 for calendar of sampling dates). Sampling was conducted in accordance with permits provided by New South Wales Fisheries, Queensland Department of Agriculture, Fisheries and Forestry and Queensland Department of Environment and Resource Management. All capturing and handling of fish, in both field sampling and
laboratory procedures were conducted as per a Griffith University Animal Ethics Committee approval (ethics approval number: ENV/01/11/AEC).

Sites were sampled 1-2 days following the last flooding tide of the monthly tidal peak (usually within 2-3 days of the peak high tide). This timing allowed optimal sampling of fish and mosquito populations for the aims of this study; it sampled resident fish in the mangrove basin following maximum connectivity across the basin, as per Sheaves and Johnson (2008), and coincided with the critical period of mosquito larval hatching development, in the 1-2 weeks immediately following tidal inundation (larvae develop fully in 5-13 days following inundation of the substrate, depending on temperature). Sampling was also conducted on days with no rainfall, and before any significant rainfall post tide, to prevent rain and surface runoff from altering water levels or water chemistry. Specific sampling procedures for each of the experiments are described in detail in their relevant chapters.
Chapter 4  Composition and abundance of resident fish species in mangrove basin forests

4.1 Introduction

Mangroves play a critical role as a habitat for many species of fish (Vidy et al 2004; Wang et al 2009), but different fish species use mangrove forests in different ways (Laegdsgaard and Johnson 1995). Fish may be classified as resident or transient, based on the length of time they spend in mangroves (Taylor et al 1998). Transient species enter mangroves for relatively short periods, such as for specific life stages (Sheridan and Hays 2003), while resident species remain in mangroves for their entire lives (Nagelkerken et al 2000; Faunce and Serafy 2006).

These definitions focus largely on situations where free exchange between the mangrove and the estuary is possible, such as the opened culverts in breached mangrove and saltmarsh impoundments (Harrington and Harrington 1982; Brockmeyer et al 1997; Taylor et al 1998). This is not a characteristic of subtropical, A. marina dominated mangrove basin forests in eastern Australia (especially in the SEQ/NNSW region), which may be tidally isolated for weeks, or even months, depending on tidal ranges and their position within the landscape (Knight et al 2008). For these mangrove basins exchange between the mangroves and estuary is brief and infrequent, and consequently fish populations that occupy the basin are confined to pooled water that persists in the basin.

In these situations the aforementioned definitions used to describe transient and resident species are inadequate. Rather, in these ecosystems transient populations comprise species that that enter and leave mangroves on tidal events, entering mangroves when the tide over-tops the berm and inundates the mangrove basin.
and leaving on the ebb of the tide to avoid being trapped in the mangrove between tides. Resident populations comprise species that are present in the mangrove basins between tides, either entering mangroves during a tidal flooding event and leave during another, or conduct their entire life cycle within mangrove basin pools.

Most studies of fish within these ecosystems (and other intertidal habitats, eg. saltmarshes) focus on fish entering and leaving mangroves during tidal flooding events, and therefore have focussed on transient species (Morton 1990; Hindell and Jenkins 2004; Smith and Hindell 2005; Hollingsworth and Connolly 2006; Platell and Freewater 2009; Valinas et al 2010; Mazumder et al 2006a). Tidally isolated mangrove basin pools experience significant reductions in water quality between tides as the period since tidal connection increases (Knight et al 2013). Consequently resident fish populations are likely to be different to transient populations. This has been seen in SEQ/NNSW saltmarshes, for example, in a SEQ saltmarsh system, where completely different resident fish populations was found in pools between tidal flooding events (Morton et al 1988) compared to transient populations moving into the saltmarsh with tidal flooding (Morton et al 1987).

However, a review of relevant literature found no studies that examined resident fish populations in intermittently connected sub-tropical, A. marina dominated mangrove basins in the SEQ/NNSW region. This knowledge gap may be linked to the difficulties in sampling in these ecosystems. Morton (1990) and Hindell and Jenkins (2004) deliberately avoided the internal basins of A. marina mangrove forests, considering the dense pneumatophore stands and pools too shallow and complex to study using conventional sampling methods. Methods such as gill, fyke, seine and cast nets cannot be used in small, shallow, snag-ridden, structurally complex mangrove pools.

Also, methods that have been used to sample in other types of mangroves could potentially cause significant environmental damage. Methods such as stake netting, which have been successfully used in different types of mangrove (Vance et al 1996; Huxham et al 2008) may significantly alter A. marina mangrove basin structure, potentially causing environmental damage and sampling inaccuracies. Methods that drag across or dig into the bottom of pools also risk disturbing MBOs which, if mixed into the water column rapidly deoxygenates pools and disrupts fish and other aquatic organisms (Howarth and Merkel 1984; Bush et al 2004a; Bush et al 2004b). Therefore, sampling fish in mangrove basin pools requires methods that
are practical in shallow, snag-ridden water, but which also have minimal impacts on the environment.

The lack of studies examining resident fish in SEQ/NNSW mangrove basins presents a significant knowledge gap. Resident fish are far more likely to be involved in fish-mosquito interactions as they are present within the mangrove basin between flooding events, when mosquito larvae hatch and develop. This has been observed in the diets of resident fish in tidally isolated saltmarshes (Morton et al 1988) and intermittently connected estuarine channels (Becker and Laurenson 2007), where mosquito larvae (and similar prey) comprise a significant portion of fish diets. Resident fish may therefore significantly impact *Ae. vigilax* populations, but we require knowledge of the fish populations that are present in the mangrove basin between tidal connections. This information is critical for determining the extent to resident fish may influence mosquito populations, and thus the potential of fish to act as biological control agents of mosquitoes. The research presented in this chapter addresses this knowledge gap by describing surveys of resident fish populations within intermittently connected SEQ/NNSW region mangrove forest basins, examines the composition of resident fish populations and describes their annual and spatial abundance.

### 4.2 Methods

#### 4.2.1 Data collection

To resolve sampling issues previously discussed this study used small, collapsible ‘baitfish’ traps to capture fish (Fig 4.1). These measured 40 cm (L) x 20 cm (H) x 20 cm (W), with a stretched mesh size of approximately 0.3 cm. The traps had two conical funnels with 3 cm openings at each end (Fig 4.1a), which opened into an inner collection chamber (Fig 4.1b) where fish can enter, but cannot escape. These traps enabled sampling in confined, shallow mangrove pools, without disturbing the MBOs in the pool substrate. Similar (albeit larger) traps have recently been used to trap fish at the Terranora study site (Lee et al, unpublished data), and similar traps have been used to sample fish in freshwater lakes (Carter and Wilson 2006), creeks (Milton and Arthington 1983) and saltmarsh pools (Edgar 1990).
Fish were sampled monthly between April 2011 and April 2012, at each of the 12 sample pools at the two study sites (see Chapter 3). Thirteen samples were taken at each pool, providing 156 samples at each site over the study period, providing 312 samples in total. One trap was deployed at each pool, at approximately the same time of day during each sampling trip, from 10am to 2pm. Sampling timed to take place at low tide (or when tides are unable to overtop the berm and flood the basin), and took place on days when no rainfall occurred, to prevent any fluctuations in water level from disrupting sampling.

Traps were deployed in the same position for all samples. Traps were set ~1m from and parallel to the edge of the pool, positioned so the two entry funnels were not blocked by pneumatophores or fallen logs or branches. Traps were also positioned with the entry funnels lying just beneath the water’s surface, as fish were only observed in the top 1-3cm of the water column.

Traps were deployed for one hour. This length of time was chosen as a compromise between allowing the trap to capture enough individuals from the pool population (to achieve the research aims in this chapter) and preventing complete digestion.
and evacuation of recently consumed prey (to achieve the research aims in Chapter 5). Upon retrieval of each trap any fish caught were removed and immediately euthanised in an ice-water slurry, both to ensure humane treatment of individuals and to minimise digestion of stomach contents, and stored for laboratory analysis (See Chapter 5). Only a small number of fish per species were originally planned to be kept, however widely varying numbers of fish caught in the traps in different areas and the large numbers of exotic species caught made this impossible. All fish caught were identified to species, with the exception of one species that has not been formally classified (see 4.3.1).

4.2.2 Data processing and analysis

Two analyses were conducted on fish catches, focussing on two aspects of the fish population: annual variation and spatial distribution. Due to significant differences in catch yields between the Terranora and Coombabah the two sites were analysed separately.

Annual distribution of fish

Fish populations were compared over the entire study period, by season: summer (December-February), autumn (March-May), winter (June-August) and spring (September-November). The fish population was expressed in two forms: mean number of fish caught and number of pools containing fish. Means of each value were pooled across all three months of each season, and due to the large numbers of samples containing no fish at both sites (see section 4.3.1) only samples containing fish were used in the analysis.

Mean fish counts per season and mean number of samples containing fish were compared between the four seasons at each site using Analysis of Variance (ANOVA), testing the null hypotheses that there is no seasonal difference in a) mean catch yield, and b) mean number of samples containing fish. Due to the dominance of G. holbrooki (see section 4.3.1) fish populations were analysed as a whole. Post hoc T LSD tests were used to distinguish between seasons.
Annual variations in species composition was analysed using by comparing the Shannon species diversity index (H'), which gives a value between 0 and 1, with a higher number representing a more diverse, even populations (0 representing only a single species present and 1 representing an even number of 2 or more species) between seasons. H' of each sample containing at least one fish was calculated using the formula:

\[ H' = -\sum_{i=1}^{s} p_i \log_e p_i \]

Where \(p_i\) = the proportion of the ith species present in sample s. H' values for each sample were pooled by season, and were compared between seasons using a one way ANOVA to test the null hypothesis that species evenness does not significantly differ between seasons. Levene’s Test was carried out to confirm homogeneity of variances, and all data was log transformed prior to analysis (\(\ln(x+1)\)).

Spatial distribution of fish

Samples were pooled by basin type to give a mean value for the different basin treatment types at each site. At the Terranora site four basin treatments were examined: front connected (2 pools), front unconnected (3 pools), back connected (3 pools) and back unconnected (4 pools). At Coombabah three basin treatments were examined: front (3 pools), deep (4 pools) and dieback (5 pools). As with annual abundance analysis, the two sites were analysed separately.

The number of samples containing fish in each pool were pooled by basin treatment to give a mean frequency of positive (fish present) samples. This was compared between the four Terranora treatments and three Coombabah treatments with ANOVAs to test the null hypothesis that mean number of positive catches does not significantly differ between mangrove basin treatments at each site.

Two values were calculated to analyse distribution of fish populations across the mangrove basin: fish density/m² and species diversity (H'). Fish density was calculated by dividing the total number of fish by the surface area (m²) of the pool in which they were caught. Fish density incorporated all species, due to the dominance of *G. holbrooki* (see section 4.3.1). Species diversity (H') was calculated
as per the methods described in the previous section. These values were calculated for each pool sample that contained fish, and were combined by basin to give a mean value for each of the four Terranora and three Coombabah basin treatments.

There were large differences in the number of sample sites per treatment, so the two fish population values (mean fish density and mean species diversity) were analysed using Kruskal-Wallis non-parametric tests to test the null hypotheses that a) mean fish density and b) species diversity did not significantly differ between mangrove basin treatments. Any significant differences were analysed post hoc using Dunn’s test to distinguish between treatments.

4.3 Results

4.3.1 Composition of resident fish populations

Table 4.1 summarises sampling at both sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Species</th>
<th>Freq. of occurrence (F)</th>
<th>Number of fish caught</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N (overall)</td>
<td>F (samples containing fish)</td>
</tr>
<tr>
<td>Terranora</td>
<td><em>G. holbrooki</em></td>
<td>33 0.21</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td><em>P. signifer</em></td>
<td>8 0.05</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td><em>Pseudogobius sp</em></td>
<td>12 0.08</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td><em>H. galii</em></td>
<td>9 0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>Coombabah</td>
<td><em>G. holbrooki</em></td>
<td>34 0.22</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td><em>P. signifer</em></td>
<td>15 0.10</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td><em>Pseudogobius sp</em></td>
<td>8 0.05</td>
<td>0.22</td>
</tr>
</tbody>
</table>

In total 1714 fish were caught during the sampling period, Sample yields were significantly higher at Coombabah (1238 individuals) than at Terranora (476 individuals). Sampling found four resident species of fish in the systems (Fig 4.2). Three of these were native species, two being well-known species: the Pacific Blue-Eye (*P. signifer*) and the Firetail Gudgeon (*Hypeleotris galii* (Ogilby)), and one an
undescribed species belonging to the genus *Pseudogobius*, known locally as the Blue-Spot Goby (Allen et al 2002) and referred to hereafter as *Pseudogobius* sp.. The fourth species was the introduced Mosquitofish (*G. holbrooki*).

Fig 4.2 The four species captured in mangrove pools throughout this study: a) *G. holbrooki*, b) *P. signifer*, c) *H. galii* and d) *Pseudogobius* sp.. (Illustrations modified from: Pollard 1980).
Overall, relatively few samples contained fish. Of the 312 samples in total across all sites only 44 at Terranora and 38 at Coombabah contained fish. *Gambusia holbrooki* was the most frequently encountered species, caught in 75% and 92% of successful fish catches at Terranora and Coombabah respectively, and comprised ~80% of fish caught at both sites. There were far fewer specimens of native species; at Terranora the three species were present in comparable abundances, between 18% and 27% of successful fish catches and comprising 5.46% to 7.56% of fish caught. At Coombabah *H. galii* was not caught at all, and *Pseudogobius* sp. was only present in small numbers (less than 1% of all fish caught). *Pseudomugil signifer* was present in much higher numbers, in 41% of successful fish catches and comprised 19.55% of all fish caught.

### 4.3.2 Seasonal variation of fish population

Fig 4.3 summarises mean monthly fishes catch at each site across the 12 study pools throughout the study period.
Mean monthly fish catches at the two sites showed similar patterns. At Terranora fish catches were high in autumn 2011, before rapidly decreasing to zero by August 2011. No fish were caught until November 2011, when fish catch yields began to increase through to March and April 2012. The mean number of pools containing fish was significantly different between seasons ($F=4.943$, df=3, $P=0.027$), being significantly higher in autumn (6.75) than in winter (1.33), spring (1.00) or summer (3.33).
At Coombabah there were small fish catches in autumn 2011, but no fish were caught in from June 2011 to January 2012 (winter through to mid-summer), except for a small catch in September 2011. As for Terranora, fish catches increased dramatically in March and April 2012. The mean number of pools containing fish was significantly different between seasons (F=5.949, df=3, P=0.02), being higher in autumn (6.00) than winter (0), spring (2.00) and summer (2.33). Seasonal sampling results are shown in table 4.2.

Table 4.2 Comparison of mean seasonal pool fish counts at the Terranora and Coombabah sites. Entries with different letters are statistically significant (p≤0.05) from the T-LSD tests. Winter is not shown at Coombabah as no fish were present. Also, the two studies sites were analysed separately and so T LSD groupings between sites are independent.

<table>
<thead>
<tr>
<th>Site</th>
<th>Season</th>
<th>Fish Counts</th>
<th>Species Diversity (H')</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Terranora</td>
<td>Winter</td>
<td>0.74A</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>0.23A</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>1.69A</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>7.13B</td>
<td>2.31</td>
</tr>
<tr>
<td>Coombabah</td>
<td>Spring</td>
<td>1.19A</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>7.19B</td>
<td>5.46</td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>19.45C</td>
<td>11.04</td>
</tr>
</tbody>
</table>

At the Terranora site ANOVA identified significant differences in mean fish counts between seasons (F=5.166, df=3, P=0.024). Post hoc T LSD tests found mean fish counts were significantly higher in autumn than the other three months, which were not significantly different to each other. Results of ANOVA comparing mean species diversity (H’) found significant differences between the four seasons (F=5.502, df=3, P=0.003), as winter, spring and summer sampling had a mean H’ of 0, comprising a single species (G. holbrooki in winter and summer and Pseudogobius sp. in spring). Autumn mean H’ was significantly higher (0.34) but still suggested significant unevenness between species, as pools were dominated by G. holbrooki.

At the Coombabah site the ANOVA identified significant variation in mean pool fish counts between seasons (F=9.459, df=3, P=0.007). As with the Terranora site autumn mean fish counts were significantly higher than all other seasons.
Additionally T LSD tests identified summer mean fish counts being significantly higher than spring fish counts, but also significantly lower than Autumn counts. Mean species diversity (H') significantly differed between seasons where fish were found (spring, summer and autumn) (F=3.601, df=2, P=0.038). Mean H’ values were low for all seasons, especially spring, where H’=0 (only G. holbrooki were caught). *Gambusia holbrooki* dominance of pools also resulted in low mean H’ values in summer (0.38) and autumn (0.18).

4.3.3 *Spatial distribution of fish population*

Frequency of samples containing fish

Results of the ANOVAs showed no significant difference in mean number of samples containing fish at either Terranora (F=0.02, df=1, p=0.891) or Coombabah (F=0.516, df=2, p=0.614).

Mean fish density and mean species diversity

Distribution of fish results at the Terranora and Coombabah sites are shown in table 4.3.

Table 4.3 Mean fish density in the basin type treatments at the two study sites. Entries with different Dunns test letters are statistically significant (p≤0.05). Note that the two studies sites were analysed separately, and so Dunn’s groupings between sites are independent.

<table>
<thead>
<tr>
<th>Site</th>
<th>Basin area</th>
<th>Density (per m²)</th>
<th>Species diversity (H’)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Terranora</td>
<td>Back</td>
<td>Unconnected</td>
<td>0.16A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Connected</td>
<td>0.44A</td>
</tr>
<tr>
<td></td>
<td>Front</td>
<td>Unconnected</td>
<td>0.46B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Connected</td>
<td>0.04C</td>
</tr>
<tr>
<td>Coombabah</td>
<td>Front</td>
<td></td>
<td>0.54A</td>
</tr>
<tr>
<td></td>
<td>Deep</td>
<td></td>
<td>2.58B</td>
</tr>
<tr>
<td></td>
<td>Dieback</td>
<td></td>
<td>2.97B</td>
</tr>
</tbody>
</table>
At the Terranora site the Kruskal-Wallis test identified significant differences in mean fish density between the four basin treatments ($\chi^2=12.692$, df=3, 0.005). Dunn’s post hoc tests showed that density in the front unconnected areas were significantly higher than the other three areas, and the front connected area was significantly lower. The back connected area had a mean density similar to that of the front unconnected area, but this was due to one very large sample (167 individuals). H’ was lowest in the back connected area (0.07) and highest in the front unconnected area (0.37), and the back unconnected and front connected area fell in between (0.19 and 0.14 respectively). The Kruskal-Wallis test failed to find any significant difference in H’ between the four areas ($\chi^2=4.692$, df=3, P=0.196).

At the Coombabah site the Kruskal-Wallis test found significant variation in mean fish density between the three basin treatments ($\chi^2=5.980$, df=2, p=0.05). Dunn’s post hoc test demonstrated the deep and dieback areas were and significantly higher than the front area, and while mean fish density was higher in the dieback area it was not significantly different to the deep area. H’ was lowest in the front area (0.08), and higher in the dieback area (0.21) and highest in the deep area (0.27), but these were not significant ($\chi^2=4.23$, df=2, p=0.12).

### 4.4 Discussion

Surveys of the populations of resident fish have provided insight into the structure of resident fish populations within two SEQ/NNSW mangrove basin forests. At both sites significant resident populations remained within mangrove basin pools between tidal connections. The results demonstrate that fish are present in the mangrove basin in the critical warmer, wetter months (in the SEQ/NNSW region this is usually late Spring (November) to mid-Autumn (April)), when mosquito larvae are present in pools, and therefore may be predators of mosquitoes and potential biological control agents.

The structure of resident fish populations was relatively simple, comprising only four species: three native species (P. signifer, H. galii and Pseudogobius sp.) and a fourth exotic species (G. holbrooki). The latter dominated pools at both sites, comprising ~80% of the fish caught at both sites. This was completely different to transient fish populations in the area; a study concurrent with this one demonstrated that transient populations at the Terranora site are dominated by
native species, including toadfish (*Marilyna pleurostica* (Günther) and *Tetractenos hamiltoni* (Richardson)), glassfish (*Ambassis marianus* (Günther)) and mullet (*Myxulus elongatus* (Günther) and *Mugil cephalus* (L.)) (Lee et al, unpublished data).

Similar results have been seen in other studies conducted on resident species in intermittently connected coastal wetland habitats. Morton et al (1988) found two, (possibly three) of the species identified in this study in SEQ saltmarsh pools, in similar proportions - *G. holbrooki* being the dominant species, and smaller numbers of *P. signifer* and an unidentified Gobiid species (which may be *Pseudogobius* sp. in this study) in a saltmarsh ~7km downstream from the Coombabah study site. In another study Harrington and Harrington (1982) found impounded saltmarshes in Florida were dominated by a small number of similar fish species, including *G. holbrooki*.

The dominance of *G. holbrooki* demonstrates the distressed nature of SEQ/NNSW mangrove basin fish populations. Since its introduction in the 1920s *G. holbrooki* has caused considerable ecological damage to native fish populations in Australia (Walton et al 2012). Its high fecundity (Milton and Arthington 1983), wide environmental tolerance (Carter and Wilson 2006; Nordlie 2006), and extremely aggressive behaviour towards native species has enabled it to quickly dominate ecosystems (Keane and Neira 2004), including, it seems, SEQ/NNSW region mangrove basin forests. *Gambusia holbrooki* is well established in eastern and southern Australian waterways (Walton et al 2012); for example a survey of fish populations in Brisbane in the late 1970s and early 1980s found *G. holbrooki* in ~90% of fresh and brackish creeks (Arthington et al 1983). It has been reported that just its presence can put significant strain on native species, which, can cause significant growth defects and reduced reproductive capacity of native species, in particular species that share a similar ecological niche to *G. holbrooki*, in particular *P. signifer* (Howe et al 1997; Warburton and Madden 2003; Macdonald et al 2012). The dominance of *G. holbrooki* at both study sites indicates that it has largely displaced, but has not completely eradicated, native species from the system.

### 4.4.1 Seasonal abundances of resident fish populations

The fish population varied throughout the study period, with fish absent or rare in Winter and Spring, becoming more common in Summer, and reaching peak
numbers in late Summer and into Autumn. This suggests that while fish remain in the mangrove between tidal connections, they are not present in large numbers in the mangrove forests for large parts of the year, and may not be permanent residents. Instead the fish population results suggest patterns of semi-residence, where fish occupy mangrove pools for longer periods, but not necessarily permanently. These populations are still clearly distinguishable from transient species, as they remain within mangrove basin pools between tidal connections, whereas transient species do not.

