The behaviour of settling coral reef fishes and supplementary management tools

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Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text.

This work has not been submitted for any other degree or professional qualification except as specified.

Adel Heenan, 11th May 2010.

Abstract

Coral reef fish larvae take an active role in selecting their settlement site and sensory cues may help them to orientate during this process. As settlement is a period of transition through which the majority of individuals do not survive, it is often a focal point for the management of coral reef populations, which are of high conservation concern. In this thesis, I used choice tests and *in situ* techniques to assess the response of settlement-stage larvae to a range of odour, light and acoustic cues and I found that larvae are more selective in their response to sensory stimuli than previously thought. Micro-habitat odours are not likely to be used during settlement orientation, and odour cues may be used to avoid inappropriate settlement sites. The photopositive behaviour of larval fish is likely to match their spectral sensitivity but this proved difficult to assess *in situ* because of the high amount of spatial and temporal variation in larval distribution. The positive response of settlement-stage fish to played back reef noise is location specific as well as being highly specific to the reef sound recording. To understand whether it might be the composition of reef sound that drives the selective response of larvae to acoustic cues, I took sound recordings while collecting visual data on fish diversity and the behavioural activity of a sound producing, or soniferous, fish species. I found that the variation in intensity of reef noise matches the activity patterns of a soniferous species, and when reef noise is most intense is when visual estimates on the diversity of the reef fish assemblage are decreased. This information provides the basis for understanding how changes in the reef soundscape may effect larval recruitment and has exciting implications for using sound recordings as a method to monitor coral reefs. Finally, I tested the viability of releasing reared larvae to boost depleted populations and found that collecting and holding settlement-stage fish for a week can increase survival, relative to natural settlement. These data demonstrate that applying our knowledge of the settlement behaviour of coral reef fish will make a significant contribution to developing tools for management.

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To Tom Heenan for teaching me how to swim

and giving me a mask and snorkel

Publications

The following published papers have arisen from this thesis and they are included with permission from the relevant publishers in Appendix A.

- Heenan A., Simpson S. & Braithwaite V. (2008). Testing the generality of acoustic cue use at settlement in larval coral reef fish *Proceedings of the 11th International Coral Reef Symposium* **16** 554–558.
- Heenan A., Simpson. S.D. Meekan M., Healy S. and Braithwaite V. (2009) Restoring coral reef fish populations through recruitment enhancement: a proof of concept *Journal of Fish Biology* **75** 1857–1867.

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1. Introduction

For coral reef fish, like most marine organisms, settlement is a part of their complex life cycle. Their life history is described as complex because it involves two distinct phases, the pelagic (open water) larval phase and the benthic (associated with the substrate) juvenile and adult phase. Settlement is the transition between these two phases and it occurs when larvae leave the pelagic waters and join the reef community (Leis & McCormick 2002). With few exceptions, such as the spiny puller, *Acanthochromis polyacanthus*, and the bangaii cardinalfish *Pterapogon kauderni*, the majority of coral reef fish species have a pelagic larval stage, the duration of which can vary from a few days to a few months. There may be multiple benefits in spending time away from the reef during early development: in the open water there is less competition for resources, less exposure to predation and there is also the potential for dispersal (Bonhomme & Planes 2000, Doherty *et al.* 1985, Johannes 1978, Leis & Carson-Ewart 1998). Settlement, however, signifies the end of the potentially dispersive pelagic phase and is essential for the recruitment of new individuals to the adult population (Leis & McCormick 2002, Caley *et al.* 1996). As such, settlement is intrinsically linked to the replenishment of marine populations and because of this it is a subject of intense interest in coral reef studies.

Much of the interest in settlement has focussed on what causes larvae to settle in a particular location not least because the distance between the settlement site and the reef where larvae originated can vary massively. The extent of larval dispersal ranges from the exchange of individuals among populations i.e. open populations over large distances (hundreds to thousands of kilometers: Doherty *et al.* 1985, Shulman 1998) to the recruitment of individuals to their natal reef, i.e. closed populations (Jones *et al.* 2009, 1999, Almany *et al.* 2007, Swearer *et al.* 1999). Whether coral reef populations are open or closed or whether larvae play an active part in the settlement process were once heavily debated but as it is now clear that dispersal varies from open to closed populations, it has

been recognised that both biological (active) and physical (passive) factors will determine where settlement actually takes place (Jones *et al.* 2009).

Of these bio-physical processes, the importance of physical factors, such as the transport of larvae by tides and water currents, diminishes as larval development and the pelagic period progresses (Armsworth 2000, Armsworth *et al.* 2001, Leis 2006). Larvae may start out as plankton i.e. being small and insignificant swimmers that are moved around by hydrographic processes, but by the time larvae are ready to settle they are able to move and orientate independently of the ambient water current (Leis 2006). As a result, attention has moved to how larval behaviour, specifically their swimming and orientation abilities might influence settlement.

Larval behaviour has been studied using laboratory and *in situ* techniques and in both cases observational and manipulative approaches have been taken (e.g. Lecchini *et al.* 2005*b*). What has emerged from these studies is the view that larvae are active, settlement-site selectors (Leis 2006). This is apparent from the evidence that larvae can detect the reef using reef-based cues, will alter their swimming direction in relation to the reef, and can do this in spite of the flow of the surrounding tidal regimes and water currents (Stobutzki & Bellwood 1998, Leis *et al.* 1996, 2003).

For a larval fish to orientate independently of the water current, it needs to be able to swim faster than the speed of the water current. By making fish swim against a counter-current in swim chambers, it is clear that larvae can swim faster than the water current for days on end (Stobutzki & Bellwood 1994, 1997, Stobutzki 1998). The ability to swim faster than the typical rate of the ambient current speed (e.g. 13.5 cm s^{-1} at Lizard Island; Frith *et al.* 1986) is common in coral reef fish larvae (95% of the 89 species that have been tested so far: Fisher 2005). But the extent to which larvae can choose where they go and where and when they settle will depend on their swimming speed and endurance. There is considerable variation in the swimming speed of different families, ranging from the fastest, the Holocentridae (mean speed of 75 cm s^{-1}) to the slowest, the Apogonidae (mean speed of 20 cm s^{-1}; Fisher 2005). The sustained swimming ability of larvae also varies quite substantially among species within the same family, for example, *Dischistodus prosopotaenia* can cover a mean distance of 7

km before exhaustion, compared to *Abudefduf vagienesis*, which also belongs to the Pomacentridae family, and can cover 55 km (Stobutzki 1998). Species whose larvae can out swim the local water currents will have the potential to control their movement during the dispersive pelagic phase and where they settle (Fisher 2005).

That settlement-stage larvae can control where they go comes from *in situ* observations on the swimming behaviour of fish. Releasing larvae into the open water and estimating their swimming speed has confirmed that in open water larvae can maintain their swimming speeds measured under laboratory conditions (Leis & Carson-Ewart 1997). If anything, the speed at which larvae swim in swim chambers might underestimate their swimming ability because often in open water larvae were recorded as swimming even faster (Leis & Carson-Ewart 1997). Indeed, it appears that larvae can remotely sense the reef and alter their swimming direction in relation to it. During the day when larval apogonids, pomacentrids and chaetodontids are released into pelagic water 1 km from the nearest reef, these larvae swim away from the reef (Leis *et al.* 1996), and do so irrespective of the distance (between 100-1000 m) or bearing they are released relative to the location of the reef (Leis *et al.* 2003). This avoidance behaviour appears to be time of day dependent, as at night the larvae appear to orientate towards the reef. As it is not practical to follow fish at night in open water, the nocturnal orientation of larval apogonids and pomacentrids has been investigated using binary choice chambers placed perpendicular to a reef 30 m away. Regardless of the bearing of the choice chamber in relation to the reef, larvae swam in the direction of the reef. From these data it was evident that the orientation of larvae is likely to be facilitated by reef-based cues. Of the potential cues that might emanate from a reef (e.g. wind and wave induced turbulence, gradients in temperature, fish, reef detritus and plankton abundance: Leis & McCormick 2002), those that are experimentally proven to be relevant in larval orientation are acoustic and odour cues.

Evidence that larval fish may use reef-based acoustic and odour cues to move towards or away from a reef come from three different experimental approaches. The first is electrophysiological evidence that fish can hear and detect odours at this point in their development (e.g. the coral trout *Plectropomus leopardus*: Wright *et al.* 2008, and the speckled damselfish *Pomacentrus nagasakiensis*:

Wright *et al.* 2005). The second approach involves laboratory and *in situ* experiments in which fish are offered a choice between specific sounds and odours and have shown that larvae will modify their swimming behaviour in response to sensory stimuli. The response of larval fish to sound cues in the field has also been tested using light traps to collect phototactic larvae as they approach the reef. The pairing of light traps, with underwater speakers appears to increase the number of larval fish caught by such traps compared to when traps are presented without the sound speaker playing reef noise (Tolimieri *et al.* 2000). This method has the advantage of testing the response of larvae to sensory cues in their natural environment, but it is the third approach that has provided the most convincing evidence that larvae can use reef-based odours and sounds to locate a settlement-site. These experiments have used small experimental patch reefs and compared and found higher rates of natural settlement on the patches that were supplied with either water conditioned with specific odours, or speakers broadcasting a recording of reef noise (odour: Sweatman 1988, sound: Simpson *et al.* 2005).

Both acoustic and odour cues can be used by larvae to detect a reef. In contrast to reef odour, which is current-dependent so larvae have to be downstream of the reef to sense the scents, reef noise is an omni-directional cue indicating the location of a reef, irrespective of the position of a fish with respect to that reef. The distance over which a larval fish may detect the sound of a reef will, in part, depend on how sensitive its hearing is. In four coral reef fish species tested over a range of frequencies (100-1200 Hz) the hearing sensitivity of the larval fish tends to increase as frequency decreases (Wright *et al.* 2005, 2008, 2010). Using these data, Wright *et al.* (2008) estimated that coral trout *Plectropomus leopardus* larvae should be able to detect low frequency sounds from over 4 km. Estimates of hearing thresholds of fish at settlement need to be coupled with measures of how loud reef noise is and how that noise attenuates with distance (Wright *et al.* 2010). Concerns that human-related activities might alter the soundscape around coral reefs, and in turn the ability of larvae to detect reef noise stem from data that show boat traffic noise can mask sound communication between adult fish (Codarin *et al.* 2009, Vasconcelos *et al.* 2007). It remains to be seen whether larval orientation using reef noise might be affected by the 3-10 dB increase in low frequency noise caused by shipping activity and traffic that has occurred in the ocean (Andrew *et al.* 2002). Anthropogenic impacts on the soundscape and

larval orientation using acoustic cues will be difficult to test before it has been established how larvae are affected by the natural changes in reef noise, and how reef noise may change in intensity over time.

What is clear is that settlement-stage larvae will swim towards reef noise (Leis & Carson-Ewart 2002). This is evident from the attraction that larvae have for reef recording playback and their ability to resolve the direction of the sound source (Leis & Lockett 2005, Tolimieri *et al.* 2004). The phonotactic response of larvae may be more specific than just to 'reef noise'. For example, *Chromis atripectoralis* have a different directional swimming response when exposed to played back reef noise compared to a played back artificial pure tone sound (Leis & Carson-Ewart 2002). The general attraction of larval fish to reef noise, that is evident from the higher catch rate of larvae in light traps broadcasting reef noise (Leis *et al.* 2003, Simpson *et al.* 2004, Tolimieri *et al.* 2000), may also be specific to particular features of the reef sound that is broadcast. For example, more settlement-stage fish were caught in light traps coupled with a playback containing the higher frequency components of a reef recording (the sounds made predominately by invertebrates) than were caught in traps coupled with a playback of the original, unfiltered recording or with the filtered, lower frequency sounds (Simpson *et al.* 2008). In all cases, however, the catch of larval fish in light traps broadcasting reef noise, filtered or unfiltered, was greater than it was in silent light traps. While settlement-stage fish may show a stronger attraction to particular components of reef noise, however, little else is known about how larvae may use acoustic-based cues during the settlement process. For example, it is not known whether larvae can detect specific information about a reef from acoustic cues, such as the resident reef community, nor is it known whether larvae may find aspects of reef noise unattractive, causing them to orientate away from a reef site.

In contrast to reef sound more is known about the type of odour cues to which a settlement-stage larvae may orientate. For example, unlike reef sound, the different components of biochemical stimuli present in the environment that are known to elicit a sensory response in adult fish have been identified: (1) amino acids; (2) steroid hormones; (3) bile salts; and (4) prostaglandins (Hara 1994). A mixture of these chemical cues will create the odour landscape around a reef and larvae are likely to be able to detect these odours because even though their

nasal epithelium may not be fully developed, it is evident that at settlement larvae have a functioning sense of smell (Lara 2008). The odour landscape that larvae will encounter will change with distance from the source, as, for example, photosynthetic components of the odour plume might start to break down and chemical cues will be increasingly mixed about with the ambient water currents (Atema 1996). It has been suggested that if settlement-stage fish can orientate with respect to the water flow (rheotaxis) using their lateral line system, and also to chemical cues (chemotaxis) using their olfactory senses, then they could sample the layers of water within the water column and follow a reef odour plume, or concentration gradient, to navigate to the reef source (Myrberg & Fuiman 2002, Atema 1996, Lara 2008).

Larval fish do appear to be able to orientate using odour cues at a range of spatial scales. Starting at the broadest level, larvae can discriminate between the different odour types present in flowing water and prefer the odour of water from a reef lagoon over that of pelagic water (Atema *et al.* 2002). The spangled emperor *Letherinus nebulosus* and post-larval french grunts *Haemulon flavolineatum*, which both recruit to seagrass beds and mangroves as their nursery ground before moving to the reef as juveniles, both prefer the odour of their nursery habitats, over the odour of coral reefs (Arvedlund & Takemura 2006, Huijbers *et al.* 2008). Such a preference for a specific habitat odour might then enable a fish to locate and swim towards a preferred settlement-site. There is also the potential that settlement-stage larvae use the odour of the reef from which they were spawned to return to their natal reef (Gerlach *et al.* 2007). Once the reef has been located larvae could then use more specific, local odours to find a settlement site. While larvae will swim towards the odour of their conspecifics and the odour of their preferred settlement substrate (Sweatman 1988, Lecchini *et al.* 2005*a*, Elliott *et al.* 1995, Dixson *et al.* 2008, Munday *et al.* 2009), it has yet to be established whether larvae reacting to broad-scale odour cues, like the odour of a reef, are responding to reef odour as a whole, or to the specific presence of particular odour types that could indicate a settlement-site on a reef.

Larval fish clearly have the potential to use reef-based cues, however specific, to control where they will settle. It is of considerable interest to both scientists and coral reef managers to understand how larval behaviour affects settlement processes, because settlement precedes recruitment, which is key to adult fish

populations. There are several aspects of larval settlement behaviour that are of relevance to the management of coral reef fish populations. But first, the reason management is necessary is because worldwide, reef ecosystems are under threat (Gardner *et al.* 2003, Bellwood *et al.* 2004, Hughes *et al.* 2003, Carpenter *et al.* 2008), due to an assortment of factors that can be divided into those that operate globally and locally. Global threats include ocean acidification, and climate-change induced coral bleaching and sea-level rise, but despite their local consequences, such as reduced coral-based services and a subsequent loss of livelihoods, local management efforts will not be able to address these global issues (Mumby & Steneck 2008). In contrast, local threats, such as pollution, habitat destruction, and chronic and destructive fishing, can be managed.

Coral reef managers aim to protect the ecosystem goods and services of reef systems, including coral reef fish populations, for their sustainable use by humans (Moberg & Folke 1999) and larval settlement orientation may be relevant to the management of the local threats coral reef fishes may face. For example, the optimal design and location of marine reserves, which restrict human access to reef resources, requires an understanding of how larval orientation may contribute to the connectivity of populations (Jones *et al.* 2009). Although marine reserves are the most widely implemented management initiative (Mumby & Steneck 2008), the sustainable use of reef resources will not be achieved by restricting access alone (Bellwood *et al.* 2004). Additional or supplementary management tools offer alternate solutions to restricting human access to resources, often by creating methods to enhance or diversify the livelihoods of people reliant on the reef. One such method is the implementation of early life history phase fisheries, or post-larval capture for culture (PCC) (Bell *et al.* 2009, Dufour 2002, Lecchini *et al.* 2006, Lourie & Lecaillon 2005, Ziemann 2001). This involves catching fishes when they are in the late-larval stage. By doing so, fish are extracted before they go through the high amount of natural mortality that is typical of settlement. There is an extreme mismatch between the number of larvae that are spawned, which can be in the order of 1000s, and the number of fish that successfully recruit to the reef (Leis 1991). Of those larvae that make it through the pelagic phase, an estimated 56-76% are lost to predation during their first few days on the reef (Doherty *et al.* 2004, Almany & Webster 2004, Planes & Lecaillon 2001). In PCC programmes, fish are caught while they are

still larvae, before they have gone through this natural population bottleneck. The target species can, therefore, still be collected but with a reduced impact on the adult population. This can reduce the fishing pressure from individuals that have successfully recruited to the reef, leaving them to reproduce for future generations (Bell *et al.* 2009). For the marine aquarium trade, collection of the larval stage provides an alternative method to the more damaging techniques that are used to capture fish, such as cyanide fishing (Bell *et al.* 2009, Lourie & Lecaillon 2005, Dufour 2002). The main cause of mortality in fish that are collected for the marine aquarium trade, however, is not from capture itself but rather due to the failure of captured fish to feed on artificial aquarium food. If fish are captured before or during metamorphosis, as they are in PCC, then larvae have the morphological and physiological ability to adapt to a new diet. Therefore, an additional benefit of PCC is that larvae may be transferred to an aquarium diet with much greater success than adults (Lecchini *et al.* 2006).

In some cases, larvae that are captured for PCC are released back onto the reef after being held for a period in captivity (Dufour 2002). This occurs when too many target fish are caught or when non-ornamental species are caught as by-catch. However, while returning captured individuals to the reef may lead to a smaller negative impact on fish biomass on the reef than if they were not returned, it is not yet clear how well those released fish survive (Dufour 2002, Bell *et al.* 2009).

The methods used to collect settlement-stage fish to study the biology and behaviour of this life-history stage are also used to collect larvae for post-larval capture for culture programmes (Dufour 2002), namely light traps, crest nets, towed and purse seine nets. Light traps and crest nets have the advantage of capturing fish in better condition as larvae tend not to get damaged, which can happen when caught in towed nets (Leis & McCormick 2002). Crest nets have an advantage over light traps, in that they can be used to collect all of the species of reef fish that come over the reef crest and into the lagoon. Crest nets, however, are restricted in that they have to be placed on the reef crest, where waves break over the reef barrier. This limits their use to certain parts of the world, like French Polynesia, where the reef crest is easily accessible and the tides are weak (Dufour & Galzin 1993). Light traps, on the other hand are used much more widely, but they do only collect reef fishes that are phototactic at the

larval-stage. Of the reef fish caught in light traps, 99% are pomacentrids (Hair *et al.* 2002). Despite pomacentrids contributing to almost 50% of the global trade in ornamental species of fish (Wabnitz *et al.* 2003), they are viewed as fish of low economic value. Only 5% of light trap caught fish are valued ornamental fish species (Bell *et al.* 2009), therefore, methods to diversify and augment the catches of light traps would be beneficial.

1.1. Thesis aims

In this thesis, I aimed to investigate the sensory cues that fish orientate with during settlement and assess how understanding the way larvae perceive and response to the sensory environment around a reef can be applied in the design of supplementary management tools for coral reef fishes. I pose the following questions:

1. **Is the attraction of late-stage larvae to reef odour an indiscriminate response that allows fish to orientate to any reef, or can larvae use odour cues to ensure that they approach a suitable settlement-site?**

   I predicted that larval apogonids would be attracted to the odour of water conditioned with a broad range of reef associated fish and invertebrate species. This prediction was based on the evidence that previously these apogonids have been shown to prefer water collected from the reef lagoon compared to pelagic water (e.g. Atema et al. 2002). It is not clear what it is specifically within broad odour types that settlement-stage fish may respond to; however, as species with specialized settlement requirements, such as the anemonefish, *Amphiprion percula* are attracted to the odour of their settlement substrate (e.g. Elliot *et al.* 1996), I predicted that larval apogonids would be attracted to the odour of water conditioned with the microhabitat to which they usually settle, coral rubble (Finn & Kingsford 1996).

2. **Can the collection of fish in light traps be augmented by using different coloured light, rather than the white-light that is typically used?**

   The light traps that collect settlement-stage fish only attract fish that orientate towards broad-spectrum white light (Doherty 1987, Choat et al. 1993). I tested whether the catch of larval fish in light traps can be modified by emitting light of different wavelengths. I predicted that light

traps emitting light that matched the spectral sensitivity of larval fish would catch more of those species (Job and Shand 2001). This prediction was based on the evidence that the more intense the light, the stronger the phototactic response of larval fish (e.g. Blaxter 1969), and therefore it seems plausible that should light traps emit more light that falls within the spectral sensitivity of larvae, then fish may exhibit a stronger orientation response towards these traps.

3. **Can the attraction of settlement-stage larvae to reef noise be used to bolster the collection of fish for post-larval capture for culture?**

   The attraction of larval fish to reef noise is well established (Tolimieri et al. 2000, Leis et al. 2003, Simpson et al. 2004, 2005, 2008). I investigated whether the addition of an underwater sound system to broadcast reef noise from the light traps that a post-larval capture for culture company were using could increase the abundance or diversify the catch of larval fish. I predicted that if larvae have a general attraction to the sound of a reef, then playing reef noise alongside light traps would augment the capture of larval fish, as in some cases this had led to a 70% increase in light traps catches (Leis *et al.* 2003, Simpson *et al.* 2004).

4. **How does reef noise vary in time, and does reef noise convey any information about the reef?**

   Reef fish may be attracted to reef noise at settlement, but it is not known whether larvae are attracted to the sound of a reef as a whole, or whether there have to be specific sounds present for them to be phonotactic. I predicted that the sound of the reef in two different locations would follow the same temporal patterns of a dusk and dusk peak in intensity that have been demonstrated on coral reefs previously (e.g. Cato 1978). To establish whether the sounds fish produce may make a significant contribution to reef noise, I collected underwater visual

census data whilst recording the reef noise. Using this data, I could assess the relationship between the noise of a reef and the temporal diversity and abundance of the fish assemblage, and consider the contribution that fish associated sounds make to the reef soundscape in relation to the known sensitivities of larval hearing (e.g. Wright *et al.* 2005).

5. **If settlement-stage fish that are collected but not required for post-larval capture for culture are then released back onto the reef, is this an effective method of increasing a locally depleted fish population?**

   Releasing fish onto the reef, after catching them at the settlement-stage and holding them in captivity for a short period, is believed to increase fish recruitment, because fish can bypass the vulnerable period of settlement. This is a technique that is practiced, but has yet to be tested (e.g. Sadovy 2005). I captured larval *Pomacentrus amboinensis* using light traps and held them in aquaria for a week before releasing them onto patch reefs. Since *Pomacentrus amboinensis* undergo predator induced size-selective mortality at settlement (e.g. Hoey & McCormick 2004, Gagliano & McCormick 2007), I predicted that the fish held in captivity would be more likely to survive than would fish that were released immediately, because they would be older, larger and potentially in better condition and therefore less susceptible to predation.

2. Odour cue use by coral reef fishes with generalist settlement requirements

This chapter is being prepared for submission as the following manuscript: Heenan, A., Simpson, S., Johansson, C., Healy, S. & Braithwaite V. (in prep) Odour cue use by coral reef fishes with generalist settlement requirements. Journal of Experimental Marine Biology and Ecology.

I collected the data with Charlotte Johansson and wrote the manuscript in collaboration with S. Simpson, S. Healy and V. Braithwaite.

2.1. Summary

Settlement-stage reef fish may use odour cues to locate a site where they can end their pelagic larval phase and begin their benthic juvenile and adult life. Once the reef has been located, a fish still needs to locate a specific microhabitat. In this chapter I investigated the response of a common coral reef fish, belonging to the *Apogonid doederleini* group, to broad scale (whole reef) and micro scale (a specific settlement habitat) odour cues. Only after prolonged exposure to reef-conditioned water, did fish exhibit a directional response, which was to move away from the reef odour. This outcome is not consistent with previous studies that have addressed the role of olfactory preferences in settlement-stage larval fish orientation. It seems plausible that settlement-stage apogonids are not sensitive to micro-habitat odours, and may use odour cues to avoid as well as to choose a site for settlement.

2.2. Introduction

At the end of their pelagic larval phase, coral reef fish are active settlement habitat selectors. From the open ocean they move towards a reef where they will metamorphose into their site-attached juvenile and adult form. As settlement-stage larvae can perceive different types of sensory cues, they may use a range of information to orientate with respect to their environment. For example, fish use the sight of conspecifics and specific microhabitats to locate, and move towards, a settlement site (Booth 1992, Danilowicz 1996, Lecchini *et al.* 2005*a*,*b*, 2007, Leis & Carson-Ewart 1999). However, the majority of settlement-stage fish locate a reef under low light conditions, or at night, under the cover of darkness (Booth 1991, Dufour & Galzin 1993, Irisson & Lecchini 2008). Furthermore, as larvae are found in greatest abundance in the pelagic waters away from the reef (Leis & McCormick 2002), they require information about potential settlement sites in conditions where visual cues emanating from settled conspecifics are not available. The acoustic cues from natural reef sound (Leis *et al.* 2003, Montgomery *et al.* 2006, Simpson *et al.* 2004, 2005) and the chemical cues present in reef odour plumes may provide relevant reef-specific information for use by larvae in directing settlement decisions (Vickers 2000). It is not clear, however, how the specific components within these loose terms 'reef sound' and 'reef smell' might actually influence larval settlement behaviour. It is difficult to assess auditory cues as investigations are limited to *in situ* field experiments because the physical property of underwater sound is not suited to aquarium or tank work (Montgomery *et al.* 2006). Odour cues are, however, amenable to manipulation in the laboratory.

Teleost fish have olfactory receptors in the paired pits or nares on their snout (Hara 1994). Settlement-stage fish draw water through their nasal cavity, drawing any chemical compounds dissolved in the surrounding water past these receptors (Atema *et al.* 2002). Scanning electron microscopy has shown that prior to settlement, wrasse (Labridae) larvae have anatomically complete olfactory apparatus (Lara 2008). No similar data is available for other species before settlement, although recently settled and juvenile Pomacentridae and Gobidae species have nasal cavities that are anatomically well developed (Arvedlund *et al.* 2007, Lara 2008) and pre- and post-settlement stage pomacentrids *Pomacentrus*

nagasakiensis are equally sensitive to the odour of conspecifics (Wright *et al.* 2005). The coral trout *Plectropomus leopardus* can detect amino acids at concentrations of 0.1 mM, which is similar to the concentration of amino acids that has been measured in seawater (Wright *et al.* 2008). It seems, therefore, plausible to infer a general level of olfactory competence in coral reef fish prior to and during the settlement process.

Once detected, these olfactory cues could 1) indicate an island or reef to colonise, and more specifically 2) indicate a suitable settlement site (as categorised by Lecchini *et al.* 2005*a*). Settlement-stage cardinalfish (Apogonidae) prefer the odour of lagoon to ocean water (Atema *et al.* 2002) and inshore or beach water to offshore water (Dixson *et al.* 2008). These preferences might allow fish to navigate in the general direction of a reef at the end of their pelagic phase. Recognition of individual reefs by larval fish is supported by the ability of settlement-stage larvae to discriminate between water collected from their home and adjacent reefs (Gerlach *et al.* 2007). This preference for the odour of their natal reef suggests that olfactory homing could assist self-recruitment (Gerlach *et al.* 2007) and supports the idea that each reef could have its own unique odour signature (Lara 2008). However, if larvae can detect specific components of a reef odour, it is not surprising that they are attracted to their natal reef, as their parents are likely to live in a settlement site where the conditions proved suitable for survival and reproduction (Almany *et al.* 2007).

In some cases, we know what larval fish are attracted to within a reef scent. For example, clown anemonefish *Amphiprion percula* are only found on reefs associated with vegetated islands. When newly settled juveniles were presented with a choice between water collected from island-associated reefs and non-island associated or emergent reefs, fish spent more time in the former than the latter (Dixson *et al.* 2008). Late-stage larvae reared in the laboratory without prior experience of vegetative odour cues prefer water conditioned with coastal rainforest leaves over blank seawater and over water conditioned with swamp tree leaves, which they would not naturally encounter (Dixson *et al.* 2008). These, potentially innate odour preferences may allow fish to locate a specific reef, and once this has been identified, fish are then presumed to home in on chemical cues that indicate their preferred settlement habitat. However, relatively little is known about the specific components within a reef odour that late-stage larvae

use to orientate towards a settlement site. It seems likely that, if species with obligate habitat associations have strict settlement requirements, they may be receptive to specific olfactory cues. This is the case for larval anemonefish, which are attracted to the odour of their anemone symbionts (Dixson *et al.* 2008, Munday *et al.* 2009). They also prefer the odour of their natural host anemones to that of other species (Elliott *et al.* 1995). This attraction is driven by a combination of an innate response to the odour (Arvedlund & Nielsen 1996) and prior experience of the anemone during the egg stage (Arvedlund *et al.* 2000). It is not known if less specialized settlers are receptive to olfactory cues that would indicate a specific settlement site. For example, it is not clear whether apogonids that preferred the odour of the lagoon water over open ocean water (as in Atema *et al.* 2002) and the odour of home reef water over non-natal reef water (as in Gerlach *et al.* 2007) were responding to the water type as a whole, or to the presence of particular components within these collective odours that could indicate a specific settlement site. Potentially, these fish may have responded to an odour within these water types that indicated the presence of a particular settlement substrate. It is not known, however, whether these fish can actually differentiate between the odours of different microhabitats.

In this study, I investigated the possible role of olfactory cue use in the settlement of apogonid larvae by testing whether they can distinguish one settlement substrate from another. In comparison to anemonefish, which have quite specialist settlement requirements, apogonids belonging to the *Apogonid doerderleini* group settle onto sandy coral rubble habitat that is readily available along the reef edge, before moving onto the live reef after a few days (Finn & Kingsford 1996). In this study, settlement-stage apogonids were presented with reef water (containing coral and heterospecific odours) and control seawater in parallel currents to see whether the fish were attracted to the scent of a reef. I predicted that settlement-stage larvae would prefer reef water to a blank seawater control, because irrespective of where they settle on the reef, they need to move in towards the reef. I then presented fish with reef water and blank seawater control, after prior experience of the reef water. If olfactory cues are used to stay within the vicinity of the reef until either the fish are ready, or the conditions are right to settle, then I predicted that late-stage larvae would maintain a preference for the reef odour, even after prior experience of it. Finally, to determine whether

fish use microhabitat odour cues after the reef has been located, I presented fish with water conditioned with live coral and coral rubble. I predicted that fish would prefer the odour of coral rubble to that of the blank seawater control as this is their settlement habitat.

2.3. Materials and methods

Fish collection, identification and housing

The experiment was carried out between 1st and 22nd December 2006 at Lizard Island Research Station on the Great Barrier Reef, Australia. Settlement-stage fish were collected daily using light traps set one meter from the sea surface from moorings anchored in water between 8-12 m depth. The moorings were located at the front of the research station (14°40'S, 145°28'E), approximately 500 m from shore and 100 m from any reef. The light traps used were a modified version of the Doherty 1987 and Stobutzki & Bellwood 1997 design (see Figure 2.1). These were set at dusk, left to collect fish overnight and were taken in at dawn. The predominant fish families caught were Pomacentridae, Apogonidae and Blennidae (representing 93% of the larval reef fish catch). I selected a commonly caught apogonid as the study subject. Fish were identified based on general features of body shape common to apogonids (being elongate in shape, having 2 dorsal fins and a large mouth and eyes) and pigmentation that was specific to this *Apogonid* sp.: a dark stripe starting on the fishes' snout and running through the middle of the eye across the length of the body to the caudal peduncle. Individuals measured approximately 12-14 mm in standard length and based on these pigment patterns, the focal species is likely to belong to the *Apogon doederlini* group.

Fish were housed in outdoor glass aquaria with approximately 40 fish per tank (dimensions: 30 x 15 x 20 cm). Each tank was filled with the research station's aquarium seawater supply with an airstone for aeration. Fish were tested within 12 hours of collection and were not fed during this period. After the experiment, they were sacrificed by immersion in an icy bath of water and preserved in 70% ethanol for a separate study.

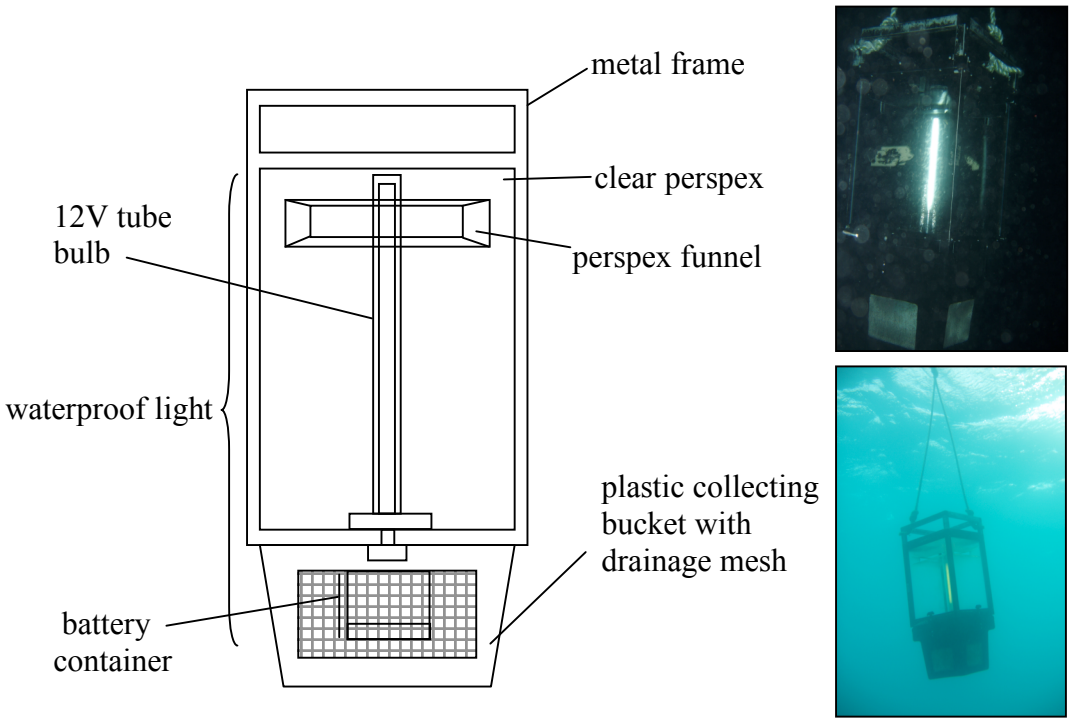

Figure 2.1.: Diagram and photo of the Stobutzki and Bellwood light trap used at Lizard Island to collect settlement-stage larval fish as the approached the reef at night

The choice flume

To investigate the response of settlement stage *Apogon* sp. to water that contained different odour cues, I used a choice flume that was modified from the experimental design of (Atema *et al.* 2002). Although the construction, methodology and protocol were adapted (in minor ways, for example the dimensions and water supply) the approach remained similar: two independent streams of water flowed alongside one another, down the length of the flume into the experimental arena, where the fish were located. Fish could sample both water types, as they were free to move from one side of the tank to the other, and I noted the frequency with which they occurred within each.

