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Ambient Underwater Sound:

Measuring the importance of spatial variability and its effect on late-stage larval crabs

Jenni Anne Stanley

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Marine Sciences

The University of Auckland, 2011
“The sea, once it casts its spell, holds one in its net of wonder forever”

Jacques-Yves Cousteau
Abstract

Recent studies have shown that underwater sound emanating from coastal reefs may be used for guiding the movements of a wide range of reef organisms to suitable settlement habitats. However, it is not known whether this underwater sound is also capable of mediating the settlement and metamorphosis processes in these organisms. The present study used laboratory- and field-based methods to determine whether ambient underwater sound is used as a settlement and metamorphosis cue in 10 species of larval crabs.

The settlement stage larvae of five common crab species showed marked changes in swimming behaviour consistent with settlement and showed a significant decrease in time to metamorphosis (TTM) when exposed to replayed ambient reef sound compared with a silent control.

Ambient underwater sound has the potential to convey valuable information about the type and suitability of the habitat at its source to settlement stage pelagic larvae provided different habitats produce distinctive underwater sound. Analyses of recordings from several different habitat types along the coast of north-eastern New Zealand showed that the sound emanating from different habitat types had marked differences in terms of gross character, i.e., spectral composition and sound level. When habitat specific sounds were used in laboratory- and field-based experiments a significant decrease in TTM was observed for settlement stage crab larvae exposed to favourable settlement habitat sound when compared to unfavourable habitats.

Behavioural thresholds for habitat sound were determined experimentally by exposing settlement stage larvae to a range of sound levels from both favourable and unfavourable habitat types for settlement. Larvae did not respond to sound from unfavourable habitat types. However, for sound from favourable habitat types for settlement most crab species showed increasing reductions in TTM as sound levels were increased, suggesting that proximity to the sound source or settlement habitat is important in inducing faster settlement.

The results presented in this thesis demonstrate that ambient underwater sound originating from coastal habitats mediates the settlement processes of the megalopae of many
common coastal crab species in both temperate and tropical waters. It provides evidence that differences in the spatial and biological characteristics of underwater sound play a significant role in this process. Overall, the results of this research greatly extend our knowledge of the importance of underwater sound to recruitment processes of coastal larvae.
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I dedicate this thesis to Alice (Yue Gui) who’s time was tragically cut short. We will all miss you greatly.
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Chapter One: General Introduction

1.1 INTRODUCTION

1.1.1 Dispersal and sensory abilities of larval fish and decapod crustaceans

Many marine organisms, including decapod crustaceans, have a bipartite lifecycle consisting of a planktonic egg and/or larval stage and a relatively sedentary benthic juvenile and adult phase (Caselle & Warner, 1996). In reef dwelling organisms the pelagic larval phase can be as little as a few days in some crab species, eight days in some anemone fish (Leis & McCormick, 2002), to over 18 months in the open ocean environment for some spiny lobster species, which can lead to dispersal of tens or hundreds of kilometres (Gaines & Roughgarden, 1985; Chiswell & Booth, 1999; Montgomery et al., 2006). These larvae must be able to balance dispersal to new locations to avoid competition with parental stock, with the necessity to settle into a habitat which is optimal for survival, growth and reproduction (Bradbury & Snelgrove, 2001). Due to the relatively small size of marine larvae there are many difficulties around returning to a coastal reef to settle. In the past, pre-settlement larvae were regarded as passive particles and were thought to disperse with the currents until sufficient development allowed them to settle onto the first suitable habitat they encountered. Many authors termed it the “simplifying assumption” and added that behaviour was not to be considered because it had negligible influence on the dispersal course of larvae (Colman, 1933; Williams et al., 1984; Frank et al., 1993; Roberts, 1997).

More recently there has been a dramatic change in our understanding of larval dispersal and settlement processes in reef organisms, largely due to the results of research on the presettlement stages of reef fishes (Stobutzki & Bellwood, 1994; Stobutzki, 1997; Stobutzki & Bellwood, 1997; Leis & Carson-Ewart, 1999; Fisher et al., 2005; Leis et al., 2007) and decapod crustaceans (Shanks, 1995; Valero et al., 1999; Jeffs & Holland, 2000). This research has revealed remarkable sensory and physical capabilities of settlement stages of fish and crustaceans, including hearing, vision, olfaction, magnetic and electrical sense, as well as impressive swimming capabilities sufficient to overcome ambient water currents. This is
especially true for the late-stage larvae of many species of fishes and crustaceans that possess multiple sensory capabilities as well as great swimming abilities (Kingsford et al., 2002) (Figure 1.1).

The first studies describing sustained swimming abilities in the late pelagic stages of a number of families of reef fishes (Acanthuridae, Apogonidae, Chaetodontidae, Lethrinidae, Lutjanidae, Monacanthidae, Nemipteridae, Pomacanthidae, Pomacentridae) found some to have impressive swimming capabilities (Stobutzki & Bellwood, 1997). They found the average time until exhaustion ranged from 7.4 to 194 h and swimming distances ranged from 3.6 to 96 km (swum at 13.5 cm s$^{-1}$). The role of active swimming has also been described for the pre-settlement larvae of some decapod crustaceans (Forward et al., 2004; 2005). For example, the puerulus stage of the western rock lobster (*Panulirus longipes*) were observed to have swimming speeds of approximately 33 cm s$^{-1}$ (maximum) and 15 cm s$^{-1}$ (mean) during the night at Seven Mile Beach in Western Australia (Phillips & Olsen, 1975). The puerulus stage of the spiny lobster (*Jasus edwardsii*) have also been examined in the laboratory and found to be capable of sustained forward swimming, with swimming speeds between 13.0 – 30.7 cm s$^{-1}$ (Jeffs & Holland, 2000). In four crab species (*Uca uruguayensis, Chasmagnathus granulate, Cyrtograpsus angulatus* and *Cyrtograpsus altimanus*) megalopal swimming speeds ranged from 1.2 to 20.8 cm s$^{-1}$, depending on species and size, with the maximum occurring in *C. altimanus* (Valero et al., 1999). These impressive swimming abilities observed in both larval fishes and decapod crustaceans will be of maximum benefit for a settling stage larva when it can be directed towards suitable settlement habitat sites. This would require the presence of reliable sensory cues that these marine organisms can detect and use to orientate or adjust their swimming behaviour accordingly.
Some pre-settlement fishes and crustaceans are active dispersers with the ability to show orientated swimming behaviour and the capacity to orientate relative to reefs from distances of at least 1 km (Leis et al., 1996). There have been a large number of studies that focus on the methods of habitat selection and settlement cues by larval fish and invertebrates at both close range and at greater distances offshore (Williams et al., 1988; Carr, 1991; Fernandez et al., 1993; Doherty et al., 1996; Hedvall et al., 1998; Lecchini, 2005). The three sensory cues thought to be useful in orientating larvae in the direction of a suitable settlement
habitat are thought to be visual cues, such as visual location of reefs, olfactory cues, such as adult odour, and acoustic cues, such as ambient underwater reef sound (Kobayashi, 1989; Leis & Carson-Ewart, 1999; Atema et al., 2002; Kingsford et al., 2002; Simpson et al., 2004; Montgomery et al., 2006).