Fish populations may not be able to survive throughout winter and spring because of lower water quality and availability. Water availability was certainly an issue at the Terranora site; the site was completely dry in November and December 2011, and a study concurrent with this one demonstrated that standing water in pools was consistently anoxic between tidal flooding events (Knight et al 2013). Water availability is also linked to rainfall (or the lack thereof) (Eslami-Andargoli et al 2009). Periods of low fish abundance correlated with the drier months of the year, and a spike in fish populations was observed following significant rainfall and flooding in January 2011. Fish may be able to survive within the mangrove in the short term, but adverse water quality and/or lack of water may eradicate or drive fish from the system. Movement of fish was outside of the scope of this study, so it is unclear whether fish populations leave the mangroves for certain times of the year or simply perish when conditions become unsuitable (and presumably are replenished when conditions improve). This warrants future research, and presents a significant future research pathway.

Seasonal differences in fish populations may also be unrelated to environmental conditions, and may instead be due to natural fluctuations in population size, driven by factors such as reproductive behaviour. Milton and Arthington (1983) documented *G. holbrooki* populations in a SEQ freshwater creek declining in late Autumn and into Winter, and increasing into Spring, reaching a peak in reproductive activity in October, which may lead to a larger population of *G. holbrooki* in Summer and Autumn. Davis (1988) found similar results that may also apply to resident or semi-resident fish, attributing annual changes in the abundance of fish moving into a tropical mangrove to reproductive and juvenile behaviour, rather than environmental factors. However these studies did not document a complete disappearance of fish for long periods, and so water quality and environmental conditions may still be at least partly responsible.
4.4.2 Resident fish abundance across the mangrove basins

Fish populations demonstrated significant heterogeneity, both temporally and spatially. A pool could have no fish following one tidal peak, but have several hundred individuals the next. Additionally, while one pool may have no fish, a nearby pool in the same basin area may contain many fish.

This heterogeneity may be due to the inherent variability of sub-tropical, *A. marina* dominated mangrove basin forests. Slight variations in topography and tide (of a magnitude of only several centimetres) can create different hydrological conditions, flooding one pool but not flooding another nearby (Griffin et al 2010). Further, variations in tidal penetration into pools may mean that certain tidal events only flood particular pools to an extent where fish (both transient and resident) can move into mangroves, leaving other nearby pools isolated and unreachable. Rainfall may also play a significant role, linking areas of the basin between tidal flooding and altering water quality in areas otherwise isolated from tidal flooding. This was not considered in this study, and so the finer resolution impacts of hydrological connection on fish utilisation of mangrove basin pools needs further research, and presents a significant future research pathway.

Another explanation of the high variability across the basin is the schooling behaviour of fish in large groups. The two dominant species identified in this study, *G. holbrooki* and *P. signifer*, are known to display schooling behaviour (Hurst et al 2004; Willems et al 2005), especially in complex, disorienting or ecologically unfamiliar habitats (Goodyear 1973). During tidal flooding events, when the entire mangrove substrate is inundated, fish may spread out across the basin, but remain clustered in schools and therefore would not be evenly distributed across the basin. When fish are forced into pools by the ebb tide, schools of fish may only be concentrated in specific pools. Again, as this study focussed on the fish-mosquito interaction between tidal connections, fish movement across the flooded basin was not examined. Therefore the source of the high degree of variability observed is unknown, and remains a significant future research pathway.

Despite the significant variation in the distribution resident fish populations, patterns were observed across both mangrove basin sites. Areas with extremes in tidal connectivity had significantly lower resident fish populations. At one extreme,
low resident fish populations in areas with too infrequent tidal connectivity, such as the unmodified area of the back basin at the Terranora site, may be a result of the balance of immigration and extinction factors seen in metapopulation theory in patchy habitats (Freckleton and Watkinson 2002; Sheaves and Johnston 2008), where tides are too infrequent to regularly replenish resident populations (ie. decreased immigration factors) or provide clean water to counterbalance water quality issues (extinction factors) in the pools.

At the other extreme, lower resident fish populations seen in areas of high tidal connectivity, such as the front connected basin at Terranora and front area at Coombabah, may be caused by improved water quality and more frequent connections allowing larger, predatory fish into the basin more frequently, thus reducing the refuge function provided by mangroves for small fish. Similar effects have been observed in impounded Florida mangroves and saltmarshes, where the breaching of dykes to increase tidal flooding greatly improved water quality and allowed larger, transient fish species, many of which were predators of smaller fish, to enter mangroves more frequently and survive in greater numbers (Brockmeyer et al 1997; Taylor et al 1998). Considering the dominance of *G. holbrooki* in mangroves this may be environmentally advantageous, as the results may suggest that larger fish may be applying significant predation pressure to *G. holbrooki* populations.

Significantly higher resident fish populations in areas with intermediate frequencies of tidal flow, such as the unconnected front basin at Terranora and the deep and dieback areas at Coombabah, may represent a balance in tidal flushing frequency favourable to resident fish. Flooding is frequent enough to reduce extinction pressure and allow immigration of new individuals into pools, but infrequent enough to restrict access of larger transient fish species to pools. To confirm this proposition requires investigation of the movement of fish across the basin, and how this movement correlates with different tidal conditions. This study did not examine this, as it primarily focussed on the post tidal peak activities of fish, and therefore fish movement across the basin and its relationship with different tidal conditions represents a future research pathway.
4.5 Conclusions

4.5.1 Summary and contribution to knowledge

This chapter examined the composition and abundance of resident fish populations in SEQ/NNSW mangrove basins, which may contribute to mosquito control. It addresses the first research aim of this study: to identify resident fish populations in mangrove basin forests. Survey of the two mangrove basins fish populations using small baitfish traps was an effective method of sampling fish populations in a shallow, structurally complex environment. Resident fish populations were dominated by *G. holbrooki*, an exotic fish species. They were also highly variable, fluctuating both spatially and temporally, suggesting that these populations may be semi-resident. However the results also demonstrated broader scale patterns; fish were mainly present in late summer and autumn, and were found in much larger numbers in areas that had frequent enough tidal flushing to allow immigration and maintain water quality, but infrequent enough to provide refuge from larger transient fish species.

4.5.2 Implications for mosquito control

The findings reported in this chapter indicate that mangrove basin fish could potentially influence mosquito populations, and thus contribute to mosquito control. Fish populations, while highly variable, were more abundant during the mosquito producing seasons, especially in March and April. As a result, fish were present in the mangrove basin pools during the mosquito larval development period immediately following the tidal peak, and therefore predatory interaction between fish and mosquitoes is possible seems likely.

However, while biological control is possible, the extent to which it may affect the mosquito population is not clear. The highly variable nature of fish populations observed in this study means that these resident fish, regardless of their individual impact on mosquitoes, may not be sufficient to reliably reduce mosquito populations. Biological control may be possible when fish are present, but unless they are abundant and ubiquitous across the mangrove basin, the *Ae. vigilax* population as a whole is unlikely to be reduced significantly by predation. This is a
recurring theme that will be discussed throughout the thesis, in particular in Chapters 8 and 9.

Despite this, the presence of potential mosquito predators within the mangrove basin during peak mosquito season means that resident fish populations warrant further investigation as biological control agents. Now that resident mangrove basin fish populations have been described, their feeding habits within mangrove basin pools can be examined, particularly the extent to which fish feed on *Ae. vigilax* larvae, which will be addressed in the following chapter.
Chapter 5 Dietary habits of resident mangrove basin fish species

5.1 Introduction

Mangrove environments provide a wide variety of potential prey items for fish. The protruding roots and pneumatophores provide a source of food for grazing fish (Robertson 1991) and provide a large surface area for algal, cyanobacteria and fungal growth (Potts 1980; Hyde 1990; Aikanathan and Sasekumar 1994; Swe et al 2009). This also provides food for large populations of grazing arthropods (Proches et al 2001; Proches and Marshall 2002; Proches 2004), creating a significant food source for many predatory fish species (Verweij et al 2006; Nagelkerken et al 2008).

Mangroves can support significant mosquito populations (Knight et al 2012) which have the potential to be a significant food source for fish. Larvae hatch from eggs deposited on tidally inundated substrate (Kerridge 1971) and congregate in isolated pools, grazing on algae, detritus and bacteria (Walker et al 1988; Thiery et al 1991; Wallace and Merritt 2004). Many studies that have examined fish dietary habits in habitats similar to mangroves, such as saltmarshes, have recorded significant predation of mosquitoes (Hess and Tarzwell 1942; Harrington and Harrington 1961; Morton et al 1988). The degree to which mosquito larvae comprise fish diets varies considerably between studies, from being completely absent from diets to being the dominant prey item.

As discussed in the Chapter 4, resident fish (defined in this study as fish remaining within mangrove basin pools between tidal flooding events) are far more likely to be predators of mosquitoes than transient species (defined as fish that enter and leave mangroves during tidal flooding events). Because mosquito eggs hatch and larvae develop in the period following flooding events, transient species are simply not
present in mangroves when larvae hatch and congregate in pools, and so cannot significantly prey upon mosquito larvae. Diets of transient species reflect this, showing little predation of mosquito larvae (Nemerson and Able 2004; Valinas et al 2010), even in well-established Ae. vigilax habitats such as saltmarshes (Morton et al 1987; Hollingsworth and Connolly 2006; Platell and Freewater 2009).

In comparison, resident species are present in larval habitats in the critical larval development period following tidal flooding, and can therefore feed on large numbers of mosquitoes. Studies conducted in habitats similar to mangroves, such as saltmarshes, document significant feeding on mosquito larvae by resident fish (Hess and Tarzwell 1942; Harrington and Harrington 1961; Morton et al 1988). For example Morton et al (1988) found mosquito larvae comprised as much as 90% of diet of resident fish in a SEQ region saltmarsh.

In the review of fish as mosquito predators (Chapter 2) several key factors that influence the degree to which resident fish may feed on mosquito larvae were identified. Small, generalist species were identified as those most likely to feed on mosquito larvae. These are fish that can cope with wide fluctuations in mosquito populations (Harrington and Harrington 1961; Murdoch 1969; Pen and Potter 1991; Schleuter and Eckmann 2008; Laufer et al 2009), and can shift their diets to other prey items when mosquitoes are unavailable (Bence 1988; Chesson 1989; Knight et al 2004; Walton 2007; Manna et al 2008). They are also morphologically suited to feed on mosquitoes, being small enough for mosquito larvae to be a worthwhile food source and having upturned or centralised mouthparts suitable for feeding on mosquitoes floating on the water’s surface (Chandra et al 2008).

Small, generalist, resident fish species may therefore be effective predators of mosquitoes, but prior to this study they had not been described in sub-tropical, A. marina dominated, SEQ/NNSW region mangrove basin forests. Consequently the extent to which resident fish feed on mosquitoes is a significant knowledge gap, especially relating to the biological control potential of mangrove fish populations. The research presented in the previous chapter demonstrated that as resident fish populations are present in pools during the critical larval growth period a predator-prey interaction between fish and mosquitoes is possible. The research presented in this chapter describes the dietary habits of the four resident fish species in SEQ/NNSW mangrove basin forests, with particular focus on predation on mosquito larvae and seasonal variations in diet.
5.2 Methods

5.2.1 Data collection

Fish used in the research presented in this chapter were collected as described in Chapters 3 and 4 (see sections 3.4 and 4.2.1). Briefly summarised, rectangular baitfish traps were deployed in 12 mangrove pools at the Terranora and Coombabah sites, monthly from April 2011-April 2012, 1-2 days after the end of the monthly tidal flooding (see section 3.3). Traps were deployed for 1 hour, after which any fish caught were immediately euthanized in an ice-water slurry to immediately halt digestion and excretion of stomach contents. All fish were identified to species and stored frozen for subsequent laboratory analysis of stomach contents.

5.2.2 Analysis of dietary habits

Dietary habits of fish were determined by fish gut content analysis (Berg 1979; Hollingsworth and Connolly 2006). The approach has been used in many studies for determining dietary habits of fish (Sumpton and Greenwood 1990; Arthington and Marshall 1999; Hollingsworth and Connolly 2006; Moreau et al 2008; Raby et al 2010; Valinas et al 2010; Vaslet et al 2012). Because the fish examined in this study were all relatively small (<5cm) distinguishing the stomach from the rest of the digestive tract proved difficult, and so the entire digestive tract was analysed. A section of the side of the fish was cut away, along the underside of the fish, from the mouth to the anus, up to the spine, and along the spine to the start of the gills. The entire digestive tract was then cut away and removed.

The digestive tracts were analysed under a dissecting microscope. Three measurements were made:

1. Gut fullness – Gut fullness was visually estimated as the proportion of the entire digestive tract containing swallowed food (expressed as a percentage), which was used to determine the extent to which fish fed within mangrove pools. The number of fish containing no food was also recorded.
2. Identification of prey items: prey items were removed from the digestive tract and spread onto a petri dish. Each prey item was identified to their lowest
possible taxonomic level. Any mosquito larvae or pupae found were identified to species. All prey items consumed appeared to be swallowed whole, which the exception of larger instar mosquito larvae, which was often partly broken down or found separate body parts. These were identified by matching easily recognisable, sclerotised heads and siphons

3. *Estimation of diet composition:* the proportion each individual prey item contributed to the total stomach content of each fish was determined using the estimated volumetric method (Hyslop 1980). The method involved evenly spreading all prey items on a small section of the petri dish to remove spaces between individual prey items, and then overlaying the dish with a 3x3mm graticule. The area occupied by each prey item was then estimated as a proportion of the total area of stomach contents.

5.2.3 *Data processing and analysis*

Dietary behaviour was examined over two treatment levels – season and species. Rather than comparing by traditional seasons, the study period was divided into two six month periods, based on broad patterns of mosquito activity in Australia: mosquito season, comprising the warmer, wetter late summer to early autumn when mosquitoes are generally more active (November-April), and non-mosquito season, the cooler, drier, late autumn to early spring when mosquitoes are far less active (May-October). Comparisons between species were made within each season at each site, and between seasons when a specific species was present in both seasons. The two sites were treated separately, due to large differences in fish sample sizes (Coombabah had approximately three times the number of fish caught as Terranora). Two components of dietary behaviour were examined: gut fullness and diet composition.

**Gut fullness**

Gut fullness was compared between species and season (mosquito and non-mosquito season) at each of the two sites. For each species, within each season, gut fullness was pooled to give a mean gut fullness per species, per season, for each site. A two way ANOVA was used to compare mean gut fullness between species
and season treatments, testing two null hypotheses: a) that mean gut fullness does not significantly differ seasonally, and b) that mean gut fullness does not differ between fish species. Post hoc T LSD tests were used to distinguish differences between treatments. Overall mean stomach fullness was also calculated for each site, and compared between the two study sites using a Student’s T-test. Levene’s test for homogeneity of variances was conducted prior to all analyses, and required data to be log transformed ($\ln(x+1)$) for analysis.

**Diet composition**

Prey items comprising the diet of the different fish species were analysed in two ways:

1. **Frequency of occurrence** - this was used to identify the most frequently occurring prey items found in fish diets. At each site, for each fish species, across the two seasons, the proportion of fish containing at least one individual of each prey item was calculated.

2. **Prey composition** – this was used to determine the proportion each prey item contributed to the total stomach contents of fish. This was calculated for each individual fish, and was pooled at each site, across each species and season, to give a mean proportion for each prey item. Schoener’s index was used to compare the similarity of diet composition (or dietary overlap) between groups. This index provides an effective way of examining overlap between groups of species (Schoener 1970) and has been used to compare overlap of particular prey items within the diets of fish (Morton et al 1988; Natsumeda et al 2012). Shoener’s index ($x$) is calculated using the formula:

   $$ x = 1 - 0.5 \left( \sum_i [px_i - py_i] \right) $$

Where $px_i$= proportion of prey item $i$ in fish group $x$, and $py_i$= proportion of prey item $i$ in fish group $y$. Shoener’s index was calculated to compare dietary overlap between all four fish species, both within and between seasons. Comparisons gave a value between 0 and 1, where 1 is a perfect overlap between diets and 0 is a completely dissimilar diet. Diets are considered to be similar when index values are over 0.6. This tested the null
hypothesis that fish diets overlap between species and between species present in both seasons.

5.3 Results

5.3.1 Stomach fullness

In total 1714 fish digestive tracts (guts) were retrieved and dissected. Mean stomach fullness across the entire study period was slightly lower overall at Terranora (31.68% compared to 33.86% at Coombabah), but a T-test showed that this difference was not significant (t=2.103, df=1, p=0.147).

Terranora

Results of gut fullness analysis at the Terranora site is shown in table 5.1.

Table 5.1 Summary of gut fullness data from fish caught at Terranora.

<table>
<thead>
<tr>
<th>Season</th>
<th>Species</th>
<th>Total number of fish</th>
<th>No. Empty guts (%)</th>
<th>Mean gut fullness (%)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosquito</td>
<td>G. holbrooki</td>
<td>178</td>
<td>39 (21.91%)</td>
<td>42.14^A</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>P. signifer</td>
<td>29</td>
<td>16 (55.17%)</td>
<td>46.15^A</td>
<td>5.46</td>
</tr>
<tr>
<td></td>
<td>Pseudogobius sp</td>
<td>37</td>
<td>8 (21.62%)</td>
<td>52.93^A</td>
<td>5.34</td>
</tr>
<tr>
<td></td>
<td>H. galii</td>
<td>22</td>
<td>2 (9.09%)</td>
<td>65.90^B</td>
<td>7.34</td>
</tr>
<tr>
<td>Non-mosquito</td>
<td>G. holbrooki</td>
<td>201</td>
<td>8 (3.98%)</td>
<td>27.08^C</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>P. signifer</td>
<td>9</td>
<td>0 (0%)</td>
<td>45.56^A</td>
<td>6.48</td>
</tr>
</tbody>
</table>

At Terranora few fish had empty guts. Only mosquito season P. signifer had more empty guts than full or partially full digestive tracts. Both null hypotheses were rejected, as the two way ANOVA identified significant variation in mean gut fullness between species and season (F=12.91, df=1, 470, p<0.001). T LSD tests identified that mean gut fullness of H. galii was significantly higher than G. holbrooki in both seasons, and non-mosquito season G. holbrooki was significantly lower than any other species in either season.
Results of gut fullness analysis at the Coombabah site is shown in table 5.2.

<table>
<thead>
<tr>
<th>Season</th>
<th>Species</th>
<th>Total number of fish</th>
<th>No. Empty guts (%)</th>
<th>Mean gut fullness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosquito</td>
<td>G. holbrooki</td>
<td>898</td>
<td>118 (12.58%)</td>
<td>40.5^A 0.95</td>
</tr>
<tr>
<td></td>
<td>P. signifer</td>
<td>242</td>
<td>42 (17.36%)</td>
<td>37.73^A 1.81</td>
</tr>
<tr>
<td></td>
<td>Pseudogobius sp</td>
<td>10</td>
<td>3 (30%)</td>
<td>27.87^B 8.84</td>
</tr>
<tr>
<td>Non-mosquito</td>
<td>G. holbrooki</td>
<td>88</td>
<td>9 (10.23%)</td>
<td>32.82^A 2.58</td>
</tr>
</tbody>
</table>

At Coombabah only one null hypothesis was rejected, as the two way ANOVA showed significant variation between mean gut fullness between the three fish species, but not between seasons (F=2.88, df=3, 1230, p=0.035). T LSD tests showed that mean gut fullness of Pseudogobius sp. was significantly lower than that of G. holbrooki during the mosquito season, however P. signifer was not significantly different to either G. holbrooki or Pseudogobius sp. Mosquito season G. holbrooki mean gut fullness was higher than non-mosquito season G. holbrooki (the only species present in non-mosquito season), but not significantly so.

5.3.2 Diet composition: occurrence of prey items

Classification of prey items was made difficult by mastication and digestion; however 7 prey items were able to be identified to class, 4 to order, 4 to family, 1 to genus and 3 to species. Both larval and pupal stages of Chironomidae and Cx. sitiens mosquitoes were identified, whereas only Ae. vigilax larvae were found.

Terranora

Sixteen prey items were found in stomachs of Terranora caught individuals. Frequency of occurrence of each prey item for each fish species is summarised in table 5.3.
Table 5.3  Frequency of occurrence of prey items in Terranora caught fish. Frequencies are shown as % of individuals containing a particular prey item. Mosquito larvae and pupae are bolded. Entries marked “-” denote no prey item was found in the particular species.

<table>
<thead>
<tr>
<th>Prey item</th>
<th>Mosquito season</th>
<th>Non-mosquito season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G. holbrooki</td>
<td>P. signifer</td>
</tr>
<tr>
<td>Ae. vigilax (Larvae)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cx. sitiens (Larvae)</td>
<td><strong>13.48</strong></td>
<td>13.79</td>
</tr>
<tr>
<td>Cx. sitiens (Pupa)</td>
<td>3.37</td>
<td>-</td>
</tr>
<tr>
<td>Culicidae: Other</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chironomidae (Larvae)</td>
<td>10.67</td>
<td>6.90</td>
</tr>
<tr>
<td>Chironomidae (Pupa)</td>
<td>8.99</td>
<td>-</td>
</tr>
<tr>
<td>Coleoptera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrophilidae (Larvae)</td>
<td>6.74</td>
<td>3.45</td>
</tr>
<tr>
<td>Formicidae (Adult)</td>
<td>3.37</td>
<td>3.45</td>
</tr>
<tr>
<td>Other Insecta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>7.30</td>
<td>-</td>
</tr>
<tr>
<td>Arachnida</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acari</td>
<td>12.36</td>
<td>6.90</td>
</tr>
<tr>
<td>Annellida</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polychaeta</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mollusca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastropoda</td>
<td>2.81</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>2.81</td>
<td>-</td>
</tr>
<tr>
<td>Crustacea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ostroidea</td>
<td>1.12</td>
<td>-</td>
</tr>
<tr>
<td>Copepoda</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harpacticoida</td>
<td>26.40</td>
<td>13.79</td>
</tr>
<tr>
<td>Plant Material</td>
<td>16.29</td>
<td>17.24</td>
</tr>
<tr>
<td>Debris/Unidentified parts</td>
<td>8.43</td>
<td>3.45</td>
</tr>
</tbody>
</table>
Mosquito season *G. holbrooki* had the most varied diet, containing 13 of the 16 prey items, followed by *Pseudogobius* sp. (11 food items), mosquito season *P. signifer* (8 prey items), non-mosquito season *G. holbrooki* (6 food items), *H. galii* (5 food items) and finally non-mosquito season *P. signifer* (3 food items). Microcrustaceans and plant material were frequently encountered food items in all species, with the exception of mosquito season *P. signifer*. Other invertebrates were frequently present in fish stomachs, in particular *Acari* (mites) in non-mosquito season and Chironomidae (biting midges) in mosquito season. Mosquito larvae were encountered frequently - *Cx. sitiens* larvae and pupae were found in three out of the four fish species (not in *H. galii*), and *Ae. vigilax* larvae were only found in *Pseudogobius* sp. stomachs.