The flume (dimensions: 100 x 30 x 19 cm, Figure 2.2) was made from opaque Perspex to minimize external disturbances on the behaviour of fish. The water was supplied to the flume through a gravity fed system. On a stand 30 cm above the flume were three header tanks, which supplied the experimental water types. Each header tank had constant input and output of water that drained either into the flume or was diverted to a sinkhole. At any one point the right and left hand side of the flume was supplied with water from two of the header tanks while water from the third was diverted. As the volume of water in all three tanks was maintained at a constant level (30 l), it was possible to switch the diverted header tank supply for one of the other two header tanks with minimal disruption to the constant flow on the flume.

In the first half of the flume, water on the right and left hand side was kept separate by a barrier, after which the water passed though a block of tightly packed straws that were placed lengthways. This created a laminar flow and meant that when the two bodies of water entered the experimental arena where the fish were, it did not mix despite the lack of physical barrier. The rate of flow was kept steady by keeping the total water volume and the drainage rate constant. Water depth was maintained at 9-10 cm using taps attached to drainage pipes at the end of the flume. The flow speed was calculated measuring the time taken for individual *Artemia* to move 10 cm downstream when released in the experimental arena. The mean flow speed was 0.5 cm s^{-1} and, based on the volumetric area of the experimental arena (30 x 9.5 x 19 cm), the volume flow rate was 3 ls^{-1}. Although this flow speed was slower than the average current

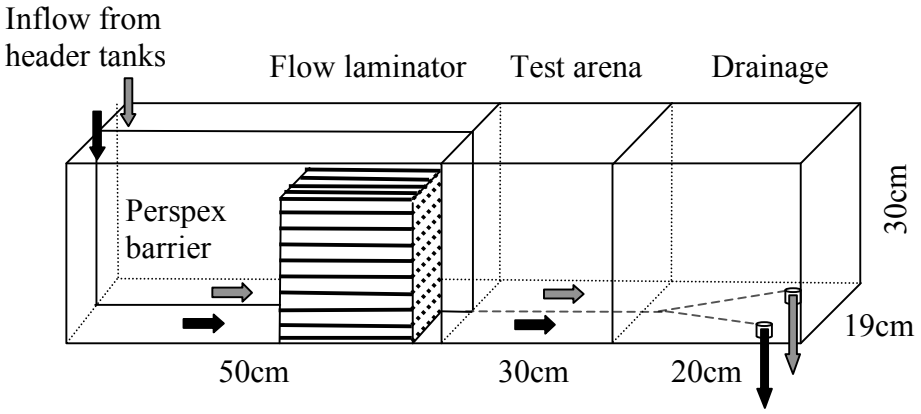

Figure 2.2.: Diagram of choice flume. Water flowed along either side of the barrier then through the tightly packed drinking straws creating a laminar flow. The flowing water remained separate in the test arena so test fish where presented with two distinct bodies of water, on the left and the right hand side (as indicated by the faint dashed line). The test arena was enclosed with mesh barriers and the water bodies remained separated beyond the downstream barrier. Water outflow was through two drainage standpipes fixed on the underside of the flume, which were connected to taps to regulate outflow rate.

speed measured at Lizard Island (10-30 cm s^{-1}; Frith *et al.* 1986), fish were still presented with two distinct types of flowing water, and so I thought this was suited to testing their response to the water treatments. During pilot trials and at intermittent periods between experimental trials, separation of the right hand and left hand streams of water in the choice flume was confirmed by the addition of coloured dye to the header tanks supplying the flume. If the water in the header tanks differed in temperature, this caused mixing across the boundary layer in the arena. To ensure fish were presented with a dichotomous flow, I monitored the temperature of the water and if the two streams differed by more 0.5°C, sealed bags of ice were placed in the appropriate header tank to bring the water to within the same temperature range (typically 27-27.5°C).

Choice tests

The experimental test treatments were: (1) reef odour (2) coral odour (3) coral rubble odour (4) reef odour after having prior experience of this water type. These test water treatments were presented simultaneously alongside 'blank seawater' in the flume. The 'blank seawater' was the research station aquarium seawater, which came from the same supply as the water in which fish (in treatments 1-3) were held before being tested in the flume. To see whether this prior exposure had any effect on the behaviour of fish in the flume, the forth treatment was created (4) reef odour acclimated.

The research station aquarium water was pumped from an inlet on the sandy flat immediately in front of the station. It was supplied via three 10,000 l tanks, the flow through rate of which was approximately 10,000 l per hour. Wright *et al.* (2005) performed electro-olfactograms on coral reef fish larvae (*Plectropomus leopardus*) using the Lizard Island aquarium water as a control solution because it did not illicit an olfactory response (as measured by a change in the negative electrical potential of the nasal epithelium) in the fish being tested. Based on this information, I assumed that the aquarium water was devoid of significant olfactory cues and therefore suitable to be used as blank seawater control. For the reef odour water that was used in Treatment 1 and 4, water was pumped from the research station's display tank into the flume header tank. The display tank was a 1000 l tank containing a selection of coral, fish and invertebrates

Table 2.1.: Inventory of the conspicuous species present in the aquarium station's reef display tank. Water from this tank was pumped to header tank of the choice flume to provide the reef conditioned water treatment.

Inventory	Family	Species (if recorded)	Common name
Coral	Acroporidae	Acropora sp.	
	Fungiidae	*Heliofungia actiniformis*	
	Pocilloporidae	*Pocillopora damicornis*	
	Poritidae	Porites sp.	
Other invertebrates	Holothuriidae	*Holothuria atra*	Sea cucumber
		Stichopus chloronotus	Sea cucumber
	Ophidiasteridae	*Linckia laevigata*	Starfish
	Tridacnidae	*Tridachna gigas*	Giant clam
Fish	Acanthuridae	*Zebrasoma veliferum*	Sailfin tang
	Apogonidae	Apogon sp.	Cardinalfish
	Chaetodontidae	Chaetodon sp.	Butterflyfish
	Pomacanthidae	*Centropyge bicolor*	Bicolour angelfish
	Pomacentridae	*Amphiprion frenatus*	Tomato clownfish
		Dascyllus aruanus	Humbug dascyllus
	Tetraodontidae	*Canthigaster bennetti*	Bennet's toby

(Table 2.1). Treatments 2 and 3 were produced by placing six pieces (c. 8 x 8 x 8 cm) of live or dead rubble of the cauliflower coral *Pocillopora damicornis* into the header tank, immediately prior to the trial. Between trials with different water treatments, the header tanks were scrubbed, washed and air-dried.

Fish used in Treatment 4 were housed in the display tank so that they had experienced the reef odour water prior to being tested in the flume. They were held in a water permeable container (a lidded plastic tub (24 x 18 x 10 cm) with mesh windows that allowed free movement of water) holding approximately 40 individuals.

Choice flume trials

As I discovered in the pilot trials that fish were more likely to move around when in a group than when alone in the flume, the fish were tested in groups. This may have been because being in a group reduces the time taken to recover from a stress response (Allen *et al.* 2009), that could be caused by the transfer from

the holding tank to the choice flume (done here using a beaker of seawater). Fish were transferred as a group, and each group contained three similar sized fish. I visually estimated fish size to keep the time spent handling them to a minimum. I attempted to avoid size differences in the groups so as to reduce the potential for hierarchical interactions influencing behaviour during the experiment, such as the chasing of smaller fish by their larger conspecifics (Yue *et al.* 2006).

After fish were placed in the choice flume they were left to acclimatize for 10 minutes in the control water. In the first five minutes of this period the water was not moving, followed by five minutes of constant flow on both sides of the flume. The control water type was the water in which the fish had been held prior to being put in the choice flume (Treatments 1-3: blank seawater, Treatment 4: reef odour water). After this acclimatization period, the experimental trial started. The trials consisted of five different stages, each lasting three minutes (Table 2.2). The stages were (with L and R indicating the left and right hand side of the tank):

1. L control, R control;
2. L test, R control;
3. L control, R control;
4. L control, R test;
5. L control, R control.

A new group of fish was used for each trial, and Stages 2 and 4 were alternated between groups, so that the side of the flume on which the test water appeared was counter balanced across trials. Based on the rate of flow, it took approximately one minute for a change in water regime to run through the whole flume. The fish were left to experience the new water environment for a further minute, at which point, during the final minute of each stage the position of fish (left or right hand side of the tank) was recorded every 15 seconds.

For each trial a score was taken of the number of fish per observation on the right or left hand side (when investigating side preference within the flume) and on the test or control side (when investigating the effect of water treatment). From the total number of replicate trials (n), I removed any in which fish showed a consistent side bias (defined here as remaining on the same side of the flume

Table 2.2.: Sequence of water treatments in the flume trials. Before each trial, fish were placed in the flume for a 10 minute acclimatisation period, the first five minutes in standing control water, the next five minutes in continuous flow of control water. Stages 2 and 4 were switched between trials in successive groups, in order to avoid the first introduction of the test water consistently being on left (LHS) or right (RHS) side of the flume.

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Stage	Experiment time point (minute)	Test type	Flow	Water treatment	
				LHS	RHS
-	10 mins before start	Acclimatisation	No	Control	Control
-	5 mins before start	Acclimatisation	Yes	Control	Control
1	1-3	Control	Yes	Control	Control
2	4-6	Test	Yes	Test	Control
3	7-9	Control	Yes	Control	Control
4	10-12	Test	Yes	Control	Test
5	12-15	Control	Yes	Control	Control

for the duration of the whole trial), the remaining number (n_1) were used for the data analysis. For each treatment, I compared the score of fish on the test and control side using a non-parametric Wilcoxon's signed rank test (after removing tied scores, number of replicates referred to n_2). A summary of the resulting number of trials per experimental water treatment can be seen in Table 2.3.

2.4. Results

Fish did not exhibit a right or left hand bias in the choice flume in any of the four experimental treatments (reef: $n_1 = 25$, $Z = -0.28$, $p > 0.05$; coral: $n_1 = 14$, $Z = -0.83$, $p > 0.05$; coral rubble $n_1 = 17$, $Z = 1.27$, $p > 0.05$; reef held: $n_1 = 11$, $Z = 0.42$, $p > 0.05$).

When fish were held in blank seawater before the choice trials, they had no preference in the flume, they were equally likely to be on the test water side as the blank seawater side (reef: $n_2 = 20$, $Z = -0.14$, $p > 0.05$; coral: $n_2 = 14$, $Z = 0.61$, $p > 0.05$; coral rubble: $n_2 = 14$, $Z = 0.37$, $p > 0.05$).

Fish that had been held in reef water before they were tested in the flume were

Table 2.3.: Summary of the number of replicate groups per experimental test water treatments. Of the total number of trials run (n), some groups of fish exhibited a consistent side bias, these were excluded from the analysis for side preferences in the flume (sample size n_1). Trials with tied scores between test and control sides were removed, leaving the remaining replicates (n_2) for the analysis of water type preferences in the flume.

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Test water treatment	Total trials (n)	(n_1)	(n_2)
Reef	29	25	20
Coral	16	15	14
Coral rubble	19	18	17
Blank seawater	13	11	10

presented with a choice between reef water and blank seawater and they spent more time on the side with the newly introduced water type, the blank seawater, than they did in the reef water (Figure 2.3; $n_2 = 10$, $Z = 1.91$, $p < 0.05$).

2.5. Discussion

Settlement-stage apogonids were not affected by the introduction of odour cues that could indicate specific microhabitat sites: when test water types were presented alongside control water, settlement-stage apogonid larvae appeared unaffected by the introduction of odour cues that could indicate specific microhabitats (live coral or coral rubble). In addition, fish did not prefer to swim in reef water rather than the control seawater. This result is not consistent with the outcomes of previous experiments in which larval apogonids preferred reef water over water collected from the ocean (Atema *et al.* 2002).

It seems unlikely that the lack of preference for either the broad-scale (whole reef) or micro-scale (specific sites on the reef) odours in this study was caused by the fish responding poorly to being in the flume or being unable to discriminate between water types, as after prior exposure to reef water, the fish preferred blank seawater to the reef water. A more likely explanation for the lack of preference seen in Treatments 1, 2 and 3 is that the water treatments in this experiment did not contain the appropriate olfactory stimuli used by late-stage apogonids

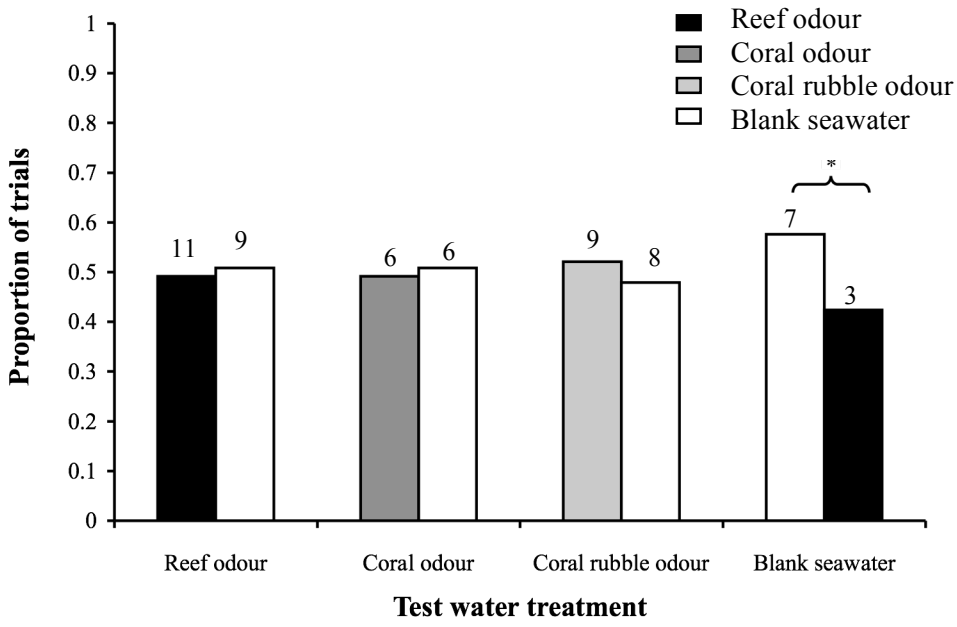

Figure 2.3.: The number trials (expressed as a proportion of the total) where the score of fish distribution was greater in the test (left hand bar of each pair) or control (right hand bar) water treatment. When fish were observed in equal numbers in the test and control, these data were excluded. The remaining sample sizes are indicated at the top of each bar. The test water type is indicated on the x axis.

when making settlement decisions. The most obvious candidate is the olfactory presence of conspecifics. When pumped onto unoccupied coral heads, the odour of conspecifics is sufficient to trigger higher rates of natural settlement in the pomacentrid *Dascyllus aruanus* (Sweatman 1988). Additionally, reef fish larvae of another pomacentrid species (*Chromis viridis*) respond positively to visual, acoustic and olfactory cues of conspecifics, but not to cues from heterospecifics or the coral substrate (Lecchini *et al.* 2005*a*). It is not surprising that in this study larval apogonids did not respond positively to general reef odour cues, as their attraction to reef odour has since been shown to be specific to their natal reef, or the reef from which they were spawned, relative to a foreign reef (Gerlach *et al.* 2007). It, therefore, seems plausible that it is the odour of conspecifics within natal reef water that apogonids cue in to when selecting a settlement-site. It remains to be established whether settlement-stage larval apogonids, like adults (Døving *et al.* 2006), can discriminate and prefer the odour of conspecifics collected from their home site when compared to conspecifics collected from an adjacent site.

An unexpected finding was that the settlement-stage fish avoided reef water after having spent some time in that water. We assume they used odour to do this, suggesting they may use odour cues to avoid unsuitable settlement-sites as well as to home in on appropriate sites. There are few data on the role of odour as a cue for avoiding non-preferred locations. This is because it is common to present larval fish with a choice between olfactory information that could indicate they are moving in the 'right' or the 'wrong' direction with respect to a settlement site i.e. lagoon collected water versus ocean collected water (Atema *et al.* 2002), home reef collected water versus foreign reef collected water (Gerlach *et al.* 2007), water conditioned with conspecifics versus water conditions with heterospecifics (Lecchini *et al.* 2005*b*). In these cases, a preference for one water type would also be achieved by an avoidance behaviour of the other, therefore it can be unclear whether it is a positive or negative behavioural response. Clearly, larval fish can respond both positively and negatively to odours (e.g. anemonefish are attracted to the odour of their anemone and avoid the scent of their parents; Elliott *et al.* 1995, Dixson *et al.* 2008, Munday *et al.* 2009), however for fish without obligate habitat associations, it may be a more efficient strategy to avoid water containing negative odour cues, as once they approach the reef, potential settlement sites

are more readily available.

It is not clear why the fish preferred the blank control water after prior exposure to the reef water treatment. It may be that fish preferred water that lacked reef olfactory cues. Indeed, if choice results from avoidance, it is possible that testing the fish during daylight hours may underlie their preference for the blank control water. In post-settlement *Dascyllus albisella*, the time of day effects their response to odour cues, as significantly fewer fish selected their preferred coral habitat using odour cues during daytime trials in comparison to night trials (Danilowicz 1996). It is plausible that a similar effect exists in settlement-stage fish, as most reef fish settle at night, and when larvae are released into water during the day, it is common for fish to swim offshore, away from the reef (Leis & Carson-Ewart 1999, 2002), which may reduce the risk of interactions with predators and aggressive resident fish (Leis & Carson-Ewart 2002). Further experimentation is needed to determine whether the time of day and the duration of exposure to odour cues has a significant impact on water choice decisions of settlement-stage fish.

Recent research has found that settlement-stage fish lose their ability to differentiate between odour cues relevant to settlement when larvae are reared in low pH conditions, conditions which simulate the decrease in seawater pH forecast by the effects of ocean acidification (Munday *et al.* 2009). It is likely that more work will focus on the impact of changing conditions on larval sensory abilities. As this study has highlighted, there are still considerable gaps in our understanding of odour cue use by larvae during settlement, and it will be important to also address these questions relating to the timing and specificity of larval olfactory abilities if we are to fully understand larval settlement behaviour in the face of a changing environment.

3. Can colour augment the capture of reef fish in light traps?

3.1. Summary

Light traps are used both to collect settlement-stage coral reef fish larvae for the marine aquarium trade and to gather information about this ephermeral life history stage. Usually traps emit broad spectrum white light but here I investigated whether broadcasting different coloured light affects the capture of larval reef fish. In the majority of cases, the more intense the light emitted, the more families, at greater abundances, were caught. The exception was for red wavelengths of light, which appear to increase the catch of some families, even though red traps emit light of lower intensity and, therefore, contact a smaller area. The spatial and temporal variation that is characteristic of light trap catch data made it difficult to detect the effects of using colour-treated traps on the total abundance of fish caught. However, if the light intensity of colour-treated traps was the same as the white light traps typically used, then using coloured-treated traps could augment light trap catches, provided the emitted light matches the spectral sensitivity of the target species.

3.2. Introduction

There is more than one way to sample larval fish before they recruit to the reef (Choat *et al.* 1993). Light traps and crest nests are favoured in post-larval capture for culture (PCC) programmes because they are passive methods that catch late-stage larvae exclusively and keep them in good condition (Dufour 2002, Bell *et al.* 2009). Crest nets collect larvae just behind the reef crest, which is the interface between deep ocean water and the reef lagoon (Dufour & Galzin 1993). As waves break over the crest and barrier reef, larvae are collected as water flows through the cod end of the net, which means all species in the water column can be sampled (Lecchini *et al.* 2006). However setting nets in the surf zone is notoriously difficult (Nolan & Danilowicz 2008). As a result, despite their advantage of being non-selective, the successful use of crest nets has been limited to amphidromic parts of the world, such as French Polynesia, where there is a small tidal range leading to very weak tides (Dufour & Galzin 1993). Light traps, on the other hand are easy to deploy and are commonly used to collect settlement-stage larval fish when placed just off the reef (Bell *et al.* 2009, Lourie & Lecaillon 2005). The disadvantage of light trapping is that only phototactic species are collected and the traps are quite inefficient as they have high escape and low recapture rates (Meekan *et al.* 2000).

The selective nature with which light traps collect larval coral reef fish has been demonstrated by comparing the catch composition of light traps with other passive collecting techniques. When placed just off a reef, light traps primarily catch settlement-stage apogonids, lethrinids and pomacentrids in greatest abundance and also typically blennioids, serranids and chaetodontids (Thorrold 1992, Choat *et al.* 1993, Fisher & Bellwood 2002, Leis & McCormick 2002, Leis *et al.* 2003, Simpson *et al.* 2004). The number of families caught in light traps is significantly less than those caught by netting techniques, such as the bongo net (Choat *et al.* 1993). Choat *et al.* (1993) suggested that less abundant families may be less likely to be caught in a light trap that is in a fixed position, as opposed to a towed net which will sample a greater volume of water. However, the common absence of some families (e.g. scarids, carangids and lutjanids) suggests that at this larval stage, some fish are not phototactic (Choat *et al.* 1993), or at least are not attracted to the white light that is typical

of light traps.

This white light is emitted from the broad spectrum, fluorescent light bulbs that are the standard type used in light traps when collecting settlement-stage coral reef fish (Doherty 1987, Stobutzki & Bellwood 1998). The reason why late-stage larvae are attracted to white light is not understood. Photopositive behaviour in teleost fish typically occurs just before settlement and metamorphosis (Evans & Browman 2002, Shand 1993, 1997). A potential reason why some but not all late-stage larvae are caught in light traps may reflect species-specific differences in the onset of this photopositive behaviour (Lara 2001). Additionally, the wavelengths of light emitted from the white-light bulbs may trigger a stronger phototactic response in larvae if the emitted light compliments their spectral sensitivity. Determining the spectral sensitivity of settlement-stage larval fish is made difficult by the temporary nature of this life history stage, however, it is known that larval pomacentrids and apogonids become more sensitive to colour with age, and their maximum sensitivity shifts towards longer wavelengths of light (Job & Shand 2001). Given this difference in the spectral sensitivity of some larvae, it seems reasonable to ask whether the catch composition of light traps could be altered by emitting different coloured light. Particularily, because the variable and selective nature of light traps is a major drawback to using them as a collecting tool (Bell *et al.* 2009), and any method to diversify or increase the catch composition would be beneficial.

In this chapter, I investigate the potential of augmenting the abundance and diversity of light trap catches by broadcasting different coloured light. I aimed to compare the taxa and numbers of coral reef fish larvae captured in light traps with five different coloured-light treatments from across the visible light spectrum: blue/green, green, orange, red and an untreated white light. Underwater, the appearance of colour is mainly determined by scatter and absorption by both water molecules and dissolved organic matter and suspended particles (Baker & Smith 1982). There is a low concentration of the latter in tropical seas and ocean water, which means that with increasing depth, blue light (450-475 nm) predominates. This is because seawater selectively absorbs the shorter and longer wavelengths of the light spectrum. As a result in shallow water broad spectrum light is available but with increasing depth, red light is lost and blue light predominates (McFarland 1986). This means that the catchment area of

traps will be smaller if traps are emitting longer (red and orange) wavelengths of light in comparison to the mid spectrum wavelengths (blue and blue/green). Therefore in this experiment, when I compared the abundance of fish caught in each colour treatment, I considered the catch relative to the intensity of the emitted light. I predicted that more apogonids and pomacentrids would be caught in the traps emitting the red and orange light, as these are families which are known to be more sensitive to longer wavelengths of light. The second prediction was that the number of families in the white light traps would be greater than in the colour-treated traps, as the white light trap emit light from across the whole visible light spectrum, which should attract all families that are photopositive.

3.3. Materials and methods

The experiment was carried out from the 10th November to the 25th December 2006 at Lizard Island, on the Great Barrier Reef in Australia. Moorings were anchored adjacent to the research station (14°40'S, 145°26'E) on a sandy sea floor in water that was 7-12 m deep. From these moorings, which were separated by a minimum distance of 150 m, light traps were set 1m below the sea surface and marked with a surface-marker buoy. The moorings were set close to but off the reef (following Meekan *et al.* (2001)), and were positioned approximately 50 m away from any reef structure. This placement was confirmed by nautical charts and SCUBA surveys. Fisher & Bellwood (2002) found that at a distance of 18 m from a trap, the broadcast light is less than the ambient light conditions typical of a new moon evening and as I used the same fluorescent tube bulbs (8W, 12V) as they had, I assumed that the distance between the moorings was sufficient to prevent overlap between the areas illuminated by adjacent traps.

To create the colour treatment the light bulbs were covered with coloured acetate. The acetate sheets were cut to fit, so that they were wrapped twice around the outside of the cylindrical bulb without any overlap. For each colour treatment the spectral output of light through the acetate was measured using a spectrometer (S2000, Ocean Optics, Florida). This was done in the laboratory by measuring the transmission of a reference signal through two layers of the coloured acetate sheets (as they were wrapped twice around the bulb). The

Table 3.1.: The light intensity readings measured *in situ* at 0, 80 and 160 cm from each light trap. Readings were taken after the moon had set during the first quartet of the lunar cycle (28th November), so ambient light conditions would be similar to a new moon evening.

Colour treatment	Light intensity (μ Em^{-2} s^{-1}) at trap	Intensity relative to light intensity at trap (distance 0 cm)		
		0 cm	80 cm	160 cm
White	2.45	100	4.7	1.89
Blue	2.25	100	4.58	3.72
Orange	2.11	100	5.9	4.08
Red	0.27	100	4.8	0.03
Blue / Green	0.14	100	77.46	9.31

light broadcast for each of the colour treated traps had peak emissions in the following wavelengths blue/green (533 nm), green (530 nm), red (727 nm) and orange (687 nm) (Figure 3.1). With the exception of the addition of the coloured acetate sheets, the light traps were the same as those commonly used to sample settlement-stage coral reef fish (Doherty 1987, Stobutzki & Bellwood 1998, Meekan *et al.* 2001). Every night for 45 nights, five light traps were deployed, four colour-treated (blue/green, green, orange and red) and one untreated control trap. The control trap emitted broad-spectrum white light. I measured the light intensity emitted from each traps *in situ* with a Li- Cor light meter (Model: LI-1400 data logger, Li–Cor Inc. Environmental, Lincoln, Nebraska). Readings were taken once the moon had set on the 28th November 2006. These were taken at three set distances, 0 cm (right beside the light trap), 80 cm and 160 cm away for each trap. Immediately in front of the light traps, the white light was the most intense and the green treatment the least, however it was the red light that decreased in intensity most rapidly with distance; at 1.6 m away from the trap the red light dropped to 3% of the intensity that was recorded immediately in front of the trap (Table 3.1).

Each night light trap treatments were randomly assigned to a mooring. Traps were set at dusk, left to collect fish for approximately 11 hours and were then retrieved at dawn. At retrieval, the catch was transferred to a bucket and transported to the laboratory by boat where the catch was preserved in alcohol

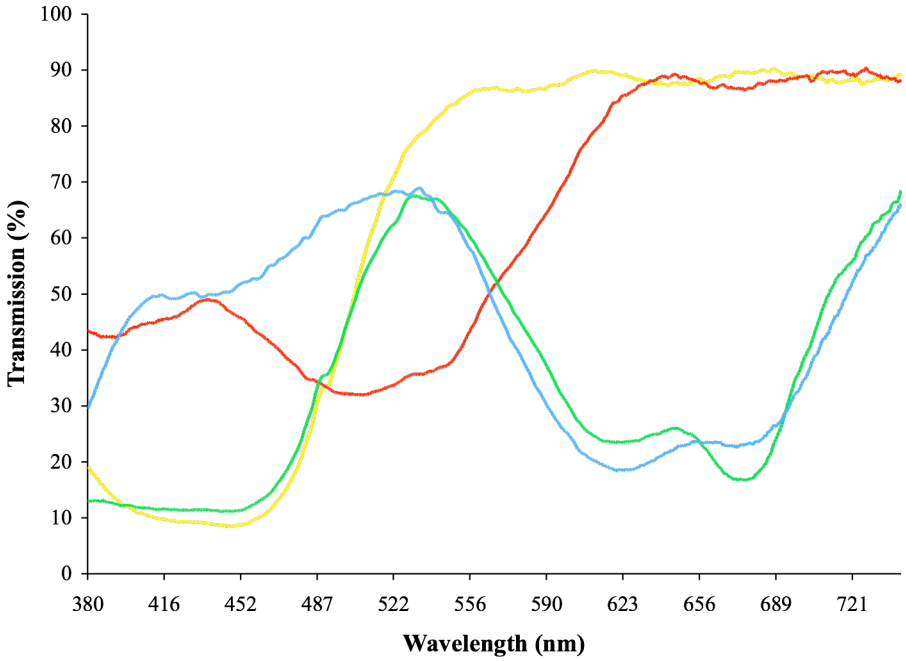

Figure 3.1.: The spectral transmission curves for the different coloured acetate used to create the colour treated light traps. The colour treated traps did not emit a monochromatic light, however the spectral peak of light through the coloured acetate was 533 nm for the blue-green acetate (blue line), 530 nm for the blue/green acetate (green line), 687 nm for the orange acetate (orange line) and 727 nm for the red acetate (red line).

(70% ethanol). Larval fish were sorted from the rest of the catch (mainly crustaceans and clupeids), identified to the family level and counted.

From the 45 nights that traps were set, only 15 nights of data were used in the analysis because of trap failure and loss of samples. To make a balanced comparison where colour treatments were equally represented in the dataset, only nights for which data were available for every colour treatment were analysed. The data were typical of species counts, as they were not normally distributed (Shapiro-Wilk normality test, $W = 0.16$, $p < 0.01$). The abundance of some families was an order of magnitude greater than that of others. In addition, counts of fish varied substantially from night to night and among mooring locations. Therefore, I used non-parametric analyses. To assess the effect of emitting coloured light on the abundance and composition of light trap catches, I used non-parametric ANOVA Kruskal-Wallis tests to compare (1) the number of fish caught per colour treatment per night; (2) the number of families caught per colour treatment per night; and because of the stochastic nature of light trap catch data, I also used (3) a ranked score of the catch per colour treatment per night. The ranked score was used to look for variation in the traps emitting different coloured light to consistently catch more or less fish. In all three cases nights were analysed as replicate blocks. When a significant effect of colour treatment was found, I used multiple comparison Tukey-Kramer-HSD to find out which colours were differed from each other. To assess the effect of mooring location, only data from the three most abundant families were used, and to test the effect of the colour treatment on the number and ranked scored abundance of fish, only families for which greater than 20 individuals were caught over five nights (five being the minimum number of replicate blocks recommended for a Kruskal-Wallis test) were assessed. The analysis was done using R (R Development Core Team 2007).

3.4. Results

A total of 15, 844 settlement-stage coral reef fish larvae from 24 families were caught. The Pomacentridae ($n = 11{,}725$), Apogonidae ($n = 3{,}318$) and Blennidae ($n = 348$) were the most abundant families in the catch (Table 3.2). These three families comprised 97% of the total catch. The number of fish caught from the

three most abundant families did not differ across the different moorings (Table 3.3).

If the colour treatment had no effect on the abundance of larval caught, the expected catches in each trap treatment should be equal to 20% of the total catch. There was a tendency for the white traps to catch more than 20% of the total catch, however this effect was not significant (Table 3.4; Figure 3.2), possibly due to the high amount of variation typical of light trap catches. However, when the data were expressed as using the rank score of the fish abundance per treatment per night, there was an effect of colour on the number of animals caught in the following families: Apogonidae, Labridae, Lethrinidae, Nemipteridae and Pomacentridae (Table 3.5; Figure 3.3). The untreated / white light traps, which emitted the most intense of the light treatments, caught the most larval fish: apogonids and pomacentrids were caught in higher numbers over significantly more nights in comparison to the other colour treated traps (Table 3.6). The labrids were caught in higher numbers on more nights in white traps but only in comparison with the traps emitting green and red light. In comparison with green traps, white traps caught more Lethrinidae over more nights. The colour emitted by the light traps did not appear to have an effect on the number of nemipterids caught (Table 3.5). Although a main effect of colour was found for this family, a statistical difference between the individual colour treatments was not apparent in the Tukey-Kramer HSD test. The reason for this discrepancy is likely to be due to the high number of nights where there were tied zero counts of nemipterids amongst the colour-treated light traps.

White-light traps caught fish from significantly more families than did the red and green traps (Kruskal-Wallis test, $\chi^2 = 14.34$, df = 5, p <0.01; Tukey-Kramer HSD, q = 2.80, alpha = 0.05).

Table 3.2.: Summary of the total number of settlement-stage coral reef fish caught in each of the colour treated light traps in November / December 2006 at Lizard Island Research Station, Great Barrier Reef, Australia. Nights were excluded if counts were not available for each colour treatment.

Family	Blue	Blue/Green	Orange	Red	White	Total
Acanthuridae	1	2	5	1	2	11
Apogonidae	683	677	671	501	786	3318
Blennidae	39	54	74	62	119	348
Carangidae	1	0	2	5	4	12
Gobidae	0	0	5	5	1	11
Holocentridae	0	0	0	0	1	1
Labridae	14	12	15	22	25	88
Lethrinidae	13	1	7	5	18	44
Lutjanidae	12	18	8	3	10	51
Monocanthidae	0	0	0	0	2	2
Mullidae	0	0	0	0	2	2
Nemipteridae	4	1	1	2	11	19
Platycepalidae	0	1	0	0	0	1
Plesiopidae	1	0	1	0	1	3
Pomacanthidae	0	0	1	0	0	1
Pomacentridae	1924	1012	1884	2844	4061	11725
Pseudochromidae	26	24	16	18	37	121
Serranidae	3	3	2	1	3	12
Siganidae	3	0	0	0	5	8
Sphyraenidae	0	0	0	1	3	4
Syngnathidae	13	3	2	4	5	27
Synodontidae	0	0	0	1	1	2
Tetradontidae	1	0	1	2	2	6
Tripterygiidae	6	2	5	12	9	34
Total	2744	1810	2700	3489	5101	15844

Table 3.3.: Proportion of the total catch at each mooring for the three most abundant families. Only nights where data for each mooring was available were used in the analysis. There was no mooring which caught more fish on significantly more nights, based on the ranked abundance of fish caught per night for these three most commonly caught families.

Family	Mooring						Kruskal-Wallis		
	A	B	C	D	E	F	χ^2	d.f.	p
Apogonidae	0.13	0.14	0.2	0.16	0.15	0.23	9.86	5	0.07
Blennidae	0.21	0.15	0.29	0.07	0.15	0.14	6.9	5	0.23
Pomacentridae	0.25	0.13	0.3	0.01	0.14	0.07	0.94	5	0.97

Table 3.4.: The total counts for the 10 families that met the criteria set for inclusion in the statistical analysis (see Methods). For each family, the total number of fish caught per colour treatment was compared across the 15 different nights. There was no statistical difference in the number of fish caught in light traps that were emitting different coloured light.