There have also been a number of studies investigating the processes involved in the induction of settlement in reef organisms. Lecchini et al., (2004) conducted a series of laboratory and field experiments using the pre-settlement larvae of the coral reef fish *Chromis viridis* to determine the ecological determinants of settlement choice. The experimental fish were found to respond to the visual, acoustic/vibratory, and olfactory cues expressed by conspecifics resident on the reef but failed to show any significant responses to heterospecifics or coral substrates. Invertebrate larvae such as decapod crustaceans, often have very specific requirements for the initiation of settlement and metamorphosis, these cues can include salinity, depth, substrate rugosity, as well as a wide range chemical cues that are often linked to their optimum habitat as adults (Forward et al., 2001).

### 1.1.2 Life history, settlement and metamorphosis in decapod crustaceans

Brachyuran crab species are common decapod crustaceans on most marine coasts and are an important component of many coastal communities, especially reef habitat. Most brachyuran crab species have distinct habitat requirements, whether it is a complex rocky reef which provides shelter from predators while providing a good supply of food, or a soft bottom estuary providing a sheltered area with ample burrowing substrate (McLay, 1988; Jones & Morgan, 2002). All brachyuran crabs are brood spawners, but the ovigerous females release their broods as soon as they are able to hatch, so that a large majority of their larval development is planktonic (Wear & Fielder, 1985; McLay, 1988). Brachyuran larval development can be divided into three distinctive phases. With a few exceptions the first free-living phase of larval development is the ‘pre-zoea’ or nauplii. This phase of larval development is non-planktonic and very short lived, with moulting into the next phase occurring within approximately one hour of hatching from the egg. The second phase is the early pelagic dispersive ‘zoea’ which actively swims in the water column, and is morphologically very dissimilar to the adult of the species (Wear & Fielder, 1985). The zoeal phase is suited to life in the plankton because they have well developed carapace spines to discourage predators and have limited swimming abilities that are most suited to vertical migration, prey capture and predator avoidance (Shanks, 2001). The zoeal phase usually consists of three to twelve developmental stages (lasting weeks to months), which varies
greatly within the Decapoda and is only somewhat consistent at the family level (Shanks, 2001). The third and final larval developmental phase in brachyurans is the post-larval ‘megalopa’ and is reached after a moult from the final zoeal stage. The megalopae is morphologically crab-like in appearance and has highly developed horizontal swimming abilities with natatory setae on the abdominal pleopods and an extended abdomen with telson (Shanks, 2001). The horizontal swimming ability of the megalopae greatly assists them with onshore migration and locating a suitable settlement (McLay, 1988). The duration of the planktonic megalopal phase in brachyurans, which usually consists of only one developmental stage, can vary between eight and twenty five days (Wear & Fielder, 1985) (Figure 1.2).

![Figure 1.2: Brachyuran life cycle, illustrating the different morphology during each phase of development (Not to scale). Source: with permission from the Smithsonian Environmental Research Centre.](image)

There are two processes which occur during the important transition by pelagic brachyuran crab megalopa to the benthic habitat; “settlement”; a behavioural process which includes movement out of the water column to a potential benthic settlement habitat, and “metamorphosis”; a physiological process which includes the loss of larval characteristics retained in the megalopa, such as the extended telson, and the transformation to the reptant body form that is typical for a benthic juvenile (Forward et al., 2001; Hadfield et al., 2001).
Although there are marked morphological changes accompanying the moult from the last zoeal stage to the megalopal phase that could be considered metamorphic, previous investigators have consistently considered metamorphosis to occur only when the megalopae moult to the first juvenile instar (Weber & Epifanio, 1996; Hadfield, 2000; Forward et al., 2001).

There is usually a period of time in the early larval phases where a brachyuran larva is morphologically and/or physiologically incapable of responding to settlement cues and passing through metamorphosis (Gebauer et al., 2004). Once a larva has reached competency, settlement and metamorphosis have commonly been found to be triggered by environmental inputs, such as chemical and physical cues that reliably identify a suitable settlement habitat (Gebauer et al., 2004). The major function of settlement process is the response to cues that will result in the settlement and metamorphosis in a habitat which presents the best probability of subsequent survival and ultimately, successful reproduction (Rittschof et al., 1998). During the megalopal phase many benthic dwelling species will come into contact with specific cues which will initiate downward swimming to locate a settlement site where they settle out of the water column and potentially metamorphose (Forward et al., 2001). During settlement, the behaviour of the megalopae will change noticeably, with reduced swimming activity followed by exploratory crawling behaviour on the substrate which exposes the megalopae to surface associated stimuli including texture, surface chemistry, pheromones and vibrations (Rittschof et al., 1998; Forward et al., 2001). If adequate stimuli are present, settlement is then closely followed by the physiological process of metamorphosis, a metamorphic moult to the first benthic juvenile instar (Forward et al., 2001). If the megalopae settle to an unfavourable site they may delay metamorphosis and return to the plankton and repeat the process of locating and selecting a suitable settlement habitat, this process is often referred to as delayed metamorphosis (Pechenik, 1990; Gebauer et al., 2003).