**Coombabah**

Eighteen prey items were identified in stomachs of Coombabah caught individuals. Frequency of occurrence of each prey item for each fish species is summarised in table 5.4.
Table 5.4 Frequency of occurrence of prey items found in Coombabah caught fish. Frequencies are shown as % of individuals containing a particular prey item. Mosquito larvae are bolded. Entries marked “-” denote no prey item was found in the particular species.

<table>
<thead>
<tr>
<th>Prey item</th>
<th>Mosquito season</th>
<th>Non-mosquito season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G. holbrooki</td>
<td>P. signifer</td>
</tr>
<tr>
<td>Diptera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cx. sitiens (Larvae)</td>
<td><strong>0.45</strong></td>
<td>-</td>
</tr>
<tr>
<td>Chironomidae (Larvae)</td>
<td>6.47</td>
<td>11.16</td>
</tr>
<tr>
<td>Chironomidae (Pupae)</td>
<td>6.47</td>
<td>8.26</td>
</tr>
<tr>
<td>Ceratopogonidae</td>
<td>0.22</td>
<td>5.37</td>
</tr>
<tr>
<td>Insecta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coleoptera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrophilidae (Larvae)</td>
<td>3.23</td>
<td>25.21</td>
</tr>
<tr>
<td>Other</td>
<td>0.89</td>
<td>-</td>
</tr>
<tr>
<td>Hymenoptera (Formicidae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formicidae (Adult)</td>
<td>1.90</td>
<td>2.07</td>
</tr>
<tr>
<td>Other</td>
<td>0.33</td>
<td>-</td>
</tr>
<tr>
<td>Other Insecta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larvae</td>
<td>1.00</td>
<td>0.41</td>
</tr>
<tr>
<td>Adult</td>
<td>1.11</td>
<td>4.13</td>
</tr>
<tr>
<td>Arachnida</td>
<td>Acari</td>
<td>-</td>
</tr>
<tr>
<td>Mollusca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastropoda</td>
<td>Planktonic (&lt;0.2mm)</td>
<td>1.34</td>
</tr>
<tr>
<td>Other</td>
<td>2.56</td>
<td>0.83</td>
</tr>
<tr>
<td>Crustacea</td>
<td>Ostroidea</td>
<td></td>
</tr>
<tr>
<td>Calanoida</td>
<td>1.11</td>
<td>-</td>
</tr>
<tr>
<td>Harpacticoida</td>
<td>41.69</td>
<td>38.02</td>
</tr>
<tr>
<td>Plant Material</td>
<td>40.91</td>
<td>10.74</td>
</tr>
<tr>
<td>Debris/Unidentifiable parts</td>
<td>1.56</td>
<td>0.83</td>
</tr>
</tbody>
</table>
Mosquito season *G. holbrooki* had the most varied diet, with 17 of the 18 food items identified. Mosquito season *P. signifer* was second highest (13 food items), followed by non-mosquito season *G. holbrooki* (7 food items) and mosquito season *Pseudogobius* sp. (5 food items). Of all prey items micro-crustaceans, in particular Harpacticoida, were the most frequently consumed prey item in all species. Plant material was also found in a significant number of mosquito season *G. holbrooki* and *Pseudogobius* sp.. Other invertebrates, such as Acari, Chironomidae and Formicidae (ants), were found relatively infrequently (generally in <10% of fish), with the exception of Hydrophilidae (diving beetle larvae), which were encountered in ~25% of mosquito season *P. signifer*. Mosquito larvae were very infrequently encountered; only one species of mosquito (*Cx. sitiens*) was eaten in small amounts by only one species of fish (0.45% of mosquito season *G. holbrooki*).

5.3.3 Diet composition – Prey composition and dietary overlap

Terranora

Of the 476 fish caught at the Terranora site 403 had food items in their guts, which were used to determine diet composition. Results are summarised in Fig 5.1.
Fig 5.1 Composition of diet of Terranora caught species. Proportions of each prey item in fish species and season is expressed as a % of total diet. To simplify, categories have been grouped by family (for mosquito and Chironomid larvae/pupae) and order (for crustaceans).

Gut composition of fish from Terranora included a large proportion of plant material and microcrustaceans (mainly Harpacticoida) consumed by all species and both season: 40% of the diet of *G. holbrooki*, 62% of *Pseudogobius* sp. and *P. signifer* and 92% of *H. galii* in mosquito season, and 66% of *G. holbrooki* and 95% of *P. signifer* diet in the non-mosquito season. The remainder of the diet of fish from Terranora comprised invertebrates including Chironomids and mosquito larvae/pupae in the mosquito season and Arachnida (Acari) in the non-mosquito season. Mosquito larvae/pupae comprised 15%, 16% and 17% of the diets of *G. holbrooki*, *P. signifer* and *Pseudogobius* sp, respectively. Mosquito larval predation was almost exclusively *Cx. sitiens*, except for *Pseudogobius* sp, where *Ae. vigilax* larvae comprised 70% of mosquitoes eaten, but only 11% of total *Pseudogobius* sp. diet.
Shoener’s index values showed significant dietary overlap between mosquito season *G. holbrooki* and *Pseudogobius* sp. (0.63), *P. signifer* (0.7), but not *H. galii* (0.52). However *H. galii* showed diet overlap with both *Pseudogobius* sp. (0.72) and *P. signifer* (0.65). *P. signifer* and *Pseudogobius* sp. also showed significant dietary overlap (0.73). Non-mosquito season *G. holbrooki* and *P. signifer* also showed significant dietary overlap (0.71). Comparison of the two species present in both seasons however showed no significant dietary overlap between *G. holbrooki* (0.54) or *P. signifer* (0.47).

**Coombabah**

Of the 1238 individuals caught at the Coombabah site 1066 individuals had food in their stomachs, which were used to determine diet composition. Results are summarised in Fig 5.2.
Fig 5.2 Composition of diet of Coombabah caught species. Proportions of each food item in fish/season population is expressed as a % of total diet. To simplify, Categories have been grouped by family (for mosquito and Chironomid larvae/pupae) and order (for crustaceans).

Diet of all three mosquito season species was dominated by plant material and microcrustaceans, which when combined comprised 82% of *G. holbrooki*, 83% of *Pseudogobius* sp. and 46% of *P. signifer* diets. The remainder of the diets consisted of small numbers of larger invertebrates, in particular Chironomidae in *G. holbrooki* and *P. signifer* and Hydrophilidae larvae in *P. signifer*. Crustacea (Harpacticoidae) made up the majority of non-mosquito season *G. holbrooki* diet (65% of stomach contents), along with smaller amounts of plant material, Chironomidae and Arachnida (Acari). Mosquito larvae (*Cx. sitiens* only) comprised a very small fraction of only one species (0.2% of mosquito season *G. holbrooki*).

Shoener’s index comparisons between the four groups found dietary overlap between species/season groups. Significant overlap was found between mosquito
season *G. holbrooki* and *P. signifer* (0.61), *Pseudogobius* sp. (0.63), and non-mosquito season *G. holbrooki* (0.61), however not between mosquito season *P. signifer* and *Pseudogobius* sp. (0.44).

### 5.4 Discussion

Small resident fish species found in mangrove basin pools were found to be generalist feeders, able to feed on a wide range of food items, dominated by plant material, and microcrustaceans, but including larger terrestrial invertebrates. Importantly, the three fish species, *G. holbrooki*, *P. signifer* and *Pseudogobius* sp. fed on mosquito larvae. A generalist feeding habit is likely critical for survival within mangrove basin pools; as has been described for similar habitats, such as saltmarshes (Harrington and Harrington 1961; Morton et al 1988). The ability to exist on a varied diet would increase survivability within a highly heterogeneous environment, where food type and availability may change significantly over short periods.

#### 5.4.1 Comparison of dietary behaviour between species

Overall there was very little difference in dietary behaviour between fish species. Comparisons of gut fullness showed some differences, however these were mostly between native species that were present in very small numbers. Diet composition was very similar between species. There were only a few exceptions to this, primarily where small numbers of native fish species showed significantly different dietary habits, for example mosquito season *H. galii* at Terranora and *Pseudogobius* sp. at Coombabah. The similarity of dietary habits, combined with the relatively small number of empty stomachs, suggests that food is relatively abundant in mangrove basin pools, and there is relatively little or no food-based competition. This is surprising, especially considering the dominance of *G. holbrooki* at both of the study sites. *Gambusia holbrooki* is clearly replacing native species, however this does not appear to be based on competition for food resources. The dominance of *G. holbrooki* may instead be linked to the its high fecundity, wide environmental tolerances and highly aggressive behaviour *G. holbrooki* shows towards native fish populations.
5.4.2 Seasonal variation in dietary behaviour and impacts on mosquito larvae

A significant seasonal shift was observed at the Terranora site; the two fish species present in both seasons (\textit{P. signifer} and \textit{G. holbrooki}) showed significant variation in dietary habits between seasons. Non-mosquito season diets were comprised predominantly of plant material and microcrustaceans. Microcrustaceans appear to be an abundance non-mosquito season food source in mangroves, and studies conducted in similar habitats, such as salt marshes, show a similar reliance on microcrustaceans and crab larvae in resident and transient fish species (Morton et al 1988; Hollingsworth and Connolly 2006; Mazumder et al 2006b) while mosquito season diets comprising larger numbers of larger invertebrates, such as Chironomids (midges), Hydrophilidae (beetles) and mosquito larvae and pupae. However there was still a strong reliance on the non-mosquito season prey items, comprising 50-60\% of mosquito season diet. Coombabah resident fish populations (with the exception of \textit{P. signifer}, a significant predator of Hydrophilidae larvae) showed similar reliance on plant material and microcrustaceans all year round, which suggests that fish at this site did not alter their diets seasonally.

Overall, dietary shifts were not of the magnitude expected, especially compared to studies conducted in comparable environments, such as saltmarshes and intermittently connected tidal creeks. In these studies fish diets showed a major seasonal shift, to mosquito larvae and other larger invertebrates (Harrington and Harrington 1961; Becker and Laurenson 2007). For example Morton et al (1988) identified a shift from almost exclusively microcrustaceans in winter to almost exclusively large invertebrate larvae, especially mosquito larvae in summer.

The relatively small dietary shifts observed in this chapter may be linked to the degree of mosquito larvae predation throughout the study period. Very little predation of larvae was recorded at either site. At Terranora mosquito larvae and pupae only accounted for 15\%-20\% of the diet, and were effectively non-existent as a prey item at the Coombabah site. Dietary shifts in other studies may reflect a sudden increase in the availability of, and subsequent feeding on mosquito larvae, which clearly did not happen at either site during the study.
The lack of significant shift to larval predation in warmer months may initially indicate that resident mangrove fish have little interest in mosquito larvae as a food source. However mosquito activity throughout the study period needs to be considered. Significant rainfall and flooding in early 2012, as described in Chapter 3 (see section 3.2.3 and 3.3.3), significantly disrupted *Ae. vigilax* populations across the SEQ/NNSW region, including at the Terranora and Coombabah study sites. As a result, mosquito populations at both sites were greatly reduced, to the extent that larval sampling concurrent with fish sampling in this study found very low mosquito larvae numbers (Easton and Falkner, unpublished data; also see Chapters 6 and 8). This is a likely reason for both the overall lack of larval predation and the lack of significant dietary shifts observed.

Despite issues resulting from low mosquito populations in the mangrove basins during the study, the findings greatly improve our knowledge of fish-mosquito interactions in mangroves. Fish dietary habits, while not directly involving significant mosquito predation, strongly indicate that predation of larval populations is potentially significant, for several reasons. Firstly, mosquitoes were present in fish diets, despite the low populations observed throughout the study. This is especially true at Terranora, where mosquitoes made up a low, but still sizeable (15%-20%) proportion of the diets of three of the four fish species. This indicates that, despite their scarcity in relation to other food sources, fish actively sought out mosquito larvae as a food source, supporting the view that mosquito larvae are an important prey item.

Secondly, though smaller than expected, shifts in diet were recorded between mosquito and non-mosquito seasons, especially at Terranora, where a shift from plant and crustacean dominated diets in the non-mosquito season to larger prey items such as Formicidae, Hydrophilidae and Chironomidae in the mosquito season was noted. The shift to these other prey items may be in response to the absence of mosquitoes in the system; fish may have been feeding on prey of a similar size and/or nutritional value to that of mosquito larvae to compensate for the lack of mosquitoes present in the basin. While a dietary shift was observed, the non-mosquito season prey items continued to be a substantial portion of diets, which suggests that the alternate prey items sought in the absence of mosquitoes may not completely compensate for the lack of mosquito larvae, and so could not be relied upon as heavily as mosquito larvae. Densities of alternate prey were not
observed in this study, nor were the relative nutritional value of different fish prey items, and so this presents a significant future research pathway.

Thirdly, the findings of this study, compared with existing studies, may demonstrate the impacts of different mosquito abundances on fish dietary habits, where the extent to which feeding on mosquitoes is directly proportional to the size of the mosquito population. Mosquito predation was effectively non-existent at Coombabah, which suggests that the Coombabah mosquito population was even lower than that at Terranora. At Coombabah fish diets may therefore demonstrate feeding habits during an absence of mosquito larvae, at Terranora during a poor mosquito season, compared to studies recording significant mosquito predation which demonstrate feeding during a high abundance mosquito season.

Although the findings of this study add to our understanding of fish and fish-mosquito interactions, the small populations of mosquito larvae during the study remains a significant limitation of this research. The dietary habits of fish during a “normal” mosquito season were not able to be examined, which presents a serious impediment to achieving new understanding of the biological control of mosquitoes in mangroves. In order to confirm many of the findings presented in this chapter the experiments need to be repeated during a “normal” mosquito season. Conducting further studies when mosquito populations are abundant, and observing dietary habits in conjunction with mosquito and alternate prey abundance would provide a comparison of the influence of the size of mosquito populations on the dietary habits of mangrove fish species.

5.5 Conclusions

5.5.1 Summary and contribution to knowledge

The lack of knowledge of fish populations in mangrove basin forests and their dietary habits has been a significant factor limiting our understanding of fish as biological control agents of mosquitoes in mangroves. The research presented in this chapter showed that the diets of three of the species that live in mangrove basin forest pools included mosquito larvae, as well as a wide array of other prey items. Mosquito larvae comprised a relatively small proportion of diet, however this
was likely due to the dearth of mosquitoes throughout the study period, rather than a lack of interest in mosquito larvae as a food source.

5.5.2 Implications for mosquito control

While there was relatively little predation of mosquito larvae, even during the mosquito season, a shift in diet to prey items similar to mosquitoes was observed in several of the fish species, which suggested that predation would be more substantial if larger mosquito populations were present. In fact, the level of predation observed, especially at the Terranora site, appears to be quite significant considering the relatively small size of the mosquito population present during the study.

However the findings in this chapter also suggest that other factors may be far more critical in determining the size of mosquito populations than biological control by fish. Low mosquito populations, such as those observed over the study period, have in the past been attributed to the impact of heavy rainfall on fish. For example, Ritchie (1984) suggested that heavy rainfall increased survival of coastal wetland fish, which led to a decreased in *Ae. taeniorhynchus* population by predation. However the findings of this chapter demonstrate that this may not necessarily be the case. Rather than acting indirectly by encouraging predation of mosquitoes by fish, increased rainfall appears to affect mosquito populations directly, by disrupting mosquito life cycle and thus mosquito production.

This chapter addresses the second research aim of this study, and combined with the previous chapter addresses the first major knowledge gap identified in Chapter 2. Four species have been identified in SEQ/NNSW region mangrove basin pools; *G. holbrooki, P. signifer, Pseudogobius* sp. and *H. galii*, and three of these (*H. galii* being the exception) demonstrated predation of mosquitoes, and may therefore contribute to biological control of *Ae. vigilax*. However, as previously mentioned, the low mosquito population during the study means that further research is required to establish the potential of these species to control mangrove *Ae. vigilax* populations. Predation efficiency of mosquitoes needs to be directly examined using a laboratory approach, and this is the focus of the following chapter.
Chapter 6  Predation of *Ae. vigilax* larvae by mangrove basin fish

6.1  Introduction

Predation of larvae is a major component of the interaction between fish and mosquitoes. Many studies document a significant predator interaction between fish and mosquito larvae across a variety of habitats and between a wide variety of different fish and mosquito species (Connor 1922; Hess and Tarzwell 1942; Davey et al 1974; Morton et al 1988; Blaustein 1992; Taylor et al 1992; Hurst et al 2004; Becker et al 2005; Louca et al 2009). Studies describing predation of mosquito larvae by fish demonstrate that this predator-prey relationship plays a critical role in influencing mosquito populations (Chandra et al 2008). However there is a lack of research focussed directly on mangrove basins and the role of resident fish predation on mosquitoes. Ritchie and colleagues (e.g. Ritchie 1984 and Ritchie and Addison 1992) provide the few exceptions, by speculating on the role of fish in influencing mosquito habitat in Florida mangroves.

Apart from the lack of evidence relating to specific habitats, studies of fish-mosquito predator-prey relationships are usually either fish or mosquito based, focussing specifically on either predator or prey. Fish based studies observe the extent to which fish populations feed on larvae, describing the contribution larvae make to the diets of fish (as in Chapter 5). This approach identifies fish that may be predators of mosquitoes, and describe factors that significantly contribute to the fish-mosquito interaction (Hess and Tarzwell 1942; Davey et al 1974; Morton et al 1988; Blaustein 1992; Louca et al 2009). But, apart from the current research only one other study has examined fish predation of mosquitoes in mangrove forests; Kokkinn et al (2009) made qualitative observations of small Atherinid fish feeding
on *Ae. vigilax* and *Ae. camptorhynchus* larvae coastal wetland pools (including in mangroves) immediately behind the advancing front of the flood tide. The authors asserted that predation was sufficient to exclude all mosquito larvae from pools (although it is not clear what is meant by the term “excluded”). However, the study did not focus on resident fish existing in pools between tidal connections (Kokkinn et al 2009).

Mosquito based studies have incorporated the impacts of predators, including fish, into models of mosquito populations. These are often conducted from a mosquito control perspective, where studies link significant decreases in mosquito populations to the introduction of predatory fish into larval habitats (Bence 1988; Ritchie and Montague 1995; Kumar and Hwang 2006; Van Dam and Walton 2007; Walton 2007; Chandra et al 2008). Only one of these studies was carried out in mangroves; Ritchie (1984) attributed changes in a Florida mangrove mosquito population to increased fish populations following heavy winter rainfall. Ritchie also identified a number of environmental factors, including higher-trophic level predation on larvivorous fish, directly affecting the impact of predation on mosquitoes.

However focussing solely on either fish or mosquitoes has limitations. Fish based studies provide little on the extent to which specific fish predation impacts mosquito populations, and mosquito based studies provide little on the specifics of mosquito predation. The result of either approach is an incomplete picture of the fish-mosquito interaction; one that does not directly examine the predation efficiency of fish, nor the impact it has on mosquito populations. This is particularly true in mangrove basins. In order to better understand the potential for biological control of fish in mangrove basins, the fish-mosquito interaction needs to be quantified.

Because of the difficulties in quantifying the impact of fish predation on mosquitoes, as discussed in previous chapters, laboratory based empirical studies may provide the necessary information to describe the predation efficiency of fish species and corresponding changes in mosquito populations. Fish-mosquito interactions are highly species specific, and an empirical approach can identify the suitability of particular fish to act as biological control agents (Taylor et al 1992; Hurst et al 2004). Certain mosquito species may be more effective at avoiding predation by fish, altering their behaviour in the presence of a predator (Kesavaraju
and Juliano 2004) or being less detectable than other prey items (Okorie and Abiodun 2010). Likewise, certain fish species may be more adept at detecting prey, including mosquitoes (Diehl 1988).

Australian laboratory-based studies have directly examined the larvivorous predation efficiency of fish, and have included a wide range of native fish species, including several of those present in SEQ/NNSW mangrove basin forest pools. For example Hurst et al (2004; 2006) measured and compared predation efficiency of a number of Australian native fish, (including P. signifer and H. galii) and the exotic fish G. holbrooki, looking at predation on Cx. annulirostris larvae (a freshwater mosquito). They found that native Australian fish species were as efficient predators of mosquito larvae as was G. holbrooki. They also found that habitat complexity (such as presence of vegetation) and the presence of alternate prey could adversely affect the predation efficiency of some fish species, including G. holbrooki. In another Australian study, also examining predation by P. signifer and G. holbrooki on Cx. annulirostris, Willems et al (2005) focussed on the feeding patterns of the fish, finding that fish reached a satiation point, where became full and predation of larvae subsequently declined.

However none of these Australian studies describe predation on Ae. vigilax larvae. Resident fish populations in SEQ mangrove basin pools may be effective predators of mosquito larvae, based on fish activity in other situations, and therefore contribute significantly to biological control. However the species specific nature of the fish-mosquito interaction means that in order to accurately determine their impacts in mangroves, predation of Ae. vigilax larvae needs to be quantified. The research presented in this chapter establishes predation rates of Ae. vigilax larvae by resident mangrove basin fish species from a series of laboratory experiments.

6.2 Methods

6.2.1 Data collection

Collecting fish

Individuals of G. holbrooki, P. signifer, Pseudogobius sp. and H. galii were collected from both the Terranora and Coombabah sites for use in laboratory experiments.
Local strains of native fish were also sourced from aquarium stores in the SEQ/NNSW region. Live fish were stored in separate 30L plastic buckets filled with mangrove pool water for transportation back to the laboratory. All fish, with the exception of the exotic *G. holbrooki*, were released back into the mangrove at the conclusion of the experiments. *Gambusia holbrooki*, being a highly noxious, introduced pest, were euthanized.

In the laboratory fish were stored in four separate 40L tanks, equipped with undergravel filters and kept at a temperature range of 23-25°C. The fish were maintained on a 14:10 day:night cycle, by using a 100W heat lamp on a timer. Fish were slowly acclimatised to a standardised brackish salinity environment (15ppt) by adjusting salinity levels by approximately 2ppt every two days either by adding freshwater or aquarium salt. Separate tanks were used for each species to avoid fighting between species, especially *G. holbrooki* (both Howe et al (1997) and Warburton and Madden (2003) report aggression by *G. holbrooki* toward Australian native fish). Fish were fed daily with standard tropical fish food flakes.

A further 21 individuals of each species were euthanized for correlation experiment fish weight. These individuals were kept alive in 30L plastic buckets for 3 hours, until stomach contents were excreted, before being euthanized. It was necessary to estimate fish weights of live fish because of the risk of adverse impacts on live fish during weighing.