Family	Blue	Blue/Green	Orange	Red	White	K-W χ^2	p value
Apogonidae	683	677	671	501	797	1.91	0.753
Blennidae	39	54	74	62	122	4.98	0.289
Labridae	14	12	15	22	25	3.86	0.426
Lethrinidae	13	1	7	5	18	6.67	0.154
Lutjanidae	12	18	8	3	10	1.02	0.906
Nemipteridae	4	1	1	2	10	8.71	0.069
Pomacentridae	1924	1012	1884	2844	4087	3	0.559
Pseudochromidae	26	24	16	18	41	6.65	0.156
Syngnathidae	13	3	2	4	5	5.26	0.262
Tripterygiidae	6	2	5	12	9	2.62	0.624
Total	2734	1804	2683	3473	5121	2.67	0.613

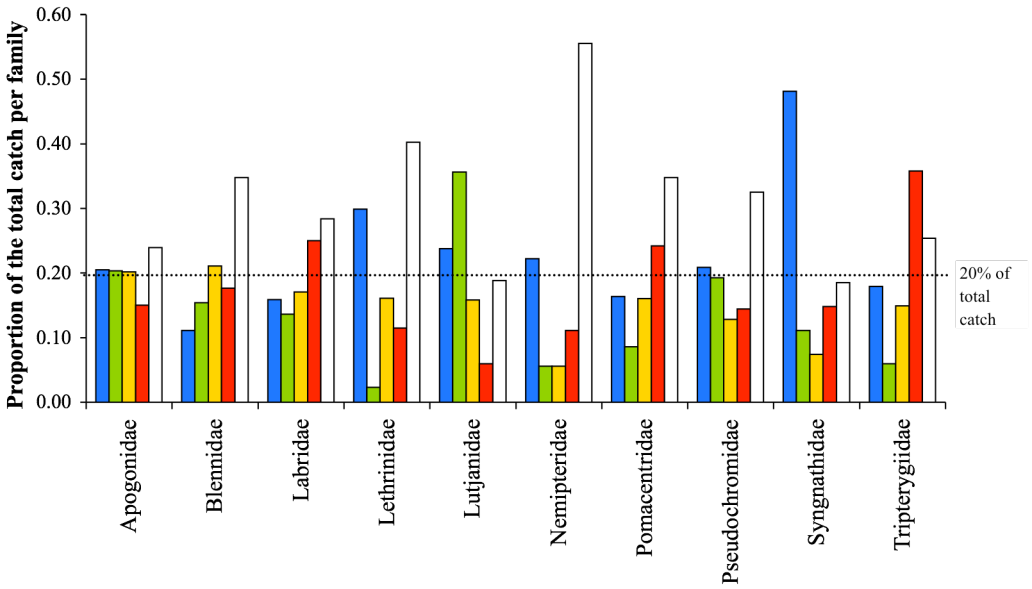

Figure 3.2.: Fish caught in each colour treatment displayed as a proportion of the total number caught per family over all nights. Only families for which more than 20 individuals were caught over a minimum of five nights are displayed. Only nights for which data were available for each colour treatment were included. The bars are coloured according to the coloured treatment of the light trap. If the colour treatment have no effect on the capture of these larval fish families, each trap would catch, on average catch 20% (dotted line) of the total number of larval caught per family.

Table 3.5.: For each family, counts of fish were scored and ranked by abundance per night per colour treatment. The ranked summed scores per colour treatment is displayed, but to test whether the numbers of fish caught was consistently affected by the colour treatments, the scores were compared across the 15 replicate nights. Five families (marked with *) were not caught in equal abundance across the colour treatments, which for these families, suggests a consistent effect of the colour treatment on larval catch rates.

Family	Blue/Green	Green	Orange	Red	White	K-W χ^2	p value	
Apogonidae	45	48	44.5	46	65.8	15.51	0.001	*
Blennidae	41.5	56	45.5	45.5	63.3	15.71	0.074	
Labridae	52	47	51.5	48	58.3	7.8	0.007	*
Lethrinidae	51.5	43	51.5	50	59.5	11.79	0.014	*
Lutjanidae	52.5	51.5	56.5	49.5	52.5	0.92	0.837	
Nemipteridae	54	48.5	48	49.5	57.5	5.74	0.052	*
Pomacentridae	44.5	46.5	50.5	36	68.8	27.91	<0.001	*
Pseudochromidae	49.5	46.5	51	47.5	60.3	6.36	0.082	
Syngnathidae	61.5	50	49.5	49.5	52.3	6.81	0.118	
Tripterygiidae	53.5	47	50.5	58	53	5.26	0.415	
Total	505.5	484	499	479.5	591	9.98	0.076	

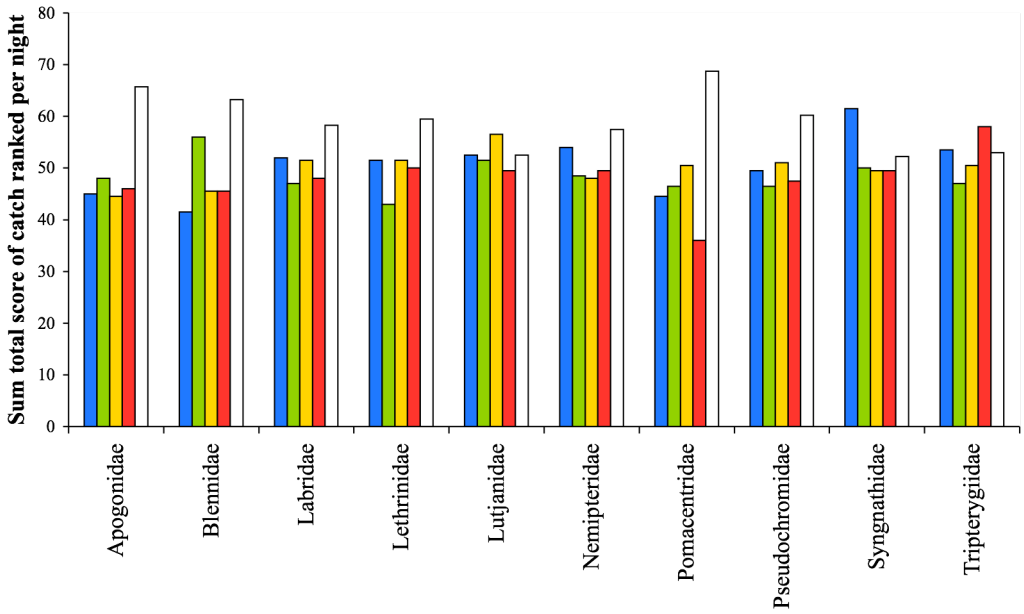

Figure 3.3.: The ranked score of fish abundance caught in each colour treated trap, summed across nights for the 10 most common families. The coloured bars represent the colour treatment of the light traps.

3.5. Discussion

The light traps that emitted the most intense light (i.e. the untreated traps emitting broad spectrum white light) caught fish larvae in greater abundance and from more families than did the traps emitting the less intense colour-treated light. The larger catch rates could be due to two reasons. Firstly, the more intense the light, the larger the area illuminated around the trap from which phototactic larval fish can be attracted. Secondly, the more intense the light, the stronger the phototactic response of larval fish (e.g. herring larvae; Blaxter 1969). If intensity of the broadcast light was solely responsible for the greater number of larvae captured in the light traps emitting more intense light, then the order of catch abundance per colour-treated-light trap should have been: white, blue, orange, green with red-emitting traps catching the fewest larval fish. However, this seems not to be the explanation for the variation in catch number as the labrids were caught in equal abundance across the different colour-

Table 3.6.: When a significant effect of colour treatment was found on the ranked abundance score (Table 3.5), Tukey-Kramer-HSD tests were carried out to determine which of the colour treatments differed from each other. Within each family, if colours are not connected by the same letter (A or B) then there was a significant difference ($p < 0.05$) in the ranked abundance of reef fish larvae caught in traps emitting those colours.

Colour	Apogonidae	Labridae		Lethrinidae		Pomacentridae
Blue/Green	B	A	B	A	B	B
Green	B		B		B	B
Orange	B	A	B	A	B	B
Red	B		B	A	B	B
White	A	A		A		A

treated traps, and the variation in the abundance of apogonids, lethrinids and pomacentrids in the different colour-treated traps did not match the order as predicted by light intensity.

It is unlikely that the equal number of labrids caught in the different coloured light traps is due to fish responding to the spectral composition of the emitted light. This is because the labrids tend only to have cone photoreceptors present within their retinae prior to settlement, the appearance of rods (and so, their ability for low light level vision and to discriminate between colours; Bowmaker 1995) does not occur until after settlement, when, during metamorphosis, their eyes undergo rapid rod differentiation (Lara 2001). The equal catch rates of labrids in the different colour-treated light traps is, therefore, likely to be because they were responding to the contrast between the illuminated area of the trap and the surrounding ambient light levels. Although the white light traps did catch apogonids and pomacentrids in greater numbers than did the traps emitting other colours, the number of these fish caught did not vary according to the light intensity of the remaining colour-treated traps. The red and green light traps emitted light that was approximately 12% of the intensity of light from the orange and blue traps and yet they did not differ in the abundance of fish they caught. A stronger attraction of some larval fish to red light is also suggested by the equal ranked abundance of lethrinids in the red-treated traps compared to the other traps emitting more intense light (blue/green, orange and white).

Considering the decreased light intensity of the emitted light, it would appear that apogonids, lethrinids and pomacentrids are caught in unexpectedly high abundancies in the red-treated light traps.

The catch rate of larval fish in colour-treated traps, did, as expected, vary according to the spectal sensitivity of apogonids and pomacentrids. Species from these two families undergo a developmental shift during the larval stage: they become more sensitive with age to a broader range of light throughout the colour spectrum and at settlement, are maximally sensitive for feeding beaviour at longer wavelengths of light (Job & Shand 2001). In this study, the apogonids, pomacentrids and lethrinids showed a stronger phototactic response to light traps that emitted longer wavelengths of light, potentially because the traps emitted light that matched the spectral sensitivity of these fish.

Given the tendency of white light traps, which emit the most intense light, to collect more fish from more families than do colour-treated traps, it seems that using coloured rather than white light in traps will not overcome the taxonomic selectivity of light traps as a collecting tool. It appears, however, that the abundance of particular species in light trap catches could be maximised, if the emitted light compliments the spectral sensitivity of that species. As information becomes available on the spectral sensitivity of a broader range of species at the settlement-stage, using coloured-light in traps that matches the sensitivity of a target species could be used to increase their catch abundance, or potentially the attraction of different species to different coloured-light could be used to selectively sort the catch within the light trap itself.

4. The response of settlement-stage coral reef fish to recordings of reef noise

I presented the first experiment in this Chapter as an oral presentation at the 11th International Coral Reef Symposium and a version of this has been published in the conference proceedings as:
Heenan A., Simpson S.D. & Braithwaite V.A. (2008) Testing the generality of acoustic cue use at settlement in larval coral reef fish. 11th International Coral Reef Symposium pp. 554-558, Fort Lauderdale, Florida, USA.

Author contributions were as followed: Adel Heenan, Stephen Simpson and Victoria Braithwaite planned the work. Adel Heenan and Stephen Simpson conducted the research. Adel Heenan, Stephen Simpson and Victoria Braithwaite prepared the article.

4.1. Summary

It appears that combining light traps with sound systems to broadcast reef noise can lead to an increased catch of larval fish compared to the numbers caught in silent light traps. To determine whether this coupling of reef noise playback with light traps could augment the collection of reef fish for post-larval capture for culture, I carried out two acoustic playback experiments. In the Philippines, fewer larvae were caught in the light traps broadcasting reef noise compared to the number caught in the silent light traps. It was possible that the use of a single recording both in the experiment in the Philippines and in the previous studies at Lizard Island were responsible for the opposite effect reef noise playback had on the light trap catches in these two locations. I, therefore, carried out a second acoustic playback experiment at Lizard Island, in which I played multiple reef recordings. The lower catch in the Philippines may have also been due to the

design of light trap I had used. In the second playback experiment I presented two types of light trap, the Ecocean traps I had used in the Philippines and the AIMS traps which had been used in the previous playback studies at Lizard Island. Although I collected too few data to examine the effect of varying the reef recording that was broadcast, the success of the trap type used depended on the presence or absence of sound. At Lizard Island, the AIMS silent traps caught more larval fish compared to the AIMS sound traps, whereas the Ecocean sound-treated traps caught more than the Ecocean silent. Settlement-stage larval fish appear to be attracted to and repelled by different coral reef noise. The effect of reef noise playback on light trap catches will vary with the recording that is broadcast, the design of light trap that is used, and potentially the ambient conditions into the recording of reef noise is broadcast.

4.2. Introduction

Every year, between 14 million to 30 million fish are caught to supply the marine ornamental trade (Wood 2001). Not only is there concern as to whether this number is sustainable (Sadovy & Vincent 2002), the methods of capture may themselves cause a serious problem. For example, cyanide fishing is used to asphyxiate target fish (marine ornamentals) temporarily to make them easier to catch (Mak *et al.* 2005). Too high a dose of cyanide poisons both target and non-target species, including invertebrates such as anemones and the coral substrate (Cervino *et al.* 2003). As fishes caught in this way often die later, more are caught than is necessary to accommodate for this loss (Bell *et al.* 2009). Even when using less detrimental legal netting techniques, post-transport mortality is common: an estimated 70% of aquarium fish die within one year of collection (Wood 2001).

There are several alternative methods for fish capture that can reduce the negative impact on the biological and physical environment of that capture. Post larval capture for culture (PCC) is one alternative that has been approved by the Marine Aquarium Council and the International Coral Reef Initiative (Bell *et al.* 2009). Approval has been granted because it appears that PCC allows for the collection of fish with minimal environmental impact while also providing a livelihood option that is likely to benefit coastal communities. The reduction in impact on adult stocks is due to fish being removed before the high rates of natural mortality that coral reef fish with a bipartite life cycle normally experience during settlement while leaving the adult brood stock of the target population intact to seed future generations (Doherty 1991, Doherty *et al.* 2004, Almany 2004, Bell *et al.* 2009). Furthermore, as PCC involves collecting species before they have reached the reef, non-target species that are usually damaged during the collection of marine ornamentals also benefit. Given these advantages and the fact that PCC offers an alternative to capturing adult fish that is both environmentally and economically sustainable (Lecchini *et al.* 2006), the number of PCC programmes has grown since the first was established in French Polynesia in 2002 (Dufour 2002). There are, currently, six PCC facilities in operation in the Indo-Pacific, which collect settlement-stage fishes using a variety of tools (e.g. light traps, crest nets and plankton nets).

Ecocean (http://www.ecocean.fr/en/) is a consultancy that designs cost-effective methods to collect settlement-stage larval fish for PCC. Of those that Ecocean offer, light traps will collect fish in good condition, unlike plankton nets, and can be used in a broader range of geographic locations than can crest nets, which are set in the surf zone. Light traps do, however, only collect phototactic coral reef fish, such as apogonids, lethrinids, pomacentrids, blennoids, serrandids and chaetodontids (Leis & McCormick 2002). Regardless of the collection technique, larval fish vary in the numbers that move towards the reef across the lunar cycle, peaking around the new moon, and in the precise location along the reef of recruitment (e.g. Meekan *et al.* 1993, Victor 1984).

While the variation in larval supply is unavoidable there is the potential for the catch of reef fish larvae in light traps to be increased: it seems plausible that, given the attraction of larval fish to reef noise, the addition of reef noise to light traps may increase the attraction of settlement-stage fish to light traps by as much as 70% (Simpson *et al.* 2004, Leis *et al.* 2003). However, to date the enhanced attraction of larval coral reef fish to light traps by the addition of reef noise has been shown only at Lizard Island and it is not clear whether sound would increase light trap catches in other locations. To determine whether the effect of sound was general, I carried out an acoustic playback experiment in the Philippines, where I presented light traps with underwater speakers. I predicted that if the attraction of larval fish to reef sound is a general and widespread response, more fish would be caught in those traps broadcasting reef noise.

4.3. Experiment 1: An acoustic playback experiment in the Philippines

4.3.1. Methods

The experimental treatments

Each night four light traps (designed by Ecocean, St Clément de Rivére, France; Figure 4.1) were deployed. Two were unmodified, broadcasting only light, and referred to as the 'silent' traps, and two were adapted to house an underwater sound system. These 'sound' traps broadcast light and sound.

The sound system consisted of an MP3 player, a 12V lead-acid battery, 18W Universal Amplifier Module (Kemo-Electronic GmbH, Lanhen, Germany), and an Electrovoice UW30 underwater speaker (Lubell Labs, Columbus, OH, USA). The sound recording was taken using an Edirol R1 recorder, and a HTI-96-MIN omni-directional hydrophone with a built in preamplifier (High Tech, Inc., Gulfport, MS, USA). The original recording was taken at 8.40 am on the 16th June 2007, at Black Forest Reef, a marine protected area located to the southwest of Bohol (09°31.23'N, 123°40.99'E). To produce a clean one-minute reef sound clip, non-reef sounds were deleted from the original recording (e.g. the sound of water slapping the hull of the boat). This was done in Audacity 1.2.6. (a free digital audio editor available at http://audacity.sourceforge.net/). This recording was played on continuous loop when the traps were deployed.

Figure 4.1.: The light traps used in the acoustic playback experiment in the Philippines. These traps were designed by Ecocean to be used by artisanal fishermen who were collecting settlement-stage larval coral reef fish for a post-larval capture for culture facility.

To investigate how the broadcast recording attenuated with distance from the sound system, an assistant was dropped off the boat into a sea channel near the experimental playback site. The assistant used a GPS to keep the sound system in the same position while broadcasting a sound file on continuous loop. The playback was a broad spectrum mix of sounds, that included a reef

recording, followed by a pure tone sound. At the same time, while the boat drifted downwind I tracked our position relative to the sound system by using a second GPS on the boat, taking recordings of the played back sound at known distances from the sound system. Recordings were taken using the handheld recorder, with the hydrophone placed off the side of the boat, 2 m below the water surface. As a reference point for the ambient sound, a recording was also taken at the site where the light traps were set. At the source, the broadcast sound was 13 dB louder than ambient, at 10m it was 4 dB louder, and past 20m it was 3 dB quieter (Figure 4.2). From this we estimated that the broadcast sound was louder than the ambient background up to 15 m away from the speaker.

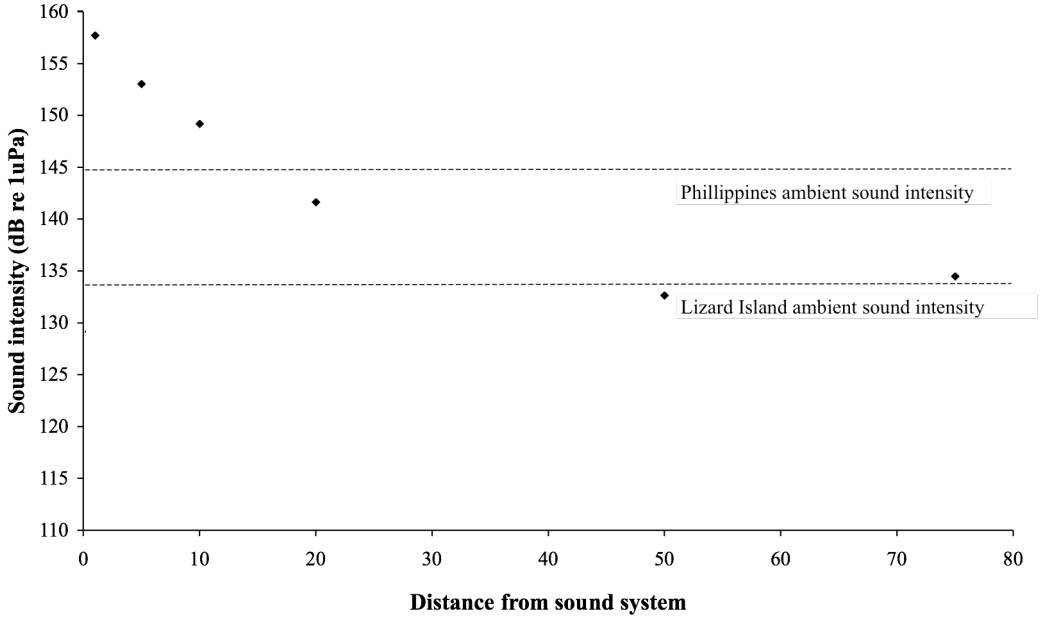

Figure 4.2.: The distance a pure tone sound could be detected over the ambient sound intensity. Dashed lines indicate the ambient sound intensity taken from recordings at experimental site in the Philippines and at Lizard Island.

Trap deployment and collection

The experiment was conducted over 21 nights from the 4th-24th July 2007. Light traps were set at surface moorings located in a sea channel to the northeast

of Pangapasan Island, Bohol, central Phillippines (10°01.1'N, 123°56.2'E). The moorings were anchored on a sandy substrate in water of 10-12 m depth. There was no reef present within 50 m of each mooring and they were separated by c. 400 m, to prevent acoustic overlap of the different traps broadcasting sound. The area that the broadcast sound was detectable was estimated to be 20-50 m (Figure 4.2). Each night, the traps were pseudo-randomly assigned to a mooring, so that the sound and silent treatments were tested multiple times at each position during the experiment. I deployed the traps at dusk (1800 hrs), left them overnight and collected at them at dawn (0530 hrs), when the catches were transferred to separate polystyrene cool boxes and transported by boat to the nearby aquarium facility in Matabao, Bohol. Reef fish were separated from the rest of the catch (primarily of invertebrates and clupeids) and identified to family, or when possible, species level and counted. I then handed the fish over to Ecocean for a rearing-for-release scheme.

Analysis

As it was not known whether it was appropriate to treat each captured fish as a statistically independent data point, two approaches were taken for the analysis. A sign test, which makes no assumptions on the independence of fish caught, was used to test whether the silent and sound-treated traps differed in the number of nights on which each caught the largest number of fish. As this test has a low power to detect a treatment difference when the number of testable nights per family is low (after excluding ties), I used a second approach to estimate the effect of the sound treatment on the number of larval fish caught by fitting a generalised linear mixed effects model (GLMM). While this method does assume larvae entered the trap independently (which is probably not the case), it will take into account the temporal and spatial variation that is characteristic of larval fish capture by light traps.

Counts of larvae were grouped by family and families from which fewer than 10 individuals were captured over the experiment were excluded from the analyses. Counts of fish per family were not normally distributed (Shapiro-Wilk test, $W = 0.1878$, $p < 0.001$), so a logarithmic link function and Poisson error distribution was specified in the GLMM. This was because the data set was bounded by

zero and the variance in counts per family was not equal. Mooring, and day were fitted as random effects, as the distribution of larval fish off the reef is patchy and their abundance changes over the lunar cycle. An interaction between sound treatment and family was fitted as a main fixed effect. The models were fitted using maximum likelihood. Deviance statistics (estimates of how well the model captures the data) were generated for each model with and without the explanatory variables. To obtain the significance levels of the explanatory variables, the deviance statistics were compared using Chi-square tests. All analyses were implemented in R (R Development Core Team 2007).

4.3.2. Results

Only twenty nights of data were collected as bad weather on one night caused all traps to be retrieved. A sound system failed on one occasion and on another a mooring was stolen, preventing a silent trap from being set, leading to a total of 18 nights data with sound-treated and silent-control trap deployments.

A total of 317 larval coral reef fish from 14 families were caught (Table 4.1). The seven most common families (Apogonidae, Holocentridae, Lethrinidae, Lutjanidae, Pomacentridae, Siganidae and Tetraodontidae) comprised 92% of the total catch. In six of the seven families more fish were caught in the silent traps than were caught in the sound traps, but only one (the Lutjanidae) significantly so Table 4.1; Figure 4.3). Additionally, the effect of adding reef noise to light traps was apparent on the light-trap catches even when the day-to -day and mooring variation in larval catches was included in the analysis. The effect that reef noise had on the catch rate of larval fish in light traps varied by family (GLMM χ^2: 14.41, $p < 0.05$). When six of the seven most abundant families (Apogonidae, Holocentridae, Lethrinidae, Lutjanidae, Pomacentridae and Siganidae) were grouped together, more larvae were caught in the silent traps compared to the number caught in the sound traps (GLMM χ^2: 15.24, $p < 0.001$), therefore the effect that reef noise had on the catch rate of larvae in the sound compared to the silent traps was evident over and above the day to day variability in larval distribution across the different mooring locations. For the last family, the Tetraodontidae, there was no difference between the number caught in the sound or silent traps (*post hoc* Mann-Whitney, W: 118.5, $p > 0.05$).

Table 4.1.: Summary of catches of settlement-stage coral reef fish larvae caught in light traps with broadcast reef noise (sound) and without (silent). Low catch rates prevented analysis of some of the families. Results (significance levels) of the sign tests per family are shown (see methods for details).

Family	Silent	Sound	Total	Sign test
Apogonidae	31	12	43	0.226
Holocentridae	22	13	35	0.133
Lethrinidae	8	2	10	0.125
Lutjanidae	14	4	18	0.015
Pomacentridae	13	1	14	0.062
Siganidae	57	26	83	0.5
Tetraodontidae	45	44	99	0.5
Blennidae	1	1	2	
Carangidae	2	2	4	
Chaetodontidae	0	1	1	
Mullidae	2	5	7	
Scaridae	0	2	1	
Sphyraenidae	1	4	5	
Syngnathidae	2	2	4	

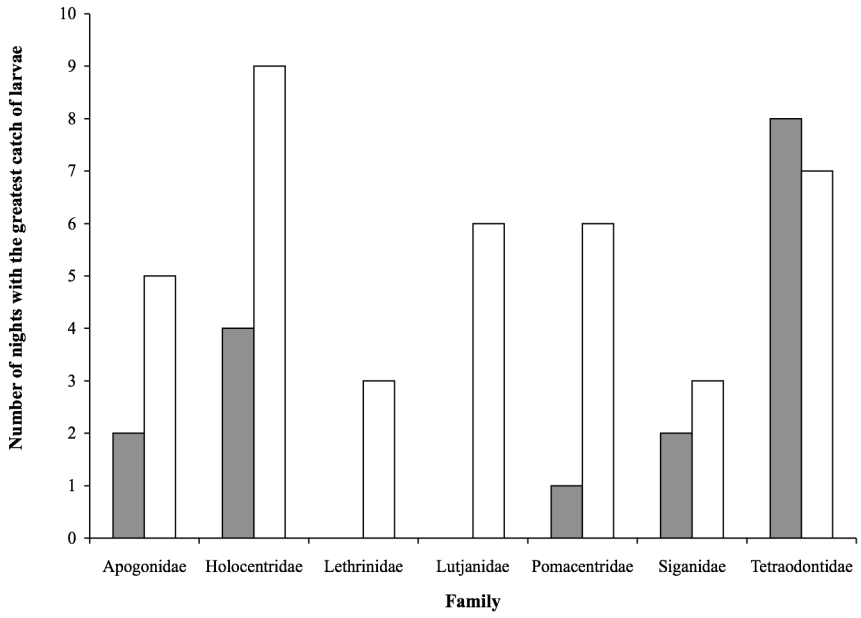

Figure 4.3.: Number of nights with the greatest catch per treatment deployed with speakers (grey) and without (white) from the 4^{th}-24^{th} July 2007, Bohol, the Philippines.

4.3.3. Discussion

Settlement-stage larval fish were not attracted to the recording of reef noise when it was broadcast from light traps. Contrary to our prediction that the sound-treated traps would attract more fish, more individuals of six of seven families were caught in the silent traps than were caught in the sound-treated traps. These data contrast those from four previous acoustic playback studies in which fish (of the families caught in this study: Apogonidae, Holocentridae, Lethrinidae and Pomacentridae) were attracted to the broadcast recording of reef noise, resulting in a greater abundance being caught in the sound-treated traps (Leis *et al.* 2003, Simpson *et al.* 2004, 2005, 2008). These data from Experiment 1 are, however, consistent with those of Leis *et al.* (2003): settlement-stage fish may respond negatively as well as positively to reef noise broadcast from light traps.

Little consideration has been given to the possibility that settlement-stage fish may choose to avoid reef noise rather than go towards it, even though both Leis *et al.* (2003) and Simpson *et al.* (2004) found significant avoidance of reef noise by two families. It is not clear why the fish avoid the reef noise. It could be that the fish prefer a quiet environment, which is consistent with apogonids and pomacentrids preferring quiet traps in quiet environments (offshore) but preferring sound traps when in a noisy environment (just off the reef; Leis *et al.* 2003. In our experiment, the ambient acoustic conditions of the playback site appeared to be substantially quieter than the area at which the sound recording was taken, which was 60 km away in a marine protected area. There the noise of the reef sounded to the human ear, at least, substantially different from the reef noise in area where it was played back. The playback region was a channel flanked by two reefs that had degraded to urchin and algal dominated.

It is also possible that fish avoided the particular recording that was broadcast. Firstly, the sound was taken 60 km away and it is plausible that it was either unfamiliar or unattractive to reef larvae because it did not resemble sufficiently closely the local ambient acoustic conditions. Structural analysis of those sounds would be necessary to establish whether this is the case. Secondly, it is possible that larval fish avoided the sound traps because of time of day effects. It appears that larvae will move away from the reef during the day and move towards it at

night (Leis & McCormick 2002, Stobutzki & Bellwood 1998). By avoiding the reef during the day, and settling at night, larvae could decrease their exposure to predators and aggressive resident fish (Leis & Carson-Ewart 1998, Stobutzki & Bellwood 1998). The noise of the reef might enable the successful return to the reef at night (Leis & McCormick 2002). This seems an unlikely explanation for the fish avoiding the sound traps in this experiment because I played reef noise at night, unless it is the case that the time of day effects of reef noise are more subtle than this. Coral reef noise varies in intensity across the day and there is some suggestion that the playback needs to be recorded from a reef at night as well as being played at night (Cato 1978, Radford *et al.* 2008). So far, settlement-stage fish have been shown to be attracted to nocturnal reef recordings at night (Leis *et al.* 2003, Leis & Lockett 2005, Simpson *et al.* 2004, 2005, 2008, Tolimieri *et al.* 2004). The playback that I used was recorded from a reef in the morning, so it is plausible that the fish recognised this as morning noise and therefore avoided it.

Finally, it is possible that use of a single playback, here and in all previous acoustic playbacks to coral reef fish larvae, i.e. pseudoreplication, may explain the success of a Lizard Island recording to attract fish to sound traps at Lizard Island and the failure of a Philippine sound recording to attract larval fish to sound traps in the Philippines (Slabbekoorn & Bouton 2008, Plowman 2006). In both cases it is possible that there was a specific feature or features that were attractive (Lizard Island) or repellant (Philippines). In the following experiment I attempted to address this by using multiple recordings.

4.4. Experiment 2: An acoustic playback experiment at Lizard Island

The content of the recording may actually determine whether larvae are attracted to or repelled by reef sound. One explanation for the avoidance by fish of sound traps in the Philippines was that I used a single recording and that recording was unattractive to settlement-stage fish. Pseudoreplication, or the use of a single playback recording limits the interpretation of the results from Experiment 1 (Hurlbert 1884, Kroodsma *et al.* 2001, Slabbekoorn & Bouton 2008). It is still

not clear what effect the addition of reef noise playback may have on the capture of larvae in Ecocean traps, as the phonotactic power of only one reef recording has been tested. I had followed the experimental protocol as detailed in seven other studies in which acoustic playback was used to test settlement-stage fish decision making and resulted in preference for coral reef noise over silence. Of these, four used the same sound recording for playback (Simpson *et al.* 2004, 2005, 2008, Tolimieri *et al.* 2004), and the remaining three used one other (Leis & McCormick 2002, Leis *et al.* 2003, Leis & Lockett 2005). While larval fish strongly preferred the test noise in these studies it is not clear whether this attraction was specific to the chosen test recording. It is, therefore, not yet clear whether the attraction or avoidance of reef larvae to reef noise is a general behaviour or one specific to the recordings used in the studies at Lizard Island. The same problem arises with interpreting the data from Experiment 1. To test whether pseudoreplication might have explained the results from Experiment 1 I carried out a second acoustic playback experiment. In this second experiment, I used eight different recordings as the test playback.

The avoidance by larval fish of sound traps in the Philippines could have been due to the traps I used. These Ecocean traps were designed to be used by artisanal fishers to collect fish for the post-larval capture for culture facility and have a completely open entrance at the top, which could mean that fish entering the trap were more vulnerable to predation. It is plausible that the reef noise was in fact attractive but that it was attractive to predators as well as to larval fish. The fewer larvae caught in the sound-treated traps relative to the silent traps may have been due to predator activity rather than anything to do with the response of larval fish to reef noise. In an attempt to determine whether predation may have led to these reduced catches in Experiment 2, I used two trap types in addition to the sound playback. The second trap type was a light trap of the Stobutzki & Bellwood (1997) design. This trap which has a more enclosed entrance may provide greater protection from predators. It was these Stobutzki and Bellwood traps that were used in the Lizard Island studies in which more larvae are caught in light traps broadcasting reef noise (Leis *et al.* 2003, Simpson *et al.* 2004, 2005, 2008). While I could not quantify predation I could use the abundance of larval reef fish caught in these two light traps designs to determine whether trap design explained the results from Experiment One.

In this experiment I made two predictions: (1) if larval fish are attracted to reef noise at Lizard Island, then the attraction to reef noise would not be dependent on the recording used; (2) if predation does cause a reduction in the number of larvae caught in Ecocean sound treated traps, then fewer larvae would be caught in the Ecocean traps than in the Stobutzki and Bellwood traps.

4.4.1. Methods

The experimental treatments

Four traps were used in this experiment, two of the Ecocean and two of the Stobutzki and Bellwood design (Figure 2.1). The Australian Institute of Marine Science (AIMS) kindly allowed the latter to be used for the study and they will be referred to as the AIMS traps. Every night, 2 'silent' unmodified traps and 2 'sound' treated traps of each design were deployed. The sound treated traps were created by coupling an underwater sound system to the light unit (for specification of the sound system see Experiment 1). The sampling period of this experiment was centred around the December new moon (9^{th} December 2007), and the recordings used were taken one month prior, during the November new moon period (8^{th} -11^{th} November 2007). The time series of recordings taken in Chapter 5 provided the reef recordings for this experiment. These were all taken at a fixed location, on a reef adjacent to where the light traps were deployed.

There were a total of 8 recordings that were of sufficient length (one minute) that were used for the sound treatment. Each night one of these recordings was pseudorandomly assigned (Table 4.2), and was broadcast on continuous loop throughout the night from both of the sound treated traps. A recording was taken at the site where the sound was being played back and used a reference for the sound intensity of the ambient reef sound. This ambient sound intensity was compared with the broadcast sound attenuation curve from Experiment 1. At the speaker, the broadcast sound was 23 dB louder than the ambient sound, and at 20 m away, it was still 14 dB louder. The played back sound should have been quieter than the ambient reef noise at a distance greater than 50m (see Figure 4.2).

Table 4.2.: Date and time that each recording was taken and when it was used as the test sound treatment in December at Lizard Island.

Recording	Day taken (Nov)	Time taken	Date played (Dec)
1	8th	23.00	6th , 16th
2	9th	5.30	12th , 14th
3	9th	23.00	11th
4	10th	2.00	7th
5	10th	5.00	9th
6	10th	20.00	10th , 13th
7	10th	23.00	8th
8	11th	5.00	5th , 15th

Trap deployment and collection

The experiment ran over 12 consecutive nights from the 4th-15th December 2007, which includes the new moon period, the time when settlement-stage larval fishes peak in abundance in light trap catches at Lizard Island (Meekan *et al.* 1993). The traps were set from moorings which were anchored in water of 10-15 m depth. These moorings were arranged in a line that ran parallel to shore, with a minimum of 200 m separating each mooring anchor. There was no reef present in the 100 m^2 around each anchor, as confirmed by nautical charts and SCUBA surveys. Each night the trap type and sound treatment was randomly assigned to a mooring, hence each treatment was tested at the different mooring positions (Table 4.3). Light traps were deployed at dusk (1830 hrs), left to collect fish overnight and brought in after dawn (0530 hrs). The trap catches were transferred into separate 10 l buckets filled with seawater and were transported back to the research station by boat. Coral reef fish were separated from the rest of the catch (mainly invertebrates and clupeids) and were given a supply of aerated flowing seawater in the bucket. Fish were identified to family level and counted, after which they were returned to the reef by boat.

Data analysis

Although four traps were set for 12 consecutive nights, catch data were available from only 45 of these. Traps failed on three occasions: twice due to battery

Table 4.3.: The number of nights that fish were collected and analysed (in parentheses) from each mooring by trap type and sound treatment. Traps were deployed a total of 12 times, however three traps failed. The two nights on which this occurred, were removed from the data set prior to analysis. This meant that although each trap type and treatment was represented on each night in the dataset, they were not distributed equally across the moorings.