A variety of cues have been identified which decrease time to metamorphosis (TTM) that are both species specific and general, including environmental factors, such as temperature (Costlow, 1967) and salinity changes (Forward et al., 1994; Forward et al., 1997), and habitat specific cues such as substrate type (Fernandez et al., 1994; Weber & Epifanio, 1996; Gebauer et al., 1998), estuarine water (Forward et al., 1994), aquatic vegetation, biofilms, conspecific odour, and potential prey odour (O'Connor, 1991; Weber & Epifanio, 1996; Gebauer et al., 1998; O'Connor & Gregg, 1998). Most crab species that have
been studied undergo larval development offshore but metamorphose in estuaries, therefore it has been hypothesised that in these species metamorphosis should be accelerated by environmental factors characteristic of an estuary (Forward et al., 2001; Wilcox, 2010). In estuaries, salinity is usually low in comparison with offshore water due to fresh water input, and water temperatures during the summer are high and are cool in the winter. Studies on the blue crab, *Callinectes sapidus*, found that the duration of the megalopal stage decreased as salinity decreased (Forward et al., 1994; Wolcott & Devries, 1994; Forward et al., 1997) and as temperatures increased from 15 to 30º C (Costlow, 1967). The opposite was found with the Harris mud crab, *Rhithropanopeus harrisi*, where TTM increased with decreasing salinity (Fitzgerald et al., 1998).

The chemical cues present in estuarine water are also known to effect metamorphosis. *Callinectes sapidus* has also been found to demonstrate a reduction in TTM when exposed to increasing concentrations of humic acids extracted from river water (Forward et al., 1997). Humic acids are abundant in estuaries and are thought to be included in the active molecules in estuarine water that accelerated TTM in the blue crab (Forward et al., 1996; Forward et al., 1997). These acids are decomposition products of the structural elements of aquatic plants (e.g., saltmarsh cordgrass, *Spartina alterniflora*) and terrestrial plants (Moran & Hodson, 1994). The concentration of humic acid in waters tends to decrease with increasing distance from the head of an estuary and is in very low concentration in offshore waters (Forward et al., 2001). Therefore, the presence of dissolved humic acids in surrounding water is a reliable indicator of being in an estuary for a pelagic larva.

As many species of brachyuran crabs settle to specific habitats needed for adult life, it is logical to expect that cues from these preferred habitats could be involved in the induction of metamorphosis. For many crab species contact with benthic substrate from adult habitats reduce TTM, and the megalopae are frequently able to differentiate between different benthic substrates, with only some typical settlement substrates acting to reduce TTM (Forward et al., 2001). A consistent result among brachyuran species tested so far is that artificial mimics of the adult substrates fail to influence TTM, most probably due to the lack of the chemical cues associated with the surface biofilms on the artificial mimics (Forward et al., 1996; Weber & Epifanio, 1996; Gebauer et al., 1998; O'Connor & Gregg, 1998). For example, clean glass fragments and marbles did not affect TTM in the common mud crab, *Panopeus herbstii*, although, when glass slides were conditioned with adult odour where a biofilm developed, TTM was found to decrease (Rodriguez & Epifanio, 2000). Many habitat cues which reduce
TTM appear to be species specific; for example, studies on the sympatric species, the blue crab and the salt-marsh fiddler crab (*Uca pugilator*) have different settlement and metamorphosis cues (Welch *et al.*, 1997; O'Connor & Gregg, 1998). Studies on the blue crab have shown that megalopae show no response when exposed to conspecific adult odour and showed a strong response with a reduction in TTM, when exposed to the odours of salt marsh grasses and sea grasses (Forward *et al.*, 1994; Welch *et al.*, 1997). Adult blue crabs are found in many habitats throughout estuaries, therefore it is unlikely that the presence of adults will indicate to the megalopae that the settlement site is suitable, unlike the odour of certain vegetation which indicates an optimal settlement site. This contrasts to the megalopa of the salt-marsh fiddler crab, which undergo a large reduction in TTM when exposed to sea water containing chemicals released by adult conspecifics. The TTM of the megalopa have also been found to be reduced in the presence of sediments collected near the burrows of adults, implying that chemicals released from the adults have been absorbed into the sediment particles surrounding the borrow (Christy, 1989). However, for this crab species, chemicals from *Spartina alterniflora* has no effect on the TTM in megalopa, whereas it does in blue crabs (O'Connor, 1991). These results show that although the species share geographical areas and are often seen sharing an ecosystem (e.g., an estuary), they choose to preferentially settle at different microhabitats with the ecosystem (e.g., mud flats versus saltmarsh cordgrass), and therefore require different settlement cues. Characteristically, many of the successful settlement and metamorphosis cues studied to date have been water-soluble and infer structural protection and/or other characteristic that infer suitability of a habitat for settlement.

There have been a range of techniques that have been used to test the effects of specific factors on time to metamorphosis with differences in larval collection and rearing methods, as well as various experimental housing, test conditions, monitoring regimes, and statistical analyses (Forward *et al.*, 1994; Wolcott & Devries, 1994; Forward *et al.*, 1996; Forward *et al.*, 1997; Gebauer *et al.*, 1998; Gebauer *et al.*, 1999; Rodriguez & Epifanio, 2000). However, all of the techniques have all been based around a behavioural assay involving exposing an experimental animal to a potential metamorphosis cue and observing changes in TTM (Forward *et al.*, 2001). The behavioural assay can be modified in many ways to adjust the test environment, e.g., in the laboratory or in the field (explored in Chapter Four) and to identify additional behavioural responses to a diverse range of stimuli (explored in Chapters Four and Five).
Ambient underwater sound shares similar characteristics to chemical cues that would theoretically make it an effective settlement cue for late-stage larval crabs. Underwater sound has the potential to provide information on the type and suitability of the habitat where it is being created. Although being found to be temporally variable, ambient underwater sound is continuous in nature, this allows for constant exposure to the stimulus and the possibility of repetitive revaluation by an organism capable of detecting this stimulus. Underwater sound also has the potential to travel large distances, irrespective of water current direction or strength with only small amounts of attenuation, especially in open water (Au & Hastings, 2008).