**Collecting mosquito larvae**

The *Ae. vigilax* larval stage comprises four instars, ranging from ~1.2mm body length and ~0.3mm head capsule width for 1st instars to ~5.8mm body length and ~1.15mm head capsule width for 4th instars (Shinkarenko et al 1986). Only 2nd and 4th instar stages were used in this study, as 1st instar larvae were too small to be counted accurately, and 3rd instars are too similarly sized to 4th instars to make comparisons of predation rates between instar stages valuable (Shinkarenko et al 1986).

Sampling to collect 2nd and 4th instar mosquito larvae was conducted throughout the study period (concurrent with fish sampling) from April 2011 to April 2012. At each pool a standardised 240 ml larval dipper was used to collect water samples
were taken every 2 m around the edge of each pool, where larvae generally cluster (Kerridge 1971), and any larvae collected were counted in-situ. However, larval sampling did not produce *Ae. vigilax* larvae due to heavy rainfall and flooding during the study period. Consequently, laboratory raised *Ae. vigilax* larvae were obtained from which a colony was established (as described below). *Aedes vigilax* individuals for the colony were collected from three sources:

1. Saltmarsh and mangrove substrate samples, containing *Ae. vigilax* eggs were collected from Terranora and Coombabah study sites. Samples consisted of 10-15 scrapings of soil and plant material, stored in zip-lock bags. These were dried in the laboratory for one week to allow eggs to develop, before being hatched by the methods described below.

2. $1^{st}$-$3^{rd}$ instar *Ae. vigilax* larvae were collected following a small, rainfall-triggered *Ae vigilax* hatch in several saltmarsh pools along the landward fringe of the Coombabah site in early August 2012. This was one of the few hatches of *Ae. vigilax* during the study period. Approximately 500 *Ae. vigilax* larvae were collected from three saltmarsh pools, using the larval collection methods described above.

3. Two oviposition pads, each containing 1000-1500 *Ae. vigilax* eggs, were supplied from an existing colony belonging to the NSW Arbovirus Surveillance and Vector Monitoring Program. Oviposition pads were hatched using the same methods described below.

Eggs on soil and oviposition pads were hatched using the evacuation technique (Kerridge 1971; Dale et al 1986; Shinkarenko et al 1986). Samples were placed into 5L glass jars and flooded with brackish water (~15ppt), sufficient to completely submerge the soil/pad. Water was not sourced from mangroves due to the possible presence of large numbers of contaminant organisms that may affect predation rates in the experiments. Instead a mangrove pool analogue was created by mixing aquarium marine salt into demineralised freshwater. Jars were sealed with cork stoppers and oxygen was evacuated from the airspace via a vacuum pump, lowering DO in the water and stimulating hatching. Samples were held in evacuation for 1 hour (a sufficient length of time to hatch the maximum number of eggs (Kerridge (1971) found almost all (~98%) eggs hatched with 1 hour of evacuation stimulus), after which samples were removed from evacuation and left
for 24 hours to allow larvae to emerge and develop to a stage where they could be visible.

After 24 hours jars containing samples and water were emptied into 35x20x4cm white larval trays. 1st instar Ae. vigilax larvae were removed using a 5mL pipette, divided into groups of 200 and placed into larval trays filled with brackish water (15ppt). Larvae were fed on a diet of ground fish food pellets every 2 days, or until all food was eaten. As larvae developed they were divided into groups of 100 at 3rd instar and groups of 50 at 4th instar. These larvae were kept in trays until they reached life stages necessary for experiments. Any additional larvae were left to mature to pupal stage (which took 5-7 days after hatch), at which time they were transferred into colony cages to emerge into adults. Larvae caught in the field were placed into larval trays and reared using the same method.

Adults that emerged were used to create and maintain a colony, from which larvae would be taken for additional experiments. Setup and maintenance of the colony is described in Appendix 3.

6.2.2 Experiment protocol

A series of laboratory experiments were undertaken to identify maximum predation efficiency of the fish. Experimental methodology was based on the method used by Hurst et al (2004) where the fish were housed in identical fish tanks, containing 8L of brackish (15ppt) water, measuring 35x15x20cm. The use of fish tanks provided an entire water column for predation, enabling the optimal feeding behaviour of all four species. White gravel was used in all the experiment tanks, so larvae in the tank would be clearly visible and easy to count.

Although several of the species examined in this study exhibit strong schooling behaviour, this has been shown in previous studies to have no impact on larval predation efficiency (Hurst et al 2004). Therefore, one fish was used in each experiment replicate. Each fish was placed into each of the experiment tanks 24 hours before each experiment to acclimatise. Fish were given food (tropical fish food flakes) for 15 minutes, after which all food was removed from the tank using a small aquarium net. This standardised hunger levels across all individuals. At the commencement of each experiment Ae. vigilax larvae were added to the tank in
quantities described below, and the experiment was run for 8 hours. If all larvae were consumed before the end of the experiment additional mosquito larvae were added in batches (depending on the instar level used in the experiment). Over the course of the study numbers of remaining larvae in the tank were recorded at set intervals: 15 minutes in the first hour and every half hour for the remaining 7 hours. Larval consumption at each point was recorded as the change in the number of larvae in the tank from the previous observation. At the conclusion of the experiment fish length was measured as fish were removed from tanks and deposited into a separate holding tank to prevent accidental re-use in the same experiment, and any remaining larvae, both living and dead were removed from the tank. Any living larvae were returned to the colony.

Three different experiments were conducted where each experiment was repeated for each species, with six replicates for each species. Different individual fish were used for each experiment replicate. Tanks were used multiple times, but were cleaned thoroughly and water was replaced between each experiments. The three experiments were run for each species including:

1. **2\textsuperscript{nd} instar experiment** - 100 late 2\textsuperscript{nd} instar larvae were added per tank, with additional larvae being added in batches of 50 if all larvae were consumed.
2. **4\textsuperscript{th} instar day experiments** - 50 4\textsuperscript{th} instar larvae were added per tank, with additional larvae being added in batches of 25 if all larvae were consumed.
3. **4\textsuperscript{th} instar night feeding** - 50 4\textsuperscript{th} instar larvae, with additional larvae being added in batches of 25 if all were consumed. Larvae were added to the tanks and the experiment was run for 8 hours in complete darkness. A red LED lamp was used to monitor predation activity.

### 6.2.3 Data processing and analysis

**Data processing**

Consumption counts in all experiments were expressed as a standardised index: consumption per gram of fish weight (pgfw). The lengths (mm) and dry weights (g) of the 21 wild length/weight individuals of each fish species were measured, and a regression analysis was performed to identify the relationship between length and
dry weight of each species. This relationship was used to estimate the weight of each live fish used in the experiments.

All larval consumption counts were standardised to account for natural larval mortality using Abbott’s method (Abbott 1987), which estimates a daily attrition rate of a natural larval population based on a sample of 50 larvae kept in the same conditions as the experiment tanks. Levene’s test for homogeneity of variance was conducted on all data prior to analyses, and no data transformations were required.

Comparing 2nd and 4th instar predation

The 2nd and 4th instar consumption (pgfw) was expressed as a ratio, and a two-way ANOVA was conducted comparing the mean standardised consumption rate (consumption pgfw) data of 2nd and 4th instar larval stages between the four mangrove fish species. Two null hypotheses were tested: a) larval consumption did not differ between mangrove fish species, and b) larval consumption was not affected by larval size (between 2nd and 4th instars). Post hoc T LSD tests were undertaken to identify differences between 2nd and 4th instar consumption within and between species.

Describing consumption patterns of fish

Predatory behaviour of fish over the course of the experiment was assessed in two phases; the initial gorging period and the background feeding period. Initial gorge period consisted of the first hour of each experiment, and the rate of larval consumption (hereafter referred to as gorge rate) was calculated by adding the number of larvae consumed across the four observations made within the first hour. Background feeding consisted of the remaining 7 hours of each experiment. The total number of larvae consumed over the background period (consumption pgfw) was averaged across the 7 hours to give a mean background feeding rate per hour (hereafter referred to as background rate). Gorge and background rates were calculated for both 2nd and 4th instar day experiment replicates and averaged across all six replicates of each species to give a mean gorge and mean background
feeding rate for each species. Gorge and background consumption rates were expressed as a ratio for each species.

For each larval stage, two-way, repeated measure ANOVAs were undertaken comparing mean consumption rates (pgfw) between the two feeding period treatments (gorge and background) and the four fish species, to test two null hypotheses: a) gorge feeding rates and background feeding rates were not different, and b) gorge and background feeding rates did not differ between species. Post hoc T LSD tests were used to identify differences within and between fish species for each larval stage.

**Day versus night predation**

Predation of mosquito larvae by fish were compared during simulated day and night conditions using mean 4th instar larval consumption (pgfw) for the four fish species. This was compared using a two way ANOVA to test two null hypotheses: a) larval consumption rates did not differ between day and night, and b) night larval consumption rates did not differ between fish species. Post hoc T LSD tests were used to identify differences in larval consumption rates between daytime and night time conditions and between the four fish species.

### 6.3 Results

#### 6.3.1 Estimation of fish weights

Statistically significant positive regression between dry weight and length of each species were found, as summarised in Table 6.1 and Fig 6.1.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>R²</th>
<th>df</th>
<th>F</th>
<th>P</th>
<th>Best-fit equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. holbrooki</td>
<td>21</td>
<td>0.649</td>
<td>1, 19</td>
<td>35.18</td>
<td>&lt;0.0001</td>
<td>y = 0.013x - 0.182</td>
</tr>
<tr>
<td>P. signifier</td>
<td>21</td>
<td>0.58</td>
<td>1, 19</td>
<td>28.09</td>
<td>&lt;0.0001</td>
<td>y = 0.014x - 0.197</td>
</tr>
<tr>
<td>H. galii</td>
<td>21</td>
<td>0.96</td>
<td>1, 19</td>
<td>220.31</td>
<td>&lt;0.0001</td>
<td>y = 0.017x - 0.256</td>
</tr>
<tr>
<td>Pseudogobius sp.</td>
<td>21</td>
<td>0.92</td>
<td>1, 19</td>
<td>216.7</td>
<td>&lt;0.0001</td>
<td>y = 0.017x - 0.274</td>
</tr>
</tbody>
</table>
Fig 6.1 Length versus weight plots of: a) *G. holbrooki*, b) *P. signifer*, c) *H. galií* and d) *Pseudogobius* sp.. A line of best fit and linear equation is reported for each species.
6.3.2 2nd versus 4th instar predation

Table 6.2 summarises larval consumption rates (actual number consumed and standardised per gram fish weight [pgfw]) for 2nd and 4th instar larvae experiments, plus the ratio of consumption of 2nd to 4th instars.

<table>
<thead>
<tr>
<th>Species</th>
<th>2nd instar consumption rate</th>
<th>4th instar consumption rate</th>
<th>2nd vs. 4th ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (per fish)</td>
<td>Mean (pgfw)</td>
<td>Mean (per fish)</td>
</tr>
<tr>
<td>G. holbrooki</td>
<td>239</td>
<td>1166.82^A</td>
<td>46.33</td>
</tr>
<tr>
<td>P. signifer</td>
<td>161.17</td>
<td>845.27^A</td>
<td>31.5</td>
</tr>
<tr>
<td>H. galii</td>
<td>58.67</td>
<td>227.64^B</td>
<td>22.67</td>
</tr>
<tr>
<td>Pseudogobius sp.</td>
<td>43</td>
<td>241.83^B</td>
<td>4.51</td>
</tr>
</tbody>
</table>

Both null hypotheses were rejected, as the ANOVA showed that a significantly higher number of the smaller 2nd instar larvae were eaten than the larger 4th instars, and that consumption rates (pgfw) were significantly different between species (F=7.183, df=3, 40, p<0.001). Post hoc T LSD tests showed that the difference between 2nd and 4th instar consumption rate was significant across all four fish species. T-LSD tests identified two significant groups; one (comprising G. holbrooki and P. signifer), showing significantly higher levels of 2nd and 4th instar predation than the second group (comprising H. galii and Pseudogobius sp.). Also, G. holbrooki and P. signifer consume proportionally more 2nd instar to 4th instar larvae than H. galii and Pseudogobius sp.
6.3.3 Gorge versus background feeding rates

2nd instar experiments

Table 6.3 summarises gorge and background larval consumption rates (pgfw) for 2nd instar larvae experiments, plus the ratio of gorge to background consumption rates.

Table 6.3 Summary of gorge and background consumption rates (pgfw) for 2nd instar Ae. vigilax larvae. Entries with different T LSD test letters are statistically significant (p≤0.05). A * next to the gorge vs. background ratio denotes a significant difference (p≤0.05) between consumption rates.

<table>
<thead>
<tr>
<th>Species</th>
<th>Gorge consumption</th>
<th>Background consumption</th>
<th>Gorge vs. Background</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>G. holbrooki</td>
<td>486.83A</td>
<td>129.24</td>
<td>99.56A</td>
</tr>
<tr>
<td>P. signifer</td>
<td>525.09A</td>
<td>92.22</td>
<td>45.74A</td>
</tr>
<tr>
<td>H. galii</td>
<td>93.27B</td>
<td>24.76</td>
<td>19.18B</td>
</tr>
<tr>
<td>Pseudogobius sp.</td>
<td>182.35B</td>
<td>52.13</td>
<td>7.34B</td>
</tr>
</tbody>
</table>

Both null hypotheses were rejected, as the two-way repeated measures ANOVA showed significant differences in mean larval feeding rates between the four fish species, and that gorge rates were significantly higher than background feeding rates in all species (F=5.514, df=3, 40, p=0.006). T-LSD tests found significant differences within and between species. Two significantly different groups were identified in gorge consumption rates, with one group (comprising G. holbrooki and P. signifer) significantly higher than the other (comprising H. galii and Pseudogobius sp.). G. holbrooki had a significantly higher background rate than the other three species. Proportionally, the largest difference between gorge and background was recorded in Pseudogobius sp. and the smallest in G. holbrooki and H. galii.
4th instar experiments

Table 6.4 summarises gorge and background larval consumption rates (pgfw) for 4th instar larvae experiments, plus the ratio of gorge to background consumption.

Table 6.4 Summary of gorge and background consumption rates (pgfw) for 4th instar Ae. vigilax larvae. Entries with different T LSD test letters are statistically significant (p≤0.05). A * next to the gorge vs. background ratio denotes a significant difference (p≤0.05) between consumption rates.

<table>
<thead>
<tr>
<th>Species</th>
<th>Gorge consumption</th>
<th>Background consumption</th>
<th>Gorge vs. Background ratio (pgfw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>G. holbrooki</td>
<td>101.91A</td>
<td>14.47</td>
<td>12.66A</td>
</tr>
<tr>
<td>P. signifer</td>
<td>84.30A</td>
<td>9.66</td>
<td>9.39B</td>
</tr>
<tr>
<td>H. galii</td>
<td>34.81B</td>
<td>4.18</td>
<td>4.00C</td>
</tr>
<tr>
<td>Pseudogobius sp.</td>
<td>49.88B</td>
<td>12.05</td>
<td>7.45B</td>
</tr>
</tbody>
</table>

Both null hypotheses were rejected, as the ANOVA showed significant differences in mean larval feeding rates between the four fish species, and that gorge rates were significantly higher than background feeding rates in all species (F=6.972, df=3, 40, P=0.002). T-LSD tests found significant differences within and between species. Two significantly different groups were identified in gorge rates, with one group, (comprising G. holbrooki and P. signifer) significantly higher than the other (comprising H. galii and Pseudogobius sp.). Three groups were shown in background consumption rates, with one G. holbrooki significantly higher and H. galii significantly lower. Proportional differences were similar across all four fish species.
6.3.4 Day versus night experiments

Table 6.5 summarises day and night larval consumption rates (pgfw) for 4th instar larvae experiments, plus the ratio of day to night consumption. The day consumption rate values are the same as those shown in table 6.2.

Table 6.5 Summary of day and night consumption rates (pgfw) for 4th instar *Ae. vigilax* larvae. Entries with different T LSD test letters are statistically significant (p≤0.05). A * next to the gorge vs. background ratio denotes a significant difference (p≤0.05) between consumption rates.

<table>
<thead>
<tr>
<th>Species</th>
<th>Day consumption</th>
<th>Night consumption</th>
<th>Day vs. Night Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SE</td>
<td>Mean  SE</td>
<td></td>
</tr>
<tr>
<td><em>G. holbrooki</em></td>
<td>178.72A 25.83</td>
<td>58.71A 24.95</td>
<td>3.04:1*</td>
</tr>
<tr>
<td><em>P. signifer</em></td>
<td>150.01A 12.42</td>
<td>114.26B 9.91</td>
<td>1.31:1</td>
</tr>
<tr>
<td><em>H. galii</em></td>
<td>62.81B 10.73</td>
<td>9.85C 4.18</td>
<td>6.38:1*</td>
</tr>
<tr>
<td><em>Pseudogobius sp.</em></td>
<td>102.02B 22.63</td>
<td>9.96C 4.90</td>
<td>10.25:1*</td>
</tr>
</tbody>
</table>

Both null hypotheses were rejected, as the two way ANOVA showed significant differences in 4th instar larval consumption between day and night experiments, and between the four fish species at night (F=4.757, df=3, 40, P=0.006). T-LSD tests showed that night consumption rates were significantly higher in *P. signifer* and significantly lower in *H. galii* and *Pseudogobius* sp.. The proportional difference between day and night consumption rates was significant in all fish species except *P. signifer*.

6.4 Discussion

This study demonstrates significant predation of *Ae. vigilax* larvae by the four resident mangrove basin fish species examined. Between 2.37 and 6.53 times more 2nd instar larvae were consumed than 4th instar larvae. 2nd instars of *Ae. vigilax* are considerably smaller than 4th instar larvae, as described by Shinkarenko et al (1986), who found that 2nd instars of *Ae. vigilax* are approximately half the length
and head capsule width of 4\textsuperscript{th} instars, therefore higher consumption of 2\textsuperscript{nd} instar larvae would be expected. This assumes the different instars have equivalent food value but just represent different sized meals. Efficient feeding on 2\textsuperscript{nd} instar larvae is also an advantage when considering that natural larval mortality reduces mosquito populations over their development cycle.

Although consumption of 2\textsuperscript{nd} and 4\textsuperscript{th} instar larvae differed significantly between instar stage and across all four fish species, \textit{G. holbrooki} and \textit{P. signifer} consumed substantially more larvae than did \textit{H. galii} and \textit{Pseudogobius} sp., and \textit{G. holbrooki} and \textit{P. signifer} are therefore likely to be more effective biological control agents.

\textbf{6.4.1 Gorge versus background feeding rates}

All four fish species showed a similar pattern of feeding throughout the experiment. A significant gorging period was observed within the first hour of the experiment, where fish ate mosquito larvae at a much higher rate than later on. One factor contributing to this period of intense feeding was the withholding of food before the experiment to standardise hunger. The results show that fish will eat until completely full and try to maintain a high level of fullness. This behaviour may be beneficial in isolated environments such as mangrove pools, where patterns of food availability will vary considerably. Short periods of 3 or 4 days during which mosquito larvae are plentiful are followed by much longer periods of weeks to months after mosquitoes have emerged as adults when there will be little if any mosquito-based food available. Also when utilising an ephemeral food source such as mosquito larvae, it would be beneficial for fish to feed as quickly and fully as possible before the food source disappears. Similar behaviour has been documented in other studies; for example Willems et al (2005) identified a feeding pattern where fish predation rates decreased over time, despite the density of mosquitoes. This suggested that fish reached a ‘satiation point’ where they were too full to keep eating.

In the experiment the gorging period was preceded by manipulation of fish hunger levels, and so it is not clear whether gorging is natural behaviour in basin pools. The previous chapter found that relatively few individuals had empty stomachs despite low mosquito larval abundance, as fish were able to feed on a wide variety of alternative prey items. Fish may simply not experience the levels of food
deprivation necessary to trigger such elevated predation rates. However fluctuations in food availability, such as following a mosquito larval hatch, or unexpected flooding (such as via heavy rainfall) washing larvae into pools containing fish (or vice versa) may result in elevated predation rates, as fish take advantage of a sudden, highly abundant food source. The exposure of large populations of mosquitoes to fish is essential for allowing mosquito control by fish (Hess and Tarzwell 1942; Ritchie 1984). Whether exposure to very large mosquito populations produces a similar gorge rate in field conditions is a knowledge gap that may be addressed in future research. Further, it is unclear at what instar stage fish commence feeding on larvae, however the results suggest some fish species may consume smaller instars more effectively when available and others may be less likely to do so, which means they may be able to apply significant predation pressure early on in the Ae. vigilax development period.

After the initial gorging period predation rates diminished and remained consistently low until the conclusion of the experiment. This pattern was similar for all individuals of the four fish species, suggesting that background feeding rates observed here are typical feeding rates under normal conditions when there are no food scarcity issues. While it is useful to observe the initial gorging period, especially if it is typical behaviour, the longer term background predation rate may be far more useful for understanding the biological control potential of these fish species.

6.4.2 Differences between species

Of the four species examined G. holbrooki showed the highest feeding rates, over both instar stages. This species appears to be capable of eating comparatively high numbers of mosquito larvae. Of the three native species, P. signifer showed the most potential as a predator of Ae. vigilax larvae, exhibiting feeding rates similar to G. holbrooki, and rates considerably higher than H. galii and Pseudogobius sp.. A number of studies have compared the rates of mosquito larvae predation between G. holbrooki and P. signifer, but results vary widely. Hurst et al (2004) found that G. holbrooki ate considerably more Cx. annulirostris larvae in a 24hr period than did P. signifer, and they concluded that other native fish, including H. galii, were far more effective than P. signifer as biological control agents. However Willems et al (2005)
found consumption of *Cx. annulirostris* by *G. holbrooki* and *P. signifer* was very similar. Reasons for the differences in results between these two studies are unclear, however the results of this study support the findings of Willems et al.

Considering the length of each experiment (8 hours), predation of *Ae. vigilax*, especially by *P. signifer* and *G. holbrooki* was much higher than the observations of predation on other Australian mosquito species, such as *Cx. annulirostris* (as described above). Higher predation of *Ae. vigilax* is not likely due to size differences between it and *Cx. annulirostris*, as measurements taken of body length and head capsule width of *Ae. vigilax* (Shinkarenko et al 1986) and *Cx. annulirostris* (McDonald et al 1977) show that instars of the two species are of similar size. Instead, behavioural/physiological differences may be responsible for the differences. Different mosquito species have different responses to predation. For example Kesavaraju and Juliiano (2004) found that larvae of *Ochlerotatus triseriatus* (Say) altered their behaviour patterns (movement speed, resting and feeding behaviour) in the presence of predatory insect larvae, whereas *Aedes albopictus* (Skuse) did not. In another study, Anyaele and Obembe (2011) suggested that differences in predation by a fish on *Anopheles* and *Culex* was likely due to the difference in how the two genera rest on the water’s surface (*Anopheles* lie flat on the surface and *Culex*, like Aedine mosquitoes, rest at an angle to the surface, with siphons extending above the water). Similarly, *Ae. vigilax* may have behavioural or physical characteristics that make them more susceptible to predation. No such characteristics have been identified in *Ae. vigilax*, although the ‘balling’ behaviour seen in Aedine mosquitoes (where larvae form dense clusters) may be a response to an increased threat of predation (G. Gilmore, pers. comm.). This presents another future research pathway.