Treatment	Mooring				Total
	A	B	C	D	
AIMS silent	4 (4)	3 (3)	2 (1)	2 (2)	11 (10)
AIMS sound	2 (2)	3 (2)	3 (3)	3 (3)	11 (10)
ECOCEAN silent	2 (2)	2 (1)	4 (4)	3 (3)	11 (10)
ECOCEAN sound	3 (2)	4 (4)	2 (2)	3 (2)	12 (10)
Total	11 (10)	13 (10)	10 (10)	11 (10)	45 (40)

power loss and once due to the system flooding after the surface bouy holding the sound system above the water was deflated. Data from nights when traps failed (6^{th} and 12^{th} December) was excluded from the analysis, so that each trap type and sound treatment was represented for the same number of nights. This left a total of 10 nights for which data were available for all four treatments, a total of 40 trap deployments, 10 per trap and sound treatment (Table 4.3). There was substantial variation in the total number of fish caught per night of the experiment, which prevented using parametric analyses. Binomial tests were run on the number of nights with the greatest catch with separate tests run to test for effects of moorings, trap types and sound treatments on the number of larval reef fish caught. All of the analyses was implemented in R (R Development Core Team 2007).

4.4.2. Results

In total, 63, 610 reef fish larvae were caught. The number of fish caught per night varied from a minimum of 419 to a maximum of 14,853 (Figure 4.4). Overall, 70% of the total number of reef fish larvae were caught over three days of the experiment. Larvae from 23 different families of coral reef fish were captured (Table 4.4) and the two most abundant families, Pomacentrids and Apogonids

made up 98% of the total catch. This composition is typical of light trap catches from Lizard Island (Simpson *et al.* 2005, Leis *et al.* 2003). If the total number of larval fish caught over the whole experiment is considered, then playback of reef noise around both trap types increased the catch (χ^2: 20.56, df = 1, p< 0.001). Overall the AIMS traps caught more larval fish than the Ecocean traps (Table 4.4, Figure 4.5; χ^2: 15852, df = 1, p< 0.001).

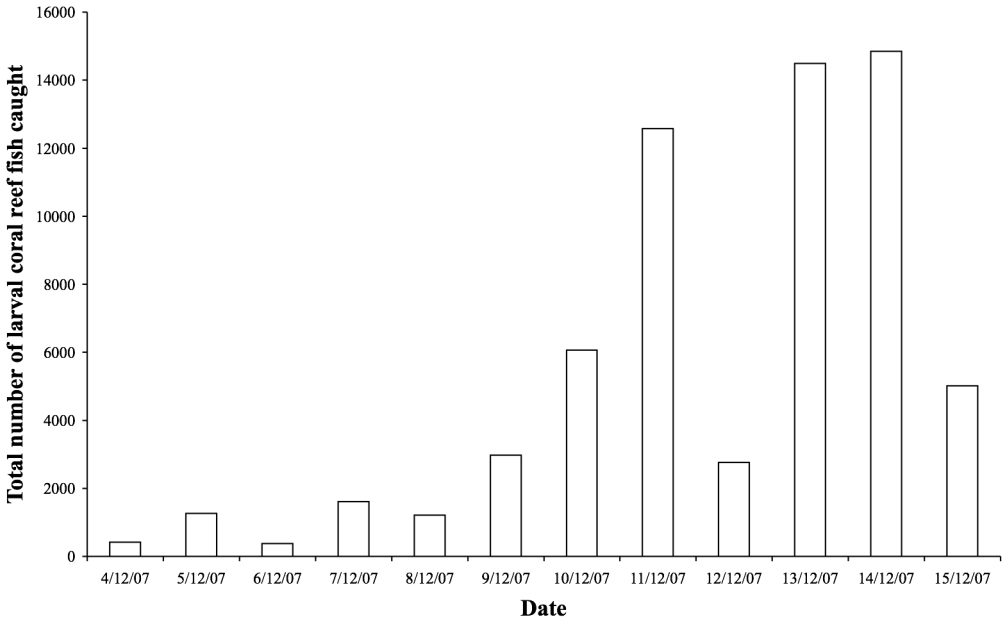

Figure 4.4.: The total number of larvae caught each night of the experiment. Each night larvae are grouped together by trap type and sound treatment. On the 6th December one trap failed (Ecocean Silent) and on the 12th December two traps failed (AIMS Sound due to battery failure and AIMS Silent due to the system flooding

The larval catch was not equally distributed across moorings (Table 4.5). When the total number of fish (grouped by family) is compared across the moorings, more were caught from moorings A (32% of the total catch) and B (39%), than were caught at moorings C (16%) and D (13%). There was, however, no clear pattern where moorings consistently caught more or less fish. Although moorings A and B caught more larvae overall, 13% of the total catch came from one night (14th December) on mooring A, and 22% of the total catch

Table 4.4.: The number of larvae reef fish caught by family per trap type and sound treatment. Families where more than 20 individuals were caught are ranked in order of abundance in the first half the table.

Family	Trap type and sound treatment				Total
	AIMS silent	AIMS sound	Ecocean silent	Ecocean sound	
Pomacentridae	20477	23114	1707	8393	53691
Apogonidae	885	749	1076	2830	5540
Blennidae	105	46	20	89	260
Lutjanidae	34	4	78	128	244
Caesionidae	15	4	44	105	168
Syngnathidae	81	33	18	0	132
Lethrinidae	12	7	18	70	107
Monocanthidae	46	7	12	8	73
Siganidae	0	0	14	39	53
Gobidae	8	13	8	19	48
Synodontidae	12	15	4	8	39
Carangidae	6	1	9	21	37
Nemipteridae	3	6	9	14	32
Serranidae	12	5	1	2	20
Acanthuridae	1	0	6	4	11
Belonidae	0	0	0	2	2
Chaetodontidae	2	2	0	1	5
Holocentridae	2	1	0	0	3
Muraenidae	0	0	0	1	1
Pseudochromidae	6	5	0	2	13
Scombridae	0	1	1	0	2
Sphyraenidae	1	3	0	1	5
Tetradontidae	3	3	1	0	7
Total	21711	24019	3026	11737	60493

was on another night (15th December) on mooring B (Table 4.5). If moorings A and B consistently caught more fish through the whole experiment, then I would expect the median values to follow the same pattern. In fact, mooring D which had the lowest total catch, had the second highest median value and mooring B with the highest total catch had the lowest median value (Table 4.6).

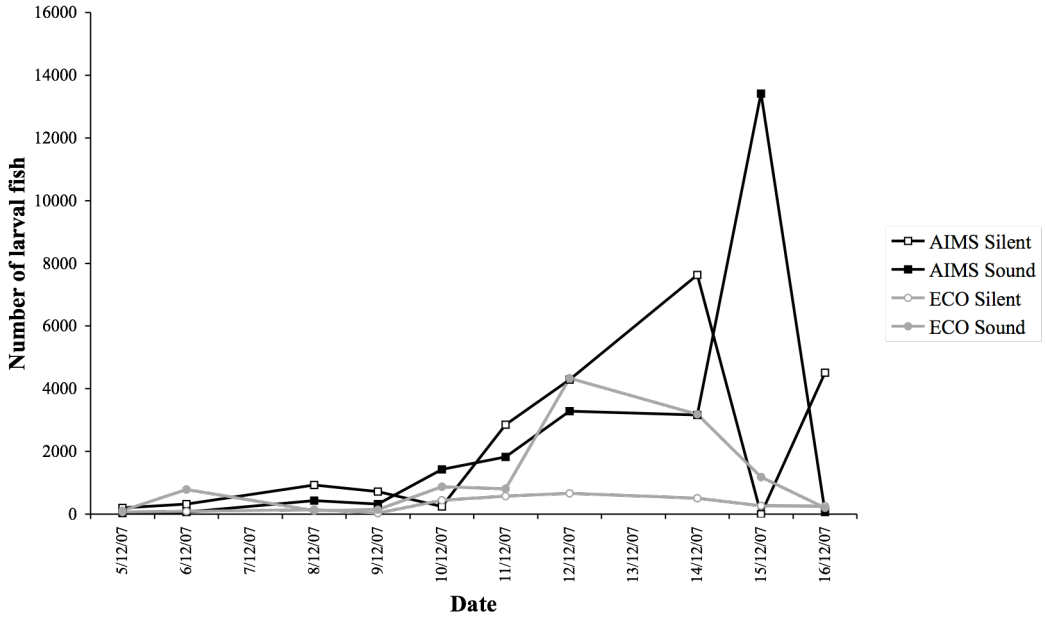

Figure 4.5.: The total number of larvae caught per light trap type on each night of the experiment at Lizard Island. Each night larvae are grouped together by trap type (AIMS: black line, Ecocean: grey line) and sound treatment (silent: open symbols, sound: closed symbols). On the 6th December one trap failed (Ecocean Silent) and on the 12th December two traps failed (AIMS Sound due to battery failure and AIMS Silent due to the system flooding

To determine the effects of mooring and of the sound treatment on the observed catch rates, I used sign tests to compare the number of nights with the greatest catch and tested for the effect of mooring location, trap type and sound treatment separately. This was because trap types and treatments and sound recordings were not represented equally across the mooring location (Table 4.3). Tests were run with the total catch data (grouped across all families) and again with the data from the two families that were most prominent in the catch, the Pomacentridae

Table 4.5.: The number of nights that silent and sound traps (AIMS and Ecocean grouped together) had the greatest catch relative to the other moorings across the 10/11 replicate nights of the experiments. The total number of nights differ because of three traps that failed.

Treatment		Mooring				Total
		A	B	C	D	
Total catch	Silent	5	2	1	2	10
	Sound	4	3	0	4	11
Pomacentridae	Silent	6	2	1	1	10
	Sound	2	2	3	3	10
Apogonidae	Silent	2	2	3	3	10
	Sound	2	4	1	4	11

Table 4.6.: The total number and median value of reef fish caught per mooring (A-D), with fish grouped across all families, and pomacentrids and Apogonids, which contributed 98% to the total catch. Mooring A caught more significantly pomacentrids (indicated by * , binomial test, p = 0.03), however, the median values do not follow the same pattern, so there was no consistent effect of mooring location of the abundance of fish caught.

Treatment		Mooring			
		A	B	C	D
All families	Total	19182	23861	9808	7622
	Median	799	283	456	469.5
Pomacentridae	Total	18631	23294	9014	5402
	Median	459 *	151	208	187
Apogonidae	Total	1960	1332	668	1972
	Median	95	88	44	81

and Apogonidae. For both the sound and silent traps, there was no difference between the number of larvae caught at the different moorings. This was evident when all families were grouped together, and when the catch of Apogonidae were analysed separately (Table 4.6). The Pomacentridae differed, as more fish were caught in the silent traps on mooring A on significantly more nights compared to the other 3 moorings (Binomial test, $p < 0.05$), however the number of nights with the greatest catch of Pomacentridae in the sound-treated traps was spread evenly among the moorings (Binomial test, $p < 0.05$).

To test for the effect of the trap design, I compared the number of nights with the greatest catch from the AIMS and Ecocean traps, separating the data for each of the experimental treatments (sound and silent). The two trap types caught a similar total number of fish and a similar number of Pomacentridae (Figure 4.6.A) but the Ecocean traps had caught more Apogonids than did the AIMS traps (Binomial test, $p < 0.05$, Figure 4.6.A).

Although for none of the three comparisons (total catch, the Pomacentridae and the Apogonidae) did the sound manipulation increase the number of fish caught (Figure 4.6B), the AIMS silent traps tended to catch more fish on more nights than did the AIMS sound traps while the opposite was true for the Ecocean traps, in which more fish were caught consistently in the sound traps compared to the silent traps (Figure 4.6.B).

There were not enough data to investigate what effect the sound recording used for playback had on the larval catch. From the data available, on four out of ten nights the playback recordings had the same effect on the catch of larval fish in both the AIMS and Ecocean traps. On two of these nights, the sound-treated traps caught more larvae than the silent traps, and on the other two nights, the silent traps caught more than the sound-treated. There were three recordings that were used on more than one night and the same pattern was observed on both nights in the sound and silent traps for only one of these recordings.

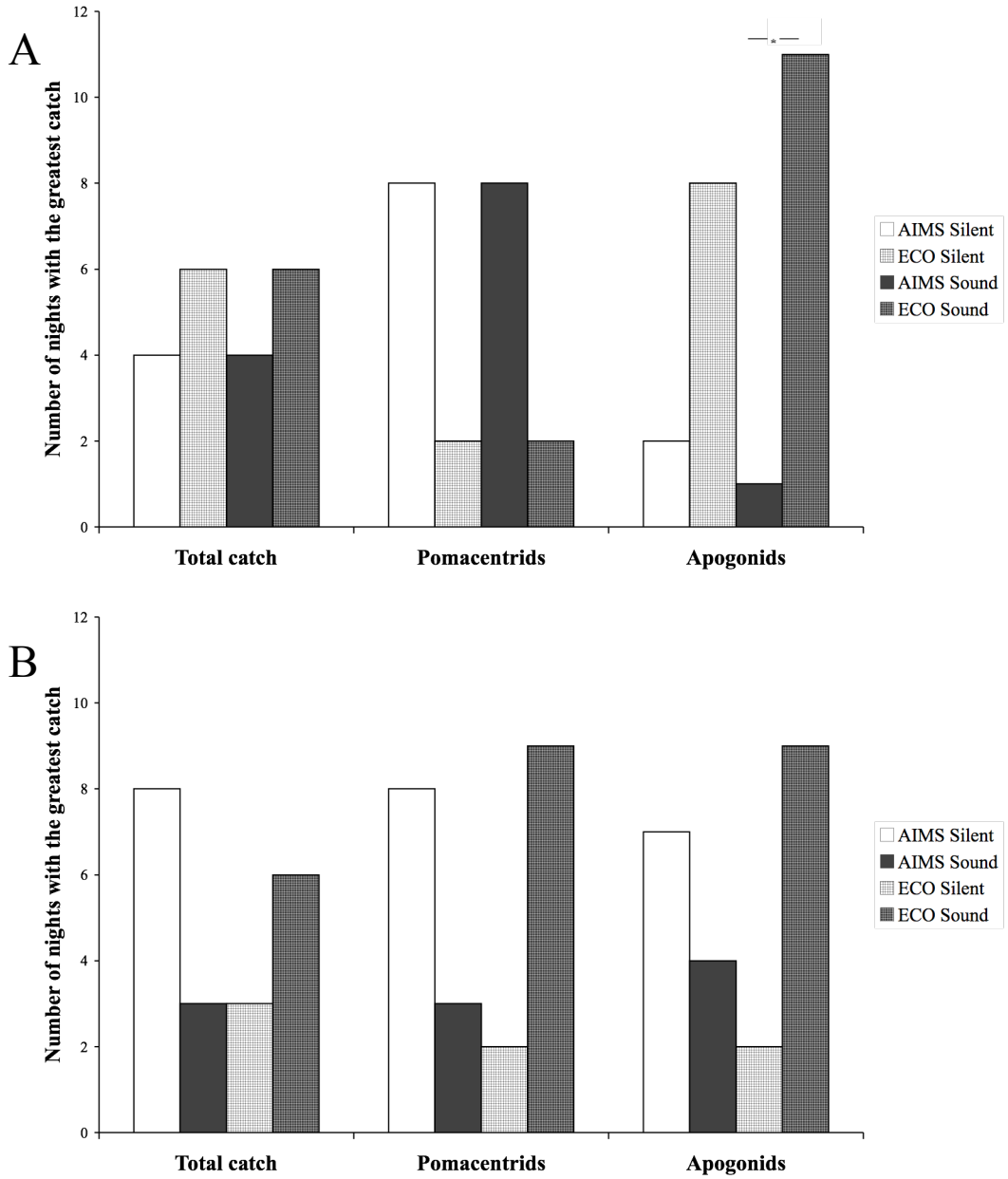

Figure 4.6.: The number of nights for which trap types (A) were compared and had the greatest catch and (B) sound treatments were compared and had the greatest catch. If the catches within a night were equal, then data from that night were not included in the test or in this graph. For the trap design comparison (A) traps were grouped by sound treatment, so AIMS silent compared with Ecocean (ECO) silent, and the same for the sound.

4.4.3. Discussion

Overall settlement-stage fish were caught in greater abundance in light traps that were broadcasting reef sound. This was true for the two traps that differed in design. The Ecocean traps caught far fewer larvae in total than did the AIMS traps. However, the addition of reef noise playback had a much greater impact on the catch of the Ecocean traps, as with sound these traps caught over three times as many larvae than did the Ecocean silent traps. These data, which suggest that the total abundance of settlement-stage fish is greater in light traps playing back reef noise, are consistent with the preference of larval fish for reef noise seen in previous Lizard Island experiments (Leis *et al.* 2003, Simpson *et al.* 2004, 2005, 2008).

In these previous playback experiments at Lizard Island one sound recording was used as the sound treatment. Seven of the ten families which were caught in higher total catches in the sound treated traps, were not caught in higher abundance over significantly more nights than the silent traps (Blennidae, Lethrinidae, Nemipteridae, Pomacentridae, Pseudochromidae, Syngnathidae, and Trichonotidae). Only four families (Apogonidae, Pomacentridae, Mullidae, Holocentridae) have higher catch rates for both measures (Leis *et al.* 2003, Simpson *et al.* 2004). Contrary to the prediction that the attraction of larval fish to reef noise at Lizard Island would not depend on the recording used, it appears that of the families previously reported to be attracted to a single test recording of reef noise, only the Apogonids and Pomacentrids are more generally attracted to reef noise leading to higher catch in sound treated traps.

The effect that reef noise playback has on light trap catches appears to depend on what measure is used to compare the catch from traps with and without sound. More larvae may have been caught in the AIMS traps in total, however there was no consistent difference between the number of nights where the AIMS traps caught more larval fish compared to the Ecocean ones. In fact, there was a tendency for the Ecocean traps to catch more larvae on more nights. Comparing the number of nights with the greatest catch may be a more suitable measure of whether light trap catches can be consistently affected by reef noise. Using this measure, the AIMS traps appear to collect more Pomacentrids than the Ecocean traps, whereas the Ecocean traps collect significantly more Apogonids.

Playback of reef noise around the AIMS traps led to a reduction in the number of fish caught of all families, whereas playing reef noise by the Ecocean traps led to a greater catch in the sound traps compared to the silent ones. The attraction of larval fish to reef noise at Lizard Island appears to be context dependent.

The ability to increase the catch of Apogonidae and Pomacentridae in light traps by the addition of reef noise playback may not be specific to just the two recordings nor the design of light traps used previously (Leis *et al.* 2003, Simpson *et al.* 2004, 2005, 2008). Firstly, this is contrary to my prediction that if predation causes a reduction in the number of larvae caught in Ecocean sound-treated traps, then fewer larvae would be caught in the Ecocean than the AIMS traps. Given the potential for Ecocean traps sound traps to catch more than the silent ones at Lizard Island, it seems unlikely that the reduced larval catch observed in the sound traps in the Philippines was due to the design of the Ecocean traps. The possibility remains that fewer fish were caught in the sound traps in the Philippines because the reef noise that was played back sounded sufficiently different from quieter ambient acoustic conditions of the playback site. Secondly, only a tendency for a greater catch in the Ecocean sound traps was apparent in these data, rather than a statistically significant difference. It is possible that the effect that reef noise playback has on the light trap catches is likely to vary by the reef recording that is used for the sound treatment. Although Ecocean traps tended to collect more larvae in the sound traps, whilst the silent AIMS traps caught more than the sound AIMS, on four out of ten nights, four separate reef recordings elicited the same effect in both the trap types. Taken together, it appears that not only will the effect of reef noise playback on light trap catches depend on the response of larvae to the reef recording that is used, it may also depend on the ambient acoustic conditions into which the reef noise is played back, and also the type of light trap that is used to catch reef fish.

4.5. Conclusion

The variation in the supply of larval fish is a major obstacle for people whose livelihoods depend on post-larval capture for culture of marine ornamentals (Bell *et al.* 2009). Part of this variation will be temporal, as was evident in both the Philippines and Lizard Island when the peak in larval catch rates roughly co-

incided with the new moon. These lunar cyclical patterns in larval supply are common and geographically widespread (Meekan *et al.* 1993, Victor 1984, Wilson & Meekan 2001, Hendriks *et al.* 2001, Aburto-Oropeza *et al.* 2009). It appears that the addition of reef noise playback to the Ecocean traps has had a significiant and consistent effect on the abundance of larvae caught in two different study locations. Although reef noise caused more larval fish to be caught at Lizard Island and less to be caught in the Philippines, in both cases the effect that broadcasting reef noise around light traps in both locations was apparent over and above the highly variable supply of larvae to the reef.

The potential for reef fish to be repelled by the recording used as the playback sound means that broadcasting reef noise from light traps will only bolster the catch of larval fish if a reef recording that is attractive to settlement-stage fish is used. At present, it is not clear what features within reef noise may cause larvae to be attracted to or repelled to the sound recording. Until this is established there will be the potential that reef noise playback around light traps will lead to a decreased catch of larval fish.

The two trap types offer quite different approaches to collecting settlement-stage larval fish. AIMS traps, with or without reef noise playback, offer the potential to obtain a greater total catch that is likely to be driven by one or two nights when larvae are captured in large quantities. Ecocean traps, if deployed with a recording that reef larvae are attracted to, offer the potential to capture a greater abundance of fish on a more reliable night by night basis. Whether a smaller but more constant, or a much larger but more fluctuating supply of larvae is best will depend on the post-larval for capture for culture operation.

5. The temporal variation in coral reef soundscapes

5.1. Summary

To investigate the natural variation in reef noise and to assess whether information about the resident community is present in the acoustic cues coming from a reef I collected data over a time series of sound recordings at a fixed position from a reef in each of two locations: Hoga Island in Indonesia and Lizard Island on the Great Barrier Reef. The reef noise at these two sites appears to vary across time in a similar way, day to day the noise of the reef peaked in intensity at dawn and dusk and over the lunar cycle around the new moon reef noise more intense than the full moon. Previously, the cyclical changes in invertebrate activity have been linked to the temporal patterns in reef noise, and larval fish may be attracted to invertebrate, high frequency sounds. In this experiment I collected underwater visual census data and observed the behaviour of a sound-producing fish species and it appears that the diel changes in the fish assemblage and the sound production of fish may also make a considerable contribution to the way reef noise varies with time. Given that larval fish are more sensitive to lower frequency sounds, which are typical of fish associated noise, it seems plausible that the fish associated sounds are a feature of reef noise that larvae use to orientate at settlement.

5.2. Introduction

During the pelagic phase, fish larvae may gain information about a potential settlement site from the noise of a reef (Tolimieri *et al.* 2000, Leis *et al.* 2003, Simpson *et al.* 2004, 2005, 2008). Just as larvae may use sound to remotely assess the reef as a potential settlement site, taking sound recordings of reef noise could be used in a research and management context to passively monitor ecosytem health (Sirovic *et al.* 2009, Sueur *et al.* 2008). Whether passive

acoustics can be feasibly used as a monitoring tool is being addressed with the design of underwater recording systems that have sufficient battery life and memory capacity to be deployed to collect data on the reef (Rountree *et al.* 2006, Lammers *et al.* 2008, Meyer *et al.* 2007). Research on both passive acoustics and larval fish orientation using reef-based acoustic cues require an understanding of what information is present within the 'acoustic footprint' of a reef, because it is not yet clear what information might be available from listening to reef noise (Montgomery *et al.* 2006). Contary to what the term acoustic footprint may imply, reef noise is not a static signal and it will change over time and with small movements within the recording location (Moulton 1958). This makes investigating reef noise from more than one site more complex than a direct comparison of how one reef sounds relative to another. The ability to compare reef noise from multiples site is essential to both understanding how larval fish may percieve the acoustic environment they encouter around different reefs, and if reef noise is to be used to monitor more than one coral reef location. For the potential to compare reef noise from multiple sites to be realised, it first has to be established how reef noise varies in one location and what information this may relay about that one site.

Reef noise, or the ambient reef soundscape, will be determined by the presence of abiotic and biotic sources of sound. Abiotic sounds are broad-ranging in frequency (from 100 Hz - 25 kHz), and include noise generated from waves, bubbles, and the wind and rain on the sea surface (Wenz 1962, Cato 1978, Wilson *et al.* 1985, Locascio & Mann 2005). In a reef soundscape, however, it is the biological sound sources that are the defining feature apparent in sound recordings taken of reefs (Wenz 1962, Cato 1978). Changes in the production of biological sound can alter the intensity of noise around shallow coastal waters by 20 dB, which represents a 100 fold increase in sound energy (Radford *et al.* 2008). There are several reasons for the biological component of reef noise to change in intensity. The crackling sound of snapping shrimp is an omnipresent feature to shallow, warm coastal waters around the world, and changes in the activity level of snapping shrimp makes a major contribution to changes in reef noise intensity (Johnson *et al.* 1947, Radford *et al.* 2008). Noise generated by other invertebrates such as the rasp of sea urchins feeding, and the sounds created by fish will also contribute towards the soundscape (Castle & Kibblewhite 1975,

McCauley & Cato 2000). It is, however, not yet known how changes in sound production by fishes may modify the reef soundscape.

Sound production by fishes is common, over 800 species of sound producing or soniferous species have been identified (Kaatz 2002). However, the soundscape will not just be determined by the presence or absence of sound producing species on the reef, it will also depend on the frequency of behaviours associated with sound production. Fishes can produce sound as a by-product of another behaviour, for example, the sound of a parrotfish biting coral, and changes in the feeding behaviour of parrotfish will determine the contribution that this sound has to the reef soundscape. Additionally, if a fish is a sound signaller, meaning it produces patterned and consistent sounds during inter- and intra-specific interactions, then the contribution that a sound signalling species makes to the soundscape will depend on the frequency of these sound-producing interactions. For example, the longspine squirrelfish *Holocentrus rufus* is most territorial at dawn and dusk and this is the time when the stocatto sounds produced by this species during aggressive interactions is most frequently present in recordings taken on the reef (Winn *et al.* 1964). Diel changes in the composition of the fish assemblage and fish behaviour are a regular feature of coral reefs. It, therefore, needs to be established how these diel changes in the fish community relate to daily changes in reef noise (e.g. Hobson 1965, Doherty 1983).

Frequent, cyclical, changes in sound production are common in underwater environments. Daily, lunar and seasonal effects can be detected in sound recordings of a variety of habitats (e.g. temperate rocky reefs: D'Spain & Batchelor 2006, Radford *et al.* 2008, coral reefs: Cato 1978, McCauley & Cato 2000, seagrass beds: Breder 1968 and river estuaries: Fine 1978). Sound intensity increases at dawn and dusk, is greater at night than during the day, is more intense around the new moon than the full moon, and is greater during the summer and spring than in the winter (Cato 1978, McCauley & Cato 2000, Radford *et al.* 2008). As yet, there is little evidence that can link these cyclical changes in the soundscape with the species that might be responsible, and certainly no *in situ* observations that can directly couple the presence of fish species with the changing soundscape (Sirovic *et al.* 2009). To understand whether reef noise varies with aspects of the fish community, sound recordings need to collected simultaneously with data on the fish assemblage as well as the

behaviour of soniferous species.

There are several approaches to quantifying the variation in reef noise once sound recordings have been collected. One method involves using the frequency of known vocalisations to estimate the abundance of a particular species. Although using sound recordings to monitor the abundance of a specific species has been attempted (Sirovic *et al.* 2009), and may work in the future, at present the number of unidentified biological sounds surpass the number of known sound producers underwater (Rountree *et al.* 2006). The potential for having unknown sounds within a reef recording is high, considering that coral reefs are the most species diverse underwater ecosystem type (Connell 1978). Therefore, methods are needed to quantify variation in reef soundscapes as a whole. This could be achieved by measuring the total energy of all the sounds that are detected within a sound recording of a reef and quantifying how the total energy in sound varies with time. If the heterogeneity of noise within a recording is measured, then this will capture variation in the sounds that make up the ambient soundscape and how the contribution of these sounds vary over the duration of a sound recording. Measuring the heterogeneity of sound recordings taken in different coastal habitats has been used to successfully classify recordings according to whether they were taken in an intact forest or one subject to deforestation (Sueur *et al.* 2008). Forest sites with increased biodiversity had more heterogenous sound recordings than the recordings taken at sites with reduced biodiversity (Sueur *et al.* 2008). The aim of my study was to document the temporal variation in coral reef soundscapes, and to test whether measuring the heterogeneity, or roughness within reef noise varies with the diel changes in the biodiversity of fish on the reef. To do this, I simultaneously collected underwater sound recordings with underwater visual census data on the reef fish assemblage.

To investigate how coral reef noise varies with time, I took reef sound recordings at a fixed point on a coral reef in two different locations, Lizard Island on the Great Barrier Reef, and Hoga Island, Indonesia. By taking recordings in a fixed position, I removed any variation in reef noise that might arise by taking recordings in a different position on the same reef, and so could concentrate on the temporal patterns in reef noise (Moulton 1958). By collecting recordings at two different locations, I was able to test whether the temporal patterns in reef noise were geographically widespread. I assessed the daily and lunar patterns in

reef noise at each site by measuring: 1) total noise intensity, 2) the intensity of specific frequency bands and 3) acoustic roughness. Although sound intensity can provide information on the average sound energy level within a recording or specific frequency band, it ignores the multi-dimensional nature with which sound can vary. I wanted to test whether acoustic roughness, which is a measure of the heterogeneity of sound, was a useful measure of quantifying reef noise as it should capture not only the total energy of reef sound, but also how variable this energy is within a sound recording. Using these three measures to assess the temporal variation in reef noise at Lizard Island, I was able to relate the daily and lunar changes in reef noise to the attraction of larval fish to acoustic cues, and the auditory sensitivity of settlement-stage fish that have also been studied at Lizard Island.

By comparing the recordings taken from the Great Barrier Reef with the recordings taken in Indonesia, I tested whether the temporal patterns in reef noise were the same in two different geographic regions. In Indonesia, I had the opportunity to collect underwater visual data on the fish community. Using this data, I was able to investigate how these three measures in reef noise varied in relation to the diel changes in the fish community. To do this, I collected data on the presence and abundance of different fish families on the reef, so that I could calculate the diversity of the fish assemblage. I was, therefore, able to assess the relationship between the noise of a reef, and the diversity and abundance of the fish assemblage.

The temporal patterns in underwater sound may be defined not just by the presence of sound-producing species, but also the frequency of their sound-producing behaviours. Therefore, I also collected data on the behavioural activities of a soniferous fish species, the jewel damselfish *Plectroglyphidodon lacrymatus*, which is common to the study site and throughout the Indo Pacific. This species lives in shallow water, in areas of mixed coral and rubble (www.Fishbase.org), where, as is common for pomacentrids, it maintains and defends an algal garden within a terrority (Meekan *et al.* 1995). In the laboratory, the vocalizations of this fish have been characterised as a popping sound (energy range 100-1000 Hz, average peak frequency: 328 Hz: Parmentier *et al.* 2006). These pops are produced in concert with an aggressive pectoral fin display, and are thought to be vocalised during territory defence (Parmentier *et al.* 2006,

Heenan pers. obs. 2009). I investigated whether the behavioual activities of this fish, including the territorial and aggressive interactions during which it produces sound, vary in accordance with the temporal variation in reef noise.

I predicted that reef noise would show the same cyclical patterns in intensity in the two different study locations. Acoustic roughness has not been used previously to describe sound recorded underwater, so I could make no clear prediction as to how it might vary. Instead, I aimed to establish how acoustic roughness varied with total sound intensity and the intensity of specific frequency bands, and I compared the temporal pattern in acoustic roughness at the two different recording locations. Finally, I predicted that if the jewel damsel shows diel changes in aggressive behaviour, then this would be evident as an increase in sound intensity within the low frequency bands that co-incide with this species vocalizations.

5.3. Materials and methods

5.3.1. Lizard Island

I took a series of recordings at a reef located in front of Lizard Island Research Station (14°40' 43.61"S, 145°26' 38" E) on the Great Barrier Reef, Australia. Recordings were taken at the reef edge in water that was 4-6 m deep depending on the tide. To reduce sounds produced by the boat interfering with the noise of the reef, the boat was tied off to a mooring that was located 30 m away from the reef. To take sound recordings, an omnidirectional hydrophone with a built-in pre-amplifier (HTI-96-min series, High Tech, Inc. Gulfport) that was connected to a handheld Edirol R1 recorder (Roland Systems Group, Bellingham, WA) was used. The recorder was not waterproof, so it was kept in a clear dry bag to stop saltwater corrosion. The protocol for taking a recording was the same each time: one person took the recording equipment and sat in an inflatable tube that was attached to the boat by a line. This line was was played out until the tube was positioned at the reef edge. At this point the person taking the recording placed the hydrophone 1 m below the sea surface and took a recording (24-Bit resolution, 44 kHz sampling rate). The gain level on the recorder was kept constant for all of the sound recordings that were taken so that when I returned

to the UK, they could be calibrated from relative to absolute units of sound intensity. Recordings were taken at 3-hourly intervals (0500, 0800, 1100, 1400, 1700, 2000, 2300, 0200 hrs) for four days centred around both the new moon (10^{th} November) and the full moon (25^{th} November) during the 2007 Austral summer. The recordings were transferred from the recorder to a computer and saved as uncompressed WAV files. At least thirty seconds of reef noise was required for the analysis, so the length of the recording period varied according to the weather conditions: in rough weather longer recordings were taken so that non-reef sounds (such as waves slapping against the tube or the clink of the anchor chain), could be deleted. This editing was done on the WAV files prior to the analysis, using Audacity 1.2.6 (a free digital audio editor available at http://audacity.sourceforge.net/). The non-reef sounds were then deleted. Although recordings were taken over four days around the full and new moon, for the analysis the number of recordings per time period varied from 2-4; this was because on one day it was not possible to access the site, and also rough weather conditions led to some recordings being unusable as the non-reef sounds could not be edited out to produce a clean 30 second sound clip.

5.3.2. Hoga Island

For the duration of the study recordings were taken at a fixed position (05°28'100" S, 123°45' 339" E) on the reef crest at Hoga Island, Sulawesi, Indonesia. The recording system comprised of a calibrated omni-directional hydrophone (HTI-96-min series, High Tech, Inc. Gulfport, MS, USA), which was connected to a Sony TCD-D8 digital recorder that was held in an underwater housing. In the housing, the recorder was operated via a Unidata Micrologger timing and delay unit. On each day of the experiment, I took the recording system to the study site just before first light so that it was in position in time for the first recording at 0545 hrs. A frame to hold the recorder was strapped to a coral outcrop, so that when it was attached, the hydrophone was positioned in water 1-4 m deep (depending on the tide). The system was left in position throughout the day and was programmed to record at three-hourly intervals. Each day, 15 minute (sampling rate 41 kHz) recordings were taken at 0545, 0845, 1145, 1445 and 1745 hrs, after which the system was brought back to the station and recordings were transferred to a computer and calibrated following

the same protocol as the Lizard Island recordings. Recordings were collected in blocks of days (22-24 June (new moon: 23^{rd}), 28-30 June (1^{st} quarter: 29^{th}), 6-7 July (full moon: 7^{th}) and the 9^{th}, and 14-16 July (3^{rd} quarter: 15^{th}). I was unable to access the study site by boat to deploy the hydrophone on the 8^{th} July, as all the boat drivers left the island to vote in the national elections, recordings were collected the next day (two days after the full moon). After inspecting the recordings, I discovered that a faulty connection between the hydrophone and recorder had rendered the 3^{rd} quarter and two of the new and full moon recordings unusable. In total, there were 41 recordings used in the analysis, with either two or three replicates per time interval for each lunar phase.