1.1.3 Underwater sound – coastal shallow water

The earliest studies of ambient noise in shallow water (Knudsen et al., 1948) identified the main sources of noise as biotic (marine life, e.g., snapping shrimp), abiotic (e.g., water motion at sea surface) and anthropogenic (e.g., moving vessels) (Cato, 1997). Ambient underwater sound is characterised by a wide range of temporal and spatial variability due to the variety of sources and propagation conditions. Sound propagation in shallow water is essentially different than propagation in deep ocean waters given that sound can only propagate over distance greater than the water depth by repeatedly interacting with the surface and bottom (Rodgers & Cox, 1988). Ambient underwater sound can be highly directional, however, there are few published investigations focusing on the horizontal directionality of ambient underwater sound in coastal water. In Monterey Bay, California, using hydrophones with cardioid receiving patterns, it was observed that whenever the maximum receiving axis of the hydrophone was directed towards the shoreline, noise levels in the frequency range 20 – 700 Hz were greater that those obtained when the axis was directed seaward (Wilson et al., 1985). Also D’Spain and Batchelor (2006) at a site 60 km west, offshore of San Diego, California, detected that during the night, underwater sound that was centred around 1500 Hz was occurring at a greater intensity level from the southeast (landward) than the northeast (seaward).

In reef sound many biological sources can be described as small pulsating sources, and under these conditions the sound field is composed of a pressure waves that propagate radially from the source with a corresponding radial water particle motion. Particle motion is composed of two components; the first is due to the compression of the fluid by the pressure wave and is regarded as ‘true sound’. The second is the ‘flow’ component, which for a source such as biological noise, decreases with the square of the distance. Within one or two
wavelengths of the source, the flow component dominates and this region is labelled the ‘acoustic nearfield’. The region past this is labelled the ‘acoustic farfield’ where particle motion is directly related to the propagating pressure wave (Montgomery et al., 2006).

For detecting the motional component of sound the most common acoustic receptor for aquatic animals is a motion detector. In marine vertebrates this is based on deferential density accelerometers of the otolithic inner ear and in invertebrates the statolith organ (Montgomery et al., 2006). The pelagic larvae of fishes and crustaceans are moved in synchrony with the sound field due to having roughly equal density to the surrounding seawater and compared to the wavelength of sound in water they are relatively small. However, their otoliths and statoliths are about three times denser than seawater, have less inertia, and therefore move less, which shapes the physical foundations of a differential density accelerometer. The detection of particle motion is known as using a ‘direct’ path of sound stimulation (Fay & Popper, 1975). The receptors used to detect and process this movement are typically mechanosensory hair cells which detect the discrepancy in movement between the otolith or statolith and the surrounding tissues (Montgomery et al., 2006). The ciliary bundles in the hair cell are ‘connected to’ the otolith or statolith via a direct contact or through a membrane. The bundles experience a shearing stress or force which causes deflection in the individual hairs and subsequently a physiological response that is similar to that found in other vertebrate ears (Popper & Fay, 2011). Detection of particle motion of water associated with acoustic pressure in these cells is secondary to equilibrium functions. These hair cells are extremely sensitive and due to this can respond to many types of fluid motions and mechanical disturbances (Montgomery et al., 2006). In fishes, behavioural and physiological measures give a frequency range for otolithic hearing in the tens to hundreds of hertz, and with reasonably low amplitudes (Popper & Fay, 1999).

For detecting the pressure component of sound, specialised adaptations, such as an otophysic connection is thought to be needed. Species that have some kind of morphological connection or close proximity between the inner ear and a gas inclusion, such as a swim bladder typically have higher hearing sensitivities (lower thresholds) and higher upper frequency range of hearing (up to 2000 – 5000 Hz) (Popper & Fay, 1999; Popper & Fay, 2011). Detection of sound pressure is known as using an ‘indirect’ path of sound stimulation (Fay & Popper, 1975). Here the swim bladder or other gas inclusion will vibrate by the pressure component of the sound field, as the gas is at a different density and compressibility to that of sea water, acting as a pressure-to-displacement transducer. The displacement will
reradiate the signal from the swim bladder and emit a particle motion component that can subsequently produce motion of the sensory epithelium or otolith or statloth via an otophysic connection such as the Weberian ossicles (Montgomery et al., 2006; Popper & Fay, 2011). In this way certain fish have added pressure sensitivity to particle motion sensitivity (Fay & Popper, 1975). As water is somewhat incompressible, it is the sound pressure wave that spreads and dominates the farfield (Rodgers & Cox, 1988). Therefore, sound reception in the farfield is hugely dependant on the ability to detect either the radial particle motion or changes in the pressure field. Specific auditory receptor systems have been identified that will respond to one or the other of these components of the acoustic field. These specialisations are particularly evident in systems sensitive to acoustic pressure and consequently have supplied evidence for good hearing, however, the absence of these does not imply the opposite (Montgomery et al., 2006). The sound reception principles in aquatic animals are based on very different physical properties to that of mammalian sound reception, and our usual understanding of hearing mechanisms. In most cases for fish and aquatic invertebrates no obvious external anatomy exists and therefore hearing ability is not easily determined other than via inference from behavioural and physiological experiments.

Underwater sound experiences only a small amount of attenuation in seawater and therefore can travel over long distances (Rodgers & Cox, 1988). For example, a 500 Hz sound propagating over 100 km in seawater will only lose 1 dB due to attenuation (Rodgers & Cox, 1988). Underwater sound emitted from some coastal reefs is thought to be at a level that is well within the sound detection abilities of many larval fishes and possibly crustaceans (Leis & Lockett, 2005; Simpson et al., 2005a; Wright et al., 2005; Montgomery et al., 2006; Simpson et al., 2008b; Wright et al., 2008). Previous studies have estimated that larval fish could potentially detect coastal reef noise from distances of 500 – 5000 m from the source reef (Egner & Mann, 2005; Mann et al., 2007; Wright et al., 2008; Wright et al., 2010). More recently, a new sound propagation model has been proposed and tested, and reported detection distances significantly greater to these previous estimations (Radford, pers. comm.). For example, the Spanish flag snapper, Lutjanus carponotatus, was estimated to detect the sound from the reef over a distance of 50 km.