The other two native species (*H. galii* and *Pseudogobius sp.*) showed much lower predation rates than *G. holbrooki* and *P. signifer*. The low predation observed for *H. galii* is in contrast to other studies, which have suggested that *H. galii* performs similarly to *P. signifer* (Hurst et al 2004). Throughout the experiments *H. galii* showed very little interest in feeding on mosquito larvae on the surface, instead feeding on the few larvae that had dived down to the lower areas of the water column. Given that mosquito larvae spend most of the time on the surface, suggests that this species would probably not be an effective predator of mosquito larvae.
*Pseudogobius* sp. showed more interest in mosquito larvae than *H. galli*. *Pseudogobius* sp. spent much of its time sitting on the bottom of the tank, feeding on diving *Ae. vigilax* larvae, but also frequently darted to the surface to lunge at groups of larvae, albeit often unsuccessfully. This suggests *Pseudogobius* sp. may be more effective than *H. galii* (as reflected in several of the experiments), however it may not be well suited to feed on surface dwelling prey items such as mosquitoes. No studies were found that document predation of mosquito larvae by this species, but Morton et al (1988) documented significant feeding on mosquito larvae in saltmarshes by an unidentified SEQ gobiid species, and Becker et al (2007) found small quantities of Culicid larvae in the stomachs of a similar estuarine species, *Pseudogobius olorum*.

6.4.3 “Night” feeding patterns

Experiments performed in complete darkness showed a significant reduction in predation rates in all species except *P. signifer*. As fish predation is thought to be largely sight-based (Mathavan et al 1980), this reduction in feeding is consistent with the current body of knowledge of fish feeding behaviour. Fish and mosquitoes showed no reaction to the red light, however they would scatter when exposed to a conventional white LED light. This agrees with other studies observing fish in darkness using red LED lights, such as deep-ocean fish populations (Widder et al 2005; Weiss et al 2006).

Observations made during the experiment showed that fish and mosquitoes appeared to not notice each other at all in the dark, and predation only happened when fish made accidental physical contact with mosquito larvae. Not only does this suggest that feeding on mosquitoes would be reduced at night, but also that predation would be reduced in the turbid, shaded waters of mangrove basins. This agrees with conclusions reached by Okorie and Abiodun (2010), who suggested that reduced mosquito consumption in darkness was analogous to less effective malaria vector mosquito control by fish in turbid water.

In contrast, the night feeding behaviour of *P. signifer* showed only a minor reduction due to darkness compared to the other species indicating it is more effective at detecting and catching prey in low light conditions. Different fish species have demonstrated different reactions to low light intensity/turbidity (Diehl
1988; Nurminen et al 2010; Salonen and Engstrom-Ost 2010; Einfalt et al 2012). For example, Diehl (1988) found that two freshwater fish species (Abramis brama (L.) and Rutilus rutilus (L.)) exhibited behavioural adaptations that allowed them to effectively feed in complete darkness. *Psueodomugil signifer* may have some similar capability, which allows it to be an effective night feeder, and also a more effective mosquito predator in low visibility, turbid, shady mangrove basin waters. However, there is no reported evidence of adaptation or behaviour in *P. signifer*. This presents an interesting future research pathway, particularly as this species has been demonstrated to be an alternative to *G. holbrooki* as an *Ae. vigilax* larval control agent.

The findings of this laboratory-based study differ from other field-based studies conducted in darkness in some aspects. For example, Morton et al (1988) identified significant consumption of larvae in saltmarsh fish collected after overnight foraging, including at least two species in this study (*G. holbrooki, P. signifer*, and possibly *Pseudogobius sp.*). This may suggest that fish are actually able to feed on prey in darkness. Alternately, saltmarsh pools are open, exposed environments, which, although in darkness, may still be illuminated by low intensity light sources, such as moonlight, which may provide sufficient light to allow predation, especially as Morton et al also sampled during the full moon. Mangrove basin pools may be shaded by tree canopy and therefore darker environments than saltmarsh pools. However the actual light intensity necessary to allow predation was not measured in this study, and presents another knowledge gap that may be addressed in future research.

### 6.4.4 Limitations of laboratory based studies

The laboratory studies presented in this chapter successfully quantified the predator-prey relationship between fish and *Ae. vigilax*. However there are the inherent limitations of laboratory studies that need to be acknowledged. Laboratory studies are not able to replicate the complex structural, environmental and ecological conditions of natural mangrove basin pools, some of which can be critical for influencing predation rates of fish. The physical size, scale and structure of a habitat is important (Hess and Tarzwell 1942), but cannot be replicated in laboratory conditions. Unfavourable environmental conditions for mosquito
populations in the field meant that planned field experiments of predation could not be conducted. Nonetheless, future research may be able to conduct field-based experiments of predation, where the findings these laboratory experiments may provide a baseline dataset against which the impacts of external factors can be assessed.

Another critical ecological factor that was not investigated was the role of alternate prey, which can significantly reduce larval consumption rates by fish if present in large numbers (Manna et al 2008). Laboratory derived consumption rates may not accurately reflect the actual predation rates in mangrove basin pools, where fish can feed on a wide variety of alternate prey. The extent to which larval consumption rates of the four fish species are influenced by alternate prey is not entirely clear; research presented in Chapter 5 showed that fish feed on a wide variety of prey items in mangrove basin pools, however these feeding habits were documented in the absence of large mosquito populations. Other studies, conducted in both field and laboratory conditions, show that many fish, when presented with large numbers of mosquito larvae and alternate prey, will still feed on mosquito larvae to a great extent (Morton et al 1988, Hurst et al 2006). Future research may be able to address this, examining predation of *Ae. vigilax* larvae by fish in the presence of alternate prey in field or laboratory studies. The findings of this study would play a critical role in such future research, providing a baseline maximum predation rate against which alternate prey influenced consumption rates could be compared.

### 6.5 Conclusions

#### 6.5.1 Summary and contributions to knowledge

The aim of the research presented in this chapter was to quantify the predation of *Ae. vigilax* larvae by four resident mangrove fish species. All fish predated on mosquito larvae, but two species, *G. holbrooki* and *P. signifer*, showed comparatively high predation rates of both 2\textsuperscript{nd} and 4\textsuperscript{th} instar larvae. *P. signifer* was also effective at feeding in darkness, which may translate to more effective mosquito control in turbid mangrove water.
6.5.2 Implications for mosquito control

The findings presented here have significant implications for mosquito control. *Gambusia holbrooki* and *P. signifer* were found to exhibit significant predation of *Ae. vigilax* larvae, at levels that were higher than other published estimates of predation. For example, Ritchie and Montague (1995) estimated that a single fish could eat as much as ~500 larvae a week, and when this was incorporated into mosquito population models projected populations would decreased significantly as a consequence. Here *G. holbrooki* and *P. signifer* individuals appeared to be easily capable of consuming an equivalent amount of 2nd instar larvae in less than 8 hours, the actual numbers that could be consumed during the complete period of mosquito larvae development (1-2 weeks, depending on temperature), could be much higher, and therefore potentially have a greater impact on mosquito populations than previously thought.

Despite the mosquito control potential of *G. holbrooki*, the environmental consequences of using this particular species disqualifies it as a viable biological control agent. For a fish to be considered as a component of an environmentally sound mosquito control strategy, the ecological consequences need to be weighed against the effectiveness of biological control. Although *G. holbrooki* appears to be an effective predator of *Ae. vigilax* larvae, it is a noxious fish species that has caused significant environmental harm to native fish species in Australia, and would therefore not be suitable for use as a biological control agent.

The results presented show that the native species *P. signifer* is a viable mosquito control alternative, consuming mosquito larvae at rates comparable to *G. holbrooki*, with the added advantage of being a low light feeder and without the adverse environmental impacts of *G. holbrooki*. *Pseudomugil signifer* is already acknowledged as an effective predator of mosquito larvae and is often sold at aquariums and gardening stores in the SEQ region as an ornamental pond fish providing mosquito control services (Hurst et al 2005).

The findings of this chapter address the second research aim of this study, describing the predator-prey interaction between resident mangrove fish and *Ae. vigilax* larvae. This chapter focussed on the interaction between fish and larvae, however this may not be the only mosquito life stage that fish can influence; the
presence of fish may also be able to affect the behaviour of adults, in particular their oviposition behaviour, and this will be explored in the next chapter.
Chapter 7  Deterrence of *Ae. vigilax* oviposition by mangrove basin fish

7.1 Introduction

Adults of many species of mosquitoes can detect the presence of potential larval predators and avoid ovipositing in areas where larvae would be at greater risk of predation. This ability has been identified in the presence of a wide range of larval predators, including invertebrates (Chesson 1989; Spencer et al 2002; Silberbush and Blaustein 2008; Silberbush et al 2010), tadpoles (Mokany and Shine 2003; Hagman and Shine 2007) and fish (Petranka and Fakhoury 1991; Ritchie and Laidlawbell 1994; Angelon and Petranka 2002). Invertebrates and tadpoles show great potential as oviposition deterrents in small, freshwater habitats, however they are not dominant organisms in mangrove basin pools. Invertebrates would also be at risk of predation by fish, and tadpoles cannot survive in saline/brackish mangrove environment. Therefore, oviposition deterrence needs to focus on fish in order to be practical in mangrove basin forests.

Oviposition deterrence by fish may be based on an ability of mosquitoes to visually detect predators (Chesson 1989) or sense the presence of chemicals exuded by fish (Petranka and Fakhoury 1991; Angelon and Petranka 2002; Van Dam and Walton 2008; Louca et al 2009). Whether visual or chemical, oviposition deterrence is highly species specific; some mosquitoes can detect the presence of fish, whereas others cannot (Van Dam and Walton 2008; Louca et al 2009; 2010). Likewise, some fish species may be more effective at deterring oviposition than others (Pamplona et al 2009).
However there is a complete lack of studies examining deterrence of *Ae. vigilax* oviposition. Only one study exists that suggests that fish populations in mangroves may be effective oviposition deterrents, as it found a reduction in *Ae. taenioryhchus* oviposition on saltmarsh substrate when fish were present in adjacent pools (Ritchie and Laidlawbell 1994). No studies document deterrence of *Ae. vigilax* oviposition at all, and, while mosquito oviposition deterrent by mangrove fish has been suggested (Ritchie and Addison 1992; Ritchie and Montague 1995), it has never been quantified.

This gap may be attributed to several factors. Firstly, prior to this study the fish that inhabit mangrove basin pools in Australia were not identified, and so a predator avoidance oviposition response in *Ae. vigilax* could not be examined. Secondly, while the more extensively studied *Culex* and *Anopheles* mosquitoes lay eggs on the water’s surface (Bates 1940; Christophers 1945), *Ae. vigilax* lays eggs onto exposed mangrove substrate (Kerridge 1971). As it is believed that mosquitoes detect fish and/or exuded chemicals when they come into contact with the water’s surface to rest or lay eggs (Petranka and Fakhoury 1991), the link between an aquatic predator and terrestrial oviposition may not be as, as oviposition does not require physical contact with the water’s surface.

It may be possible that fish are visible in adjacent pools, or chemicals may be detected in mangrove water saturating oviposition substrate, however no studies have documented this. It may also be possible that mosquitoes can detect chemicals exuded by fish in the air without actually touching the water or sighting fish. For example Silberbush and Blaustein (2008) provided *Culiseta longiareolata* (Macquart) mosquitoes two sets of unpolluted oviposition pools, with one surrounded by a separate channel of water containing predator chemicals (from *Notonecta maculata* (Fabricius), a larvivorous insect). Oviposition was significantly reduced in the pool surrounded by water containing chemicals exuded by fish, indicating that oviposition was influenced by adjacent, untouched pollutants. *Aedes vigilax* may detect airborne fish chemicals in a similar manner.

Oviposition deterrence may become another way (in addition to predation) that fish populations may act as biological control agents of mosquitoes. The research presented in this chapter aims to quantify, through a series of laboratory experiments, *Ae. vigilax* oviposition deterrence by resident SEQ/NNSW mangrove fish species.
7.2 Methods

7.2.1 Data collection

Collecting fish

Individuals of *G. holbrooki*, *P. signifer*, *Pseudogobius* sp. and *H. galii* were collected from both the Terranora and Coombabah sites for use in laboratory experiments. The methods of collection and storage are detailed in Chapter 6.

Collecting mosquitoes

*Aedes vigilax* adults were sourced from the mosquito colony summarised in Appendix 3. Eggs from the colony were hatched via the evacuation method described in the previous chapter, and mosquitoes were raised to late pupal stage for the experiments. Adults used in the experiments were 3\textsuperscript{rd} generation adults raised in the colony.

7.2.2 Experiment protocol

Experiment setup

Experiments were conducted in 35x15x20cm rectangular fish tanks. These tanks were equipped with undergravel filters and filled with 5L of brackish (15ppt) water. A single fish was placed in each tank and left for a week, to condition the water with fish exudates. Fish were fed daily on artificial fish food flakes, throughout the water conditioning period and experiment period. Only artificial fish food was used to avoid contamination of water with chemicals from live or frozen food.

An oviposition medium was provided in each experiment tank, in three opaque, plastic containers measuring 17cm x 12cm x 5.5cm. A cotton oviposition pad, measuring 4cm x 4cm x 2cm was placed in the centre of each of the three plastic containers. These containers were filled with water to a depth of 2cm, so the cotton pad was completely saturated and positioned just above the water level (Gerberg 1970; Service 1993).
Oviposition treatments

Oviposition treatments were designed as per Van Dam and Walton (2008), who provided *Ae. aegypti* adults with a choice between a clean and fish-contaminated oviposition substrate. However in this study three oviposition treatments were provided: one submerged in, unpolluted, demineralised water (the clean, control treatment), one saturated with water from the tank that had fish living in it for one week (polluted treatment) and one placed in a plastic container with two sides removed, which allowed free circulation of tank water to keep the oviposition pad constantly saturated by freshly fish polluted water and allowed the fish access to the oviposition substrate (tank treatment).

Adding mosquito adults

Fifty *Ae. vigilax* pupae were placed into a separate container within each tank. Pupae, rather than adults were used, as attempts to introduce large numbers of adult insects into the experiments tanks resulted in significant adult mortality and escape. The oldest pupae were chosen for the experiment, and all emerged into adults within 6 hours of being placed in the experiment tank. Once pupae were in the tank nylon fly screen was stretched over the top of the tank to prevent the mosquitoes from escaping, and a glass plate was used to hold down the fly screen and reduce evaporation from the plastic containers. One corner of the fly screen was secured, but could be lifted up to feed fish and mosquitoes without allowing adult mosquitoes to escape.

Upon emergence the number of female mosquitoes was recorded to ensure that 25 female mosquitoes were present in each tank. If not, male and mosquitoes from the colony were added or returned to the colony using an aspirator until 25 females and 25 males were present. Mosquitoes were fed on cotton balls soaked in a 10% sucrose solution, replaced daily. Adults were not fed a blood meal during the experiment. A blood meal was not necessary for oviposition, as *Ae. vigilax* has demonstrated autogeny (it can produce eggs without a blood meal, albeit at a lower rate) (Hugo et al 2003). Relying on autogeny for egg production resulted in lower overall egg deposition, but standardised oviposition across all individuals, as it
prevented the development of host blood antigens from repeated blood meals from causing discrepancies in mosquito egg production (Sutherlaand and Ewen 1974).

Experiment protocol

When all mosquitoes had emerged and 25 females were present the experiment was run for seven days. Six replicates of the experiment were performed for each of the four fish species, giving a total of 24 experiments. To prevent airborne cross-contamination of chemicals between species, experiments were undertaken one fish species at a time. At the conclusion of each experiment the nylon fly screen was removed and any fish and surviving mosquitoes were returned to their respective storage tank and colony. The tanks, filters and gravel were all thoroughly cleaned at the conclusion of experiments and all water was discarded and replaced with clean water. The three oviposition pads were removed from the experiment tanks. Mosquito egg deposition was recorded by counting the number of eggs laid on each pad, under a dissecting microscope.

7.2.3 Data processing and analysis

Two analyses were undertaken to determine the impact of fish on Ae. vigilax oviposition: total egg deposition and oviposition distribution. Levene’s test for homogeneity of variance was conducted on data prior to all analyses, and data transformations were conducted where necessary, as described below. All results in 7.3 are presented untransformed for ease of inspection.

Total egg deposition

The total egg counts for each experiment tank was calculated across all three pollution treatments, and averaged across all six replicates for each species. Mean egg counts for each species was compared using a one way ANOVA, to test the null hypothesis that Ae. vigilax egg deposition was not significantly different in the presence of different fish species. Post hoc T LSD tests were used to distinguish differences between the four species.
Oviposition distribution

The number of eggs laid on the three fish pollution treatments (clean, polluted and tank) were compared as a proportion of the total egg count for each experiment replicate. This compensated for variable egg counts between replicates. These proportions were pooled across all replicates to give a mean proportion for each pollution type, for each species. Mean proportions were compared between the three fish chemical treatments and the four species treatments using a two way ANOVA, testing two null hypotheses: a) mean egg counts do not significantly differ between the oviposition treatments in the presence of each fish species, and b) that mean egg counts on each oviposition treatment type does not significantly differ in the presence of different fish species. Levene’s test showed non-homogeneity of variances for raw data (due to the high number of zero values in the dataset), and so mean proportions were $\arcsin\sqrt{\cdot}$ transformed for analysis. Post hoc T LSD tests were conducted to distinguish differences between the three pollution treatment within and between the four fish species.
7.3 Results

7.3.1 Total egg deposition

Total *Ae. vigilax* egg deposition in the presence of the four fish species is shown in Fig 7.1.

![Graph showing mean total *Ae. vigilax* egg deposition in the presence of four fish species](image)

Fig 7.1 Mean total *Ae. vigilax* egg deposition in the presence of the four different fish species experiments. Different colours correspond with the two significantly different (p≤0.05) T LSD groupings. Bars represent standard error.

The null hypothesis was rejected as the one-way ANOVA showed significant variation in total egg deposition between the four fish species (F=6.081, df=3, P=0.004). T-LSD comparisons identified two significantly different groups, with mean egg deposition higher in the presence of *G. holbrooki* and *P. signifer* (mean egg counts of 93 and 81 respectively) than *H. galii* and *Pseudogobius* sp.(mean egg counts of 17 and 16 respectively).
7.3.2 Oviposition distribution

Table 7.1 summarises results of *Ae. vigilax* oviposition experiments on the three different oviposition treatments in the presence of the four fish species.

Table 7.1 Mean egg proportions on the three oviposition treatments in the presence of the four fish species. The statistically significant (p≤0.05) treatments are bolded, and * and ** next to mean counts denote significantly different groups.

<table>
<thead>
<tr>
<th>Species</th>
<th>Oviposition treatment</th>
<th>Mean proportion of eggs</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. holbrooki</em></td>
<td>Tank</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Polluted</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td><strong>Clean</strong></td>
<td><strong>0.84</strong></td>
<td><strong>0.08</strong></td>
</tr>
<tr>
<td><em>P. signifer</em></td>
<td>Tank</td>
<td>0.24</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Polluted</td>
<td>0.22</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td><strong>Clean</strong></td>
<td><strong>0.54</strong></td>
<td><strong>0.14</strong></td>
</tr>
<tr>
<td><em>H. galii</em></td>
<td>Tank</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Polluted</td>
<td>0.29</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td><strong>Clean</strong></td>
<td><strong>0.55</strong></td>
<td><strong>0.14</strong></td>
</tr>
<tr>
<td><em>Pseudogobius</em></td>
<td>Tank</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>sp.</td>
<td>Polluted</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td><strong>Clean</strong></td>
<td><strong>0.91</strong></td>
<td><strong>0.19</strong></td>
</tr>
</tbody>
</table>

Both null hypotheses were rejected, as the two way ANOVA showed significant variation in mean egg proportions between the three oviposition treatments, as well as between species (F=16.426, df=6, 60, P=0.001). In the presence of all four species a significantly higher proportion of eggs were laid on the clean oviposition treatment, and the tank and polluted treatments were not significantly different. Comparisons between species showed two significantly different groups, with the mean proportion of eggs on the clean treatment being significantly higher in the presence of *G. holbrooki* and *Pseudogobius* sp.(0.84 and 0.91 respectively) than *P. signifier* and *H. galii* (.54 and .55 respectively).

7.4 Discussion

This study identifies a strong oviposition deterrence response to the presence of resident mangrove fish. *Aedes vigilax* clearly has the ability to identify the presence
of a predatory fish and adjust its oviposition behaviour accordingly. An ability to detect predatory fish, both in the presence of fish and in the presence of residual chemicals, has been documented in Anopheles and Culex mosquitoes, which lay eggs on the water’s surface. However, these results show, for the first time, that Ae. vigilax can detect the presence of fish.

Results support the possibility of either visual or chemical detection as the mechanism that deters oviposition. While two polluted oviposition pads had significantly lower egg counts than the clean pad, the oviposition pad placed within the tank water generally had slightly lower egg counts (albeit not statistically significantly lower). This suggests that visual detection may be important in deterring Ae. vigilax from ovipositing, as the fish in the tank was clearly visible, and had full access to the oviposition substrate.

However, egg counts were also low on pads polluted with tank water but isolated from the fish themselves. Fish were not physically able to access the oviposition substrate and were not visible to mosquitoes ovipositing on the pad, which suggests that visible detection is not solely responsible for variations in oviposition. This indicates that that oviposition deterrence is due to factors other than visual detection, and may suggest the presence of chemicals exuded by fish that can be detected by gravid adult mosquitoes.

Whether chemical or visual, a clear oviposition deterrence effect was identified, however what is not clear is the underlying reasons behind changes in egg deposition. The reduction in oviposition could be due to an actual reduction in egg production in the presence of fish or their chemicals, or it could be behavioural, caused by changes in mosquito mating or oviposition behaviour. Other studies of oviposition deterrence have not speculated on the underlying biological or behavioural responses fish cause in mosquitoes, and so this remains a critical further research pathway.

7.4.1 Species specific effects

Oviposition deterrence was highly species specific, both in terms of overall egg deposition and oviposition distribution. Of the four species Pseudogobius sp. had the most pronounced effects on Ae. vigilax oviposition. Experiments using this
species showed the lowest overall egg deposition and highest proportion of eggs laid on the clean substrate. *Gambusia holbrooki* and *H. galii* also triggered strong oviposition deterrence, significantly influencing egg distribution and reducing egg deposition respectively.