For every sound recording, I simultaneously collected underwater visual census (UVC) data along a fixed transect to record the presence and abundance of coral reef fish. The transect line (40 m), which was marked out at regular intervals with flagging tape, ran parallel to shore along the reef crest for 20 m on either side of the recorder. UVC were conducted by snorkling along the transect twice, first to record the presence / absence of mobile fish within 5 m of either side of the transect line (10 m wide), and second to record smaller, less mobile fish within 2.5 m of each side of the transect line (5 m wide). Fish were identified to the family level. As the 40 m transect was too far to survey during the dusk period (due to the rapid loss of light), 20 m was surveyed during this period with data from 10 m on either side of the recorder used in the analyses. The abundance of fish per family per m^2 was calculated and, for each census period, the Shannon-Weaver index of diversity calculated for the analysis.

After I completed the visual survey on the abundance of fish, I then carried out visual observations, by snorkel, on the behaviour of the jewel damselfish *Plectroglyphidodon lacrymatus*. To do this, I used categories of behaviour that I had classified during pilot trials that I ran prior to the start of the experiment. For the pilot trials, I randomly selected a focal fish at the reef crest close to the recording transect and I continually recorded the behavioural activities of that fish for 10 minutes. I repeated this four times and from this, categorized the most common behaviours of the jewel damselfish as foraging, taking cover and tracking, chasing and fleeing. For the latter three categories, which involved interactions with other fish, I noted whether each event involved a heterospecific, heterofamilial, conspecific or confamilial individual. I chose a

two-minute observation period for the experimental observations. This appeared to be long enough to capture a snapshot of the behavioural activities of this species, as none of the categories of behaviour were more likely to be observed in the 10 minute pilot trials when it was divided into two-minute observation periods.

Over the 12 days of the experiment, I collected data from 48 observation trials, one after each of the 0545, 0845, 1145, 1445 visual surveys on the abundance of fish. Again the light levels were too low to accurately see what fish were doing at dusk. The protocol for the observation trials was as follows: an individual Jewel damselfish was selected within 2 m of any point along the transect line. I did this pseudorandomly to avoid using the same individual more than once a day by noting their position relative to the transect line. Once I had located an individual, I positioned myself within 2-3 m of the focal fish and then left them to acclimatize to my presence for one minute. For two minutes, I watched the fish and recorded the frequency of each behavioural trait.

Calibration of sound files

I calibrated the recordings at the Bioacoustics laboratory at the University of Bristol. Following the protocol of Kennedy et al. (unpublished data), I converted the measures of sound intensity from relative to absolute units by using the recorder (at the fixed gain level used in the field) to take a recording of a reference signal of a known voltage. The reference signal was a pure tone 1 kHz sine wave which was created and played back using Avisoft-RECORDER (Avisoft, Bioacoustics, Berlin, Germany) via a USB National Instruments data acquisition (DAQ) module (NI USB-6251, National Instruments, Austin, TX). The peak-to-peak voltage (Vpp) of the reference sine wave was measured using an oscilloscope (HP / Agilent 54602B, Santa Clara, CA) and compared against the level of the reference signal in the recorded sound file which was measured in Avisoft-SASLab Pro #2 Version 4.52 (Avisoft Bioacoustics, Berlin, Germany). From the recording, the root mean squared (rms) voltage was compared against the absolute Vpp, based on 1 Vrms = Vp/1.41 and Vp = Vpp/2. This established any additional gain within the recording system, allowing the sound pressure level of the recordings to be expressed in dB referenced against the pressure level

for sound underwater which is one microPascal (1μ Pa). Finally, the sensitivity of the hydrophone (based on the manufacturer settings; -164.3 dB re 1Vp/μ Pa, with a frequency response from 2Hz to 30 kHz), was used to calibrate the sound level of the recordings in Avisoft to dB re1μ Pa, which is the unit typically used to report underwater noise levels (Cato 1978). During the calibration, low frequency system noise was identified, therefore recordings were high pass filtered (0-100 Hz) to remove this recorder generated low frequency signal.

Data analysis

It was necessary to take subsamples from the 15 minute sound files because of the computationally intensive analysis of the recordings. I determined that a one minute subsample was an appropriate length by calculating the mean sound intensity from subsamples of increasing length taken from the same sound recording (Figure 5.1). The longer the recording taken, the smaller the standard error associated with the mean intensity of noise. The mean intensity of reef noise from a one minute subsample differed from the 15 minute recording by only 0.01 dB, therefore one minute seemed a sufficient length to quite closely represent the overall sound conditions at the time the recording was taken. Because of the transient nature, or short term variability in the sound conditions, for each recording 3 subsamples were taken, one every 5 minutes of each 15 minute recording.

From these subsamples, the sound pressure level, amplitude level (rms) in dB re 1μ Pa was calculated and averaged for each recording and in turn averaged for each time period (0500, 0800, 1100, 1400, 1700, 2000, 2300 and 0200 hrs) for each lunar phase separately. The sound pressure level gives an indication of the overall noise intensity of a recording. In addition for each subsample, the sound level (rms) was also calculated for 1/3 octave bands (centre frequencies defined by ANSI S1.6-1984) to give an indication of which frequency bands were changing over the recording time periods. The rms is calculated by averaging the power of the sound signal over a set time interval, therefore it does not provide any information on the temporal structure or variation in sound. As ambient noise varies from moment to moment the recordings may contain sounds that are constant, transient, directional, omni-directional, broadband and tonal (Miksis-

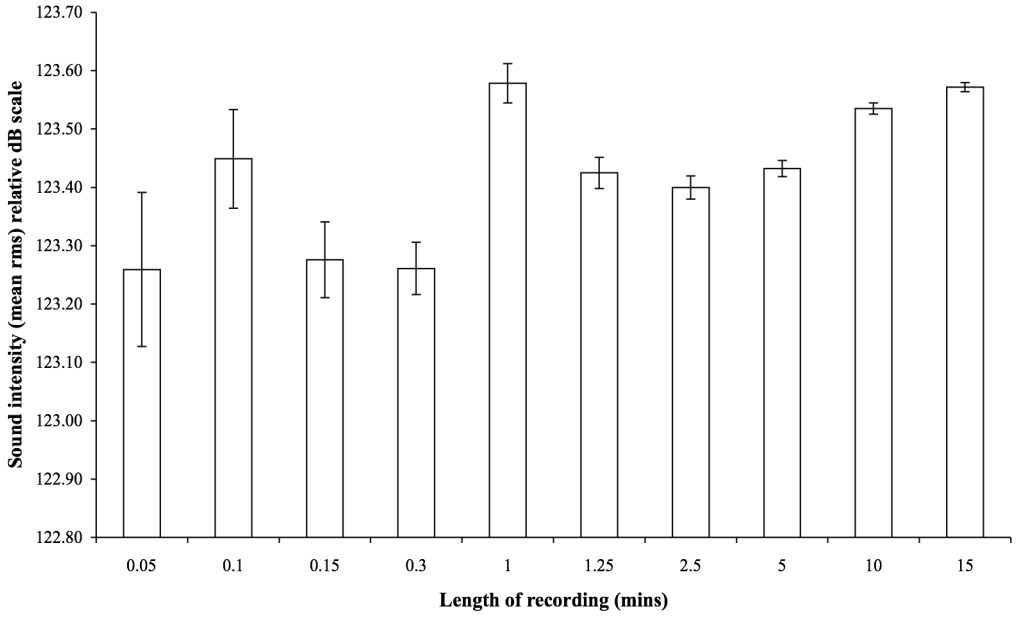

Figure 5.1.: A comparison of the sound pressure level, or sound intensity (rms) calculated from subsamples of increasing length taken from one of the 15 minute recordings from Hoga Island.

Olds *et al.* 2007), resulting in recordings with completely different characteristics having apparently similar average measures in rms or amplitude (Lei *et al.* 1994). Therefore, for each recording, I also calculated the 4^{th} moment (again using the subsamples and averaging for each recording) which measures the kurtosis for each recording and is sensitive to the sound level, the duration of transient sounds and the temporal structure in noise and therefore it captures the multi-dimensional nature with which sound can vary. It is a measure in which amplitude, frequency and how variable these are over time, are combined and high 4^{th} moment values represent infrequent and abrupt changes in the sound signal because the 4^{th} moment increases with increasing gaps between noises: this has been described as acoustic 'roughness' (Hubner & Wiegrebe 2003, Figure 5.2).

To determine how sound intensity varied with time, a two factor, non-linear multivariate ANOVA (with time nested in moon phase) was run for the sound level of each of the 1/3 octave frequency bands. In the case of the Indonesia dataset, the multivariate ANOVA also included the Shannon-Weaver measure of fish diversity, which was calculated from the visual census data. Significant effects were further explored in *post hoc* univariate tests using the least significant difference and significance levels calculated using Tukey's HSD. To visualise the changes in total amplitude of reef noise with time, non-linear regression models were fitted separately for the phases of the moon. The change in fish diversity over time was also analysed separately using a Wilcoxon rank sum test. The 4^{th} moment data were analysed with a linear model that included the sound intensity of each recording (rms amplitude) as a covariate, time of day and time of day nested in moon phase. To analyse the observational data I collected on the activity of jewel damselfish, the behavioural categories were treated as discrete events, and the frequencies with which each trait was observed were analysed separately, as a function of time, using non-parametric Kruskal-Wallis tests. The 1/3 octave filtering of the sound files was done in Avisoft-SASLab Pro #2 Version 4.52 (Avisoft Bioacoustics, Berlin, Germany), the 4^{th} moments were calculated using the R (R Core Development team 2007) package Seewave (Sueur *et al.* 2008) and the statistical analysis done using JMP Version 5.0.1.2 (SAS Institute Inc., Cary, NC).

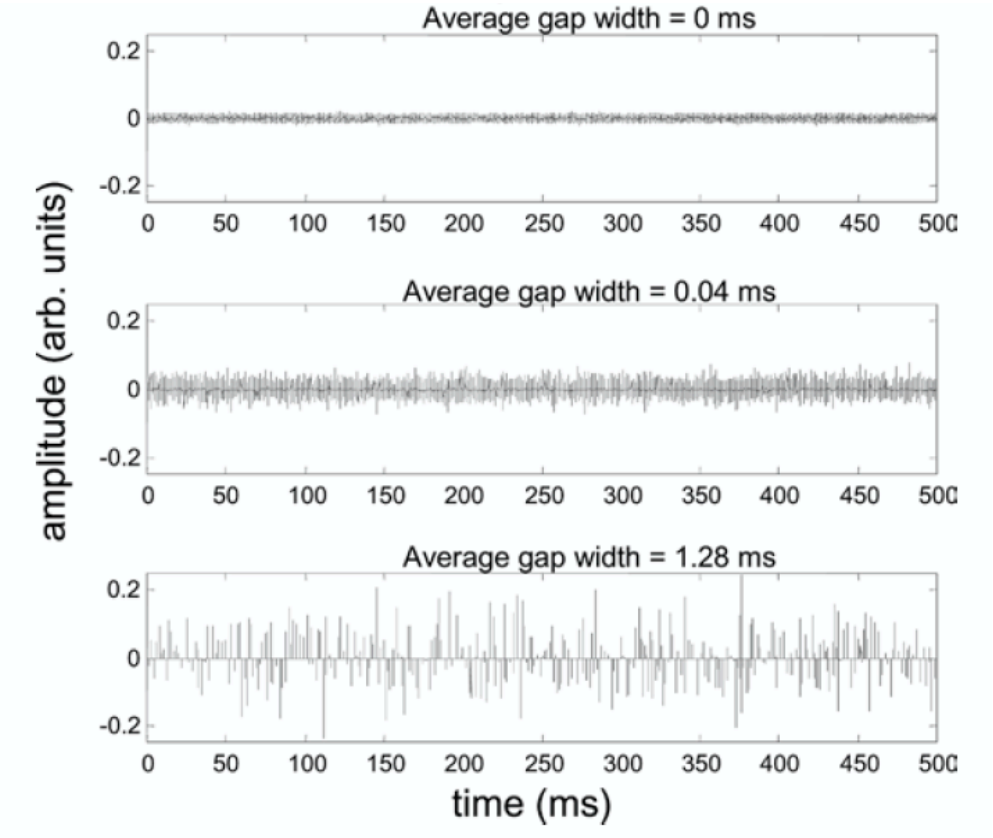

Figure 5.2.: The 4^{th} moment is a measure of kurtosis within sound. The 4^{th} moment increases with increasing gaps between noises. It combines both sound intensity and the duration of transient sounds within the recordings. Taken from Hubner & Wiegrebe (2003).

5.4. Results

5.4.1. Lizard Island

During the new moon, reef noise was more intense than it was during the full moon, there was also a greater change in amplitude from the night and crepuscular peaks to the mid-afternoon minima in reef noise intensity (Table 5.1 and 5.2; Figure 5.3; MANOVA, Roy's max root, approximate F = 5.28, df = 19, p <0.001). The reef recordings were analyzed at 19, 1/3 octave bands (250 Hz -16 kHz) across set times of day (every 3hrs, 7 times a day). The difference in noise levels between the new and full moon was driven by sounds in the 1500 Hz band, as this was the only 1/3 octave band that showed a significant moon effect: within the 1500 Hz band reef noise on the new moon was significantly louder than it was during the full moon (ANOVA, F = 5.53, df = 7,48, p <0.001). For all frequencies above 1500 Hz, the reef noise was greatest at dawn, followed by the recordings taken at night (in all bands from 1500 Hz-16 kHz ANOVA, F = 5.53, df = 7, 48, p <0.001, and *post hoc* Tukey HSD α <0.01). Reef noise was least intense during the day, with the minima occuring at 1400 hrs (in all bands from 1500 Hz–16 kHz, ANOVA, F = 5.53, df = 7, 48, Tukey HSD α <0.01). For the lower frequency bands (250-1260 Hz), there was no change in the intensity of sound over the phase of the moon, nor time of day, With the exception of sound in the 800 Hz band, which at dawn was 7 dB greater than it was during the 14.00 afternoon recording (Table 5.1 and 5.2; ANOVA, F = 2.48, df = 7, 48, p <0.05), there was no change in the intensity of sound over the phase of the moon, nor time of day for the remaining lower frequency bands (250-1260 Hz).

As the 4^{th} moment estimates did not vary consistently over time (Bartlett test for equal variance, F = 8.86, df = 7, 41, p <0.001; Figure 5.4), the data were analysed non-parametrically and the effects of the moon phase and time of day were assessed separately. Overall, reef noise around the new moon had higher values of the 4^{th} moment: the acoustic signal from the reef was rougher in comparison to the noise of the reef around the full moon (Kruskal-Wallis, χ^2: 9.73, df = 1, p <0.001). This roughness in sound also differed during the course of the day (Kruskal-Wallis, χ^2: 24.84, df = 7, p <0.001). During the night and at dawn, the noise of the reef was less rough in comparison to the day time

recordings. In particular, reef noise was most rough between 1100-1700 hrs. The decreased roughness at night and dawn means that the sound signal from the reef was more stable, and instanteous fluctuations in intensity and amplitude common, whereas in the afternoon fluctuations in the sound signal over time were more infrequent. When these data on the total sound intensity and 4^{th} moment are considered together, it appears that reef noise is more quiet and changes in intensity and amplitude more infrequent in the afternoon.

5.4.2. Hoga

Sound analysis

The intensity of reef sound varied in daily cyclical patterns for the 1/3 octave filtered bands and these differed with the phase of the moon (Figure 5.5; MANOVA, Roy's max root, approximate F = 212.95, df = 19, 21, p <0.001). *Post hoc* multiple comparisons showed reef noise was higher in amplitude at dawn and dusk and in some cases at 0845 hrs (Table 5.3). This was true for several frequency bands ranging from the lower band of 397 Hz up to 13 KHz (Table 5.3, Figure 5.5). In addition to a time of day effect, six of the 1/3 octave bands varied with moon phase (Table 5.3). Because of the regular pattern with which sound intensity would vary over the course of the day, when comparing reef noise over the phase of the moon, I only considered the statistical differences that arose between recordings taken at the same time of day during the different phases of the moon. This left the 4000 and 5040 Hz bands. Sound within these two frequency bands was significantly less at dawn on the full moon compared to dawn on the new and 1^{st}quarter of moon (Table 5.3, Table 5.4, 5.5, 5.6).

From the underwater visual census data, I found that species diversity of the fish assemblage varied over the course of the day. The fish community was least diverse at dawn and significantly lower than at the other survey times (Wilcoxon rank sum: 10.05, df = 4, p <0.05, Figure 5.6). Fish diversity did not correlate with the daily changes in amplitude of any of the 1/3 octave bands, although two bands did approach significance (397 Hz: ANOVA, F = 3.28 df = 1,40, p = 0.08; 500 Hz: ANOVA, F = 3.7, df = 1,40, p = 0.06). Reef noise in the low frequency bands of 400 and 500 Hz tended to be more intense at dawn when

the diversity of the fish assemblage was lower than any other time of day.

The roughness in the sound signal of reef noise changes with the time of day and also the phase of the moon (time of day x moon phase: $F = 9.05$, $df = 14, 40$, $p = <0.01$; Figure 5.7). During the day, reef noise was less rough and, therefore, more constant in amplitude and more constant in the distribution of noises of different frequencies, whereas at dusk reef noise was rougher and more variable in changes in frequency and amplitude over time (Tukey HSD: $Q = 2.3$, $p < 0.05$; Table 5.7). Reef noise taken at dusk around the new moon was also markedly more rough, compared to all the other recordings (Tukey HSD: $Q = 3.73$, $p < 0.05$; Table 5.7).

Fish behaviour

Foraging was the most commonly observed behaviour in the jewel damselfish. The frequency of foraging events and chasing of other heterospecific pomacentrid species differed significantly over the course of the day: foraging least at dawn, increasing during the morning to being common from noon through the afternoon (Table 5.8; Figure 5.8). The chasing of heterospecific Pomacentridae fish was relatively common at dawn, but decreased through the morning and did not occur in the afternoon (Figure 5.8). The remaining behavioural traits either did not differ across the course of the day (taking cover and the chasing of heterospecifics/familials or conspecifics) or were too infrequently observed (tracking and fleeing; Table 5.8).

Table 5.1.: Noise intensity (rms, dB re1μPa) of recordings taken over a shallow water reef at Lizard Island. Recordings were taken at 3 hourly intervals for 3-4 days around the full moon in November 2007. Each recording was filtered in 1/3 octave bins and averaged as a function of time and lunar phase. Standard errors are in parentheses.

Moon	Hz band	Time of day (hrs)							
		200	500	800	1100	1400	1700	2000	2300
Full	250	99 (1)	103 (1.4)	106 (0)	102 (1.6)	102 (19.5)	106 (8.8)	102 (5)	102 (39.6)
	315	101 (0)	107 (3.1)	109 (0.1)	106 (2.1)	102 (29.7)	109 (16.9)	106 (2.6)	107 (76.7)
	397	103 (0)	108 (2.7)	111 (0.3)	109 (11.4)	104 (22.4)	110 (20.1)	106 (6.5)	108 (55.1)
	500	106 (6)	110 (2.1)	113 (1.4)	111 (16)	106 (9.1)	112 (44.8)	109 (6.4)	109 (34.8)
	630	110 (14)	115 (0.7)	115 (1.3)	111 (18.6)	108 (15)	114 (42.5)	114 (22.8)	114 (43.5)
	794	108 (0)	116 (8.5)	115 (1.1)	112 (13.7)	108 (9.6)	111 (8.2)	112 (4.7)	114 (10)
	1000	111 (2)	114 (0.1)	116 (0.8)	113 (5.4)	109 (12.7)	112 (6.8)	112 (3.3)	112 (1.2)
	1260	114 (0)	116 (2.4)	119 (0.6)	115 (3.3)	112 (12.2)	115 (3.7)	115 (3.4)	116 (0)
	1587	118 (1)	119 (1.6)	121 (1.3)	117 (2.5)	113 (5.7)	116 (2.1)	117 (2.6)	119 (0)
	2000	120 (1)	123 (1)	124 (0)	119 (3.1)	116 (3.9)	117 (2.2)	120 (1.3)	121 (0.1)
	2519	122 (1)	125 (1.6)	126 (0.6)	122 (2.3)	118 (2.4)	119 (2.1)	123 (0.9)	124 (0)
	3175	125 (0)	128 (0.7)	127 (1)	123 (1.5)	119 (3.7)	121 (1.3)	125 (1.3)	125 (0.9)
	4000	126 (0)	129 (0.2)	128 (1)	124 (3.2)	119 (4.8)	122 (1)	127 (1.8)	127 (1.1)
	5040	128 (0)	130 (0.1)	130 (1.1)	126 (2.7)	121 (5.3)	123 (3)	128 (1.7)	129 (1.6)
	6350	128 (0)	131 (0)	130 (1.4)	127 (4.1)	121 (7.1)	124 (3.4)	129 (1.7)	129 (0.7)
	8000	128 (0)	131 (0)	130 (1.9)	127 (4)	122 (7)	124 (3.6)	129 (1.4)	129 (0.4)
	10080	128 (0)	130 (0.1)	130 (1.5)	127 (3.4)	122 (5.4)	125 (3.5)	129 (1.5)	129 (0.3)
	13000	127 (0)	129 (0.2)	129 (2)	126 (4.1)	121 (5.3)	124 (3.7)	129 (1.4)	128 (0.7)
	16000	123 (1)	126 (0.1)	125 (1.9)	123 (3.1)	117 (6.8)	120 (4.8)	125 (1.5)	124 (0.9)

Table 5.2.: Noise intensity (rms, dB re1μPa) of recordings taken over a shallow water reef at Lizard Island. Recordings were taken at 3 hourly intervals for 3-4 days around the new moon in November 2007. Each recording was filtered in 1/3 octave bins and averaged as a function of time and lunar phase. Standard errors are in parentheses.

Moon	Hz band	Time of day (hrs)							
		0200	500	800	1100	1400	1700	2000	2300
New	250	110 (1)	106 (24.6)	108 (20.1)	102 (4.8)	102 (3.7)	102 (17.7)	102 (0.3)	107 (11.1)
	315	113 (14)	110 (35.3)	111 (27.3)	105 (6.3)	104 (6.7)	103 (19)	104 (3.3)	109 (9.3)
	397	115 (13)	114 (53.2)	115 (27.2)	107 (8.9)	104 (14.3)	103 (23.5)	107 (18)	111 (5.8)
	500	114 (6)	115 (47.7)	115 (14.6)	107 (7.3)	105 (33.3)	104 (19.4)	109 (19.7)	112 (10.1)
	630	116 (0)	117 (38.2)	115 (8.2)	110 (4.6)	108 (17.6)	107 (13.4)	109 (3.4)	114 (9)
	794	116 (0)	117 (34.9)	114 (3.2)	112 (8.1)	110 (15.2)	110 (15.9)	111 (0.2)	115 (8.5)
	1000	115 (0)	117 (19.5)	115 (6.4)	113 (12.2)	112 (10.8)	112 (14.5)	113 (0)	117 (6.5)
	1260	119 (0)	120 (11.7)	117 (4.9)	115 (8.5)	113 (9.2)	115 (19.2)	116 (0)	120 (6.5)
	1587	122 (1)	123 (8)	120 (2.8)	117 (6.2)	115 (3.4)	117 (14.1)	119 (0.3)	123 (3.6)
	2000	124 (0)	125 (7.6)	122 (2.5)	120 (1.9)	116 (2.9)	119 (11.4)	122 (0)	125 (5.2)
	2519	126 (0)	128 (7.8)	124 (2.9)	121 (2.8)	118 (2.3)	120 (11.2)	125 (0)	127 (5.8)
	3175	128 (0)	130 (7)	126 (3.4)	122 (4)	119 (2.3)	122 (9.3)	127 (0)	130 (5.9)
	4000	129 (0)	131 (6.5)	127 (2.9)	123 (4.2)	121 (1.4)	123 (11.9)	128 (0.1)	131 (6)
	5040	131 (0)	132 (7.8)	129 (2.9)	124 (5.4)	122 (1.5)	125 (12.2)	129 (0.1)	132 (5.5)
	6350	131 (0)	133 (8.3)	130 (3)	125 (6.6)	123 (2.4)	126 (13)	130 (0)	133 (6.2)
	8000	131 (0)	133 (8)	129 (3.1)	125 (5.3)	123 (2.9)	126 (12.2)	130 (0)	132 (4.9)
	10080	131 (0)	132 (9)	129 (3.1)	125 (5.2)	123 (2.3)	126 (11.7)	130 (0)	132 (4.9)
	13000	131 (0)	132 (9.2)	128 (3.5)	125 (4.5)	122 (1.5)	126 (10)	129 (0.1)	131 (4.8)
	16000	127 (0)	128 (10.6)	125 (3.5)	120 (5.3)	118 (2)	123 (10.2)	126 (0)	128 (5.5)

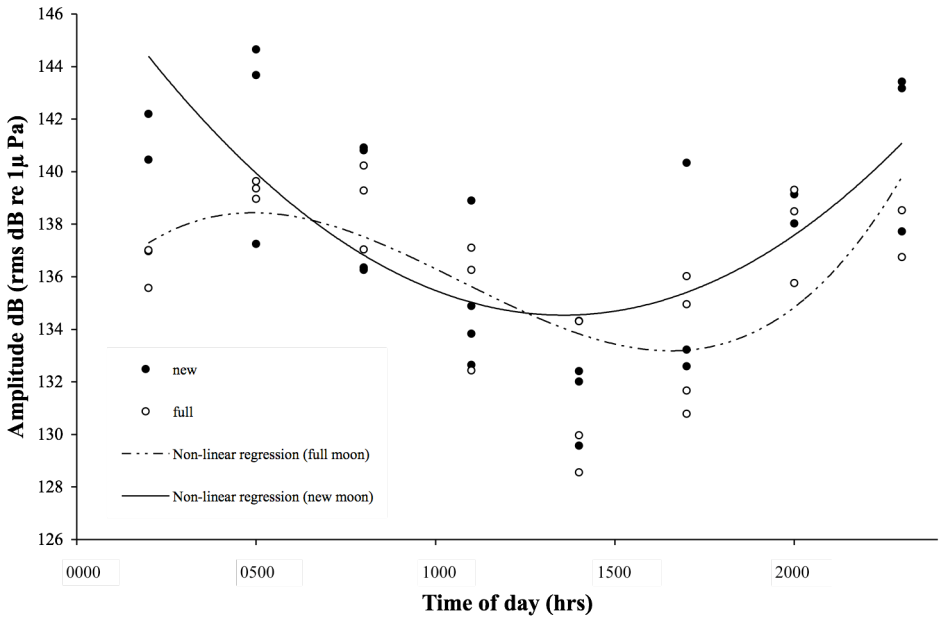

Figure 5.3.: Intensity of noise (rms, dB re 1μPa) from recordings taken on a shallow water reef at Lizard Island during the full moon (white circles) and new moon (blacked out circles). The changes in sound intensity over time were best modelled using a quadratic non linear regression for the new moon recordings, however a 3rd order polynomial regression gave a better fit in comparison to the quadratic regression for the full moon recordings (F = 8.21, df = 1, 20, p <0.01).

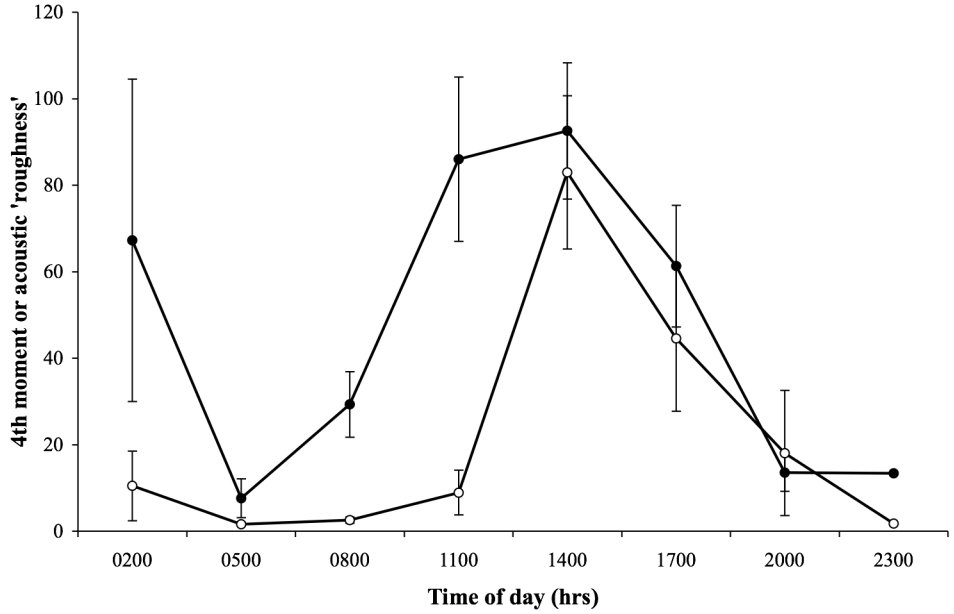

Figure 5.4.: The mean 4^{th}moments (a measure of 'roughness' or instantaneous fluctuations in the sound signal) with standard error bars for sound from recordings taken off a shallow water reef at Lizard Island during the full moon (white circles) and new moon (blacked out circles).

Table 5.3.: Significant ANOVA effects for the 1/3 octave filtered frequency bands of the sound recordings taken at a shallow reef near Hoga Island, Indonesia. Differences between levels were tested using the Tukey HSD, $\alpha = 0.05$), and are shown in the significant relationships column, when sounds were equal (=), or greater (>) in amplitude. The moon(time) effects are described in the text.

Filtered Hz band	Significant effects	Effect $F_{15,40}$ (p value)	Significant relationships (Tukey HSD, p <0.05)		
250	None				
315	None				
397	Time	7.28 (<0.01)	0545 = 1745 = 0845 >	0845 = 1145 = 14.54	
500	Time	12.28 (<0.01)	0545 = 1745 = 0845 >	0845 = 1145 = 14.54	
630	Time	11.69 (<0.01)	0545 = 1745 = 0845 >	0845 = 1145 = 14.54	
794	Time	13.31 (<0.01)	0545 = 1745 >	0545 = 0845 >	0845 = 1145 = 1445
1260	Time	12.01 (<0.01)	1745 >	0545 = 0845 = 1145 = 1445	
1587	Time	10.36 (<0.01)	1745 = 0545 >	0545 = 0845 = 1445 >	0845 = 1145 = 1445
2000	Time	10.75 (<0.01)	1745 = 0545 >	1745 = 0845 >	0845 = 1145 = 1445
2519	Time	15.57 (<0.01)	0545 >	1745 = 0845 >	0845 = 1145 = 1445
8000	Time	26.04 (<0.01)	0545 >	1745 = 0845 >	1145 = 1445
10080	Time	24.76 (<0.01)	0545 >	1745 = 0845 >	1145 = 1445
13000	Time	20.09 (<0.01)	0545 = 1745 >	1745 = 0845 >	1145 = 1445
1000	Moon(time)	9.26 (<0.01)	0545 = 1745 >	0545 = 0845 = 1145 = 1445	
3175	Moon(time)	18.97 (<0.01)	0545 >	1745 = 0845 >	1145 = 1445
4000	Moon(time)	11.78 (<0.01)	0545 = 1745 = 0845 >	1145 = 1445	
5040	Moon(time)	9.24 (<0.01)	0545 = 1745 = 0845 >	1145 = 1445	
6350	Moon(time)	19.02 (<0.01)	0545 = 1745 >	1745 = 0845 >	0845 = 1145 = 1445
16000	Moon(time)	13.2 (<0.01)	0545 = 1745 = 0845 >	0845 = 1145 = 14.54	

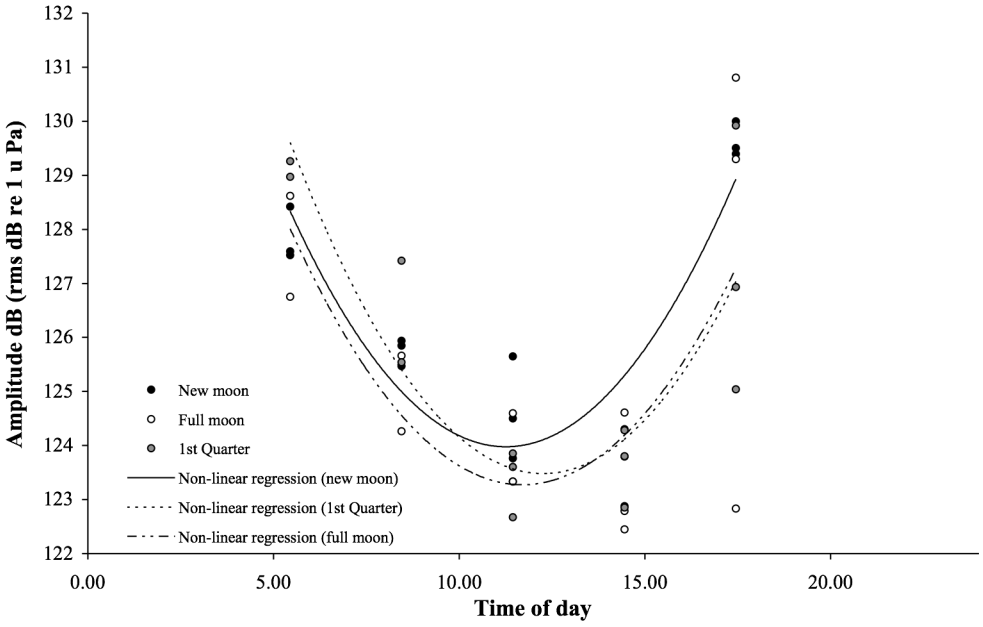

Figure 5.5.: Intensity of sound (rms, dB re 1μPa) from recordings taken at a shallow water reef at Hoga Island, Indonesia. Recordings were taken every 3 hours between dawn and dusk, for 3 days during the new moon (black circles), full moon (white circles) and 1st quarter (grey circles). The non-linear regression equations for the sound amplitude for each lunar phase over time is displayed.

Table 5.4.: Intensity of sound (rms, dB re1μPa) from recordings taken at a shallow water reef at Hoga Island, Indonesia. Recordings were taken every 3 hours between dawn and dusk, for 3 days over the new moon. Each recording was filtered in 1/3 octave bins and averaged as a function of time and lunar phase. Standard errors are in parentheses.

New moon	Time of day (hrs)				
Hz band	0545	0845	1145	1445	1745
250	100.22 (1.28)	98.56 (1.16)	98.22 (0.4)	97.33 (0.69)	100.56 (0.56)
315	103.68 (1.1)	102.1 (1.63)	99.8 (0.17)	100.23 (0.59)	104.6 (0.88)
397	108.9 (1.25)	106.21 (1.59)	103.35 (0.24)	103.16 (0.24)	109.24 (0.58)
500	113.98 (1.62)	110.36 (2.06)	105.41 (0.21)	106.52 (1.02)	113.04 (0.34)
630	115.27 (0.44)	110.76 (1.07)	106.68 (0.45)	107.14 (0.59)	117.05 (1.07)
794	110.59 (1.03)	108.86 (1.02)	105.73 (1.05)	105.39 (0.58)	118.91 (2.01)
1000	113.89 (1.22)	113.8 (0.33)	112.37 (0.62)	112.62 (1.03)	122.68 (0.17)
1260	112.05 (0.54)	111.13 (0.36)	110.03 (0.12)	110.02 (0.63)	120.03 (0.7)
1587	109.9 (0.37)	108.81 (0.43)	107.51 (0.26)	107.3 (0.5)	114.79 (0.79)
2000	110.82 (0.2)	109.15 (0.12)	108.67 (0.19)	108.05 (0.18)	112.56 (0.57)
2519	112.55 (0.26)	110.59 (0.06)	109.75 (0.03)	109.63 (0.1)	112.23 (0.41)
3175	115.01 (0.14)	113.07 (0.08)	111.97 (0.31)	111.32 (0.15)	113.69 (0.28)
4000	117.11 (0.22)	114.97 (0.12)	113.86 (0.38)	113.28 (0.13)	115.54 (0.12)
5040	117.44 (0.33)	115.3 (0.04)	114.37 (0.44)	113.68 (0.27)	116.1 (0.19)
6350	118.82 (0.15)	116.6 (0.14)	115.77 (0.41)	115.1 (0.24)	117.6 (0.11)
8000	118.43 (0.27)	116.45 (0.16)	115.64 (0.47)	114.92 (0.25)	117.39 (0.11)
10080	118.26 (0.2)	116.48 (0.24)	115.59 (0.66)	114.85 (0.35)	117.72 (0.03)
13000	117.24 (0.23)	115.77 (0.33)	114.91 (0.83)	114.02 (0.49)	117.21 (0.05)
16000	116.23 (0.41)	115.04 (0.43)	114.29 (1.04)	113.66 (0.52)	116.99 (0.08)
Total amplitude	128.43 (0.26)	126.59 (0.1)	125.42 (0.47)	124.87 (0.32)	130.06 (0.2)
Fish diversity	1.34 (0.61)	1.42 (0.28)	1.85 (0.17)	1.91 (0.05)	2.02 (0.07)

Table 5.5.: Sound intensity (amplitude rms dB re1μPa) of recordings taken over a shallow water reef at Hoga Island, Indonesia on the 1st quarter of the lunar cycle. Standard errors in parentheses. For full details see previous table.