Some marine animals make extensive use of sound for orientation, communication and prey capture (Hawkins & Myrberg, 1983; Budelmann, 1992a; Popper et al., 2001a; Montgomery et al., 2006; Miller, 2010), it is for this reason that a substantial component and dominant feature of ambient underwater sound in the oceans is of biological origin, especially
in the southern hemisphere (Cato, 1992; Cato & Tavener, 1997; Cato & McCauley, 2002). Reefs are a major source of biotic noise in shallow coastal waters due to the abundance of soniferous organisms inhabiting the reef (Cato, 1992; 1997; Cato & McCauley, 2002). Whether the sound source produced on a coastal reef is biological or physical in origin, the most important sources are likely to occur in the top 10 – 20 m of the water column (Montgomery et al., 2006). For example, waves breaking on an exposed reef are generated in the shallows and in terms of sound the upper 20 m is also thought to be the most acoustically productive part of a reef due to the concentration of invertebrates and fishes.

Therefore, the most significant source of biotic sounds is to be expected in relatively shallow water (Montgomery et al., 2006). One of the most common and widespread biological sounds in coastal waters is created by snapping shrimp, with a measured peak in the frequency between 2000 – 5000 Hz and acoustic energy extending out to 200 kHz (Cato, 1992; Au & Kiara, 1998). In New Zealand waters, the sound produced by grazing activity of the urchin, *Evechinus chloroticus*, also appears to be a major contributor to reef sound in some locations (Tait, 1962; Castle, 1974). It is possible that differences in the presence and abundance of resident soniferous animals could possibly lead to different coastal habitat types, such as a sandy beach or estuary habitats, emitting different ambient sound signatures. This possibility needs further investigation as it would have important ecological consequences for animals searching for a suitable habitat from a distance offshore (explored in Chapters Three and Four).

Dawn and dusk choruses have been found to occur in coastal waters which consist of a distinct increase in biotic sound and are associated with the emergence and increased activity of crepuscular and nocturnal organisms (Tait, 1962; Cato, 1978; 1992; Cato & McCauley, 2002; Radford et al., 2008b). Tait (1962) was the first to describe the ‘evening chorus’ after observing an increase in sound level of 7 – 10 dB in waters off the eastern side of Great Barrier Island, north-eastern New Zealand. Dusk choruses have now been observed in a wide range of temperate and tropical waters such as, San Diego (D'Spain & Batchelor, 2006), Bimini, Bahamas (Fish, 1964), East Indian Ocean, Western Pacific Ocean, and the Timor Sea (Cato, 1978; 1980). Characteristically, these evening choruses begin at, or just after, sunset and continue for between two to five hours after sunset, although choruses have also been observed just before sunrise (Cato, 1992). In New Zealand the dominant frequencies (800 – 2000 Hz) in the dusk chorus were theoretically linked to feeding by the sea urchin (*Evechinus chloroticus*) (Tait, 1962; Castle, 1974; Castle & Kibblewhite, 1975; Cato, 1978). Later it was
experimentally confirmed that this relatively narrow frequency band was due to the ovoid calcareous skeleton (test) of the urchin acting as a Helmholtz resonance chamber greatly amplifying their feeding noises (Radford et al., 2008a). These results indicate that coastal urchin populations have the potential to be a major contributor to the underwater choruses as they are ubiquitous in temperate reef systems (Tait, 1962). There has also been a range of fish identified as contributing to the dusk chorus, particularly from the families Sciaenidae (croakers), Batrachoididae (toadfish) and Ariidae (sea catfishes) around North America (Cato, 1978; D'Spain et al., 1994). The biological choruses discussed above can increase the sound level emitted from a location to allow further transmission of the signal from the source. The greater the distance offshore that the reef sound travels the more it may enhance its importance in habitat selection and as settlement cue for larval marine organisms looking for potential coastal reef settlement sites.

While the properties of underwater sound in the ocean allow transmission over large distances, it also suggests that distant (tens to hundreds of kilometres) and intense sound sources can also contribute to local ambient noise. Frequently ambient underwater noise varies over a range of about 20 dB due to changing weather conditions, shipping activity and temporal patterns of behaviour of sound producing organisms (Cato, 1992; Cato & McCauley, 2002). However, the full range of variation in ambient underwater sound can exceed 30 dB and ambient underwater sound levels in the frequency band 50 to 10000 Hz are usually in the range 90 – 120 dB re 1 µPa. Noise sources such as breaking waves at the sea surface and rain may mask useful biological information contained in the underwater sound signal (Montgomery et al., 2006). As the distance away from a reef increases the background noise levels become relatively high in relation to the source sound signal and a considerable amount of this background noise will mask the useful signals emitted from the reef (Kingsford et al., 2002). However, some biological sounds from a reef can still exceed background levels at approximately 4 – 20 km from its source location (McCauley, 1997). Therefore, the intensity of the acoustic signal must surpass background noise to be detectable and therefore be useful as an ecological signal (Kingsford et al., 2002).

### 1.1.4 Coastal underwater sound as an ecological signal/settlement cue

For larvae of benthic coastal species to locate and successfully recruit to a suitable settlement habitat it would be most advantageous to have information available that specifies the direction, distance and quality of the potential habitat site. For ambient underwater sound
to be an ecologically relevant and reliable signal there are five criteria it must meet: 1) be directional, 2) travel some distance away from the source to the receiving organism, 3) be above the detection threshold of the receiving organism, 4) be at a detectable frequency range of the receiving organism, and 5) carry biologically relevant information to the receiving organism (Higgs, 2005). Ambient underwater sound originating at shallow coastal reefs often possesses all of these criteria making it a potentially reliable ecological signal.

Electrophysiological experiments have given evidence of hearing competence in both crustaceans and fishes (Breithaupt & Tautz, 1990; Kenyon et al., 1998; Lovell et al., 2005; Wright et al., 2005; 2007; 2008; Wright et al., 2010) and have therefore proved that it is at least possible for them to detect underwater sound. However, direct behavioural experiments need to be carried out to establish that these animals are in fact using sound to guide their movements to a reef and that acoustic cues enable them to locate and identify a suitable habitat in which to settle and metamorphose. Several field studies have shown that the settlement stage larvae of many reef dwelling organisms can detect and respond to reef sound. Studies using light traps employed in conjunction with underwater loud speakers have shown that broadcast reef sound attracts more larvae than traps without sound for both temperate and tropical reef fish larvae (Tolimieri et al., 2000; Leis et al., 2003; Simpson et al., 2004). Using the same techniques the presence of this attraction to reef sound in larval decapod crustaceans has also been established, with experimental ‘sound traps’ catching significantly more crab zoea and megalopae than ‘silent traps’ depending on the moon phase (Jeffs et al., 2003). In contrast, taxa with a nocturnally emergent lifestyle avoided traps in conjunction with sound (Simpson et al., 2011).