Species specific oviposition deterrence may be caused by several factors. Differences in egg deposition and distribution may be based on differences in visual detection of different fish species, where some species may be more easily detectable than others. Different fish species may have behaviour, morphology or colouring that make them more easily detectable by mosquitoes, especially in the clear water used in the experiment tanks. For example, throughout the experiments *Pseudogobius* sp. and *G. holbrooki* were observed leaping out of the water, attempting to catch mosquitoes flying close to the water’s surface, whereas the other two species remained at the bottom of pools or within the water column. This behaviour may increase the visibility of *Pseudogobius* sp. and *G. holbrooki*, thereby reducing oviposition. However this was not examined in detail in this study, and little is known about the capability of adult mosquitoes to visually detect fish, as fish-based oviposition deterrence studies exclusively focus on chemical-based deterrence. This presents a significant future research pathway.

Secondly, differences in egg deposition may be linked to an ability of *Ae. vigilax* to better detect the presence of chemicals exuded by specific fish. Different species of fish are believed to exude different chemical compounds, some of which may be more easily detected by adult mosquitoes (Pamplona et al 2009), resulting in variation in the effectiveness of oviposition deterrence between species. The chemicals exuded by *Pseudogobius* sp. and *G. holbrooki* may be more easily detectable by *Ae. vigilax*, thereby making them be more potent oviposition deterrents than *P. signifer* and *H. galii*.

7.4.2 Air and water-based oviposition deterrence

The results of this experiment indicate a possible chemical avoidance ability present in *Ae. vigilax* adults, however the medium chemicals are detected in is not clear. Studies of chemical detection based oviposition deterrence have suggested detection of chemicals both in water (Petranka and Fakhoury 1991; Angelon and Petranka 2002) and air (Silberbush and Blaustein 2008), and *Ae. vigilax* may detect
fish exuded chemical either way. The differences observed between the clean and polluted pads indicate that chemicals were detected in the water saturated within the substrate, rather than in the air. The glass plate laid over the top of each tank trapped moisture within the tank, which meant that the air was likely saturated with water and fish chemicals, however *Ae. vigilax* adults overwhelmingly favoured the clean pad for oviposition. *Ae. vigilax* adults may be able to detect waterborne chemicals saturated in the substrate when the make direct physical contact for oviposition, which may be analogous with the physical contact necessary for chemical detection by water-surface oviposition mosquitoes (Petranka and Fakhoury 1991).

However total egg deposition varied in the presence of the four fish species, independent of substrate type, which may indicate that fish were influencing *Ae. vigilax* oviposition before gravid mosquitoes made physical contact with the substrate. This may have been due to visual detection, but may also be attributed to an ability to detect chemicals in the saturated air within the experiment tank. Other studies have found similar results, finding a significant reduction in oviposition on water adjacent to, but not saturated by, fish chemicals (Silberbush and Blaustein 2008).

This also presents a possible technical limitation of the study, as the clean control pad may have been contaminated by airborne fish chemicals. As a result, it is not possible to know the extent that fish reduce overall *Ae. vigilax* oviposition. While still significantly higher than in the presence of *G. holbrooki* and *Pseudogobius sp.*, total oviposition in the presence of *P. signifer* and *H. gali* may still be far lower than that seen in areas completely free of fish and their chemicals. Future research may be able to address this however, by attempting to establish a baseline *Ae. vigilax* egg production rate, against which the findings of this study could be compared.

### 7.4.3 Applicability of laboratory-derived results

The findings presented in this chapter suggest that resident mangrove fish species can deter *Ae. vigilax* oviposition, and therefore fish may be able to deter oviposition in field conditions. Optimal *Ae. vigilax* oviposition sites are located on substrate at an elevation where it is persistently moistened by water from the adjacent pools (Dale et al 1986; Griffin et al 2010). Therefore fish in adjacent pools may be visible to any gravid adults ovipositing at the site, especially if they are forced to
congregate at the water’s surface due to the low DO concentrations common to SEQ/NNSW mangrove basin forest pools. Furthermore, oviposition substrate adjacent to pools containing fish would be continuously soaked with waterborne fish chemicals, providing a constant concentration of chemicals that could deter oviposition.

However it is not clear whether oviposition deterrence may actually effect mosquito populations in the field. While the impacts of other factors such as tidal flooding frequency and microtopography are well established in mangroves (Knight et al 2009; Griffith et al 2010), and the role of fish as predators is well documented in field studies in other habitats (Morton et al 1988; Becker and Laurenson 2007), most studies of oviposition deterrence, including this study, have taken place in a laboratory setting (the one exception being Ritchie and Laidlawbell (1994)). Laboratory studies alone may be suitable for understanding the oviposition deterrence effects on container breeding mosquito species, however in large, complex habitats such as mangrove basin forests environmental factors may reduce the oviposition deterrence impact fish may have. Rainfall or tidal flooding may reduce the visibility of fish populations or concentrations of chemicals, thereby reduce the effectiveness or consistency of the oviposition deterrent. Ritchie and Laidlawbell (1994) observed this in saltmarsh pools, where a fish population deterred oviposition on adjacent substrate until the pool dried out and eradicated the fish. However when rainfall and tides refilled the pool with clean water (without fish) oviposition on the adjacent substrate increased dramatically.

Furthermore, airborne chemicals (if they are present) may not be as effective an oviposition deterrent as what laboratory-derived results indicate. The air in the closed experiment tanks was likely artificially hyper-saturated with water vapour and fish chemicals, which may have increased airborne fish chemicals to concentrations not replicable in field conditions. While mangrove forests are sheltered, humid environments it is unlikely that the air above mangrove pools and adjacent substrate would be sufficiently saturated with fish chemicals to achieve the reductions in oviposition observed in this study. Therefore the impacts on total egg deposition observed in this study, which were likely caused by hyper-saturation within experiment tanks, may not be as relevant as the distribution of eggs between the three oviposition treatments.
Airborne chemicals would also need to be detected in a far less stable medium than saturated substrate. Their distribution and concentration may be influenced by factors that would not affect chemicals in isolated pools or saturated soil. Wind speed and direction is known to reduce the potency of host chemicals, impairing the ability of insects, including mosquitoes, to sense these chemical cues (Brady et al 1995; Geier et al 1999; Hoffmann and Miller 2003). The effects of wind direction have been examined in other forest habitats, for example Brady et al (1989), found that air movement made host detection by tsetse flies more difficult in wooded habitats, compared to open areas. Airborne chemicals exuded by fish may be similarly effected, and be difficult to detect or trace to the specific areas that contain fish.

Ultimately the applicability of the findings of this study to field conditions is not known. Unfavourable environmental conditions for mosquito populations in the field meant that planned field experiments of oviposition deterrence could not be conducted. Nonetheless, future research may be able to conduct field or mesocosm-based experiments of oviposition deterrence, where the findings these laboratory experiments may provide a baseline dataset against which field based observations could be compared.

7.4.4 Nature of fish chemicals

The actual presence of chemicals was not examined in this study. Little is known about the chemicals that may repel oviposition. Other studies refer to fish chemicals simply as ‘fish exudates’ or ‘kairomones’ (Petranka and Fakhoury 1991; Hurst et al 2010), however no studies, this one included, have isolated the specific chemical compounds from fish that mosquitoes react to. As with other studies of oviposition deterrence, the presence of fish chemicals was assumed, based on the behaviour of the gravid mosquitoes in circumstances where the presence of fish chemicals was expected. It is not known what concentrations are necessary to deter oviposition, and how long chemicals can remain at sufficient concentrations to deter oviposition. Other studies have investigated these factors in other habitats, for example Angelon and Petranka (2002) identified a threshold limit, above which mosquitoes were able to detect fish chemicals, but still did not identify the specific chemicals involved in oviposition deterrence.
In order to determine whether or not fish chemicals could be employed in mosquito management, the actual chemicals involved need to be identified. There is only one reported study that has identified the chemicals exuded by a larval predator, albeit not a fish; Silberbush et al (2010) identified two chemical compounds (h-heneisocane and n-tricosane) exuded by a predatory insect, *N. maculata*, and determined the concentrations necessary to deter *Cu. longiareolata* oviposition. Future research may be able to similarly isolate the chemicals exuded by fish, and quantify the concentrations of these necessary to deter *Ae. vigilax* oviposition.

7.5 Conclusions

7.5.1 Summary and contribution to knowledge

The aim of the research presented in this chapter was to investigate whether resident SEQ/NNSW fish species can deter *Ae. vigilax* oviposition. A strong oviposition avoidance behaviour was identified in *Ae. vigilax* in the presence of mangrove basin fish species, particularly *Pseudogobius* sp. and *G. holbrooki*. *Aedes vigilax* largely avoids laying eggs on substrate saturated by water containing fish, even when fish were physically separated from the oviposition substrate, which suggests that *Ae. vigilax* possesses an ability to either visually or chemically detect fish.

7.5.2 Implications for mosquito control

The findings presented in this chapter may have significant implications for mosquito control, as oviposition deterrence presents a new avenue by which resident fish may reduce *Ae. vigilax* populations in mangrove basin forests. The findings of this study may be applied to mosquito control in several ways. Chemicals exuded by fish, if they can be identified and synthesised, may be used in similar manner to existing larvicides; introduced into larval pools to deter oviposition. Given that the one of the dominant forms of mosquito control in eastern Australian mangroves is Bti, a synthetic biological control agent, it is not unrealistic to consider using chemicals exuded by fish as an alternative control method. Chemicals isolated from specific organisms have been used to alter the
behaviour of other mosquitoes on a smaller scale, for example derivatives from plants have been used as adult mosquito repellents (Singh and Upadhyay 1993; Greive et al 2010), and fish chemicals may be able to be used as pesticides on a mosquito population scale to reduce oviposition. However this form of mosquito control is likely to be no less expensive or more practical than current chemical control methods.

A far more practical, sustainable and affordable use of this new knowledge is to incorporate it into an integrated control method that aims to enhance mangrove values while reducing mosquito populations. Enhancing fish populations and increasing their access to mosquito habitats, in addition to an increase in predation of larvae, may trigger a strong oviposition deterrent response in gravid mosquitoes, reducing oviposition on the surrounding substrate.

The results presented in this chapter address the fourth research aim of this study, describing *Ae. vigilax* oviposition deterrence. Combined with the results presented in the previous chapter these indicate how each resident fish species may contribute to mosquito control. *Gambusia holbrooki* and *P. signifer* appear to have a significant impact on *Ae. vigilax*, and therefore may be effective mosquito control agents. *Gambusia holbrooki* demonstrated strong oviposition deterrence and high predation rates, and *P. signifer* demonstrated less effective oviposition deterrence, but this was counterbalanced by its relatively high larval predation rates. *Pseudogobius* sp. and *H. galii* had a less significant impact on *Ae. vigilax*; *Pseudogobius* sp. showed a very strong oviposition deterrence but a low predation rate, and *H. galii* showed less effective oviposition deterrence and low predation rates. Now that the fish-mosquito interaction has been examined, both in terms of larval predation and oviposition deterrence it needs to be applied to mosquito populations in SEQ/NNSW mangrove basin forests, which will be explored in the next chapter.
Chapter 8  Impact of fish on immature mosquito production in mangrove basin forests

8.1 Introduction

Fish populations are often assumed to have a significant impact on mosquito populations in coastal wetlands. To varying degrees numerous studies identify a need for refuge from predation by fish as part of understanding the ecology of coastal wetland mosquito species such as *Ae. vigilax* in Australia and *Ae. taeniorhynchus* in the United States (Dale et al 1986; Ritchie and Montague 1995; Griffin et al 2010). The extent to which fish are believed to influence mosquito populations varies. Ritchie and Addison (1992) believed that predation and oviposition deterrence by fish was excluding certain areas of mangrove forests as mosquito habitats. Knight (2008) acknowledged that fish may be important, but placed far more emphasis on tidal patterns for creating suitable habitats.

Three key components influence the degree to which fish populations affect mosquitoes, which may be applied to SEQ/NNSW region mangrove basin forests. First, to significantly influence mosquito populations, fish need to be present in relatively large numbers, with ready access to mosquitoes. Hess and Tarzwell (1942) found a strong relationship between the size of *Gambusia affinis* fish populations with ready access to mosquito habitat and the extent that fish reduce mosquito populations, presumably by predation of larvae. Mosquito control using fish often incorporates this by introducing large numbers of larvivorous fish into larval habitats (Davey et al 1974; Bence 1988; Blaustein 1992; Kumar and Hwang 2006) or increasing fish access to mosquito habitat, such as in the case of
saltmarsh runnelling, where channels dug into the saltmarsh allow fish access to previously isolated pools containing larvae (Connolly 2005).

The second critical factor is the spatial and temporal stability of the fish population. A fish population can only have an effect on a mosquito population when present within the larval habitat, and fish populations that are frequently present in pools would therefore have a large impact on mosquito populations. For example Ritchie and Laidlawbell (1994) found that the presence of fish in pools was able to significantly deter oviposition. However, fish were not permanent in pools, and, when fish were removed oviposition resumed. Morton et al (1988) reached a similar conclusion in SEQ saltmarshes, where fish communities in pools preyed upon significant numbers of larvae, but were not present frequently enough to have a lasting impact on mosquito populations.

The third critical factor is the composition of fish communities. Different species, while occupying similar ecological niches and having similar behaviour, morphology or feeding habits, can have different impacts on larval populations and oviposition preferences. Studies have compared the predatory capacity of different fish species in small pool habitats (Morton et al 1988; Schleuter and Eckmann 2008; Laufer et al 2009) and oviposition deterrence effects of different fish species have been documented in laboratory studies (Van Dam and Walton 2007; Pamplona et al 2009; Silberbush et al 2010). For example Hurst (2004) compared fish predatory capacity of several different fish species, and found a wide variation in their ability to predate upon Cx. annulirostris mosquitoes. In another study Laufer et al (2009) found that four closely related killifish in Brazil demonstrated significantly different feeding habits, recording wide variations in consumption of larval invertebrates, including mosquito larvae.

The research presented in the previous chapters of this study has investigated these three factors in SEQ/NNSW region mangrove pools. Four resident fish species are present within the mangrove basin forest between tidal flooding events, when mosquito larvae are present in pools and adults are depositing eggs on adjacent substrate. These fish can consume large numbers of Ae. vigilax larvae and can have a strong oviposition deterrence effect. The next step is to investigate whether the size, stability and composition of resident SEQ/NNSW mangrove basin fish populations allow them to significantly influence Ae. vigilax production. This would allow the current impact of fish on mosquito populations to be determined,
and thus give insight into the biological control potential of fish within mangrove basin forests.

Examining the impact of fish requires a method of sampling mosquito populations. Sampling larval populations is one commonly used method. As larvae are relatively immobile, confined to the pools into which they hatched, the distribution and density of larval populations gives a strong indication of mosquito production in an area (Service 1993). Larval sampling is often employed to observe direct changes in a mosquito population due to external stimuli, such as the introduction of various mosquito control methods (Lawler et al 1999; Butler et al 2006). Observing changes in larval populations has been used to directly measure the impact of fish populations on immature mosquitoes, both in laboratory (Taylor et al 1992; Hurst et al 2004; Willems et al 2005; Manna et al 2008) and field experiments (Kumar and Hwang 2006; Louca et al 2009).

However larval sampling was not possible in this study, due to environmental factors significantly disrupting mosquito populations. Instead alternate methods of measuring mosquito production are required. Eggshell sampling is one such method; saltwater Aedine mosquito species such as *Ae. vigilax* lay their eggs on the substrate surrounding pools, and eggshells remain in the soil long after hatching (Linley et al 1992; Richie and Johnson 1991; Ritchie 1994). Therefore eggshells provide a historical record of oviposition activity (and thus mosquito production) at a particular site, and can be used as a surrogate for larvae, indicating suitable larval habitats (Ritchie and Addison 1992; Dale et al 1999). It is particularly useful in circumstances where mosquito populations are highly variable, and so is ideal for use in this study.

Correlating eggshell densities with the size, stability and composition of fish populations may demonstrate that predation of larvae and deterrence of oviposition by fish reduces mosquito production within mangrove basin forests, and therefore significantly contributes to biological control. The research presented in this chapter aims to do this, by exploring the extent to which fish populations impact immature *Ae. vigilax* production in SEQ/NNSW mangrove basin forests, using eggshell density as a measure of mosquito production.
8.2 Methods

Field data was collected at the 12 study pools in the Terranora and Coombabah study sites (see Chapter 3). Data collection in this chapter included mosquito eggshell sampling (as described below), which required collecting small samples of mangrove substrate, so each pool study site also included the hummocks of substrate and pneumatophores immediately adjacent to the pools.

8.2.1 Eggshell sampling

Eggshell sampling was conducted using the method of Ritchie et al (1992). Soil samples were collected from hummocks directly adjacent to each pool. Samples comprised 10-15 scrapings (~300g) of exposed soil, pneumatophores and substrate vegetation. Eggshells were sampled at all 12 pools at each site. Two samples were taken at each pool, giving a total of 24 samples collected overall at each site.

Eggshell samples were processed in the laboratory as per the method of Ritchie and Jennings (1994). Each sample was broken down in a blender, which loosened eggs and eggshells from the soil. This sample was then reduced to an aliquot and all particles in the size range of *Ae. vigilax* eggshells were filtered out using nested 300µm and 125µm sieves (*Ae. vigilax* eggshells are between 170-180µm at their greatest width (Kay and Jorgensen 1986)). The sample was then dried for three days, and then ground to a fine powder in a mortar and pestle to break up the substrate. This was then mixed with water, allowing eggs and eggshells float to the surface, where they were counted. Eggshells of all mosquito species were counted. Eggshell counts were indexed as the number of eggshells/cm³, as per Knight (2008). Each sample from each site was analysed separately, however, the two eggshell counts from each site were averaged for each pool.

8.2.2 Fish predation index

The findings presented in the previous chapters were used to create an index of predation vulnerability, which categorised each pool within the study based on the likelihood that mosquito larvae within the pool would be exposed to larvivorous
fish, and the degree to which these fish would feed on the larvae. The index used for each pool aimed to incorporate the three factors identified in 8.1:

- **Population size** - the size of the fish populations within each pool (assuming that samples captured an accurate representation of the fish population within each pool).
- **Composition** - The specific species present in each pool, and the predatory capacity of each of these species.
- **Stability** - The frequency that fish are present within each pool.

These three factors were combined to express predation vulnerability within each pool using a modified form of the formula used by Ward et al (1995) and Zimmerman and Ward (1999), which examined predation of juvenile salmonoids by a single predatory fish species by combining data collected on the abundance of the predator and its predation rate on a particular prey item. The original index was modified to accommodate the requirements of this study. Firstly, to measure across multiple species the method was modified to calculate average predation vulnerability across different proportions of all species of fish within each sample. Secondly, to incorporate mosquito predation rates as calculated in chapter 5, which were standardised to fish weight, fish populations in mangroves were similarly expressed as by weight, in terms of biomass of each species within each sample (per gram of fish biomass - pgfb). A predation index for each pool sample \( P_{li} \) was therefore calculated using the formula:

\[
P_{li} = \frac{\sum_{j=1}^{s} AI_{ij} \cdot Cl_{ij}}{s}
\]

Where: \( AI_{ij} \) = Predator abundance index of the jth fish species within the ith pool sample, \( Cl_{ij} \) = Consumption rate (pgfb) of the jth fish species within the ith pool sample and \( s \) = number of fish species present in sample. Predator abundance index \( (AI) \) for each species within each sample was calculated using the formula:

\[
AI_{ij} = \frac{W_{ij}}{SA_{i}}
\]

Where \( W_{ij} \) = total biomass of the catch of the jth larvivorous fish species in the ith pool sample, \( SA_{i} \) = surface area of the ith pool. The total biomass of each fish species caught within each sample comprised the sum of estimated weights of all
fish caught of each species, based on a correlation with fork length from a small sample (21 individuals) of each fish species (see Chapter 6).

Consumption index (CI) for each species within each sample was calculated from 4th instar larval predation experiments conducted in chapter 5. Night and day feeding rates were combined to give an estimated mean daily feeding rate (pgfb), based on the assumption of 13 hours of daylight feeding and 11 hours night time feeding (which approximately correlated with standard day length during peak mosquito season in 2011-2012). Night time feeding comprised 11 hours of background rate feeding. Daytime feeding rates comprised 12 hours of background feeding rate, with an additional 1 hour of gorge period rate feeding, to simulate a gorging period at dawn.

PI was calculated for all samples containing fish. Sample PI values from each pool were combined into a mean PI for each pool, which was multiplied by the proportion of samples taken at each site containing fish to give an overall mean PI value for each pool. This allowed PI values to incorporate the frequency of fish presence within mangrove pools as a factor affecting mosquito populations.

8.2.3 Data processing and analysis

In addition to the PI value calculated for each fish, three other values of the fish population in each pool were recorded, based on fish population data collected in Chapter 4: 1) Frequency of fish occurrence (the number of samples at each pool containing at least one fish), 2) sample yield (the number of fish per m² caught in each pool), and 3) sample biomass (biomass of samples containing fish caught at each pool). Mean values of these were calculated for each pool.

Eggshell densities (per cc) included all mosquito species eggshells found. For analysis eggshell densities were log transformed (ln(x+1)) to approximate a normal distribution, as per Dale et al (2008). Four regression analyses were conducted between the four aspects of the fish population described above and transformed eggshell density, to test four null hypotheses: a) no relationship exists between overall mean PI values and eggshell density b) no relationship exists between the frequency of fish occurrence and mean eggshell density c) no relationship exists between mean sample yield and mean eggshell density d) no relationship exists
between mean sample biomass mean mosquito eggshell density. All eggshell data in the tables below are presented untransformed for ease of inspection.