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1st quarter	Time of day (hrs)				
Hz band	0545	0845	1145	1445	1745
250	101 (0.33)	98.67 (0.67)	100.44 (1.13)	99.56 (1.39)	100.67 (1.35)
315	104 (0.08)	102.05 (0.93)	102.74 (0.95)	101.53 (1.5)	103.12 (1.58)
397	108 (0.52)	105.83 (0.13)	105.29 (0.7)	103.9 (0.88)	106.96 (1.94)
500	112 (0.21)	109.21 (0.45)	107.43 (0.29)	106.44 (0.91)	109.34 (2.29)
630	114 (0.08)	110.31 (0.66)	109.01 (0.19)	107.48 (0.55)	111.54 (1.83)
794	113 (0.81)	108.65 (1.02)	108.54 (0.06)	107.76 (0.5)	114.11 (2.07)
1000	118 (0.67)	114.59 (0.38)	113.14 (1.18)	112.93 (0.99)	117.63 (2.85)
1260	115 (0.54)	112.09 (0.14)	111.37 (0.83)	111.07 (0.81)	115.93 (2.31)
1587	112 (0.13)	109.43 (0.01)	108.78 (0.32)	108.95 (0.38)	112.06 (1.68)
2000	112 (0.22)	109.74 (0.35)	108.91 (0.42)	108.91 (0.42)	111.18 (1.25)
2519	114 (0.02)	112.02 (0.84)	110.45 (0.68)	109.94 (0.28)	112.11 (1)
3175	116 (0.05)	114.21 (1.08)	111.73 (0.46)	111.58 (0.35)	113.97 (1.07)
4000	118 (0.1)	116.32 (1.11)	113.3 (0.41)	113.21 (0.3)	115.89 (1.04)
5040	119 (0.08)	116.89 (1.15)	113.78 (0.42)	113.95 (0.34)	116.74 (0.94)
6350	120 (0.07)	117.65 (1.07)	114.63 (0.26)	114.95 (0.35)	117.51 (0.77)
8000	120 (0.04)	117.38 (0.97)	114.23 (0.23)	114.75 (0.39)	117.31 (0.75)
10080	119 (0.02)	117.03 (0.93)	113.78 (0.25)	114.39 (0.33)	116.96 (0.71)
13000	118 (0.08)	115.77 (0.97)	112.2 (0.28)	112.95 (0.39)	115.67 (0.74)
16000	116 (0.08)	114.51 (1.22)	110.51 (0.46)	111.2 (0.47)	114.07 (0.79)
Total amplitude	129 (0.09)	127.2 (0.82)	124.61 (0.38)	124.71 (0.36)	127.88 (1.27)
Fish diversity	2 (0.13)	1.97 (0.01)	2.06 (0.05)	2.15 (0.05)	1.88 (0.46)

Table 5.6.: Sound intensity (amplitude rms dB re1μPa) of recordings taken over a shallow water reef at Hoga Island, Indonesia on the full moon. Standard errors in parentheses. For full details see previous table.

Full moon	Time of day (hrs)				
Hz band	545	845	1145	1445	1745
250	95.58 (6.08)	98.67 (0.33)	98.67 (0)	98.78 (1.64)	101.33 (1.35)
315	100.11 (5.85)	101.55 (0.14)	101.21 (0.6)	101.02 (1.6)	105.22 (2.35)
397	106.44 (4.04)	105.55 (0.05)	105.12 (1.04)	103.82 (1.44)	108.63 (2.42)
500	112.56 (2.61)	108.4 (0.55)	107.6 (1.39)	106.71 (1.14)	110.62 (2.1)
630	112.56 (4.67)	110.17 (0.9)	109.43 (1.22)	108.55 (1.22)	114.55 (3.51)
794	113.16 (4.29)	107.85 (1.73)	106.97 (1.27)	107.49 (1.5)	115.08 (4.33)
1000	113.97 (0.36)	109.46 (0.37)	107.11 (0.46)	107.24 (1.39)	115.11 (5.07)
1260	113.89 (4.06)	112.95 (0.16)	111.67 (0.39)	111.69 (0.41)	119.68 (4.05)
1587	113.31 (1.43)	110.28 (0.83)	109.25 (0.4)	109.79 (0.53)	114.08 (3.4)
2000	115.05 (2.96)	110.58 (0.43)	109.18 (0.49)	109.16 (0.26)	111.84 (1.89)
2519	114.36 (1.03)	111.01 (0.56)	109.95 (0.57)	109.19 (0.43)	111.46 (1.42)
3175	113.37 (0.38)	112.07 (0.68)	111.03 (0.51)	110.15 (0.49)	112.05 (0.99)
4000	112.44 (2.56)	113.13 (0.75)	112.05 (0.54)	111.16 (0.52)	113.25 (0.78)
5040	113.11 (3.04)	114.6 (0.64)	113.41 (0.34)	112.76 (0.37)	114.7 (0.98)
6350	116.42 (1.42)	115.76 (0.66)	114.77 (0.25)	114.27 (0.42)	116.06 (1.05)
8000	119.94 (1.38)	116.09 (0.65)	115.11 (0.28)	114.58 (0.57)	116.55 (1.1)
10080	120.22 (1.48)	116.37 (0.61)	110545 (0.29)	114.83 (0.63)	116.88 (1.08)
13000	118.08 (0.47)	115.25 (0.56)	114.3 (0.3)	113.83 (0.67)	115.89 (1.28)
16000	115.66 (0.64)	113.21 (0.48)	112.64 (0.18)	112.46 (0.69)	114.69 (1.49)
Total amplitude	128.79 (0.21)	125.8 (0.59)	124.84 (0.38)	124.38 (0.59)	128.22 (2.16)
Fish diversity	2 (0.13)	1.97 (0.01)	2.06 (0.05)	2.15 (0.05)	1.88 (0.46)

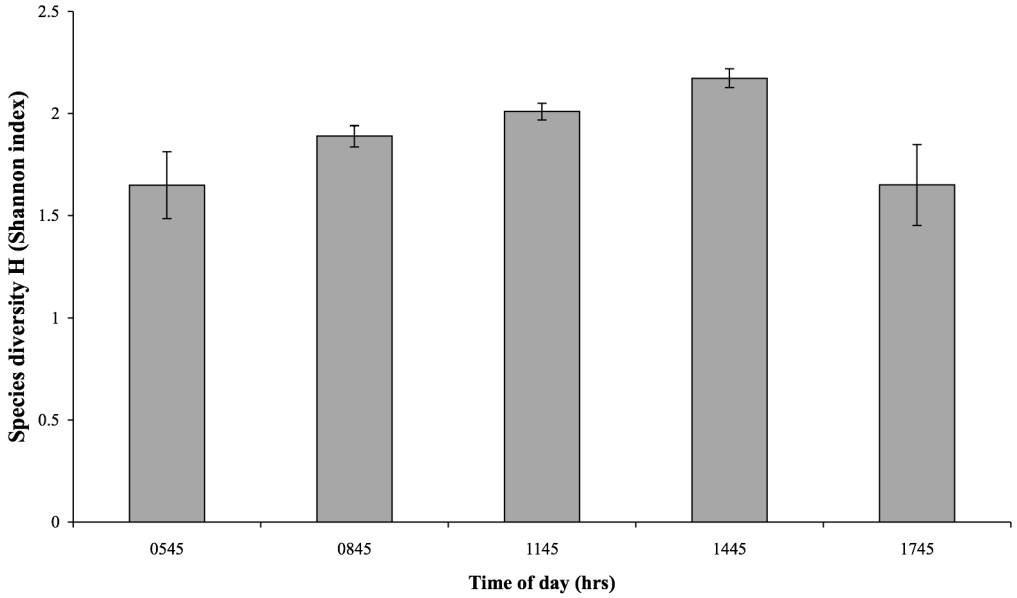

Figure 5.6.: The diversity in fish species from underwater visual census on a fixed transect running adjacent to the sound recorder. Diversity increased during the course of the day, and at dawn this was significantly lower than the other survey times.

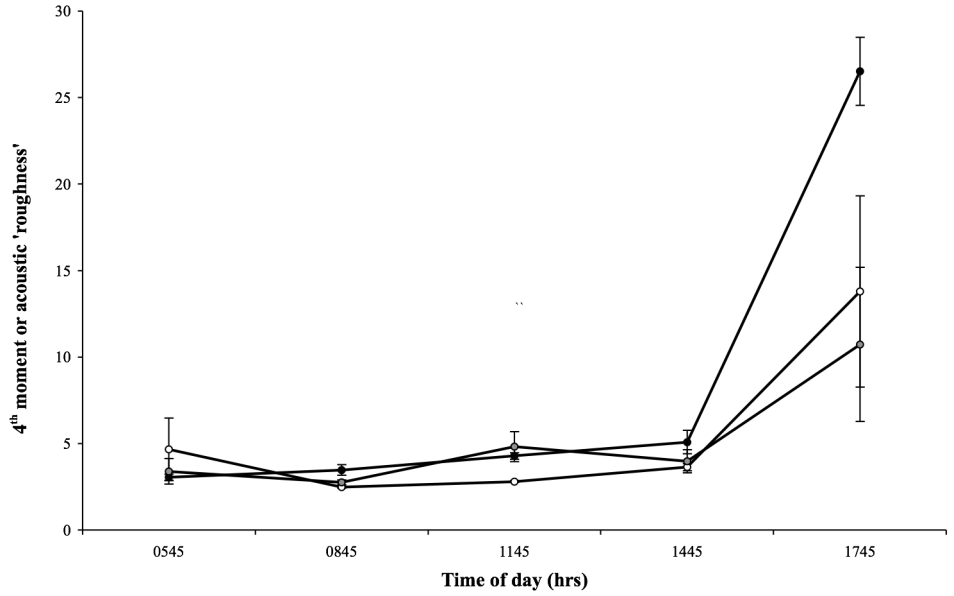

Figure 5.7.: The 4th moments (a measure of 'roughness'or instantaneous fluctuations in the sound signal) of sound from recordings taken at a shallow water reef at Hoga Island, Indonesia during the new moon (blacked out circles) and full moon (white circles) and 1st quarter (grey circles). The mean value per lunar phase is displayed per time period, with standard errors in parentheses. The kurtosis of recordings was significantly higher in the dusk recordings, and at the new moon.

Table 5.7.: The mean measures of the 4thmoments (or roughness) for each time period and lunar phase with the sample size (number of recordings) in parentheses for recordings taken at Hoga Island, Indonesia. Statistically significant differences are indicated with * (Tukey HSD, $\alpha = 0.05$).

Lunar phase	Time of day (hrs)				
	0545	0845	1145	1445	1745
1st quarter	3.39 (2)	2.75 (2)	4.83 (3)	3.97 (3)	10.72 (3)*
Full	4.66 (2)	2.48 (2)	2.8 (3)	3.63 (3)	13.79 (3)*
New	3.05 (3)	3.47 (3)	4.28 (3)	5.08 (3)	26.52 (3)*

5.5. Discussion

Day to day, reef noise peaked in intensity at dawn and dusk, and over the lunar cycle reef noise was more intense during the new moon than it was during the full moon. The change in the intensity of reef noise, over the course of day and the phase of the moon, was the same in the two study locations, at Lizard Island on the Great Barrier Reef, and Hoga Island in Indonesia. Diel changes in the reef soundscape vary in synchrony with aspects of the fish community. Reef noise is most intense at dawn, when the fish assemblage is least diverse. The frequency of sound-producing activities of fishes may also contribute to the way in which reef noise changes over the course of the day. As predicted, when the aggressive interactions between the jewel damselfish and other pomacentrid species decreased after dawn, this coincided with a reduction in the soundscape of noise within the same low frequency bands ($\sim$400 Hz) that the jewel damselfish vocalizes in during aggressive interactions. The abundance and behavioural activity of the fish community seem to vary according to the same cyclical patterns in reef noise intensity.

The cyclical change in the intensity of coral reef noise, over the time of day and phase of moon appear to be geographically widespread. These data from Lizard Island and Hoga, showed similar patterns to each other and to those recorded at coral reefs in the northern waters of Australia, the southern Great Barrier Reef, Bermuda and the Bahamas (Winn *et al.* 1964, Cato 1978, McCauley & Cato 2000, Moulton 1958). The same temporal patterns have been observed in colder temperate reefs (Southern California: D'Spain & Batchelor 2006; New Zealand: Radford *et al.* 2008) and in different habitat types (seagrass beds: Breder 1968 and river estuaries: Fine 1978). These data are, therefore, further evidence to support the case that cyclical changes in biological noise are a regular feature of underwater habitats around the world. So far, the only study that has directly linked the changing reef soundscape to changes in the biological community has shown that variation in reef noise intensity may be attributed to the changes in the activity level of invertebrates (sea urchins and snapping shrimp: Radford *et al.* 2008). These data, which I collected by recording reef noise whilst simultaneously observing the visual diversity and behavioural activity of the fish community, suggest that the diel changes in reef noise are also likely to be

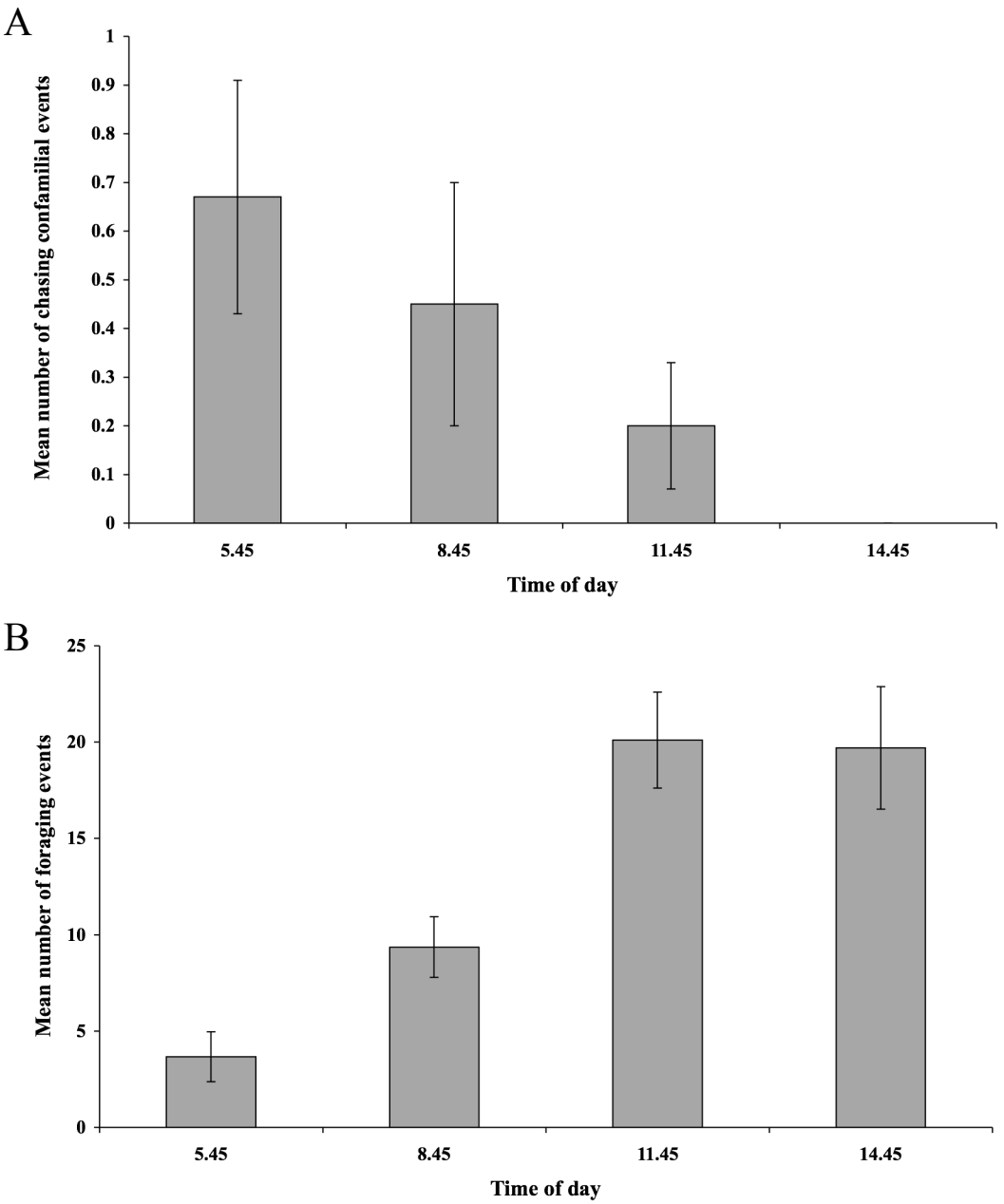

Figure 5.8.: The mean number of foraging (A) and chasing events (B) with standard error bars, of the jewel damsel at four different times of day. Chasing events were more frequent at dawn but these decreased during the course of the day while foraging events became the more common behavioural activity.

Table 5.8.: The mean frequency of behavioural traits displayed by the jewel damsel at four different time periods, with standard errors in parentheses. The frequency of foraging events and chasing confamilial fish was not equal across time periods, Kruskal-Wallis p <0.05). There was no difference in the frequency of the remaining chasing categories over the course of the day, however the power to detect statistical differences in the chase categories is likely to be low given the rarity of these events. The other categories of behaviour (i.e. tracking and fleeing) are not displayed as they were very infrequent.

Behavioral trait	Time of day (hrs)				Kruskal-Wallis	p value
	0545	0845	1145	1445	χ^2	
Foraging	3.67 (1.29)	9.36 (1.57)	20.1 (2.49)	19.7 (3.18)	20.19	<0.01
Chasing (heterspecifics)	0.22 (0.22)	0 (0)	0.1 (0.1)	0 (0)	2.2933	0.51
Chasing (heterofamilial)	0.67 (0.33)	0.73 (0.3)	0.8 (0.36)	0.3 (0.15)	1.0847	0.78
Chasing (conspecific)	0.22 (0.22)	0.36 (0.2)	0.2 (0.2)	0.1 (0.1)	1.5749	0.66
Chasing (confamilial)	0.67 (0.24)	0.45 (0.25)	0.2 (0.13)	0 (0)	7.4509	0.05
Seeking cover	1.44 (1.44)	1.36 (1.36)	2.3 (2.3)	2.1 (2.1)	1.2545	0.74

driven by the fish assemblage. This suggests reef noise, and the variation in reef noise that is apparent in the reef soundscape, may relay information about the abundance and activity of the fish as well as the invertebrate community present on a reef.

It is possible that this variation in reef noise affects larval fish approaching the reef for settlement. It has been suggested that the more intense reef noise is, the further reef noise will transmit offshore and, potentially, the greater contact that is made with larval fish that are attracted to the sound of the reef (Radford *et al.* 2008, Simpson *et al.* 2004, 2005, 2008, Leis *et al.* 2003). This seems plausible given these data from Lizard Island that show reef noise is most intense at night on the new moon, as this is when settlement-stage fish arrive at Lizard Island in greatest abundance to recruit to the reef (Meekan *et al.* 1993, 2001). Total intensity of the reef sound signal is unlikely to be the only feature of reef noise that settlement-stage larval fish are affected by. It is possible that the intensity of specific components of reef noise may cause larvae to swim towards or away from the reef. The reason reef noise around the new moon on Lizard Island was more intense than the full moon was because of an increase in sounds around the 1500 Hz band. Settlement-stage fish at Lizard Island are more strongly attracted to the higher frequency (570-2000 Hz) invertebrate-associated part of reef noise compared to the low frequency components (0-570 Hz; Simpson *et al.* 2008, Radford *et al.* 2008, which could explain why, on the new moon when there is an increase in invertebrate associated sounds, larvae arrive in greatest abundance to settle to the reef. Alternatively, features of reef noise may also cause settlement-stage larvae to avoid the reef. This appears likely, given that the intensity of reef noise, either in total or of specific components, can not explain why, during the day when reef noise is quietest at Lizard Island, larvae tend to swim away from the reef (Leis *et al.* 1996, 2003) and why, in some cases, larvae are repelled by reef sound (Leis *et al.* 2003, Simpson *et al.* 2004, 2005, 2008). The apparent preference of larvae for higher frequency, invertebrate associated sound could also arise if larvae avoid reef noise containing low frequency sounds. This seems plausible as, in Hoga at least, an increase in the aggressive activity of the common pomacentrid, the jewel damselfish, may have been apparent in reef noise as an increase in low frequency sound around 400 Hz. Avoiding reef noise when it contains features that indicate an increase in fish activity, or abundance could

allow larvae to approach the reef when fewer fish are present or active on the reef, and therefore with reduced risk of predation.

Information on the fish assemblage and the activity of fish species may be available within reef sound and this is likely to impact on how passive acoustics can be used to monitor coral reefs. These data collected at Hoga demonstrate the regular patterns of change in the intensity of reef sound, the diversity of fish species on the reef, and the frequency of sound producing behaviours of soniferous fish. In a fixed position on one reef, the intensity of reef noise was inversely related to the diversity of the fish assemblage, and this diversity varied with time of day. At dawn, when reef noise is most intense, is when the visually estimated diversity of fish is lowest. For the rest of the day, when the visual estimates of fish diversity were higher, reef noise was less intense. The fact that these patterns are regular suggests that if reef noise is to be used to monitor more than one reef, then recordings taken at different reefs should be taken at the same time of day. This is already done when visual survey data is collected for monitoring programmes on coral reefs (Hill & Wilkinson 2004). If taking reef sound recordings were combined with a monitoring programme where multiple sites are visually surveyed, this information could help establish whether the inverse relationship between the intensity of reef noise and the diversity of the fish community holds true when coral reef sites with differing fish diversity are compared. This seems unlikely, given how much quieter noise around a degraded, algal dominated reef sounds when compared to the noise of a reef in a marine protected area (Heenan, personal observation).

A great deal of variation in reef noise appears to be caused by how it changes in intensity. There are several reasons why the noise of one reef may be more intense than the noise of another. For example, it is clear from these data collected at Lizard Island and Hoga that the intensity of reef noise will vary depending on the time a recording is taken. Considerable variation in the reef noise recorded at one site may also arise just by small differences in the location the recording was taken (Steinberg *et al.* 1965). Quantifying the variation in reef noise in ways other than intensity may be useful if reef noise is to be compared across multiple sites. Additionally, it would also be beneficial to be able summarize the variation in reef noise in a way that will not be swayed by the behaviourally driven changes of sound producing species in the soundscape, or by diel changes in the diversity

of the fish assemblage. I investigated the potential to use acoustic roughness as a measure to characterise reef sound. Acoustic roughness of the reef noise signal (when there are more infrequent abrupt changes in the signal) or 'smoothness' (when the signal was constant and stable in variation in frequency and intensity) was not affected by the diel changes in fish diversity or activity. It was, however, sensitive to larger scale differences that were apparent over the lunar phase and the geographically distinct study sites.

The two locations, Lizard Island and Hoga Island shared the same pattern of change in the roughness of reef noise over the lunar cycle: on the new moon reef noise was more rough than during the full moon. The two locations differed, however, in their diel pattern of acoustic roughness and this is likely to be driven by the difference in amplitude of reef noise at the two sites: at Lizard Island, the reef sound signal was smooth at peak reef noise intensity and rough at low intensity. At Hoga Island, on the other hand, from dawn to the mid-afternoon, the reef sound signal was relatively smooth (despite changes in sound intensity), while at dusk when the intensity of reef noise increased, acoustic roughness also increased. Both reefs showed the same daily cycles in reef intensity, however at Hoga reef noise intensity was quieter overall and increased by a maximum 7 dB, whereas at Lizard Island reef noise was more intense and there was a 16 dB increase from the mid-afternoon minimum to the maximum at dawn and dusk. This tendency for increased reef noise intensity to equal increased acoustic roughness at Hoga, was also found during the 2 AM recordings of the new moon at Lizard Island. This is likely to be caused by recording at the onset of a fish chorus, before it reached the uniformly loud and stable signal that was captured in Lizard Island dusk and night sound recordings. It appears that by measuring the acoustic roughness of reef noise this will capture large-scale differences in variation in amplitude and the stability of the signal of reef noise over time at two separate coral reef locations. To test whether measuring the acoustic roughness of reef noise can capture variation in reef soundscapes that is related to the community structure or diversity of different reef would require a direct comparison of more than one site where both visual and acoustic data is available.

6. Restoring depleted coral-reef fish populations through recruitment enhancement

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I collected the data with the assistance of Daniel Bailey and Harriet Salomonson in the field. I analysed the data and wrote the manuscript in collaboration with the authors listed.

6.1. Summary

To determine whether enhancing the survival of new recruits is a sensible target for the restorative management of depleted coral-reef fish populations, settlement-stage ambon damsel fish *Pomacentrus amboinensis* were captured, tagged and then either released immediately onto small artificial reefs or held in aquaria for 1 week prior to release. Holding conditions were varied to determine whether they affected survival of fish: half the fish were held in bare tanks (non-enriched) and the other half in tanks containing coral and sand (enriched). Holding fish for this short period had a significantly positive effect on survivorship relative to the settlement-stage treatment group that were released immediately. The enrichment of holding conditions made no appreciable difference on the survival of fish once released onto the reef. It did, however, have a positive effect on the survival of fish while in captivity, thus supporting the case for the provision of simple environmental enrichment in fish husbandry. Collecting and holding settlement-stage fish for at least a week before release appear to

increase the short-term survival of released fish; whether it is an effective method for longer-term enhancement of locally depleted coral-reef fish populations will require further study.

6.2. Introduction

Worldwide, coral reefs are in decline (Carpenter *et al.* 2008), one consequence of which is the decrease in abundance and diversity of fishes (Wilson *et al.* 2006). Most susceptible are fish species that have obligate coral associations, particularly those whose larvae settle onto live coral (Jones *et al.* 2004). There are two general responses to managing this demise. The first is the use of zoning plans and marine protected areas, which provides the opportunity for natural ecosystem regeneration by restricting access and decreasing anthropogenic activities on reefs. The second is a more interventionist approach, by attempting to restore the communities that inhabit reefs. This has included efforts to repair or replace the coral matrix through transplants and the provision of artificial settlement sites (Rinkevich 2005, Shaish *et al.* 2008) and attempts to enhance depleted populations through the release of individuals into the wild. This technique is referred to as stock enhancement, which in the context of reefs, has thus far largely focused on invertebrate species (giant clams, subfamily Tridacninae, Gomez & Mingoa-Licuanan 2006; sea cucumber, e.g. *Holothuria scabra*, Purcell & Simutoga 2008). The few examples of attempts to repopulate fish communities have used species that associate with corals only transitorily, for example, the Pacific threadfin *Polydactylus sexfilis* and the red snapper *Lutjanus campechanus* (Friedlander & Ziemann 2003). Despite being an integral part of their ecosystem, there are no data on enhancement programmes for obligate coral-reef fishes.

There are reservations over active management approaches such as this because they do not directly address the primary causes of degradation, e.g. habitat and live coral loss through climate change-induced warming, pollution and overfishing (Jameson *et al.* 2002, Graham *et al.* 2006, Newton *et al.* 2007). Stock enhancement, like reef restoration, however, may be a useful supplementary management tool (Edwards 2008, Mumby & Steneck 2008). Empirical studies are required to determine whether this is the case, particularly because it remains a practiced yet unproven technique (Sadovy 2005).

Coral-reef fishes have a pelagic larval and benthic adult stage, experiencing an estimated mortality rate of c.60% during settlement (Doherty *et al.* 2004, Almany & Webster 2006). The release of juveniles from cultured wild-caught

or hatchery reared larvae into recruitment limited populations (as many coral reef fish populations are) can bypass or reduce this mortality bottleneck that occurs at settlement (Bell *et al.* 2008). This, in combination with the highly effective methods available for collecting coral-reef fishes from a great variety of families during or just prior to settlement, e.g. light traps, crest nets and hoa nets (Doherty 1987, Dufour & Galzin 1993), makes settlement larvae an ideal life-history stage on which to focus attempts to enhance depleted fish communities.

As predation is the main threat to settlement-stage fish survival (Planes & Lecaillon 2001), simply using light attracting devices to increase the recruitment rate of settlement-stage fishes to localized patches on a reef will not necessarily lead to a sustained increase in population size (Munday *et al.* 1998). Indeed, an increase in recruitment of fishes at this stage may well result in higher abundance of their gape limited predators and, therefore, increase recruit mortality (Munday *et al.* 1998). In temperate stock-enhancement programmes, predation is the main cause of the high mortality experienced by released fishes (Olla *et al.* 1994, Brown & Laland 2001, Salvanes & Braithwaite 2006). Survival of released fish can be significantly increased by holding fish in conditions that stimulate their behavioural development, e.g. exposure to predators, altering the spatial or temporal distribution of food, manipulation of the social environment and the provision of natural habitat refugia (Olla *et al.* 1998, Brown & Laland 2001).

The aim of this study was to determine whether enhancing recruitment could be used to assist depleted populations of obligate coral reef fish species. To examine whether the high level of settlement-stage mortality could be alleviated, wild caught larvae were held captive for a short period and then released onto the reef. If holding fishes captive during the vulnerable stage around metamorphosis makes them less susceptible to predation, then the prediction would be for higher survival rates in the fishes that were held prior to release, relative to those released immediately. The conditions in which fishes were held captive were manipulated, to determine whether tank variability leads to increased survival in released fishes, as it does for the North Sea cod *Gadus morhua* (Braithwaite & Salvanes 2005). If being held in psychosensorily deprived conditions leads to behaviourally deficient animals (Olla *et al.* 1998), then the prediction would be for higher survival rates in fishes that were held in tanks enriched with habitat refugia relative to those held in bare tanks.

6.3. Materials and methods

The Ambon damsel *Pomacentrus amboinensis* was the study subject. These fish can be caught in abundance during their summer breeding period in light traps, which can be used to collect fish just prior to settlement on the reef (Meekan *et al.* 2001). *Pomacentrus amboinensis* is common to the Great Barrier Reef, where like most Pomacentridae, it represents an important part of the total fish biomass (Ackerman & Bellwood 2000). As a protogynous hermaphroditic species (Jones 1987), males guard the nest in which females lay demersal eggs. The eggs hatch 4-5 days later and the larvae then spend 15-23 days off the reef in pelagic water, after which time they return to the reef to settle, typically to small reef patches on the reef base or slope where there is a mixture of live coral, sand and rubble (Kerrigan 1996, McCormick & Makey 1997). This species undergoes a high mortality bottleneck in the days immediately following settlement, when up to 75% of young fish may be removed by predators (Almany 2004). Their main predators are the dusky dottyback *Pseudochromis fuscus*, the rockcod *Cephalopholis boenak*, moonwrasse *Thalassoma lunare* and two species of lizardfish *Synodus variegates* and *Synodus dermatogenys*. All are either site-attached or home-ranging (Holmes & McCormick 2006, McCormick & Holmes 2006). A further useful feature of the *P. amboinensis* is that it remains attached around the same site once settled (McCormick & Makey 1997), allowing for the assumption that once fish were released onto patch reefs they would remain in place, unless eaten.

Settlement-stage *P. amboinensis* were caught using light traps deployed before dusk (1830 hours) and collected after dawn (0600 hours) from permanent moorings in 10–15 m depth over a sandy substratum, in the near-shore waters of Lizard Island Research Station (14°40'S, 145°26'E) from the 22nd to 27th November and the 8th to 12th December 2007. Settlement-stage *P. amboinensis* were separated from the rest of the catch and placed in shaded outdoor aquaria supplied with aerated flowing sea water at an estimated density of 200 fish per 40 l tank.

A pilot study carried out in November 2007 was used to determine the release protocol, and the frequency and duration of visual counts needed to assess post-release survival. Mixed species groups of *P. amboinensis* and the

lemon damsel *Pomacentrus moluccensis* were used, as too few *P. amboinensis* were available for these trials. *Pomacentrus moluccensis* are similar in size at settlement to *P. amboinensis* (mean standard length, *P. amboinensis*: 11.5 mm and *P. molucensis*: 11.3 mm; (McCormick *et al.* 2002). At settlement, *P. moluccensis* will only settle on live coral, typically on areas of continuous reef (Booth 2002) but also isolated coral bommies (Figueira *et al.* 2008). *Pomacentrus amboinensis* is more of a settlement generalist, settling to live coral and rubble on continuous reef and patches (McCormick & Makey 1997, Booth 2002). These broad similarities at settlement made *P. moluccensis* a sufficient substitute for the purposes of a pilot study. In this 12 day pilot trial, the greatest rate of mortality occurred during the first 2 days following release (on average 25% loss). The rate of mortality then reached a plateau, decreasing by 2% (of the original number released) over the remaining 10 days. Based on this information, survival of *P. amboinensis* in the full experiment was measured on days 1 and 2 by three visual surveys (at 0600, 1200 and 1700 hours), on day 3 by two surveys (0600 and 1700 hours) and then once daily (0600 hours) for a further 5 days, for a total of 8 days.

In December 2007, single species experimental trials using *P. amboinensis* were conducted. On the morning of capture (day 0), fish were randomly allocated to one of three treatment groups, then tagged and photographed for measurement as follows, each fish was placed into a plastic click-seal bag (size: 9 cm x 12 cm) containing aerated sea water and placed flat on its side on top of a laminated piece of graph paper. Fish were digitally photographed using an Olympus Camedia C-5000. The camera was positioned *c.* 30 cm above the fish with both the fish and the graph paper in focus. The standard length ($\pm$ 0.01) were measured from the photographs using Image-J (Rasband, 1997-2009, http://rsb.info.nih.gov/ij). The same observer measured fish throughout the experiment to reduce between observer variation. Using a 29-gauge hypodermic needle, fish were tagged through the plastic bag with a subcutaneous fluorescent elastomer tattoo (Northwest Marine Technology; www.nmt-inc.com). Tag colours (blue, orange, pink and yellow) were alternated among treatment groups to reduce any potential interaction between predation rate and colour. Tagging of fish allowed any movement between neighbouring patches to be detected and enabled the identification of released fish. Settlement-stage fish selected for the

experiment were randomly allocated to three treatment groups: (1) released the day after capture and referred to as settlement-stage, (2) held for 7 days in enriched tanks and (3) held for 7 days in non-enriched tanks, together referred to as captive held. There were four replicates per treatment group, each containing 30 fish (360 fish in total). Four aquaria were modified so that they could each house one enriched and one non-enriched replicate group separately. Silicone sealant was used to fix a single opaque Perspex divider, creating two separate holding areas per aquarium (dimensions 30 cm x 15 cm x 20 cm). Each half had an independent supply of fresh aquarium-supplied sea water and an outflow standpipe which maintained the water at 15 cm depth. On the enriched side, the aquarium was lined with sand and had a live cauliflower coral *Pocillopora damicornis* coral head (*c.* 8 cm x 8 cm x 8 cm) positioned in the centre of the tank, while the non-enriched side of the aquaria was left bare.