Larvae of both fish and crustaceans have also been found to be capable of determining the direction of a sound source. Experiments using in situ binary choice chambers showed than more pomacentrid (damselfishes) larvae orientated towards the nocturnal reef sound than away from it (Tolimieri et al., 2004), which was consistent with findings from other related studies (Stobutzki & Bellwood, 1998; Leis & Lockett, 2005). The choice chamber method was also used with the settlement stage larvae of marine invertebrates (Radford et al., 2007; Vermeij et al., 2010). In New Zealand, the post-larvae of five common species of coastal crabs were observed to orientate and swim towards a source of reef sound indicating a clear behavioural attraction to reef sound (Radford et al., 2007). It has also been found that coral larvae were not only able to detect sound, but were also able to respond to the sound by swimming towards the replayed reef sound (Vermeij et al., 2010).
The use of sound in habitat selection by tropical reef fish larvae has also been investigated (Simpson et al., 2005a). The study demonstrated that artificial patch reefs, made from dead coral, associated with replayed reef sound had higher number of larval fishes than patch reefs without sound. They also compared settlement rates among silent patch reefs, patch reefs with broadcast high-frequency (80% > 570 Hz) and patch reefs with broadcast low-frequency (80% < 570 Hz) components of reef sound. Apogonids settled on the high- and low-frequency patch reef in equal numbers, but pomacentrids were preferentially attracted to reefs with the high-frequency component of sound. Similarly the reefs without sound received less settlement and total number of families and taxa than reefs with broadcast sounds.

Together these studies have demonstrated that underwater reef sound is capable of eliciting a wide range of behavioural responses in both fish and decapod crustacean late-stage larvae from a wide range of species in both tropical and temperate waters. However, the potential for ambient reef sound to affect the process of settlement and metamorphosis in late-stage brachyuran larvae has not been assessed.

1.1.5 Sound reception in decapod crustaceans and fishes

In order for sound to be a strong candidate in the mediation of settlement and metamorphosis of marine larvae attempting to find a suitable settlement habitat it is necessary for the larvae to possess the sensory abilities needed to detect the underwater sound. They will also need the ability to interpret the information carried within the signal if attempting to find a specific suitable settlement habitat. The sound reception principles in aquatic animals are based on very different physical properties to that of sound reception in air. In most cases no obvious external anatomy exists and therefore hearing ability is not easily estimated but from behavioural and physiological experiments we know that a good sense of hearing is present in a number of species (Montgomery et al., 2006).

Sound reception in the acoustic far field (beyond about two wavelengths of the source sound) is hugely dependant on the ability to detect either the radial particle motion or changes in the pressure field. Specific auditory receptor systems have been identified that will respond to one or the other of these components of the acoustic field, in both fishes and crustaceans. Receptor systems sensitive to acoustic pressure have been well described in fishes and consequently have been used to supply evidence for good hearing, however, the absence of these receptors in an organism does not imply that they may have no hearing ability (Montgomery et al., 2006). For detecting the motional component of sound the most common
acoustic receptor for aquatic animals is a motion detector (Budelmann, 1992a; Popper et al., 2001a; Montgomery et al., 2006). In invertebrates this is based on deferential density accelerometers of the statolith organ. The pelagic larvae of crustaceans travel in coordination with the sound field due to having roughly equal density to the surrounding seawater and compared to the wavelength of sound are relatively small (Montgomery et al., 2006). The statoliths of crustaceans respectively are about three times denser than seawater, have less inertia and therefore move less, which shapes the physical foundations of a differential density accelerometer (Fay & Megela Simmons, 1999; Montgomery et al., 2006). The receptors used to detect and process this movement are typically mechanosensory hair cells which detect the discrepancy in movement between the statolith and the surrounding tissues (Montgomery et al., 2006). The ciliary bundles in the hair cell are ‘connected to’ the statolith via a direct contact or through a membrane. The bundles experience a shearing stress or force which causes deflection in the individual hairs and subsequently a physiological response that is similar to that found in other vertebrate ears (Popper & Fay, 2011).

These hair cells are extremely sensitive and due to this can respond to many types of fluid motions and mechanical disturbances (Montgomery et al., 2006). There has been substantially greater investigation into the hearing abilities of fishes than of decapod crustaceans, because of this detection abilities are largely unknown for decapods, especially in their larval phase (Jeffs et al., 2003; Radford et al., 2007). There have been a wide variety of structures identified in crustaceans that resemble (but are not homologous to) vertebrate receptors and may be involved in the ability of these organisms to respond to the physical parameters associated with underwater sound, such as hydrodynamic stimulation, particle motion and possibly, pressure changes (Cohen & Dijkgraaf, 1961; Breithaupt & Tautz, 1990; Budelmann, 1992a) (Figure 1.3). Their operation, sensitivity thresholds and behavioural significance of these receptors are unclear (Popper et al., 2001a; Jeffs et al., 2005; Montgomery et al., 2006). The receptor systems that have received the most investigation and allow reception of underwater sound (i.e., mechanical disturbance of water) can be classified into three groups: superficial receptor systems on the body surface, internal statocyst receptor systems and chordotonal organs (Budelmann, 1992a)
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Figure 1.3: Schematic illustrations to show the diversity of gravity receptor systems in Crustacea. Illustrative transverse sections (a – c) and lateral view (d) of the statocysts (not to scale). Small insets show dorsal views of the statoliths (S), with the arrangement of the cuticular hairs and their direction of polarisation (small arrows). a) Statocyst from the telson of the iospod, Cyathura polita. b) Right uropod statocyst from the mysid shrimp, Praunus flexuosus. c) Crayfish and lobster statocyst from the basal segment of the right antennules. D Statolith organ (large arrow) in the vertical canal of the crab statocyst. Note that in c) and d) the sensory hairs of the angular acceleration receptor systems of the statocyst are not shown (adapted from Budelmann, 1992 – with kind permission from Springer + Business Media).