8.3 Results

8.3.1 Summary of eggshell data

Terranora

Eggshells of two species were found at the Terranora site: *Ae. vigilax* and *Ve. funerea*. Table 8.1 summarises eggshell data collected at the Terranora site:

<table>
<thead>
<tr>
<th>Pool</th>
<th>Mean eggshell density (per cm$^3$)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Ae. vigilax</em></td>
<td><em>Ve. funerea</em></td>
<td>Total</td>
</tr>
<tr>
<td>TW01</td>
<td>0.62</td>
<td>0.02</td>
<td>0.64</td>
</tr>
<tr>
<td>TW02</td>
<td>0.78</td>
<td>0.00</td>
<td>0.78</td>
</tr>
<tr>
<td>TW03</td>
<td>0.73</td>
<td>0.04</td>
<td>0.77</td>
</tr>
<tr>
<td>TW04</td>
<td>2.07</td>
<td>0.01</td>
<td>2.08</td>
</tr>
<tr>
<td>TW05</td>
<td>1.38</td>
<td>0.06</td>
<td>1.43</td>
</tr>
<tr>
<td>TW06</td>
<td>1.15</td>
<td>0.01</td>
<td>1.16</td>
</tr>
<tr>
<td>TW07</td>
<td>0.64</td>
<td>0.00</td>
<td>0.64</td>
</tr>
<tr>
<td>TW08</td>
<td>0.06</td>
<td>0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>TW09</td>
<td>2.67</td>
<td>0.00</td>
<td>2.67</td>
</tr>
<tr>
<td>TW10</td>
<td>2.10</td>
<td>0.05</td>
<td>2.15</td>
</tr>
<tr>
<td>TW11</td>
<td>0.25</td>
<td>0.00</td>
<td>0.25</td>
</tr>
<tr>
<td>TW12</td>
<td>0.02</td>
<td>0.00</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Eggshells were found in samples collected at all 12 study pools. All samples were dominated by *Ae. vigilax* eggshells, and small numbers of *Ve. funerea* was found at 7 of the 12 pools. Mean densities differed considerably across the mangrove basin, ranging from 0.02 eggshells per cm$^3$ in T12, to 2.67 eggs per cm$^3$ in T09. All pools supported significant mosquito populations except T12.
Coombabah

Only eggshells of *Ae. vigilax* were found at the Coombabah site. Table 8.2 summarises eggshell data collected at the Terranora site:

Table 8.2 Results of eggshell sampling at the Coombabah study site.

<table>
<thead>
<tr>
<th>Pool</th>
<th>Mean <em>Ae. vigilax</em> eggshell density (per cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM01</td>
<td>0.46</td>
</tr>
<tr>
<td>CM02</td>
<td>2.00</td>
</tr>
<tr>
<td>CM03</td>
<td>0.46</td>
</tr>
<tr>
<td>CM04</td>
<td>0.92</td>
</tr>
<tr>
<td>CM05</td>
<td>0.12</td>
</tr>
<tr>
<td>CM06</td>
<td>0.81</td>
</tr>
<tr>
<td>CM07</td>
<td>0.13</td>
</tr>
<tr>
<td>CM08</td>
<td>1.00</td>
</tr>
<tr>
<td>CM09</td>
<td>8.01</td>
</tr>
<tr>
<td>CM10</td>
<td>0.83</td>
</tr>
<tr>
<td>CM11</td>
<td>0.66</td>
</tr>
<tr>
<td>CM12</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Eggshells were found at all 12 pool sites at Coombabah. As with Terranora, eggshell densities varied considerably across the basin, from 0.11 eggshells per cm$^3$ at C12, to 8.01 eggshells per cm$^3$ at C09. All pools supported significant mosquito populations.
8.3.2 Consumption index

Consumption index (CI) values for each species were based on measurements of consumption of 4th instar *Ae. vigilax* larvae in Chapter 6. Table 8.3 summarises the formation of the CI values used for each fish species.

Table 8.3 Recorded and estimated consumption rates and CI values for the four fish species used in this study. All values displayed are expressed as consumption per gram of fish biomass (pgfb).

<table>
<thead>
<tr>
<th>Species</th>
<th>Night feeding (11hrs)</th>
<th>Dawn gorge (1 hr)</th>
<th>Day feeding (12 hrs)</th>
<th>(N+G+F) (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Est. larval consumption /hr</td>
<td>Est. larval consumption /hr</td>
<td>Est. larval consumption /hr</td>
<td></td>
</tr>
<tr>
<td><strong>G. holbrooki</strong></td>
<td>0.23</td>
<td>101.91</td>
<td>12.66</td>
<td>256.43</td>
</tr>
<tr>
<td><strong>P. signifer</strong></td>
<td>0.27</td>
<td>84.30</td>
<td>9.39</td>
<td>199.86</td>
</tr>
<tr>
<td><strong>Pseudogobius sp.</strong></td>
<td>0.00</td>
<td>49.87</td>
<td>7.45</td>
<td>139.27</td>
</tr>
<tr>
<td><strong>H. galii</strong></td>
<td>0.05</td>
<td>34.81</td>
<td>4.00</td>
<td>83.40</td>
</tr>
</tbody>
</table>

Night feeding rates were all relatively low (all species showed consumption rate of less than 1 larvae pgfb), and so estimated night larval consumption comprised only a small proportion of CI (1% of *G. holbrooki* CI, 1.46% of *P. signifer* CI, 0% of *Pseudogobius* sp. CI and 0.7% of *H. galii* CI). In all species day feeding contributed the most to CI (59% of *G. holbrooki*, 56% of *P. signifer*, 64% of *Pseudogobius* sp. and 58% of *H. galii*), as consumption rates were higher than night experiments for all species. Given the low night feeding, the addition of the dawn gorge rate to the CI appears to be a suitable way of accounting for a sudden increase in predatory activity at dawn.
8.3.3 PI values and correlation with eggshell density

Terranora

Table 8.4 summarises information about the pools, fish population and estimated total PI values at the Terranora study site.

Table 8.4 Summary of fish population variables and calculated estimated total PI values for each of the study pools at the Terranora study site. Mean eggshell densities from table 8.1 above are shown for comparison.

<table>
<thead>
<tr>
<th>Pool</th>
<th>No. samples containing fish</th>
<th>Mean sample yield (m²)</th>
<th>Mean sample biomass (g)</th>
<th>Overall estimated PI</th>
<th>Eggshell density (per cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TW01</td>
<td>2</td>
<td>0.11</td>
<td>1.39</td>
<td>1.19</td>
<td>0.64</td>
</tr>
<tr>
<td>TW02</td>
<td>6</td>
<td>0.22</td>
<td>0.77</td>
<td>13.66</td>
<td>0.78</td>
</tr>
<tr>
<td>TW03</td>
<td>3</td>
<td>0.00</td>
<td>0.17</td>
<td>0.94</td>
<td>0.77</td>
</tr>
<tr>
<td>TW04</td>
<td>4</td>
<td>0.22</td>
<td>0.40</td>
<td>2.36</td>
<td>2.08</td>
</tr>
<tr>
<td>TW05</td>
<td>4</td>
<td>0.48</td>
<td>0.40</td>
<td>7.47</td>
<td>1.43</td>
</tr>
<tr>
<td>TW06</td>
<td>5</td>
<td>0.63</td>
<td>0.60</td>
<td>18.91</td>
<td>1.16</td>
</tr>
<tr>
<td>TW07</td>
<td>5</td>
<td>0.05</td>
<td>0.35</td>
<td>1.52</td>
<td>0.64</td>
</tr>
<tr>
<td>TW08</td>
<td>4</td>
<td>0.04</td>
<td>0.86</td>
<td>0.82</td>
<td>0.07</td>
</tr>
<tr>
<td>TW09</td>
<td>3</td>
<td>0.03</td>
<td>0.61</td>
<td>0.78</td>
<td>2.67</td>
</tr>
<tr>
<td>TW10</td>
<td>2</td>
<td>0.75</td>
<td>0.85</td>
<td>4.88</td>
<td>2.15</td>
</tr>
<tr>
<td>TW11</td>
<td>4</td>
<td>0.59</td>
<td>4.10</td>
<td>16.19</td>
<td>0.25</td>
</tr>
<tr>
<td>TW12</td>
<td>2</td>
<td>0.16</td>
<td>1.10</td>
<td>1.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The results fail to reject any of the four null hypotheses at the Terranora site. Regression analyses failed to find any significant relationship between mean eggshell density (transformed) and the frequency of fish occurrence ($R^2=0.014$, $F=0.145$, df=1, $P=0.711$), mean fish sample yield ($R^2=0.045$, $F=0.467$, df=1, $P=0.510$) or mean fish yield biomass ($R^2=0.141$, $F=1.647$, df=1, $P=0.228$). Regression analysis also failed to detect any relationship between eggshell density and overall estimated PI in Terranora mangrove basin pools ($R^2=0.009$, $F=0.095$, df=1, $P=0.764$).
Coombabah

Table 8.5 summarises information about the fish population and calculated estimated total PI values at the Coombabah study site.

Table 8.5 Summary of fish population variables and calculated estimated total PI values for each of the study pools at the Coombabah study site. Mean eggshell densities from table 8.2 above are shown for comparison.

<table>
<thead>
<tr>
<th>Pool</th>
<th>No. samples containing fish</th>
<th>Mean sample yield (m²)</th>
<th>Mean sample biomass (g)</th>
<th>Overall estimated PI</th>
<th>Eggshell density (per cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM01</td>
<td>4</td>
<td>0.03</td>
<td>0.60</td>
<td>0.19</td>
<td>0.46</td>
</tr>
<tr>
<td>CM02</td>
<td>2</td>
<td>0.38</td>
<td>0.48</td>
<td>3.88</td>
<td>2.00</td>
</tr>
<tr>
<td>CM03</td>
<td>2</td>
<td>0.20</td>
<td>0.97</td>
<td>0.24</td>
<td>0.46</td>
</tr>
<tr>
<td>CM04</td>
<td>4</td>
<td>0.10</td>
<td>0.89</td>
<td>0.68</td>
<td>0.92</td>
</tr>
<tr>
<td>CM05</td>
<td>4</td>
<td>1.29</td>
<td>0.88</td>
<td>24.05</td>
<td>0.12</td>
</tr>
<tr>
<td>CM06</td>
<td>2</td>
<td>0.37</td>
<td>1.06</td>
<td>0.62</td>
<td>0.81</td>
</tr>
<tr>
<td>CM07</td>
<td>4</td>
<td>0.16</td>
<td>0.47</td>
<td>2.29</td>
<td>0.13</td>
</tr>
<tr>
<td>CM08</td>
<td>4</td>
<td>0.88</td>
<td>0.78</td>
<td>42.79</td>
<td>1.00</td>
</tr>
<tr>
<td>CM09</td>
<td>4</td>
<td>3.03</td>
<td>1.43</td>
<td>7.92</td>
<td>8.01</td>
</tr>
<tr>
<td>CM10</td>
<td>2</td>
<td>0.27</td>
<td>1.24</td>
<td>1.00</td>
<td>0.83</td>
</tr>
<tr>
<td>CM11</td>
<td>2</td>
<td>2.53</td>
<td>1.25</td>
<td>8.32</td>
<td>0.66</td>
</tr>
<tr>
<td>CM12</td>
<td>4</td>
<td>0.74</td>
<td>3.55</td>
<td>0.90</td>
<td>0.11</td>
</tr>
</tbody>
</table>

The results fail to reject the four null hypotheses at the Coombabah site. Regression analyses failed to find any significant relationship between mean eggshell density (transformed) and the frequency of fish occurrence ($r^2=0.001$, $f=0.006$, $df=1$, $p=0.938$), mean sample yield ($r^2=0.326$, $f=4.834$, $df=1$, $p=0.053$) and mean yield biomass ($r^2=0.01$, $f=0.105$, $df=1$, $p=0.753$). Regression analysis also failed to find any significant relationship between overall estimated PI values and eggshell density ($r^2=0.001$, $f=0.011$, $df=1$, $p=0.919$).
8.4 Discussion

This study found no evidence that resident fish populations influence mosquito production. Eggshell density varied considerably across the mangrove basin, but did not correlate with the size or frequency of occurrence of resident fish populations, nor the risk of predation provided by resident fish populations. In addition, eggshell densities in most samples were much greater than those regarded as indicating significant mosquito habitat. An eggshell density of more than 0.05 per cm$^3$ is considered to represent significant mosquito production (Addison et al 1992; Knight 2008), and eggshell densities of up to 40 times higher were recorded in this study, which suggests that mosquito populations are not greatly affected by the presence of fish.

8.4.1 Low fish abundance versus high consumption rates

The results of this section of the study appear to conflict with the long-held view that fish influence the suitability of habitats for mosquitoes in mangroves. As described earlier in this chapter, areas more likely to have larger fish populations have been thought to put too much predation and/or oviposition deterrence pressure on gravid mosquitoes to be suitable mosquito habitat. For example, Ritchie and Addison (1992) found a strong relationship between hydrology and mosquito habitat and suggested that the less suitable habitat found in some areas was due, at least in part, to an increased presence of fish in more frequently flushed areas.

The findings also contrast with those presented in the previous chapters. All four resident mangrove species consumed large numbers of $Ae. vigilax$ larvae in laboratory experiments, and daily estimates of consumption used in this chapter incorporated significant levels of mosquito predation into the measure of predation vulnerability. $Gambusia holbrooki$, the dominant species in the mangrove basin environment consumed large numbers of 4$^{\text{th}}$ instar mosquito larvae (every gram of $G. holbrooki$ biomass present in pools was estimated to consume over 250 4$^{\text{th}}$ instar larvae per day). The three native species also showed similar effectiveness as mosquito predators, especially $P. signifer$, which demonstrated comparable effectiveness to $G. holbrooki$ (a single gram of $P. signifer$ consumed ~200 4$^{\text{th}}$ instar larvae per day). This was also likely to be an underestimate of the overall effect on
the mosquito populations, as fish feed on mosquito larvae throughout the entire mosquito larval life stage, and feed on younger instars at a much higher rate. Other support this, and suggest that these levels of predation could be sufficient enough to have drastic effects on mosquito populations (Hurst et al 2004; Willems et al 2005; Hurst et al 2006).

Despite the impacts fish can have on mosquito populations, there was no correlation between fish and eggshell density. This indicates that fish do not significantly impact mosquito habitat suitability in mangroves, especially given the nature of fish populations within mangrove basin pools. Fish populations were highly heterogeneous, both spatially and temporally, fluctuating widely in size and frequency. Most samples taken within pools had no fish, and those that did had relatively low densities of fish (most pools had mean fish catch yields of less than 1/m²) throughout the study. Large populations of fish were occasionally found in mangrove pools, however these did not persist long term. As a result measures of abundance of fish in this chapter (frequency of fish occurrence, mean fish density and mean fish biomass) were all very low, and this low abundance was incorporated into the predation vulnerability index.

This variability may have had significantly reduced the impact of resident fish populations on mosquito production. Fish, when present, could potentially influence mosquito populations, especially in the few instances when they were present in large numbers. However overall they were far too heterogeneous to provide the consistent pressure on mosquito populations necessary to reduce mosquito populations basin wide. Fish may be present in a pool to feed on hatching larvae and deter oviposition following one flooding period, but be absent the next, allowing oviposition and larval development to continue undisturbed.

8.4.2 Limitations of eggshell sampling

It is also possible that the lack of impact of fish on mosquito production is due to other factors that obscure the relationship between fish and mosquitoes, masking the actual impacts of fish. These factors relate to the inherent limitations of relying on eggshell densities to sample mosquito populations.
Firstly, eggshell data may not be capable of reflecting the impacts of fish populations. Whereas sampling of fish in pools (which were incorporated into mean biomass, sample yield, frequency of occurrence and predation vulnerability index) was undertaken at particular points in time, eggshell density data is a historical record of mosquito activity, which includes eggshells deposited over successive oviposition cycles and flooding events. It is difficult to correlate long term measurements of eggshell sampling with in situ measurements of fish populations, as the temporal disparity between the two measurements means that eggshell sampling cannot accurately identify short term or intermittent impacts on mosquito populations, such as the impacts of a highly heterogeneous fish population. Fish may be present during one oviposition cycle, preying on larvae and deterring oviposition, but be absent the next, allowing oviposition to resume uninterrupted. Eggshells cannot be differentiated over so short a time period (Ae. vigilax eggshells fade over time, from black/dark brown to yellow, however this process takes several years (Turner 2002)), so it is difficult to determine when particular eggs were laid, and consequently it is not possible to determine the nature of the fish populations at that particular time. Therefore any impacts fish did have on mosquito habitat suitability would be obscured in the long term historical record of oviposition.

Secondly, the link between eggshells and larval populations is not adequately established. High eggshell density is considered to positively correlate with higher larval populations, however only one study has attempted to quantify this, finding a correlation between higher eggshell density and larger larval populations (Addison et al 1992). This may be true in a general sense, but the actual relationship between eggshell density and larval populations may be far more complicated. Biological factors such as instalment hatching (where eggs of the same brood hatch over successive tides) (Kerridge 1971) and natural mortality of larvae can make larval populations completely unrepresentative of emerging adult populations that would deposit eggs (Abbott 1987; Barrera et al 2006). Therefore, mosquito larval populations in a pool, at a specific time may not be accurately represented with eggshell densities on adjacent soil.

For these reasons examining eggshell density may not be effective at determining the effects of resident fish populations on mosquitoes. Eggshell density is useful for identifying broad patterns across the mangrove basin (Griffin et al 2010; Knight et al 2012), or gaining a general picture of oviposition behaviour and distribution.
(Dale et al 1999). However it is not sufficiently precise or relatable to larval production to adequately describe the impacts of fish.

Incorporating larval sampling into this study would have avoided the limitations of eggshell sampling, by examining the effects of fish populations on a concurrent Ae. vigilax larval population. Sampling larvae simultaneously with fish sampling would have been an effective method of directly examining the effects of fish population on the mosquito life stage most significantly affected by the presence of fish. Simply understanding whether fish and mosquitoes are present in the same pool at the same time would represent a significant finding, and could demonstrate whether fish have the capacity to reduce mosquito production within mangrove pools.

However larval populations could not be examined in this study. Attempts were made to simultaneously sample Ae. vigilax and fish populations (see Chapter 6), however Unfavourable environmental conditions for mosquito populations meant that larval populations were greatly reduced throughout the study period, leaving eggshell sampling as the only option for analysing mosquito production. This particular limitation could not be addressed in the time frame of this study, however future research may be able to address this limitation, by sampling larval populations and fish populations simultaneously in mangrove basin forests when environmental conditions are more favourable.

In addition to sampling larvae, the effects of alternate prey need to be considered. As described in Chapter 5, the four fish species show strong generalist behaviour, and it is well known that the presence of alternate prey can significantly reduce the impact fish may have on mosquito populations (Bence 1988; Knight et al 2004; Manna et al 2008). The laboratory derived feeding rates incorporated into the CI did not take alternate prey into account, and so the larval consumption estimates used to create the predation index may be overestimates of the potential impact fish could have on immature Ae. vigilax populations. Proportions of mosquito larvae and alternate prey documented in Chapter 5 were not used in this research, as the low mosquito abundance likely resulted in dietary habits not representative of normal feeding activity or correlate with long term eggshell densities. Future research may be to increase the accuracy of estimates of the impacts of fish on mangrove mosquito populations, by sampling alternate prey present in mangrove pools simultaneously with larvae, and establishing the impact alternate prey have on larval consumption rates.
8.5 Conclusions

8.5.1 Summary and contribution to knowledge

This study aimed to combine the findings of the previous chapters, to determine the role fish play influencing *Ae. vigilax* production. Analyses failed to find a relationship between mosquito production (as evidenced by eggshell samples) and resident fish populations in mangrove basin pools. While fish may have the potential to reduce mosquito populations, populations in mangroves are too small and variable to have a long term impact on eggshell density. This may indicate that fish are not significantly influencing mosquito populations, however it may also indicate that eggshell sampling is not capable of detecting the impacts of a highly heterogeneous resident fish population. These findings address the fifth research aim of this study, describing the relationship between fish and mosquitoes in SEQ/NNSW mangrove basin forests.

8.5.2 Implications for mosquito control

The implications of this chapter for mosquito control are not entirely clear. If the lack of relationship between fish and mosquitoes is due to limitations of eggshell sampling, then the findings of this chapter reflect the need to improve our understanding of *Ae. vigilax* larval populations in mangroves. A better understanding of the size and distribution of larval populations may allow the biological control potential of fish to be described in greater detail. This could be addressed in future research.

However if fish populations are too heterogeneous to have a consistent impact on mosquito populations then it may suggest that fish based biological control is not currently feasible in mangrove basin forests. If so, then significant alterations of the fish populations may be necessary to make biological control a viable option. There may be a need to increase the stability of resident fish populations and increase fish-mosquito interactions, and this will be discussed in detail in the next chapter.
Chapter 9  Synthesis and conclusions

9.1 Introduction

The human health risks and quality of life issues associated with mosquitoes from mangrove forests may be mitigated through an integrated control strategy, which may incorporate resident larvivorous fish to control disease vectors in mangrove basin forests. Fish are capable of having a significant impact on mosquito populations, via predation (Morton et al 1988; Hurst et al 2004; Willems et al 2005) or oviposition deterrence (Petranka and Fakhoury 1991; Ritchie and Laidlawbell 1994; Angelon and Petranka 2002). However the assertion that fish are significant predators of mosquitoes in mangroves and that their presence can deter oviposition have not, until now, been tested. This gap in knowledge has implications for mosquito control in an Australian context, partly because of the impacts arising from the introduced G. holbrooki (an invasive exotic pest) and a general ignorance concerning the potential of native fish to be effective mosquito control agents in mangrove basins. The knowledge gap needs to be addressed in order to develop biological control options of mosquitoes in mangroves where few alternatives to costly and potentially environmentally harmful chemical-based treatments exist.

This study aimed to address this knowledge gap, examining the interactions between resident fish and saltwater disease vector mosquito populations in subtropical eastern Australian mangroves. In particular this study focussed on A. marina dominated basin forests of the SEQ/NNSW region, and the potential fish may have as biological control agents of the saltwater mosquito Ae. vigilax. The study addressed five research aims with results reported in individual chapters (Chapter 4 – 8). Chapter 4 investigated the composition and abundance of resident fish populations in mangrove basin forests, identifying three native fish but also the
dominance of the exotic *G. holbrooki*. Chapter 5 examined the composition of the diet of the resident fish identifying the potential for mosquito predation. Chapter 6 examined the predator-prey interaction between resident mangrove fish and *Ae. vigilax* larvae in laboratory experiments that demonstrated a native species was at least as efficient a predator of mosquito larvae as the exotic *G. holbrooki*. Chapter 7 examined the significance of resident fish in deterring gravid female mosquitoes from ovipositing. Chapter 8 integrated the two parts of the equation to examine the influence of fish populations on mosquito production.

In this final chapter a synthesis of the findings of the research is presented. The major findings of the study are discussed, with emphasis on the contributions these findings make to our understanding of mosquito-fish interactions in mangroves and the implications for biological control of mosquitoes. Future research and management pathways identified in the study are discussed to enhance mangrove resident fish-based biological control.

### 9.2 Major findings of thesis

The aims of the research, as set out above were to: 

1. identify the composition and abundance of resident fish populations in mangrove basin forests,  
2. describe the dietary habits of resident fish populations,  
3. examine predation by resident fish on larvae of *Ae. vigilax*,  
4. examine oviposition deterrence of *Ae. vigilax* by presence of resident fish,  
5. examine the impact of resident fish populations on mosquito production.