Settlement-stage fish were not fed during the time they spent in the laboratory and were released the day after capture (day 1). Fish held for < 24 hours (captive-held) received their first feed the day after capture (day 1) and were fed twice daily (0600 and 1800 hours), receiving their last laboratory meal at 0600 hours on the day of their release (day 8). They were fed 40 ml of 12–16 hours old *Artemia* sp. nauplii (density: c. 2000 individuals per 1 ml sea water). The release protocol was identical for all three treatment groups. The exception to this was that the captive-held fish were photographed again *c.* 4 hours prior to release on day 8. At 12 hours before fish were released, the patch reefs were cleared of existing *P. amboinensis* using an anesthetic consisting of a mixture of clove oil (eugenol 85–95%), alcohol (98% ethanol, 2% methanol) and fresh sea water (ratio 0.005:0.05:1) (Munday & Wilson 1997). On the day of release, fish were placed in open 8.5 l plastic click-seal bags (one bag per replicate group containing 30 fish) filled with aerated water. Bags were sealed for transport and taken to the patch reef site and fish were released between 1600 and 1700 hours. Fish were released onto small artificial patch reefs that had been built on a 4–5 m depth sandy bottom in the Lizard Island lagoon (14°41'S; 145°28'E). One treatment replicate group was released per patch. Reefs consisted of a coral rubble base (60 cm x 40 cm x 20 cm) with a live *P. damicornis* coral head (*c.* 20 cm x 20 cm x 20 cm) positioned on top. For 1 hour following release, wire cages (100 cm x 100 cm x 100 cm, mesh size: 5 mm) were positioned over each

patch reef to exclude predators, after which the cages were removed (McCormick & Meekan 2007).

Patches were arranged in rows with 5 m within and between rows. As newly settled *P. amboinensis* tend not to move > 0.5 m in the first week following settlement (McCormick & Makey 1997), the 5 m separation was assumed sufficient to prevent between-patch migration, and tagging of fish allowed this to be tested. Each treatment group was represented on every row away from the reef edge (distance 20, 25, 30 and 35 m), except 30 m where there was a settlement-stage group alone. The final number of treatment group replicates was: settlement-stage = 4, enriched = 3 and non-enriched = 3. This was due to the loss of one enriched and non-enriched replicate group during the captive period, as the tank was inadequately sealed allowing fish to pass between the enriched and non-enriched sides of the tank.

Released fish were surveyed for 8 days as described above. On the final day, survivors were collected using clove oil and transported back to the aquarium in 8.5 l click-seal bags where they were photographed again.

The treatment effect on fish survivorship was examined using survival analysis. Data were righthand censored, as some individuals outlived the study, and interval-censored as survival of released fish was recorded at set time increments, i.e. the time of death was unknown but was bounded between observation periods. To obtain the significance levels for the explanatory variables, deviance statistics generated from models with and without the explanatory variables were compared using χ^2 -tests. Survivorship is not a linear function of age, as the risk of mortality decreases with time after settlement. This was assessed in a preliminary model comparison, where the Weibull error distribution (non-constant survivorship) had a greater explanatory power for the variance in the data than an exponential (constant survivorship) error distribution (χ^2, d.f. = 1, $P \leq 0.01$). A *post-hoc* assessment of the within-tank mortality of the captive-held fish was made, where the proportion of fish remaining alive after 7 days in captivity in the different tank treatments (enriched or non-enriched) was compared using a Kruskal -Wallis test. Differences in standard lengths of fish among treatments were compared using one-way ANOVA at the different experimental stages (capture, release and recapture). Significant effects were

further explored using *post hoc* Tukey's HSD tests to determine which treatment groups differed in standard length. All analyses were implemented in the R environment (R; http://www.r-project.org), using the R package survival (S original by Terry Therneau and ported by Thomas Lumley).

6.4. Results

Irrespective of holding conditions, the survival of fish from the combined captive held treatment groups was higher than that of the settlement-stage treatment group that were released immediately onto patch reefs (Survival analysis (Weibull error distribution), $\chi^2 = 41.07$, $p < 0.01$; Figure 6.1). After 8 days on the patch reefs, 24% of the settlement-stage treatment fish had survived, while 40% of the non-enriched and 60% of the enriched individuals survived (inclusive of any within tank mortality experienced during the 7 day holding period). This difference in survival on the patch reefs between fish held in the enriched or non-enriched conditions was not significant ($\chi^2 = 1.20$, d.f. = 1, $p > 0.05$); however, a comparison of their survival during the 7 days spent in captivity showed that fish held in non-enriched tanks suffered greater mortality than those held in the enriched tanks (Kruskal Wallis, d.f. = 1, $p < 0.05$). There was no effect of tag colour ($\chi^2 = 3.11$, d.f. = 1,3, $p > 0.05$), and the distance of experimental patch from the lagoon reef edge ($\chi^2 = 5.50$, d.f. = 1,3, $p > 0.05$) also did not affect the survival of released individuals for all three treatment groups. No between patch movement was detected from the coloured tags present on the fish.

There was no difference in the mean standard length of fish at the start of the experiment when they were allocated to different treatment groups (one-way ANOVA, $F = 1.27$, d.f. = 2,7, $p > 0.05$; Figure 6.2 (capture, day 0 for all treatment groups)). A test for normality showed that the non-homogeneity in variance of size distribution of fish at the release stage was not significant (Bartlett test, K-squared = 1.35, d.f. = 2, $p > 0.05$). At the point of release, after 7 days in captivity, there was a main effect of treatment group on the standard length of fish (one-way ANOVA, $F = 24.95$, d.f. = 2,7, $p < 0.01$; Fig. 2 (release, day 1 for the settlement-stage fish and day 8 for the captive-held fish)). The settlement-stage fish that were 7 days younger at the time of release were smaller than fish held in captivity (one-way ANOVA, $F = 12.26$, d.f. = 1,6,

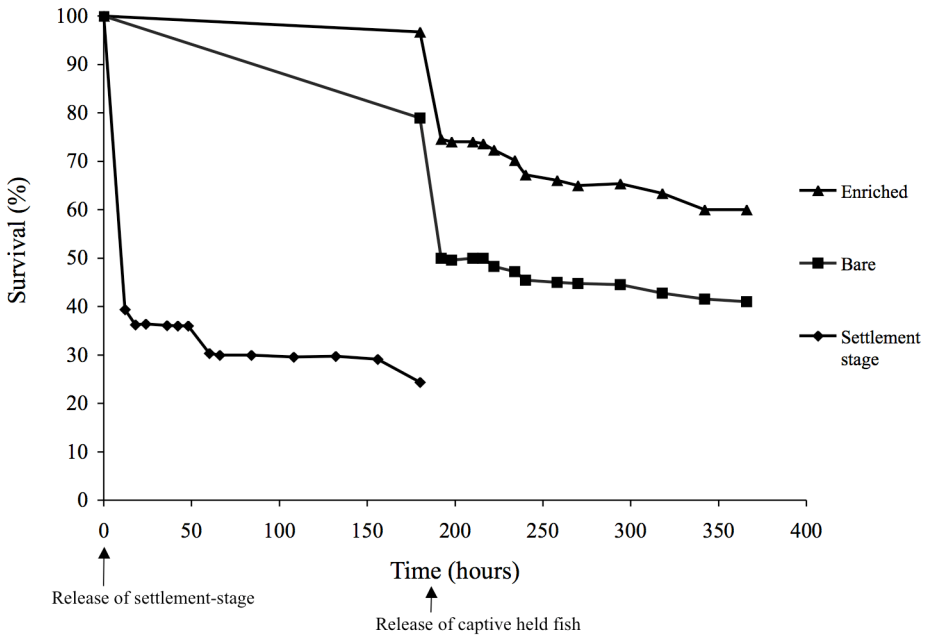

Figure 6.1.: Percentage survival per treatment group. The settlement-stage *Pomacentrus amboinensis* were released within 36 hours of capture, while the non-enriched and enriched treatment groups were released after 7 days captivity in aquaria. The total number of individuals captured and released per treatment group: settlement-stage (131 at capture, 130 at release), non-enriched (90 at capture, 71 at release), enriched (90 at capture, 87 at release).

Tukey's HSD, $p < 0.05$). Enriched and non-enriched fish did not differ in size (one-way ANOVA, $F = 0.79$, d.f. $= 25,6$, Tukey's HSD, $p > 0.05$). At recapture, after 8 days on the patch reefs, the captive-held fish (enriched and non-enriched) were significantly larger than the settlement-stage fish (one-way ANOVA, $F = 12.28$, d.f. $= 1,6$, $p < 0.05$; Figure 6.2). Recapture was 8 days after initial capture for the settlement-stage and 15 days for the captive held fish.

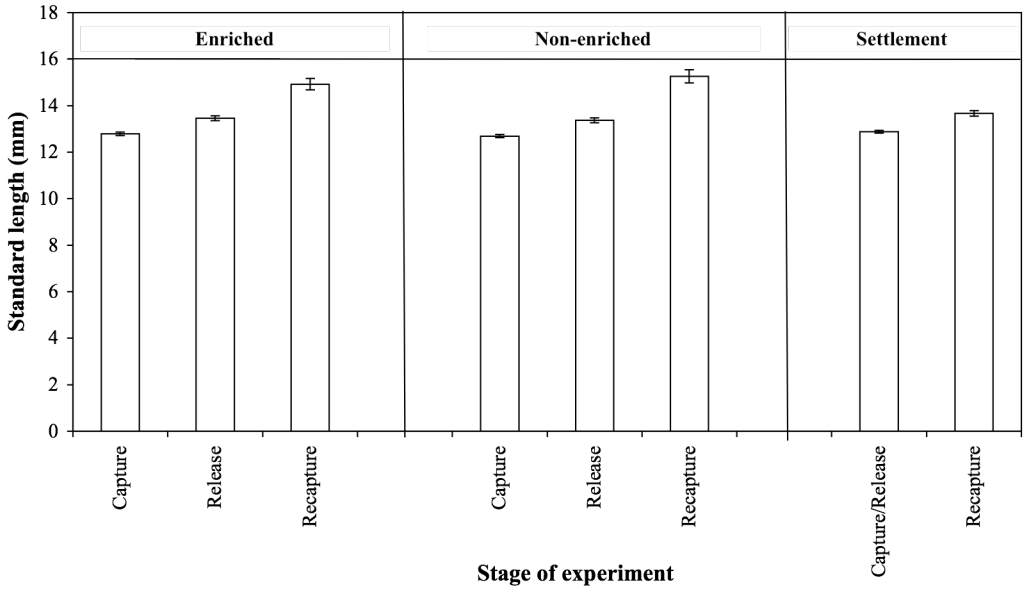

Figure 6.2.: The size of *Pomacentrus amboinensis* measured in standard length(mm) with a panel per treatment group. Fish were either held for 7 days in tanks with enriched or non-enriched conditions, or released straight after capture (settlement-stage). The standard length measurements were taken at three stages, at the time of collection from the light traps (capture), at release onto the patch reefs (release) and at recapture after 7 days on the patch reefs (recapture). As the settlement-stage treatment group were released immediately after capture, the capture and release measurements are the same (capture-release); therefore, the age of the settlement-stage fish at this stage is equivalent to the tank-held fish at the release stage.

6.5. Discussion

Fish that were held for 7 days in captivity prior to release had significantly increased survival when released onto patch reefs in comparison with fish released immediately after capture. Survivorship was improved by 16 - 36%. This major effect on survivorship following the relatively minor intervention of holding fish captive for a week led to increased survival of *P. amboinensis*. Artificial enhancement is a common technique for commercially fished species. This study demonstrates that by assisting fishes through vulnerable settlement and metamorphosis processes, the immediate survival of new recruits can be increased, and hence enhancement may be a useful tool for the conservation of coral-reef fishes. Holding conditions had no effect on survival once released onto the reef; however, during captivity fish kept in bare tanks survived less well than fish kept in tanks containing pieces of coral. This suggests there is merit in including psychosensory enrichment in the holding conditions in fish husbandry.

The chief advantage of holding fishes is that it confers higher survival once they are released onto the reef, potentially through a reduction in vulnerability to predation. This is inferred from a predator-exclusion experiment that identified predation on metamorphosing fishes as the major cause of mortality in settlement-stage pomacentrids when fishes were released into cages containing patch reefs with or without natural predators (Planes & Lecaillon 2001). Over a period of 48 hours, fishes released onto predator-free patches experienced a 14% mortality rate in contrast to fishes released on to the patches containing predators, where mortality ranged from 29 to 76%.

Releasing fishes immediately after capture directly onto a particular area (Munday *et al.* 1998), led in this experiment at least, to poorer survival in the immediately released fishes relative to fish held captive for a week. In order to enhance recruitment artificially on a small scale, it is better to hold fish captive for a short period, as *P. amboinensis* experiences its highest mortality risk at settlement (Almany 2004, McCormick & Hoey 2004). Fish were held in captivity beyond that peak (2 days following settlement; (Almany & Webster 2006) and those that survive through this critical period are likely to persist in the long-term. Irrespective of whether fish were released immediately or were held in captivity, average L_S increased during the first 8 days following initial

capture. Hence, by the time of release the captive-held fish were larger than the settlement-stage fish. *Pomacentrus amboinensis* undergoes size-selective mortality at settlement. The direction (positive and negative) of this process can vary (Hoey & McCormick 2004, Gagliano & McCormick 2007) and is thought to be driven by predation. Whether settlement mortality is selective for smaller individuals can depend on physiological and morphological traits, i.e. the individual fish condition (Hoey & McCormick 2004) and also on the predator conditions into which fish recruit (Holmes & McCormick 2006). It would appear that a period of alleviated predator-stress in captivity allowed fish to increase in size, allowing the captive-held fish to successfully evade predation once released onto the reefs.

The possibility cannot be excluded that the greater mortality suffered by the immediate-release group was a result of these fish still recovering from a stress response to the handling and tagging procedure. Although this may have had an effect, these fish experienced similar levels of mortality (76%) as those previously reported during the natural settlement of damselfish (Pomacentridae) onto patch reefs in the presence of predators (*c.* 75%; Almany 2004). If handling and tagging were detrimental, this should have resulted in an increase in mortality over and above this level.

It is not clear whether less common larger fish, which are not as site-attached immediately after settlement, would respond as positively to the experimental protocol. Furthermore, it is also not clear how the tank environment contributed to enhanced survival on release. During this study, rough weather conditions led to low larval catch rates, preventing the further replication needed to test whether the tendency for higher survivorship in the enriched holding conditions was biologically significant. As the within-tank mortality of fish held in enriched tanks was lower in comparison with the non-enriched tanks; this demonstrates that there is merit in providing fish with structure while in captivity. One theory is that artificial rearing conditions cause the production of behaviourally deficient or modified animals (Olla *et al.* 1998, Brown & Laland 2001, Hawkins *et al.* 2008). This has been demonstrated for behavioural traits likely to affect survival in the wild such as foraging behaviour in *G. morhua* (Braithwaite & Salvanes 2005). It seems plausible that providing some structure to the tank allowed fish to hide from conspecifics, leading to lower stress levels and therefore lower levels of

mortality while in captivity and possibly to lower predation upon release.

Pomacentrus amboinensis was used in this study because it can be readily caught at the settlement-stage and is amenable to experimentation. Although they do not form part of commercial food fisheries, in many countries they are an important ecological component of the reef fish assemblage, being the second most abundant family and making the greatest contribution to biomass production (Depczynski *et al.* 2007). Pomacentrids also represent 47% of the global export of marine ornamental fishes for the aquarium trade (Wabnitz *et al.* 2003). These findings are therefore relevant for the conservation of reef fishes by providing a management model that may be relevant to commercially harvested fishes, and a demonstrated tool for less commercially exploited, but ecologically important, species. Attempts to restore and enhance natural recruitment have proved successful for corals (Heyward *et al.* 2002, Amar & Rinkevich 2007), but have rarely been trialed for reef fishes (Sadovy 2005). This study has demonstrated that holding settlement-stage coral-reef fishes for as little as a week leads to a significant increase in survival. Therefore, this may be a promising method for use in attempts to increase population numbers in commercially important or endangered reef fish species.

7. Discussion

The aim of this thesis was to determine which cues larval fish might use for orientation at settlement. Understanding these could be useful for both enhancing settlement on a reef and for increasing catch of this stage of fish for post-larval capture for culture (PCC).

7.1. Summary of thesis

The first question I addressed was whether fish could use odour cues to locate a specific microhabitat type. I tested this using apogonids because these fish are attracted to reef odour but it was not clear how specific that attraction was. In a choice flume I presented settlement-stage fish with pairs of odours and found that apogonids 1) did not prefer the odours of their microhabitat, live coral or reef odour; 2) will avoid reef odour after prior exposure to that odour. One interpretation of these data is that live coral, coral rubble or reef odour are not attractive to settlement-stage apogonids. However, as settlement-stage apogonids have been shown to be attracted to reef odour previously, it is plausible that the water I used in this experiment was lacking in some way. Perhaps the most obvious missing element was the odour of conspecifics. The water in previous studies in which preference for reef odours has been demonstrated in apogonids will have contained the odour of conspecifics (Gerlach *et al.* 2007). To determine whether the lack of conspecifics in the water explained the apparent avoidance of that water in my experiment would require an explicit test. Furthermore, one could determine whether it was the odour of familiar conspecifics that is especially attractive, which, if true, would support the case for larvae using odour to locate their natal reef (Gerlach *et al.* 2007). Choice of microhabitat might be intrinsic to the reef itself and it is likely that larvae will locate their preferred microhabitat using other sources of information, such as visual cues (e.g. Lecchini *et al.* 2005*a*), although it is also possible that odours

will provide additional information other the presence of a suitable habitat. The scent of a conspecific might indicate not only a habitat where fish have previously settled successfully, but also the presence of conspecifics with which to mate, or to avoid (i.e. relatives). It is evident that the clownfish *Amphipion percula*, which have been shown to recruit to their natal reef, are attracted to the odour of conspecifics but will avoid the odour of their parents (Munday *et al.* 2009). Such refined used of odours would allow precision of philopatry and it is plausible that other species of reef fish are also capable of similar levels of precision. It will be difficult to investigate such precision in the field.

The use of some cues is more readily tested in the field than odour is. In Chapter 3 I investigated whether different light colours were more or less attractive to settlement-stage fish. I set out traps with five colour treatments: blue, green, red, yellow and white. Overall, although more fish were caught in greater abundance over more nights in the white traps, the red light emitting traps tended to catch more individuals of two families: apogonids and pomacentrids. The white light traps are likely to have caught more fish because they emitted the most intense light and therefore illuminated more of the water surrounding those traps than did any of the other colour treated traps. Inspite of the red light traps emitting the least intense light, it is probable that these traps caught more pomacentrids and apogonids because the light emitted from these traps matched the spectral sensitivity of these fish. This suggests that it is possible to catch fish selectively based on appropriate choice of light. To increase the catch of these particular fish one would need to increase the intensity of the red light. The advantage of this capacity for selectivity is that there is less bycatch, reducing the number of unwanted fish and the time taken to sort light trap catches. Such selectivity would be relevant to collectors who already use light traps to collect fish for the marine aquarium trade, as the Pomacentridae make up over 50% of the global trade in marine ornamentals (Wabnitz *et al.* 2003).

While more attention has been paid to the ways in which settlement-stage fish may respond to odour and visual cues, larvae are also attracted to reef noise. In Chapter 4 I investigated whether the attraction of larval fish to reef noise was specific to Lizard Island where all previous experiments on attraction to sound had been carried. At a site in the Philippines I paired light traps with and

without reef noise and found that more larvae were caught in the silent traps than were caught in the sound traps. There were at least three explanations for this apparently surprising result: 1) the study site in Philippines was a degraded and potentially quieter area where reef noise is not attractive to settlement-stage larval fish; 2) the playback I used contained a feature that the larvae found repellant; 3) the larvae were not avoiding the sound but the traps also attracted predators which then ate the larvae attracted to the sound treated traps. The way to investigate whether the first of these possibilites was an appropriate explanation would be to repeat another playback experiment in that area but to use a reef recording that was similar to the acoustic conditions of the playback site. I attempted to deal with possibilites 2 and 3 in the second experiment in Chapter 4. I carried out a second acoustic playback experiment at Lizard Island, this time using multiple sound recordings, to overcome the limitations of interpreting a result based on one reef sound recording. It is not clear whether the issue of pseudoreplication in reef noise playback experiments has struck a chord because no similar studies have been published since the Slabbekoorn & Bouton (2008) review that highlighted the issue. Although I was unable to test the effect of the recording itself, I could test whether the the effect of addition of reef noise playback varied with the type of light trap used. And indeed the effect reef noise playback has on light trap catches is dependent on the design of the light trap: AIMS traps are more effective when silent and Ecocean traps are more effective with reef noise. Whether this Lizard Island result could be replicated elsewhere would require further study. Research attention may be now be shifting towards questioning how changes in the environment might effect larval orientation (e.g. Munday *et al.* 2009). However, until it is established what features of reef noise particular species chose to orientate towards or avoid, it will be important to exercise caution in designing experiments that aim to investigate how changes in the environment might effect larval orientation, as the response larvae have to reef noise appears to be context dependent.

Larvae may be both attracted to and repelled by the noise of a reef. It is possible that there are particular components within reef sound that are attractive or not. In Chapter 5, I investigated the temporal variation in coral reef sound and assessed the different components that make up a soundscape of a reef. Perhaps the most conspicuous feature of reef noise is the change in intensity

both across the day and the month (loudest at night and over the new moon), which co-incide with the peaks in settlement. In Indonesia I recorded the sounds of the reef across the day, I censused visually the number of species of fish present and for one particular species, the jewel damselfish, I measured the frequency of their sound producing behaviours. By combining these data together, it appears that the intensity of reef noise is negatively related to the diversity of the fish assemblage. To determine the generality of this finding it would be necessary to collect visual and reef noise recordings at the same time of day across multiple reef sites. Additionally, the level of reef noise will change depending on the activity of sound producing fishes, specifically their sound production. For example, the jewel damselfish is active throughout the day, but they are especially noisy in the morning. To determine whether it is fish noise or its absence that settlement-stage fish may use when using reef noise for orientation, would require direct testing in the laboratory using choice experiments.

There are two major reasons to address the sensory cues larval fish use to orientate towards a settlement-site. The first relates to understanding the natural patterns in recruitment to the reef and the second relates to using the response of larvae to sensory cues to collect fish for PCC. In the final data chapter, I focussed on another aspect of PCC and that is the survival of fish that are released after capture to try and increase locally depleted fish populations. I captured settlement-stage larval pomacentrids and allocated them to one of three treatment groups; the first I released immediately back onto the reef, the second and third I kept in aquaria for a week in either enriched or non-enriched conditions. Holding larval fish in captivity prior to release onto the reef led to a higher survival compared to the fish released immediately. This suggests that releasing fish can lead to an increased biomass in the short-term, although the long term survival would need to be established. Keeping larval fish in enriched tanks makes no difference on fish survival once released into the wild, however, these fish did suffer lower within tank mortality compared to the fish held in non-enriched tanks. It would appear that, irrespective of whether larvae are captured to be exported for the marine aquarium trade or to be released back onto the reef, providing tank enrichment could benefit the survival of larvae while in captivity.

7.2. Future work

I used a combination of *in situ* and controlled aquarium approaches to investigate how larval fish might use different sensory cues during settlement orientation. Common to the three experiments in Chapters 3 and 4, where I compared light trap catches after deploying traps with different sound and light treatments, was the tendency for one extremely large catch on one night to make a substantial contribution to the overall catch. Caution should be taken when considering an increased total catch or even mean catch as evidence that larvae are more attracted to the experimental treatment because the result could be easily flipped by another large catch for the different treatment. Ways to separate the experimental treatment introduced variation from the spatial and temporal variation in larval distribution are to either consider the median rather than the mean catch per trap treatment, or to use the number of nights with the greatest catch. I used this measure as it has been used previously by Leis *et al.* (2003) to look for consistent effects of the light trap treatment being tested. An additional observation from the light trap catch data, was that in light traps set parrallel to the reef and separated by less than 100 m, the catch from adajcent moorings could vary by the order of thousands. It would appear that larvae might move around in high density clumps but whether larvae respond to orientation cues as a group or individually has yet to be established. If group orientation proved true, this would open a whole new perspective for future questions on larval settlement behaviour.

Aquarium studies will allow for the potentially more refined use of sensory cues by larvae to be tested in a way that will not be feasible *in situ*. The development of equipment that can present fish with different acoustic cues in a choice chamber will provide direct answers to which specific features within reef noise might cause larvae to move away or towards the source. Pairwise choice experiments, as used with odour cues in Chapter 2, have proved to be an effective method for testing whether larval fish alter their swimming behaviour in response to sensory stimuli, particularily because the transient nature of the settlement-stage rules out any behavioural conditioning techniques that are used on adult fish and other animals. The disadvantage to using binary choice experiments is that choice can result from both an avoidance or preference of one cue type over

another. For example, in Chapter 2, when larval apogonids spent more time in the control seawater compared to the reef water in the choice flume, it was not possible to say whether this result arose because they preferred seawater, or were avoiding reef water. A danger of this methodology is that it can introduce an inadvertent bias into which cues we think settlement-stage larvae ought to respond to. This bias can be introduced both at the experimental design stage, in chosing which odour types are presented alongside one and other, and also when considering the results, as it is unclear how choice should be interpreted. Future choice experiments could introduce more than two options for larvae to chose from, such that an avoidance, or indeed ambivalent response of settlement-stage fish to sensory cues could be detected.

A common theme from Chapters 3 and 4 was that fish from different families will react differently to sensory cues. This is not surprising given that the settlement requirements of fish will vary from species to species, therefore it might not be suitable to make assumptions at the family level. This has implications not just for designing specific methods to capture target fish for PCC, but also for the development of biophysical models which use simple rules and assumptions on larval behaviour to predict how patterns in fish recruitment might change with changing conditions (Cowen *et al.* 2006, Paris *et al.* 2005, Sanchirico & Mumby 2009). More species-specific investigations into larval orientation behaviour could be used to back up the assumptions that are made in these applied contexts.

A future benefit of learning to percieve the reef from the perspective of a settlement-stage fish is that potentially we, like larvae, can use remotely available sensory cues, such as the sound of a reef, to monitor different coral reef sites. There is huge scope in learning to use the natural soundscape of a reef as a method to monitor coral reef sites. Sound recordings are already being successfully used to triangulate the location of illegal dynamite fishers in Southeast Asia, something that is difficult to police because of the diffuse nature of coral reefs (Woodman *et al.* 2004). For the potential benefits of remotely monitoring multiple areas using sound to be realized, then further studies that build from the work in Chapter 5 and 'sound truth' the variation in reef noise across multiple reef sites, with details on the biological characteristics of each reef are now required (Rountree *et al.* 2006). Although there are changes in the sound intensity of a reef during the day and night, the temporal variation is regular and

predictable, and therefore, could be controlled for, assuming recordings are taken at the same time of day at different sites. Furthermore, using sound recordings at night could also be used to monitor a previously neglected component of the fish assemblage, the less detectable, nocturnal inhabitants.

Of the questions that have arisen from this thesis, some would be best tested at Lizard Island. The reliable catch of larvae during the Australian summer months in combination with ready access to equipment, facilities and logistical support make Lizard Island Research Station an efficient place to conduct studies on larval settlement behaviour. As a consequence, a large amount of work on settlement-stage fish orientation has already been carried out there, so returning to Lizard Island for future work would have the advantage of building from these previous studies. However, it is not yet clear how general the picture that is being developed on larval orientation behaviour is. Certainly these data in Chapter 4 from the Philippines would suggest that it may not be appropriate to transfer findings from one location directly to another. To establish the generality with which larvae may react to sensory cues, acoustic or otherwise, one could start by moving to other reefs that are relatively intact, or at least less impacted by humans. For example, the response of larvae to acoustic cues may not be general but very specific to particular features within reef noise that have yet to be empirically tested, which will make it difficult to; 1) interpret and 2) apply findings from studies that make generalised assumptions on larval orientation behaviour in different locations. This will be especially true for future studies that are carried out in areas where the orientation cues surrounding a reef might be modified by anthropogenic impacts which could another layer of complexity to larval behaviour.

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A. Publications

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Testing the generality of acoustic cue use at settlement in larval coral reef fish

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Abstract. Some settlement-stage larval fish appear to be attracted to reef sound and may, therefore, use acoustic cues when orientating towards their settlement site. However, all work on the *in situ* response of coral reef fish larvae to sound in acoustic playback experiments has been carried out in the same location (Lizard Island, the Great Barrier Reef), and in some cases, using the same reef recording. It is therefore not clear how widespread acoustic cue use is. To test whether sound is a general and reliable indicator of reef settlement site, we conducted a similar experiment in a different coral reef region, where the coral reef habitat and therefore soundscape is less uniform in quality (Bohol, Philippines). Contrary to our predictions, in some cases we found that fish were not attracted to the broadcast reef sound. We suggest that this may be due to an artefact of the reef recording, possibly the location or the time of day the recording was made. Our results indicate that larval fish are more selective in their response to coral reef sound rather than just being innately attracted to generic reef sound. This highlights the need to assess anthropogenic impacts on the natural soundscape, as this could affect the ability of larval coral reef fish to acoustically detect a suitable settlement site.

Key words: coral reef fish, cue, sound, light traps, settlement

Introduction

Ten years ago, it was hypothesised that larval reef fish could use sound to locate a settlement site (Stobutski and Bellwood 1998). There are now data that show that as early as the embryonic stage, coral reef fish can detect sound and their sensitivity to sound increases with age (Egner and Mann 2005; Kenyon 1996; Simpson et al. 2005a). At the time of settlement, damselfish (*Pomacentrus nagasakiensis*) are as equally sensitive to sound frequencies as juvenile-stage fish, and therefore are physiologically able to receive acoustic information (Wright et al. 2005). Additionally, Pomacentridae larvae can determine the direction of a sound source and will swim towards reef recordings broadcast in a choice chamber (Leis and Lockett 2005; Tolimieri et al. 2004). This is not just a general phonotactic response but appears to be specific to reef sound as fish were attracted towards reef recordings, but not artificial pure tones (Leis et al. 2002).

Acoustic playback experiments have shown that reef fish are attracted to light traps broadcasting reef sound over the ambient soundscape (Leis and Carson-Ewart 2003; Simpson et al. 2004; Tolimieri et al. 2000), and higher natural settlement rates are seen on patch reefs that were associated with underwater speakers playing reef recordings, in comparison to silent control patches (Simpson et al. 2005b). Generally, settlement-stage fish are more attracted to the higher frequency components of reef sound (made predominantly by invertebrates), relative to the original recording and the filtered lower frequencies alone, so sound appears to be more than just a broad indicator of reef location and may provide specific information used in settlement site selection (Simpson et al. 2008).

The use of sound for orientation during settlement varies among families, however, with some families appearing not to respond to sound cues (Leis and Carson-Ewart 2003; Simpson et al. 2004). What is not yet understood is how widespread acoustic cue use is. With the exception of one study carried out on sub-tropical rocky reef fish (Tolimieri et al. 2000), the remaining seven *in situ* studies that have shown positive phonotactic responses of larval fish to coral reef sound were all carried out at Lizard Island. Four of these studies shared the same single reef recording as the test sound (Simpson et al. 2004; Simpson et al. 2005b; Simpson et al. 2008; Tolimieri et al. 2004), and the remaining three used another (Leis et al. 2002; Leis and Carson-Ewart 2003; Leis and Lockett, 2005). As a result, our knowledge of acoustic cue use

in settlement-stage fish orientation is potentially very location specific and it has not been investigated in any other coral reef area, where the soundscape may be less consistent due to variability in reef quality. We questioned the generality of acoustic cue use by testing the response of larval coral reef fish to sound in a different location. Using the same techniques that have previously been used to assess the attraction of settlement-stage fish to broadcast reef sound at Lizard Island (i.e. coupling light traps with underwater speakers), we carried out a similar experiment on settlement-stage coral reef fish in the Philippines. Light traps collect phototactic larval reef fish at the end of their pelagic phase, and the comparison of catch rates in the sound treated vs. the silent traps can be used to assess the attraction of settlement-stage fish to the broadcast sound treatment (Leis et al. 2003; Simpson et al. 2004; Simpson et al. 2008; Tolimieri et al. 2000). We predicted that if sound is a general and reliable indicator of reef location, it will be used by settlement-stage fish in this different study area, therefore higher numbers of fish would be attracted to the sound, in comparison to the silent control treatment.

Methods

Traps (designed by Ecocean, St Clément de Rivère, France) were set at surface moorings located in a sea channel to the northeast of Pangapasan Island, Bohol, central Philippines (10°01.1'N, 123°56.2'E). The moorings were anchored on a sandy substrate in water of 10-12 m depth. There was no reef present within 50 m of each mooring and they were separated by c. 400 m to prevent acoustic overlap of the different traps broadcasting sound. The area at which the broadcast sound was detectable over the ambient reef sound was estimated to be 20-50 m (see Fig. 1).

Each night, two sound and two silent traps were each pseudo-randomly assigned to a mooring, so each treatment was tested multiple times at each position during the experiment. The sound systems consisted of an MP3 player, a 12V lead-acid battery, 18W Universal Amplifier Module (Kemo-Electronic GmbH, Lanhen, Germany), and an Electrovoice UW30 underwater speaker (Lubell Labs, Columbus, OH, USA). This played the sound treatment on continuous loop through the night, which was a recording taken at 8.40am on the 16th June 2007, at Black Forest Reef, a marine protected area located to the southwest of Bohol (09°31.228'N, 123°40.991'E). The recording was taken using an Edirol R1 recorder, and a HTI-96-MIN omni-directional hydrophone with a built in preamplifier (High Tech, Inc., Gulfport, MS, USA) and processed using Audacity 1.2.6. (a free digital audio editor available at http://audacity.sourceforge.net/) to delete artificial artefacts (e.g. the sound of distant boat engines) and produce a clean one-minute recording.

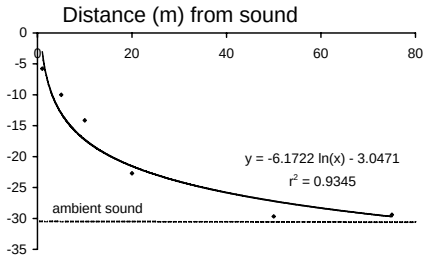

Figure 1. The distance over which the sound treatments were detectable over the ambient sound level. Sound intensity (root mean squared measured in relative dB) was measured at increasing distances from the sound system playing back a pure tone sound.

The experiment was conducted over 21 nights from the 4th-24th July. Traps were deployed at dusk, left overnight and collected at dawn, when the catches were transferred to separate polystyrene cool boxes and transported by boat to a nearby aquarium facility in Matabao, Bohol. Reef fish were separated from the rest of the catch (which consisted primarily of invertebrates and clupeids) and identified to family, or when possible, species level and counted. The fish were then given to Ecocean for rearing for a release scheme.