It has been proposed that the statocyst found in crustaceans could be used as the basic hearing organ (Budelmann, 1992a; Popper et al., 2001a). Crayfish (Orconectes limosus) statocysts have been reported to be sensitive to vibration with a peak-to-peak threshold of 0.1 μm over a range of frequencies from 150 to 2350 Hz (Breithaupt & Tautz, 1988). The Norway lobster, Nephrops norvegicus, has been reported to have a distinct set of postural motor responses elicited by sound frequencies of 20 – 180 Hz in the laboratory and then later during free field experiments found to be sensitive to particle displacement with a response threshold of 0.888 μm, independent of frequency within the range 20 – 200 Hz (Goodall et al., 1990).
It has also been demonstrated that crabs show responses to pressure pulses (Fraser & Macdonald, 1994; Fraser et al., 2003). Previously no pressure sensor had been identified in crabs and the absence of any apparent compressible gas-filled compartment had been considered a problem in making a functional sensor. It was reported that the sensory hair receptors from the statocyst of adult crabs respond to step changes in hydrostatic pressure possibly through a mechanism concerning mechanoreceptor activation by alteration of the anterior volume of cuticular hairs. Planktonic crustaceans showed orientation changes with a threshold between 5 and 25 millibar (mb) (Fraser & Macdonald, 1994; Fraser et al., 2003). However, very little information is available regarding these structures in the early stages of crustaceans.

There are two main methods in determining the auditory abilities in marine organisms; behavioural assays and electrophysiological responses such as the acoustic brainstem response (ABR) technique (Montgomery et al., 2006). From a range of sensory modalities the general finding is that electrophysiological measures for thresholds are higher than behaviourally measured thresholds (Montgomery et al., 2006). The behavioural assay used in the current study is immensely valuable in understanding the behavioural responses and hearing capabilities of late-stage crab larvae to underwater sound. The assay may enable the measurement of behavioural response thresholds to levels of underwater reef sound in a number of crab species. These threshold values in turn may indicate the spatial scale in which underwater reef sound can act as a settlement and metamorphosis cue.
1.2 OBJECTIVES AND STRUCTURE OF THESIS

The main objective of this thesis was to begin to understand how ambient underwater sound emanating from coastal habitats can influence the settlement behaviour and metamorphosis in coastal crab species. There has been little previous research on the role of underwater sound on the behaviour and development of larval marine invertebrates. Therefore, there is a need for research to improve our understanding of the responses by larval decapod crustaceans to ambient underwater sound, which is the major focus of the research presented in this thesis. The specific aims of the thesis (for each chapter) are as follows:

Chapter Two: Induction of settlement in crab megalopae by ambient underwater reef sound
- Describe changes in settlement behaviour during settlement.
- Investigate the potential for ambient underwater sound to trigger settlement behaviour and/or shorten TTM in late-stage megalopae of common species of brachyuran crabs.
- Determine whether brachyuran crabs from both temperate and tropical locations are using underwater sound as a settlement and metamorphosis cue.

Chapter Three: Unique acoustic habitat signatures
- Determine if distinct habitat types on a short length of coastline (10 km apart) produce different underwater sounds (intensity level, spectral composition and temporal variation) that have the potential to be used by larval marine organisms to distinguish suitable settlement habitats.

Chapter Four: Settlement responses to differences in ambient underwater sounds associated with different habitat types.
- Determine whether laboratory experimental methods used for measuring the response of settling crab stages to underwater sound (Chapter Two) produce any potential artefacts arising from laboratory conditions.
- Determine the potential of settlement stage crab larvae to discriminate among different settlement habitats based on differences in habitat-specific underwater sound alone in both temperate and tropical locations.

Chapter Five: Behavioural response thresholds in New Zealand crab megalopae.
- Determine the behavioural response thresholds in a number of New Zealand crab species to underwater reefs sounds.
• Determine whether the observed settlement and metamorphosis response thresholds were specific to preferred habitat type.

*Chapter Six: General discussion*

• Provide an integrated discussion of the findings from the thesis as a whole.
Chapter Two:
Induction of settlement in crab megalopae by ambient underwater reef sound

Published as:

2.1 INTRODUCTION

A pelagic larval phase in the lifecycle of many benthic marine organisms typically involves dispersal away from parental habitat with the final stage of larval development selecting a suitable benthic habitat in which to settle (O'Connor & Gregg, 1998). Settlement and metamorphosis often involve a specific cue or a combination of physical and/or chemical settlement cues (Gebauer et al., 2004). These cues include salinity, depth, substrate rugosity, as well as a wide range of chemical cues from sources such as conspecifics, settlement substrates, aquatic vegetation, estuarine water and potential prey. These cues can be both general and apply to many species, such as the specific chemical cues associated with conspecific adults (Uca pugilator, Uca pugnax, Pagurus maclaughlinae, Paguristes tortugae, Chasmagnathus granulata, Panopeus herbstii, and Sesarma curacaoence) (Gebauer et al., 2003) and species specific, such as the presence of certain macroalgae species for the blue crab, Callinectes sapidus (Forward et al., 1996).

The majority of studies on marine invertebrate larval settlement and metamorphosis have concentrated on species that are sedentary as adults, especially commercially important biofouling and aquaculture organisms, such as barnacles and oysters (Forward et al., 2001). By comparison, relatively little is known about settlement cues in mobile marine invertebrates, such as brachyuran crabs which are common and important inhabitants of coastal habitats around the world (Wear & Fielder, 1985).

The late-stage larvae of many marine organisms are known to be capable of extending their larval phase, often for considerable periods, until suitable settlement cues or habitats are encountered. For example, polychaetes (Wilson, 1977), gastropods (Paige, 1988),...
echinoderms (Strathmann, 1978) and coral reef fish (Victor, 1986; 1991) have all been shown to delay metamorphosis until appropriate settlement cues are encountered. Some larvae will metamorphose spontaneously or even die without metamorphosing in the absence of specific settlement cues (Pechenik, 1990; Zimmerman & Pechenik, 1991; Gebauer et al., 2003).