The extent to which these aims have been addressed by the research is discussed below.

1. **Composition and abundance of resident fish populations in mangrove basins**

Sampling of fish in mangrove basin pools found a small, highly heterogeneous (both spatially and temporally), resident fish population comprising at least three native species (*P. signifer, H. gali* and *Pseudogobius sp.*), but dominated by *G. holbrooki*, an exotic fish species native to the United States. This presents a major contribution to knowledge. The fact that fish were present in mangroves during peak mosquito seasons indicates that interactions between fish and mosquitoes could potentially contribute to mosquito control. However the dominance of *G.
holbrooki, a highly invasive exotic fish species also illustrated the disturbed nature of SEQ fish populations in mangroves, and the heterogeneity of the fish population may be problematic for mosquito control.

2 Dietary habits of resident fish populations

Analysis of dietary habits strongly indicated generalist behaviour in all four mangrove basin fish species, which may be advantageous for utilising a highly ephemeral food source such as mangrove mosquitoes. Diet comprised a wide variety of prey items including adults and larvae from several insect species, including larvae and pupae of Ae. vigilax and Cx sitiens. Relatively little predation of mosquito larvae was recorded, however this was likely due to the lack of Ae. vigilax larvae in the system, rather than a lack of interest in mosquitoes as a prey item. Despite this, this presents a major contribution to knowledge, as it demonstrates that resident fish feed on mosquito larvae and similar prey, and there could consume larvae at a much higher rate if more larvae were present in the system.

3 Predation by resident fish on larval Ae. vigilax.

A series of laboratory experiments described the predator-prey interaction between fish and larvae, comparing predation of 4th and 2nd instar Ae. vigilax larvae by the four fish species identified in Chapters 4 and 5. Experiments found significant predation of both larval stages by all four resident fish species. All species showed a similar feeding pattern, demonstrating a higher initial gorge rate, until reaching a satiation point, after which predation changed to a lower, but relatively constant, background feeding rate. Gambusia holbrooki and P. signifer showed the highest feeding rates in both 4th and 2nd instar experiments, and therefore showed the greatest potential for mosquito control. Pseudomugil signifer also demonstrated significantly higher predation in darkness than the other species, including G. holbrooki. This presents a major contribution to knowledge, as it identified native species that could be effective at controlling disease vector mosquitoes in mangrove basins, particularly. This suggests that mosquito control would still be viable with radical changes to mangrove basin resident fish populations (see section 9.4.3)
4 Oviposition deterrence of Ae. vigilax by presence of resident fish

A series of laboratory experiments described Ae. vigilax oviposition deterrence in the presence of resident mangrove fish species. Laboratory experiments of oviposition found that adult mosquitoes avoided depositing eggs on substrate saturated by water containing fish. Mosquitoes also largely avoided laying eggs on substrate saturated with water that previously contained fish, predominantly laying eggs on clean, isolated substrate. The three native species appeared to be effective at deterring oviposition. Although all species demonstrated significant impacts on oviposition, Pseudogobius sp. and G. holbrooki had the most acute effects on oviposition distribution and overall egg production. This presents a major contribution to knowledge, as it demonstrates that resident fish populations have a two-fold impact on Ae. vigilax (in addition to larval predation), and that native species can be effective at deterring oviposition as effectively as G. holbrooki.

5 Quantifying the impact of resident fish populations on mosquito production

Understanding the biological control potential of resident fish in mangroves ultimately rests on examining the relationship between fish and mosquito populations. Research presented in Chapter 8 focussed on this examining the influence of the size, stability and composition of fish populations in pools on immature Ae. vigilax habitat in mangrove basin pools. The findings of the study were synthesised into a predation vulnerability index, which was related to eggshell density, an indicator of mosquito production. This chapter failed to find any correlation between the presence of fish populations in pools and Ae. vigilax production, and this likely due to the high spatial and temporal heterogeneity of resident mangrove fish species, and the ineffectiveness of eggshell density to accurately observe the impacts of fish-mosquito interactions. This contributes to knowledge as it demonstrated that our understanding of immature Ae. vigilax populations in mangrove basins is not complete, and further research is necessary to understand the biological control potential of resident mangrove basin fish.
9.3 Synthesis

*Do resident fish control mosquitoes in mangrove basin forests?*

The results of the chapters of this study indicate that a significant interaction exists between fish and mosquitoes, and therefore biological control of disease vector mosquito populations is at least theoretically possible. Mangrove basin pools can support large populations of fish following tidal inundation, when larvae are developing in pools, and the numbers of resident fish are highest in summer and autumn, the period when mosquito populations are also at their peak. The resident fish population is comprised of species that can be larval predators in the mangrove ecosystem, and have the potential to significantly reduce mosquito populations by larval predation and oviposition deterrence.

However the evidence suggests that fish populations are simply not large or ubiquitous enough across the basin to put sufficient pressure on mosquito populations to reduce basin-wide *Ae. vigilax* populations. Fish present in a pool may have a significant impact on mosquito populations and larvae, however this impact is sporadic, semi-permanent and localised. The temporal variation means that fish may be present in a pool one month and provide biological control, but then be absent from the pool the next month, allowing mosquito populations to recolonise the site. The spatial variation of fish means that overall mosquito production is likely not significantly affected. As a result, while fish can reduce mosquitoes in the short term or in a small area, they are having little lasting or widespread impact on mosquito populations.

These conclusions agree with those of other studies conducted in similar, tidally isolated habitats. For example Harrington and Harrington (1982), examining impounded Florida saltmarshes found no relationship between fish activity and recorded declines in mosquito populations. The declines in mosquito population were linked to changes in hydrology rather than the interaction between fish and immature mosquitoes. In another example, Morton et al (1988), despite identifying significant predation of mosquitoes by fish in SEQ region saltmarsh pool, ultimately concluded that fish populations were too small and sporadic to reduce mosquito populations to a degree that facilitated biological control.
9.4 Future research and management implications

This study indicates that biological control by fish alone does not currently present a viable mosquito control method in mangrove basin forests. Using existing, native fish populations to control mosquito larval populations is not a form of mosquito control currently used in the SEQ region. In coastal wetland environments including mangroves, mosquito control primarily takes the form of chemical spraying or source reduction by habitat modification. Biological control using fish is limited to stocking of small, far less complex habitats, such as containers, ornamental pools and water tanks (Moffat et al 2005; Allaway et al 2012).

Despite biological control currently being unavailable in mangroves, resident fish should not be completely discounted as a useful part of an integrated mosquito control strategy. Biological control using resident fish populations would provide an ideal method of controlling mosquitoes in mangrove basin forests, being environmentally and economically low risk, especially in comparison to some forms of chemical control and habitat modification. Three major factors were identified that are responsible for limiting the impact of resident fish on mosquito populations, or limiting our understanding of the relationship between fish and mosquitoes. Addressing these issues in future research or management strategies may help improve fish-mosquito interactions in mangroves, and therefore may enable fish to contribute to integrated mosquito control programs in future.

9.4.1 Lack of mosquito populations during study period

One major issue throughout this study was the lack of observational evidence of fish directly influencing mosquito populations in field conditions. Unfortunately environmental factors (heavy rainfall and flooding) greatly reduced *Ae. vigilax* populations over the study period. This had an impact on several major parts of this study; dietary habits, interactions between fish and immature mosquitoes and the distribution of mosquitoes could not be directly examined in the field.

Alternatives were sought to substitute for direct field-based measurements, which were, for the most part, successful. Documented feeding on other prey organisms, especially those similar to mosquitoes, gave significant insight into the dietary behaviour of fish if mosquito larvae were present. Laboratory studies provided
strong evidence of predation of larvae and oviposition deterrence, which suggests that fish would disrupt mosquito populations if they were present in the field. In the absence of larval populations historical oviposition records were used to determine whether fish had an impact on mosquito production.

The lack of *Ae. vigilax* populations during the study period meant that the critical interaction between fish and mosquitoes in field conditions could not be examined in greater detail. This represented a major limitation of this study, and had implications for our understanding of fish-mosquito interactions. This was particularly problematic in the research presented in Chapter 8, as the intermittent impacts of the highly heterogeneous fish population were not observable in the historical, cumulative eggshell records. Research undertaken when *Ae. vigilax* populations recover could test the assumptions made in this study and allow a much greater understanding of the role fish may play in determining suitable oviposition and larval mosquito habitat in mangroves.

### 9.4.2 Variability of resident fish population

A second major issue identified in this study was the highly variable nature of fish populations observed throughout in this study. Fish populations showed a high degree of temporal and spatial variation, and it was not clear whether fish populations observed in this study are representative of normal mangrove fish populations. It is possible that the variation observed was due to the aforementioned environmental factors disrupting the two study sites. If accurate, this presents a significant technical issue that limits our understanding of resident fish populations. Unusual environmental conditions may have provided an inaccurate or incomplete picture of resident fish populations in mangrove basin forests. However, even if this is the case, the findings of this study are still valuable in their own right, as they still contribute to a complete understanding of mangrove basin forests.

The variation observed in this study may also be natural. This seems far more likely, as intertidal habitats, being a transitional habitat between terrestrial and estuarine systems, have a large degree of inherent variability (Knight et al 2009; Griffin et al 2010). Populations of organisms attempting to survive in these habitats are likely to demonstrate a similar degree of variability, and the few studies that
examine resident fish populations in comparable habitats show a similar degree of natural heterogeneity (Harrington and Harrington 1982; Morton et al. 1988). The relationship between the distribution of fish and environmental factors within mangrove forests, such as tidal hydrology and water quality, was outside of the scope of this study, however future research may be able to examine the role these factors play in influencing fish populations. At the time of this study the effects of tidal hydrology on larger, transient fish species are being examined in ongoing research (Lee et al., unpublished data), as are patterns of water quality in mangroves (Knight et al. 2013). Future research could similarly examine impacts of these factors on resident mangrove fish populations, investigating the movements of small, resident mangrove fish into and within mangrove forest basins, and the effects of water quality fluctuations on the survival of fish within these basins. This would provide further knowledge as to how fish populations in mangrove basins may be increased and stabilised, thereby allowing a greater and more consistent biological control.

Enhancing fish populations and increasing fish access to mangroves, in addition to improving mosquito control, would likely have additional positive environmental impacts and improve the ecological value of mangrove ecosystems. Given the value placed on mangroves as a refuge and feeding habitat for small and juvenile fish species, increasing the capacity of mangrove basins to support small fish species may significantly improve not only mangrove health, but also improve that of the adjacent estuary. This may also provide significant economic benefits, as improved access and conditions within mangroves may allow other species, including some of economic importance, to utilise the mangrove basin environment.

To this end, improving hydrological connectivity within the mangrove basin may be the key to improving fish populations in mangroves. The modification project being undertaken at the Terranora site (as described in 3.1) may be an example of this. Improving hydrological connectivity, in addition to disrupting mosquito life cycles directly, may have the secondary benefit of improving conditions for fish. More frequent tidal flushing may allow fish to more easily move into previously isolated mangrove pools, and more regular tidal flushing may also improve water quality conditions within the mangrove basin, thus increasing fish survival and potentially allowing a wider variety of fish to use mangrove basins. This has been observed following improvements to hydrology to impounded mangroves in Florida, which bear similarities to SEQ mangrove basins; improved tidal connections into impoundments resulting in drastic increases in fish populations, and allowed
mangroves to support larger, commercially important species (Brockmeyer et al 1997).

9.4.3 Dominance of G. holbrooki

The third major issue identified in this study was the dominance of the exotic G. holbrooki at both sites. The dominance of G. holbrooki, a significant exotic pest, has clearly caused major ecological disturbance in SEQ/NNSW region mangroves. While other fish species are present, the presence of a hardy, fast breeding and highly aggressive exotic species (Howe et al 1997) prevents native species from establishing in significant numbers within basin pools.

Official use of G. holbrooki as a mosquito control agent would be forbidden in Australia, due to its status as a major exotic pest. The Queensland Fisheries Act 1994 and New South Wales Fisheries Management Act 1994 both declare G. holbrooki as a noxious fish species and restrict the handling and possession of G. holbrooki. Therefore it would be impossible to use G. holbrooki dominated resident mangrove fish populations in any official mosquito control at either of the two sites examined in this study. Successful biological control using resident mangrove fish is therefore directly linked to a need to address the ecological health of resident fish populations. Fish populations not only need to be significantly improved in terms of size and stability, but the dominance of exotic G. holbrooki needs to be addressed, by exploring methods of eradicating G. holbrooki and encouraging native species to move into mangroves.

How to address G. holbrooki dominance is not clear. Official management of G. holbrooki in Australia is largely based around preventing the spread of individuals into unaffected ecosystems (New South Wales Government 2003; Queensland Government 2011). Gambusia holbrooki is notoriously hard to eradicate; nowhere in Australia has an entrenched G. holbrooki fish population been successfully eradicated from a large, complex, ecologically sensitive ecosystem such as subtropical mangrove basin forests without significant environmental disruption. For example physical removal and application of chemicals have been successful in eradicating G. holbrooki populations from small, ephemeral freshwater spring wetlands in South Australia; however without considerable increases in effort it was unlikely to be effective in larger, more complex environments (Kerezsy 2009).
Radical changes to hydrology have also ameliorated *G. holbrooki* infestations, by selectively draining wetland pools at specific times to coincide with critical *G. holbrooki* life stages (O’Meara and Darcovich 2008). This would also not be effective in SEQ mangroves, due to the high level of disturbance and risks involved in exposing acid sulfate soils. The difficulties of controlling *G. holbrooki* in mangroves are also compounded by the relatively high structural complexity within the mangrove basin, and the high connectivity with other estuarine and intertidal habitats which, in all likelihood, also contain large *G. holbrooki* populations. Eradicating *G. holbrooki* in mangrove forests would involve management not only on a basin-wide scale, but on an estuary or catchment scale. This would be a massive undertaking, but ultimately a worthwhile one, especially given the potential ecological and human health benefits that would result from its eradication.

However it also needs to be considered that in terms of mosquito control reducing *G. holbrooki* populations is only part of the picture. The ecological benefits of removing *G. holbrooki* need to be balanced with the human health benefits of mosquito control, ensuring that both ecological and human health values are met. Reductions of *G. holbrooki* need to be done without sacrificing the mosquito control capability of resident fish populations. Reducing *G. holbrooki* populations to improve the ecological value of mangroves may result in fewer fish in the system, thereby diminishing any existing biological control. Conversely, improving fish habitat and enhancing fish populations to increase mosquito control without addressing the *G. holbrooki* issue may simply increase the dominance of *G. holbrooki* in the system. This not only fails to address the ecological issues within mangrove systems, but may also pose a significant threat to the estuary, as it risks mangroves becoming a reservoir for *G. holbrooki*.

In order to satisfy both ecological and human health requirements in mangroves, efforts to reduce *G. holbrooki* populations in mangroves needs to work closely with mosquito control. This represents a major future research and/or management pathway, investigating ways to reduce exotic populations while encouraging native species to move into mangroves, especially those that show potential as mosquito control agents. This requires increased understandings of how fish move into mangroves, the factors that influence fish survival in mangroves and knowledge of the specific species that may be able to take the place of *G. holbrooki*, without sacrificing, and preferably enhancing, mosquito control. The findings of this study
may be essential for guiding attempts to accomplish this, as native species were identified in this study that have comparable mosquito control potential to *G. holbrooki*. *Pseudomugil signifer* in particular shows promise as a replacement for the ecological niche occupied by *G. holbrooki*, and has a proven track record as an effective biological mosquito control agent. Future research and management may be able to build on this and address the requirements detailed above.

### 9.4.4 Summary of future research pathways

In addition to these major issues, numerous future research and management recommendations were made throughout this study. Addressing these recommendations may further enhance our understanding of fish-mosquito interactions in mangrove basin forests, and therefore contribute significantly to the viability of biological control in future. These recommendations are summarised in table 9.1.
Table 9.1 Future research and management pathways identified in this study

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Future research pathways</th>
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<tbody>
<tr>
<td>4</td>
<td>Explore factors such as tidal connectivity, water quality and fish ecology that may influence resident mangrove fish heterogeneity. Examine resident mangrove fish movement into and within the mangrove basin during tidal inundation.</td>
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<tr>
<td>5</td>
<td>Examine dietary habits of resident mangrove fish when mosquitoes are abundant. Examine the abundance and nutritional value of alternate prey for resident species, and investigate why certain prey items would be preferable to <em>Ae. vigilax</em> larvae.</td>
</tr>
<tr>
<td>6</td>
<td>Examine predation of <em>Ae. vigilax</em> larvae by resident mangrove fish while incorporating ecological factors such as water quality, habitat structure and alternate prey. Examine the behavioural responses of <em>Ae. vigilax</em> to the presence of predatory fish. Identify behavioural or morphological characteristics of resident mangrove fish species, in particular <em>P. signifer</em>, that enable it to be an effective predator of <em>Ae. vigilax</em> larvae.</td>
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<tr>
<td>7</td>
<td>Examine <em>Ae. vigilax</em> oviposition deterrence by resident mangrove fish in field or mesocosm trials. Identify the specific chemical compounds that deter <em>Ae. vigilax</em> oviposition. Distinguish between airborne and waterborne chemical oviposition deterrence. Examine the behavioural/biological responses of mosquitoes behind of fish mediated oviposition deterrence.</td>
</tr>
<tr>
<td>8</td>
<td>Compare <em>Ae. vigilax</em> larval and resident mangrove fish abundance and distribution when <em>Ae. vigilax</em> larvae are abundant in mangrove basin forests. Quantify the relationship between <em>Ae. vigilax</em> oviposition and larval production.</td>
</tr>
<tr>
<td>9</td>
<td>Explore methods of enhancing the size and stability of resident fish populations. Examine the effects of a larger, more stable resident fish population on mosquito production. Explore methods of encouraging native species to move into mangroves, particularly those that can provide biological control of <em>Ae. vigilax</em>.</td>
</tr>
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</table>
9.5 **Summary and concluding remarks**

This study aimed to investigate the relationship between resident fish and disease vector mosquitoes in sub-tropical, eastern Australian mangrove, with particular focus on *A. marina* dominated basin forests of the SEQ/NNSW region, in order to better inform biological control. Mangroves can support significant populations of fish, which can strongly impact mosquitoes via predation of larvae and deterrence of oviposition, however resident fish populations are simply too heterogeneous across the mangrove to be currently contributing towards mosquito control.

This study has filled three significant knowledge gaps: (1) investigated fish populations within mangrove basin forests, (2) described the mosquito-fish interaction between resident mangrove fish and *Ae. vigilax*, and (3) investigated the influence of fish on mosquito production. The findings of this study have contributed significantly to our understanding of mosquito populations in mangroves, and may be able to guide future mosquito control.

The fish species identified in this study showed significant potential for biological mosquito control, demonstrating significant impacts on larvae and oviposition. Therefore biological control, while not currently viable, may be viable in future, and this may be accomplished through an integrated mosquito control strategy that makes mangroves a more attractive native fish habitat by improving water quality and improving connectivity into and within mangroves. Larger, more stable fish populations may allow increased predation of larvae and deterrence of oviposition, and thus increased pressure on mosquito populations. Additionally, the dominance of *G. holbrooki*, a significant noxious fish species, needs to be addressed, and efforts to remove this species from mangroves may allow both mosquito control and ecological values in mangroves to be addressed. Fig 9.1 shows the research space and major outputs (major findings and future research directions) of this thesis.

As fish based biological control is possible, it should be pursued as part of an integrated mosquito control program in mangroves. In the long term it could be considerably more environmentally and economically sustainable than other methods. Incorporating fish populations into mangrove modification and restoration projects is key to this, and would greatly enhance the ecological value of mangrove forests and greatly contribute towards the health of adjacent estuarine ecosystems, as well as address a major human health issue.
Fig 9.1 Research space, findings and major future research/management pathways derived from this thesis


Breitfuss, M., 2003. The effects of physical habitat modifications for mosquito control, runnelling, on selected non-target saltmarsh resources. PhD, Griffith University.


Knight, J. M., 2008. Characterising the biophysical properties of a mangrove forest to inform mosquito control PhD, University of Queensland.


Samoa. Transactions of the Royal Society of Tropical Medicine and Hygiene 75(3):426-431.


## Appendix A  Climate data

Table A.1  Terranora (Murwillambah, NSW) monthly rainfall and temperature data (source: Bureau of Meteorology).

<table>
<thead>
<tr>
<th></th>
<th>Total rainfall (mm)</th>
<th>Historical mean total rainfall (mm)</th>
<th>Mean max. temperature (°C)</th>
<th>Historical mean max. temperature (°C)</th>
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Table A.2 Coombabah (Coombabah Wastewater Treatment Plant, Helensvale, QLD) monthly rainfall and temperature data (source: Bureau of Meteorology).

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### Appendix B  Sampling calendar

Table B.1 Sampling dates at the Terranora study site. Green dates represent sampling where fish were caught and red dates represent when no fish were caught.

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Table B.2  Sampling dates at the Coombabah study site. Green dates represent sampling where fish were caught and red dates represent when no fish were caught.

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Appendix C  Mosquito colony description

Colony cage description - The colony was housed in two 46x46x46cm cages. One cage was constructed of 20x20 strands per inch\(^2\) aluminium mesh, and the other of 32 x 32 strands per inch\(^2\) lumite screening on three sides and a clear vinyl panel on the fourth. A successful mosquito colony requires ~ 1.8cm\(^2\) vertical resting space per adult mosquito (Gerberg 1970), which meant that the two cages were able to house ~4500 mosquito adults each. The cages were positioned under 100W heat lamps, which provided light and heat on a 14:10hr day:night cycle. Adults were fed a 10% sucrose solution, delivered via cotton balls and laid across the top of the cage.

Mosquito colony maintenance - Three days after emergence, adults were offered a blood meal; a human arm placed inside the cage for 30 minutes. *Ae. vigilax* has demonstrated autogenic abilities (Hugo et al 2003), and so blood feedings were not essential, but were used occasionally, especially at the early stages of establishing the colony to bolster numbers. Blood feeds were kept to a minimum, to prevent development of blood antigens affecting fecundity of adults that as has been observed in other mosquito species (Sutherlaand and Ewen 1974). As a result a blood meal from the one arm was offered only 6 times throughout the study, and no blood was provided to adults used in any oviposition experiments (see Chapter 7).

A medium for oviposition was supplied to each cage in the form of cotton pads. A single 20x10x3cm cotton oviposition pad was placed plastic container, completely saturated with brackish water, and placed inside the cage. The pad was left in the cage for 1 week, after which the pad was removed and checked for eggs. If eggs had been laid the pad was left to dry in a sealed plastic bag at room temperature for another week, after which eggs on the oviposition pad were hatched via the method described in Chapter 6 (6.2.1).