Analysis

There are no data available on the behaviour of larval fish upon entering light traps, therefore we do not know if it is a fair assumption to treat each captured fish as a statistically independent data point. For this reason, two approaches were taken for the analysis. A sign test, which makes no assumptions on the independence of fish caught, was used to test the null hypothesis that the number of nights with the largest catch would be the same for the silent and sound treated traps. As this test has a low power to detect a treatment difference when the number of testable nights per family is low (after excluding ties), the second approach estimated the effect of the sound treatment on the number of larval fish caught by fitting a generalised linear mixed effects model (GLMM). This method does assume larvae entered the trap independently, however it has the benefit of including the temporal and spatial variation that is characteristic of larval fish distribution and occurrence in light traps. Counts were grouped by family and families for which fewer than 10 individuals were captured over the experiment were excluded. Counts of fish per family were not normally distributed (Shapiro-Wilk test, $W = 0.1878$, $p < 0.001$). A logarithmic link function and Poisson

error distribution was specified as the data set was bounded by zero and the variance in counts per family was not equal. As there was inter-family variation in abundance, the number of fish caught per mooring and the number of fish caught per day over the lunar cycle, these were fitted as random effects. An interaction between sound treatment and family was fitted as a main fixed effect. As a significant trap unit effect was not found it was dropped from the model. The models were fitted using maximum likelihood.

Deviance statistics (estimates of how well the model captures the data) were generated for each model with and without the explanatory variables. To obtain the significance levels of the explanatory variables, the deviance statistics were compared using Chi-square tests. All analyses were implemented in R (R Development Core Team 2007).

Results

Twenty nights of data were collected, with 78 trap deployments (39 sound and 39 silent) as bad weather (on the 8th night) caused all traps to be retrieved early. A sound system failed on one occasion and on another a mooring was stolen, preventing a silent trap from being set, leading to a total of 18 nights data with sound treated and silent control trap deployments.

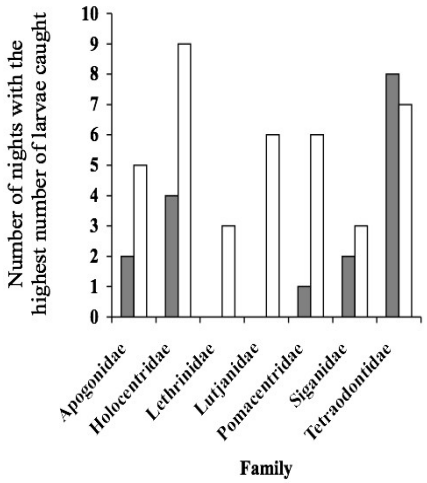

Figure 2. Number of nights with the greatest catch per treatment deployed with speakers (grey) and without (white) from the 4th-24th July 2007, Bohol, the Philippines.

A total of 326 larval coral reef fish from 14 families were caught (see Table 1). The four most common families (Apogonidae, Holocentridae, Siganidae and Tetraodontidae) comprised 75% of the total catch.

Table 1. Summary of catches of settlement-stage coral reef fish larvae caught in light traps with broadcast reef noise (sound) and without (silent). Low catch rates prevented analysis of some of the families. Results (significance levels) of the sign tests (per family) and the generalised linear mixed effects model (GLMM) (families grouped according to their direction of response to the sound treatment) are shown (see methods for details).

Family	Silent	Sound	Total	Sign test	GLMM
Apogonidae	31	12	43	0.226	
Holocentridae	22	13	35	0.133	
Lethrinidae	8	2	10	0.125	< 0.001
Lutjanidae	14	4	18	0.015	
Pomacentridae	13	1	14	0.062	
Siganidae	57	26	83	0.500	
Tetraodontidae	45	44	99	0.500	0.718
Blennidae	1	1	2		
Carangidae	2	2	4		
Chaetodontidae	0	1	1		
Mullidae	2	5	7		
Scaridae	0	2	1		
Sphyraenidae	1	4	5		
Syngnathidae	2	2	4		

Sign tests showed that one family (Lutjanidae) was caught in greater numbers on significantly more nights in the silent traps (see Table 1; Fig. 2).

In contrast, the less conservative GLMM that takes into account other spatially and temporally variable factors found that there was a variable response of fish families to the sound treatment (family: sound treatment interaction, χ^2: 14.41, p= 0.025). When the seven most abundant families were grouped according to their direction of response to the sound treatment, six (Apogonidae, Holocentridae, Lethrinidae, Lutjanidae, Pomacentridae and Siganidae) were caught in higher numbers in the silent traps in comparison to the sound (χ^2: 15.240, p<0.001). There was no difference between the sound and silent treatment in catch rates for the Tetraodontidae (*post hoc* Mann-Whitney, W: 118.5, p= 0.718).

Discussion

Settlement-stage larval fish were not attracted to the broadcast reef sound. We predicted that if fish could detect and were attracted to reef noise, there would be higher catch rates in the sound treated light traps. The opposite effect was found for the Lutjanidae, where significantly more fish were caught in the silent than in the sound treated traps. There was an overall

trend, when the abundant families were grouped together, for higher catch rates in the silent treated traps. This result is in contrast to those from four previous acoustic playback studies in which fish (of the families caught in this study: Apogonidae, Holocentridae, Lethrinidae and Pomacentridae) were attracted to broadcast reef sound (Leis and Carson-Ewart 2003; Simpson et al. 2004; Simpson et al. 2005b; Simpson et al. 2008). Our results are consistent with those of Leis et al. (2003), who demonstrated that the attraction of settlement-stage apogonids and pomacentrids to sound varied with location. In that study larvae responded positively to the sound treament at inshore but not offshore sites. There are two possible explanations for the lack of congruence with the findings that settlement-stage fish are attracted to reef sound: 1) there was a negative effect between the design of the traps used and the sound treatment 2) there was an artefact of the recording we broadcast for the sound treatment that acted as a repellant to settlement-stage larval fish.

We used a light trap that has a more open entrance than did those used at Lizard Island. This could mean that fish entering the trap were more vulnerable to predation. So if for example, the sound treatment also attracted predators, this could reduce the number of fish caught. Without any data on the rate of predation on fish entering the trap, this explanation, as with any other on a potential trap type and sound treatment interaction, is speculative. However, this is unlikely to have contributed to our finding that settlement-stage fish were caught in higher numbers in the silent traps, as when the Ecocean traps were used at Lizard Island in 2007-8, the most commonly caught families were more abundant in the sound treated traps (Heenan, pers. obs). This asymmetry also is unlikely to be the result of the fish caught in our study being unable to detect the sound treatment, as if this were the case, one would expect an equal number to be caught in the silent and sound treated traps. Without further experimentation, we do not know if this represents a general avoidance of coral reef fish larvae to sound in this region of the Phillipines or if it was specific to the recording used for the sound treatment.

There are two aspects of the recording itself that may have been repellent to settlement-stage coral reef fish. The first concerns the variation in reef sounds: they vary with time (season, moon phase and time of day); and the biological chorus has cyclical patterns in intensity, peaking during summer evenings around the new moon (Cato 1978; Radford et al. 2008). This coincides with when larval fish arrive in highest density to recruit to the reef (Dufour and Galzin 1993; Irisson and Lecchini 2008). While settlement-stage fish are attracted at night to nocturnal reef recordings (Leis and Carson-Ewart 2003; Leis and Lockett 2005; Simpson et al. 2004; Simpson et al. 2005b; Simpson et al. 2008; Tolimieri et al. 2004), they do not respond to nocturnal reef noise during the day (Leis et al. 2002; Tolimieri et al. 2004). Due to logistical reasons, the test recording we used was taken in the morning (8am), however *in situ* observations of released larvae showed that they orientate away from the reef during the day in Australia (Leis and Carson-Ewart 2002). In this study larval fish were repelled by a daytime recording, therefore this result supports the diel dependent nature of larval attraction to sound, suggesting that 1) they can perceive the difference between the sound of a reef at night and during the day and 2) they use this information to time their approach to the reef.

The second aspect of the test recording relates to the difference between the area where it was taken and played back. Located 60 km away, the recording was chosen as it was a marine protected area, with high fish diversity and abundance, and we believed it to be a biologically rich in sound. Some settlement-stage larval fish appear to imprint to their natal reef site by olfaction (Arvedlund and Nielsen 1996; Arvedlund et al. 1999; Gerlach et al. 2007), and so as embryonic stage fish can hear sounds (Simpson et al. 2005a) it is plausible to suggest that imprinting may also occur to natal reef sounds. If this were the case in this study fish may have been affected by the non local aspect of the test sound. However, in four separate studies, larval fish at Lizard Island were attracted to a recording taken at Feather Reef, which is located over 300 km away, which shows that larval fish will respond to a non local recording. Instead, we suggest that the test recording sounded sufficiently different from the ambient acoustic conditions that were characteristic of the playback site (a channel flanked by two reefs that had degraded to urchin and algal dominated reefs), that it caused fish in this area to avoid the played back sound.

This is the first *in situ* acoustic playback experiment on settlement-stage coral reef fish performed outside of the Great Barrier Reef, and we found that in contrast to these previous studies, catches of larvae decreased due to the sound treatment. This suggests that larvae are more selective in their response to reef sound, rather than having a generic innate attraction. Given the potential for habitat degradation, overfishing and anthropogenic sources of sound to modify the natural soundscape, acoustic surveys are needed to compare the soundprints of different reefs. Furthermore, experiments are required to determine the selectivity of acoustic cue use in settlement-stage fish, as it seems possible that this could affect the

ability of larvae to acoustically detect a suitable settlement site.

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Restoring depleted coral-reef fish populations through recruitment enhancement: a proof of concept

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To determine whether enhancing the survival of new recruits is a sensible target for the restorative management of depleted coral-reef fish populations, settlement-stage ambon damsel fish *Pomacentrus amboinensis* were captured, tagged and then either released immediately onto small artificial reefs or held in aquaria for 1 week prior to release. Holding conditions were varied to determine whether they affected survival of fish: half the fish were held in bare tanks (non-enriched) and the other half in tanks containing coral and sand (enriched). Holding fish for this short period had a significantly positive effect on survivorship relative to the settlement-stage treatment group that were released immediately. The enrichment of holding conditions made no appreciable difference on the survival of fish once released onto the reef. It did, however, have a positive effect on the survival of fish while in captivity, thus supporting the case for the provision of simple environmental enrichment in fish husbandry. Collecting and holding settlement-stage fish for at least a week before release appear to increase the short-term survival of released fish; whether it is an effective method for longer-term enhancement of locally depleted coral-reef fish populations will require further study.

Key words: behaviour; enrichment; *Pomacentrus amboinensis*; recruitment; restorative management; settlement-stage coral-reef fishes.

INTRODUCTION

Worldwide, coral reefs are in decline (Carpenter *et al*., 2008), one consequence of which is the decrease in abundance and diversity of fishes (Wilson *et al*., 2006). Most susceptible are fish species that have obligate coral associations, particularly those whose larvae settle onto live coral (Jones *et al*., 2004). There are two general

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responses to managing this demise. The first is the use of zoning plans and marine protected areas, which provides the opportunity for natural ecosystem regeneration by restricting access and decreasing anthropogenic activities on reefs. The second is a more interventionist approach, by attempting to restore the communities that inhabit reefs. This has included efforts to repair or replace the coral matrix through transplants and the provision of artificial settlement sites (Rinkevich, 2005; Shaish *et al*., 2008) and attempts to enhance depleted populations through the release of individuals into the wild. This technique is referred to as stock enhancement, which in the context of reefs, has thus far largely focused on invertebrate species (giant clam, subfamily Tridacninae, Gomez & Mingoa-Licuanan, 2006; sea cucumber, *e.g. Holothuria scabra*, Purcell & Simutoga, 2008). The few examples of attempts to repopulate fish communities have used species that associate with corals only transitorily, for example, the Pacific threadfin *Polydactylus sexfilis* (Valenciennes) and the red snapper *Lutjanus campechanus* (Poey) (Friedlander & Ziemann, 2003). Despite being an integral part of their ecosystem, there are no data on enhancement programmes for obligate coral-reef fishes.

There are reservations over active management approaches such as this because they do not directly address the primary causes of degradation, *e.g.* habitat and live coral loss through climate change induced warming, pollution and over-fishing (Jameson *et al*., 2002; Graham *et al*., 2006; Newton *et al*., 2007). Stock enhancement, like reef restoration, however, may be a useful supplementary management tool (Edwards, 2008; Mumby & Steneck, 2008). Empirical studies are required to determine whether this is the case, particularly because it remains a practiced yet unproven technique (Sadovy, 2005).

Coral-reef fishes have a pelagic larval and benthic adult stage, experiencing an estimated mortality rate of *c*. 60% during settlement (Doherty *et al*., 2004; Almany & Webster, 2006). The release of juveniles from cultured wild-caught or hatchery-reared larvae into recruitment limited populations (as many coral-reef fish populations are) can bypass or reduce this mortality bottleneck that occurs at settlement (Bell *et al*., 2008). This, in combination with the highly effective methods available for collecting coral-reef fishes from a great variety of families during or just prior to settlement, *e.g.* light traps, crest nets and hoa nets (Doherty, 1987; Dufour & Galzin, 1993), makes settlement larvae an ideal life-history stage on which to focus attempts to enhance depleted fish communities.

As predation is the main threat to settlement-stage fish survival (Planes & Lecaillon, 2001), simply using light-attracting devices to increase the recruitment rate of settlement-stage fishes to localized patches on a reef will not necessarily lead to a sustained increase in population size (Munday *et al*., 1998). Indeed, an increase in recruitment of fishes at this stage may well result in higher abundance of their gape-limited predators and, therefore, increase recruit mortality (Munday *et al*., 1998). In temperate stock-enhancement programmes, predation is the main cause of the high mortality experienced by released fishes (Olla *et al*., 1994; Brown & Laland, 2001; Salvanes & Braithwaite, 2006). Survival of released fish can be significantly increased by holding fish in conditions that stimulate their behavioural development, *e.g.* exposure to predators, altering the spatial or temporal distribution of food, manipulation of the social environment and the provision of natural habitat refugia (Olla *et al*., 1998; Brown & Laland, 2001).

The aim of this study was to determine whether enhancing recruitment could be used to assist depleted populations of obligate coral-reef fish species. To examine whether the high level of settlement-stage mortality could be alleviated, wild-caught larvae were held captive for a short period and then released onto the reef. If holding fishes captive during the vulnerable stage around metamorphosis makes them less susceptible to predation, then the prediction would be for higher survival rates in the fishes that were held prior to release, relative to those released immediately. The conditions in which fishes were held captive were manipulated, to determine whether tank variability leads to increased survival in released fishes, as it does for the North Sea cod *Gadus morhua* L. (Braithwaite & Salvanes, 2005). If being held in phsycosensorily deprived conditions leads to behaviourally deficient animals (Olla *et al*., 1998), then the prediction would be for higher survival rates in fishes that were held in tanks enriched with habitat refugia relative to those held in bare tanks.

MATERIALS AND METHODS

The ambon damsel *Pomacentrus amboinensis* Bleeker was the study subject. These fish can be caught in abundance during their summer breeding period in light traps, which can be used to collect fish just prior to settlement on the reef (Meekan *et al*., 2001). *Pomacentrus amboinensis* is common to the Great Barrier Reef, where like most Pomacentridae, it represents an important part of the total fish biomass (Ackerman & Bellwood, 2000). As a protogynous hermaphroditic species (Jones, 1987), males guard the nest in which females lay demersal eggs. The eggs hatch 4–5 days later and the larvae then spend 15–23 days off the reef in pelagic water, after which time they return to the reef to settle, typically to small reef patches on the reef base or slope where there is a mixture of live coral, sand and rubble (Kerrigan, 1996; McCormick & Makey, 1997). This species undergoes a high mortality bottleneck in the days immediately following settlement, when up to 75% of young fish may be removed by predators (Almany, 2004). Their main predators are the dusky dottyback *Pseudochromis fuscus* Müller & Troschel, the rockcod *Cephalopholis boenak* (Bloch), moonwrasse *Thalassoma lunare* (L.) and two species of lizardfish *Synodus variegatus* (Lacépède) and *Synodus dermatogenys* Fowler. All are either site-attached or home ranging (Holmes & McCormick, 2006; McCormick & Holmes, 2006). A further useful feature of the *P. amboinensis* is that it remains attached to the site once settled (McCormick & Makey, 1997), allowing for the assumption that once fish were released onto patch reefs they would remain in place, unless eaten.

Settlement-stage *P. amboinensis* were caught using light traps deployed before dusk (1830 hours) and collected after dawn (0600 hours) from permanent moorings in 10–15 m depth over a sandy substratum, in the near-shore waters of Lizard Island Research Station (14° 14′ S; 145° 26′ E) from the 22 to 27 November and the 8 to 12 December 2007. Settlement-stage *P. amboinensis* were separated from the rest of the catch and placed in shaded outdoor aquaria supplied with aerated flowing sea water at an estimated density of 200 fish per 40 l tank.

A pilot study carried out in November 2007 was used to determine the release protocol, and the frequency and duration of visual counts needed to assess post-release survival. Mixed species groups of *P. amboinensis* and the lemon damsel *Pomacentrus molucensis* Bleeker were used, as too few *P. amboinensis* were available for these trials. *Pomacentrus molucensis* are similar in size at settlement to *P. amboinensis* (mean standard length, L_S, *P. amboinensis*: 11·5 mm and *P. molucensis*: 11·3 mm; McCormick *et al*., 2002). At settlement, *P. molucensis* will only settle on live coral, typically on areas of continuous reef (Booth, 2002) but also isolated coral bommies (Figuira *et al*., 2008). *Pomacentrus amboinensis* is more of a settlement generalist, settling to live coral and rubble on continuous reef and patches (McCormick & Makey, 1997; Booth, 2002). These broad similarities at settlement made *P. molucensis* a sufficient substitute for the purposes of a pilot study. In this 12 day

pilot trial, the greatest rate of mortality occurred during the first 2 days following release (on average 25% loss). The rate of mortality then reached a plateau, decreasing by 2% (of the original number released) over the remaining 10 days. Based on this information, survival of *P. amboinensis* in the full experiment was measured on days 1 and 2 by three visual surveys (at 0600, 1200 and 1700 hours), on day 3 by two surveys (0600 and 1700 hours) and then once daily (0600 hours) for a further 5 days, for a total of 8 days.

In December 2007, single species experimental trials using *P. amboinensis* were conducted. On the morning of capture (day 0), fish were randomly allocated to one of three treatment groups, then tagged and photographed for measurement as follows. Each fish was placed into a plastic click-seal bag (size: 9 cm × 12 cm) containing aerated sea water and placed flat on its side on top of a laminated piece of graph paper. Fish were digitally photographed using an Olympus Camedia C-5000. The camera was positioned *c*. 30 cm above the fish with both the fish and the graph paper in focus. The L_S(±0·01 mm) were measured from the photographs using Image-J (Rasband, 1997–2009; http://rsb.info.nih.gov/ij). The same observer measured fish throughout the experiment to reduce between-observer variation. Using a 29-gauge hypodermic needle, fish were tagged through the plastic bag with a subcutaneous fluorescent elastomer tattoo (Northwest Marine Technology; www.nmt-inc.com). Tag colours (blue, orange, pink and yellow) were alternated among treatment groups to reduce any potential interaction between predation rate and colour. Tagging of fish allowed any movement between neighbouring patches to be detected and enabled the identification of released fish.

Settlement-stage fish selected for the experiment were randomly allocated to three treatment groups: (1) released the day after capture and referred to as settlement-stage, (2) held for 7 days in enriched tanks and (3) held for 7 days in non-enriched tanks, together referred to as captive held. There were four replicates per treatment group, each containing 30 fish (360 fish in total). Four aquaria were modified so that they could each house one enriched and one non-enriched replicate group separately. Silicone sealant was used to fix a single opaque Perspex divider, creating two separate holding areas per aquarium (dimensions 30 cm × 15 cm × 20 cm). Each half had an independent supply of fresh aquarium-supplied sea water and an outflow standpipe which maintained the water at 15 cm depth. On the enriched side, the aquarium was lined with sand and had a live cauliflower coral *Pocillopora damicornis* coral head (*c*. 8 cm × 8 cm × 8 cm) positioned in the centre of the tank, while the non-enriched side of the aquaria was left bare.

Settlement-stage fish were not fed during the time they spent in the laboratory and were released the day after capture (day 1). Fish held for >24 h (captive-held) received their first feed the day after capture (day 1) and were fed twice daily (0600 and 1800 hours), receiving their last laboratory meal at 0600 hours on the day of their release (day 8). They were fed 40 ml of 12–16 h old *Artemia* sp. nauplii (density: *c*. 2000 individuals per 1 ml sea water).

The release protocol was identical for all three treatment groups. The exception to this was that the captive-held fish were photographed again *c*. 4 h prior to release on day 8. At 12 h before fish were released, the patch reefs were cleared of existing *P. amboinensis* using an anaesthetic consisting of a mixture of clove oil (eugenol 85–95%), alcohol (98% ethanol, 2% methanol) and fresh sea water (ratio 0·005: 0·05:1) (Munday & Wilson, 1997). On the day of release, fish were placed in open 8·5 l plastic click-seal bags (one bag per replicate group containing 30 fish) filled with aerated water. Bags were sealed for transport and taken to the patch reef site and fish were released between 1600 and 1700 hours. Fish were released onto small artificial patch reefs that had been built on a 4–5 m depth sandy bottom in the Lizard Island lagoon (14° 41′ S; 145° 28′ E). One treatment replicate group was released per patch. Reefs consisted of a coral rubble base (60 cm × 40 cm × 20 cm) with a live *P. damicornis* coral head (*c*. 20 cm × 20 cm × 20 cm) positioned on top. For 1 h following release, wire cages (100 cm × 100 cm × 100 cm, mesh size: 5 mm) were positioned over each patch reef to exclude predators, after which the cages were removed (McCormick & Meekan, 2007).

Patches were arranged in rows with 5 m within and between rows. As newly settled *P. amboinensis* tend not to move >0·5 m in the first week following settlement (McCormick & Makey, 1997), the 5 m separation was assumed sufficient to prevent between-patch migration, and tagging of fish allowed this to be tested. Each treatment group was represented on every row away from the reef edge (distance 20, 25, 30 and 35 m), except 30 m where there was a settlement-stage group alone. The final number of treatment group replicates was: settlement-stage = 4, enriched = 3 and non-enriched = 3. This was due to the loss of

one enriched and non-enriched replicate group during the captive period, as the tank was inadequately sealed allowing fish to pass between the enriched and non-enriched sides of the tank.

Released fish were surveyed for 8 days as described above. On the final day, survivors were collected using clove oil and transported back to the aquarium in 8·5 l click-seal bags where they were photographed again.

The treatment effect on fish survivorship was examined using survival analysis. Data were right-hand censored, as some individuals outlived the study, and interval-censored as survival of released fish was recorded at set time increments, *i.e.* the time of death was unknown but was bounded between observation periods. To obtain the significance levels for the explanatory variables, deviance statistics generated from models with and without the explanatory variables were compared using χ^2-tests. Survivorship is not a linear function of age, as the risk of mortality decreases with time after settlement. This was assessed in a preliminary model comparison, where the Weibull error distribution (non-constant survivorship) had a greater explanatory power for the variance in the data than an exponential (constant survivorship) error distribution (χ^2, d.f. $= 1$, $P \leq 0{\cdot}01$). A *post hoc* assessment of the within-tank mortality of the captive-held fish was made, where the proportion of fish remaining alive after 7 days in captivity in the different tank treatments (enriched or non-enriched) was compared using a Kruskal–Wallis test.

Differences in L_S among treatments were compared using one-way ANOVA at the different experimental stages (capture, release and recapture). Significant effects were further explored using *post hoc* Tukey's HSD tests to determine which treatment groups differed in L_S. All analyses were implemented in the R environment (R; http://www.r-project.org), using the R package survival (S original by Terry Therneau and ported by Thomas Lumley).

RESULTS

Irrespective of holding conditions, the survival of fish from the combined captive-held treatment groups was higher than that of the settlement-stage treatment group that were released immediately onto patch reefs [Survival analysis (Weibull error distribution), $P < 0{\cdot}01$; Fig. 1]. After 8 days on the patch reefs, 24% of the settlement-stage treatment fish had survived, while 40% of the non-enriched and 60% of the enriched individuals survived (inclusive of any within-tank mortality experienced during the 7 day holding period). This difference in survival on the patch reefs between fish held in the enriched or non-enriched conditions was not significant (χ^2, d.f. $= 1$, $P > 0{\cdot}05$); however, a comparison of their survival during the 7 days spent in captivity showed that fish held in non-enriched tanks suffered greater mortality than those held in the enriched tanks (Kruskal–Wallis, d.f. $= 1$, $P < 0{\cdot}05$). There was no effect of tag colour (χ^2, d.f. $= 1, 3$, $P > 0{\cdot}05$), and the distance of experimental patch from the lagoon reef edge (χ^2, d.f. $= 1, 3$, $P > 0{\cdot}05$) also did not affect the survival of released individuals for all three treatment groups. No between-patch movement was detected from the coloured tags present on the fish.

There was no difference in the mean L_S of fish at the start of the experiment when they were allocated to different treatment groups [one-way ANOVA, d.f. $= 2, 7$, $P > 0{\cdot}05$; Fig. 2 (capture, day 0 for all treatment groups)]. A test for normality showed that the non-homogeneity in variance of size distribution of fish at the release stage was not significant (Bartlett test K-squared, d.f. $= 2$, $P > 0{\cdot}05$). At the point of release, after 7 days in captivity, there was a main effect of treatment group on the L_S of fish [one-way ANOVA, d.f. $= 2, 7$, $P < 0{\cdot}01$; Fig. 2 (release, day 1 for the settlement-stage fish and day 8 for the captive-held fish)]. The settlement-stage fish that were 7 days younger at the time of release were smaller than fish

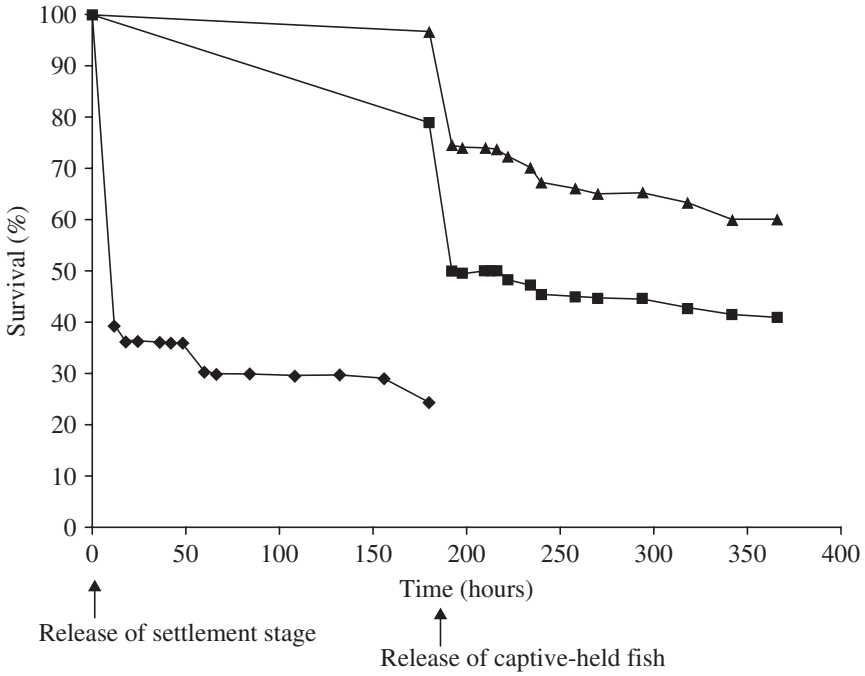

FIG. 1. Percentage survival per treatment group. The settlement-stage *Pomacentrus amboinensis* (—◆—) were released within 36 h of capture, while the non-enriched (—■—) and enriched (—▲—) treatment groups were released after 7 days captivity in aquaria (↑, release times). The total number of individuals captured and released per treatment group: settlement-stage (131 at capture, 130 at release), non-enriched (90 at capture, 71 at release), enriched (90 at capture, 87 at release).

held in captivity (Tukey's HSD, $P < 0{\cdot}05$). Enriched and non-enriched fish did not differ in size (Tukey's HSD, $P > 0{\cdot}05$). At recapture, after 8 days on the patch reefs, the captive-held fish (enriched and non-enriched) were significantly larger than the settlement-stage fish (one-way ANOVA, d.f = 1, 6, $P < 0{\cdot}05$; Fig. 2). Recapture was 8 days after initial capture for the settlement-stage and 15 days for the captive-held fish.

DISCUSSION

Fish that were held for 7 days in captivity prior to release had significantly increased survival when released onto patch reefs in comparison with fish released immediately after capture. Survivorship was improved by 16–36%. This major effect on survivorship following the relatively minor intervention of holding fish captive for a week led to increased survival of *P. amboinensis*. Artificial enhancement is a common technique for commercially fished species. This study demonstrates that by assisting fishes through vulnerable settlement and metamorphosis processes, the immediate survival of new recruits can be increased, and hence enhancement may be a useful tool for the conservation of coral-reef fishes. Holding conditions had no effect on survival once released onto the reef; however, during captivity fish kept in bare tanks survived less well than fish kept in tanks containing pieces of coral. This suggests there is merit in including psychosensory enrichment in the holding conditions in fish husbandry.

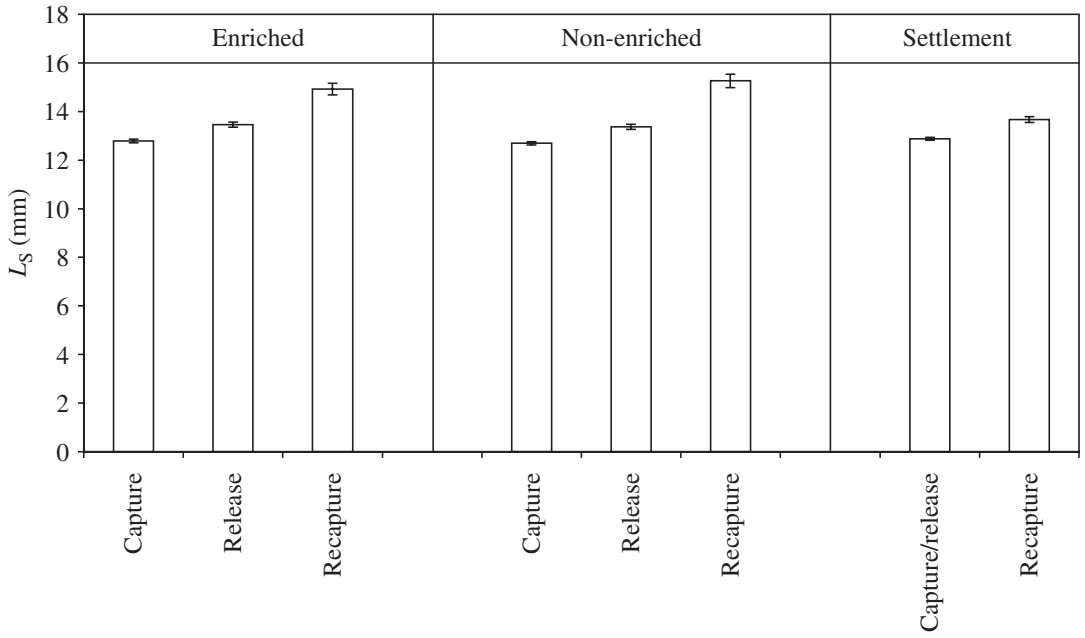

FIG. 2. The size of *Pomacentrus amboinensis* measured in standard length (L_S) with a panel per treatment group. Fish were either held for 7 days in tanks with enriched or non-enriched conditions, or released straight after capture (settlement-stage). The L_S measurements were taken at three stages, at the time of collection from the light traps (capture), at release onto the patch reefs (release) and at recapture after 7 days on the patch reefs (recapture). As the settlement-stage treatment group were released immediately after capture, the capture and release measurements are the same (capture–release); therefore, the age of the settlement-stage fish at this stage is equivalent to the tank-held fish at the release stage.

The chief advantage of holding fishes is that it confers higher survival once they are released onto the reef, potentially through a reduction in vulnerability to predation. This is inferred from a predator-exclusion experiment that identified predation on metamorphosing fishes as the major cause of mortality in settlement-stage pomacentrids when fishes were released into cages containing patch reefs with or without natural predators (Planes & Lecaillon, 2001). Over a period of 48 h, fishes released onto predator-free patches experienced a 14% mortality rate in contrast to fishes released on to the patches containing predators, where mortality ranged from 29 to 76%.

Releasing fishes immediately after capture directly onto a particular area (Munday *et al*., 1998), led in this experiment at least, to poorer survival in the immediately released fishes relative to fish held captive for a week. In order to enhance recruitment artificially on a small scale, it is better to hold fish captive for a short period, as *P. amboinensis* experiences its highest mortality risk at settlement (Almany, 2004; McCormick & Hoey, 2004). Fish were held in captivity beyond that peak (2 days following settlement; Almany & Webster, 2006) and those that survive through this critical period are likely to persist in the long-term.

Irrespective of whether fish were released immediately or were held in captivity, average L_S increased during the first 8 days following initial capture. Hence, by the time of release the captive-held fish were larger than the settlement-stage fish. *Pomacentrus amboinensis* undergoes size-selective mortality at settlement. The direction (positive and negative) of this process can vary (Hoey & McCormick, 2004;

Gagliano & McCormick, 2007) and is thought to be driven by predation. Whether settlement mortality is selective for smaller individuals can depend on physiological and morphological traits, *i.e.* the individual fish condition (Hoey & McCormick, 2004) and also on the predator conditions into which fish recruit (Holmes & McCormick, 2006). It would appear that a period of alleviated predator-stress in captivity allowed fish to increase in size, allowing the captive-held fish to successfully evade predation once released onto the reefs.

The possibility cannot be excluded that the greater mortality suffered by the immediate-release group was a result of these fish still recovering from a stress response to the handling and tagging procedure. Although this may have had an effect, these fish experienced similar levels of mortality (76%) as those previously reported during the natural settlement of damselfish (Pomacentridae) onto patch reefs in the presence of predators (*c.* 75%; Almany, 2004). If handling and tagging were detrimental, this should have resulted in an increase in mortality over and above this level.

It is not clear whether less common larger fish, which are not as site-attached immediately after settlement, would respond as positively to the experimental protocol. Furthermore, it is also not clear how the tank environment contributed to enhanced survival on release. During this study, rough weather conditions led to low larval catch rates, preventing the further replication needed to test whether the tendency for higher survivorship in the enriched holding conditions was biologically significant. As the within-tank mortality of fish held in enriched tanks was lower in comparison with the non-enriched tanks; this demonstrates that there is merit in providing fish with structure while in captivity. One theory is that artificial rearing conditions cause the production of behaviourally deficient or modified animals (Olla *et al.*, 1998; Brown & Laland, 2001; Hawkins *et al.*, 2008). This has been demonstrated for behavioural traits likely to affect survival in the wild such as foraging behaviour in *G. morhua* (Braithwaite & Salvanes, 2005). It seems plausible that providing some structure to the tank allowed fish to hide from conspecifics, leading to lower stress levels and therefore lower levels of mortality while in captivity and possibly to lower predation upon release.

Pomacentrus amboinensis was used in this study because it can be readily caught at the settlement-stage and is amenable to experimentation. Although they do not form part of commercial food fisheries, in many countries they are an important ecological component of the reef fish assemblage, being the second most abundant family and making the greatest contribution to biomass production (Depczynski *et al.*, 2007). Pomacentrids also represent 47% of the global export of marine ornamental fishes for the aquarium trade (Wabnitz *et al.*, 2003). These findings are therefore relevant for the conservation of reef fishes by providing a management model that may be relevant to commercially harvested fishes, and a demonstrated tool for less commercially exploited, but ecologically important, species.

Attempts to restore and enhance natural recruitment have proved successful for corals (Heyward *et al.*, 2002; Amar & Rinkevich, 2007), but have rarely been trialled for reef fishes (Sadovy, 2005). This study has demonstrated that holding settlement-stage coral-reef fishes for as little as a week leads to a significant increase in survival. Therefore, this may be a promising method for use in attempts to increase population numbers in commercially important or endangered reef fish species.

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