Brachyuran crabs seem to lack the ability to delay metamorphosis indefinitely as they appear to have a temporal threshold beyond which settlement and metamorphosis occurs even in the absence of settlement cues (Weber & Epifanio, 1996). To determine maximum time to metamorphosis (TTM), megalopae are typically reared in the laboratory and exposed to a control treatment of untainted seawater. The mean TTM in previous studies of brachyuran crabs has varied from 5 to 20 d depending on the species (Forward et al., 2001). In brachyuran crabs the TTM can often be shortened by 15 – 25% upon exposure to chemical cues which serve as indicators of potentially suitable settlement habitat. These chemical cues can be sourced from the presence of adults, aquatic vegetation, biofilms, conspecifics, estuarine water, humic acids, related crab species and potential prey (Forward et al., 2001). The majority of these past studies have been carried out in laboratory aquaria or compartmentalized containers. Consequently the spatial range over which these settlement cues operate in nature is largely unknown and it is assumed that other physical processes, such as tidal currents, serve to initially position the megalopae in the vicinity of these chemical cues. Therefore, the current evidence suggests chemical settlement cues are being used over small distances (m) and do not appear to acting as an orientation cue over larger distances such as on the scale of kilometres (Butman, 1987; Boudreau et al., 1993; Forward et al., 1994).

A number of experimental studies have concluded that ambient underwater sound emanating from coastal habitats may act as a long distance orientation cue for settlement stage crabs and fishes attempting to locate suitable habitats (Stobutzki & Bellwood, 1998; Tolimieri et al., 2000; Jeffs et al., 2003; Jeffs et al., 2005; Leis & Lockett, 2005; Simpson et al., 2005a; Montgomery et al., 2006; Radford et al., 2007). However, previously the role of underwater sound as a settlement cue has not been investigated. Therefore, the aim of this present research was to investigate the potential for underwater sound to trigger settlement behaviour and/or shorten TTM in late-stage larvae (megalopae) of common species of brachyuran crabs from both temperate and tropical waters.
2.2 METHODS

The study was undertaken in temperate waters near the Leigh Marine Laboratory, located in north-eastern New Zealand (36° 15’ S, 174° 47’ E), and in tropical waters near the Lizard Island Research Station, north-eastern Australia (14° 39.5’ S, 145° 26’ E) during October to December 2008.

2.2.1 Source of megalopae

Light traps were used to capture megalopae for behavioural experiments (Hickford & Schiel, 1999). Up to two light traps were deployed at night within 500 m of the shoreline, 15 m apart and submerged 2 m in water of 5 – 7 m depth. The traps were recovered within 2 h of sunrise the following morning. When large planktivorous fishes were found in a light trap, megalopae were not used in the experiments as they may have altered behaviour due to stress from being in the presence of a predator (Forward & Rittschof, 2000). The megalopae were transported in seawater to a nearby laboratory where they were counted, sorted into settlement stage and identified to lowest taxonomic level possible given the available taxonomic descriptions (Wear & Fielder, 1985; McLay, 1988). Only intermoult pre-settlement (i.e., natant and active swimming) megalopae of a similar size were selected for use in the experiments. The megalopae were held in a flowing filtered (40 µm) seawater system with natural light period and ambient temperature (14 – 31° C, dependant on timing and location) until experiments begun the evening following capture. Five species of brachyuran megalopae were used for this research. The reptant phase in the lifecycle of these three temperate species, *Hemigrapsus sexdentatus*, *Cyclograpsus lavauxi* and *Macrophthalmus hirtipes* are all known to be associated with nearshore subtidal and intertidal habitats. The two tropical species were both identified to be members of the Grapsidae family, however, more detailed taxonomic placement was not possible due to the lack of taxonomic descriptions of megalopae and first instar juveniles of crab species in this region. The researchers observed early juvenile crabs with similar taxonomic characters to both experimental species settled in nearshore subtidal reef habitats. For the purposes of this work the species have been referred to as Grapsidae sp. one and Grapsidae sp. two.

2.2.2 Behavioural assay

Each treatment and control (i.e., Sound and Silent) consisted of three replicate water baths that were used to maintain a constant water temperature for megalopae throughout the
experiment. Each replicate water bath contained one Perspex container housing a group of megalopae (up to five megalopae) and one plastic vial housing a single megalopa. Individually housed megalopae were included in the experiment as a comparison with communally housed megalopae to test for any interactive effects on settlement behaviour that may exist among individuals.

Grouped megalopae were housed in a clear Perspex container (160 × 160 × 140.5 mm deep) with a square piece of Perspex sheet (100 × 100 × 15 mm) on the bottom imitating a settlement surface. The upper surface of the sheet had been roughened with coarse sandpaper to provide a chemically inert settlement substrate for settling megalopae. Each individually held megalopa was in a plastic vial (250 ml) with a roughened base, within the same water bath as the container holding the group-housed megalopae. Each replicate for both the sound treatment and Silent control had a weighted Sony loudspeaker inside a watertight plastic bag which was submerged in the water bath. For the Sound replicates only, a Sony CD Walkman D – EJ815 was used to continually play a 4 min loop of recorded ambient underwater reef sound into the water bath and through the acoustically transparent plastic containers holding the crabs (Gerber, 1978) (Figure 2.1).

When on a single night sufficient (>30) megalopae of the same species were collected from the light traps to conduct the experiments they were randomly allocated to the experimental treatment or control and replicates within these. Both grouped megalopae and the individuals in each treatment were kept in filtered (1 µm) and UV treated seawater under natural light period and ambient water temperature (14 – 31°C, depending on local temperature) for the duration of the experiment. Sound treatment and Silent control were randomly allocated to water baths for each experiment. Both the treatment and control were located in the same laboratory, but were acoustically isolated using foam rubber mats beneath all water baths to prevent transfer of any external acoustic energy. The absence of any significant acoustic signal in the Silent control tanks was confirmed by recording with a calibrated hydrophone (High Tech, Inc., HTI–96–MIN). In the absence of an anechoic chamber, all laboratory-based experiments were conducted in a quiet concrete floored and walled laboratory.

The megalopae were added to the experiment at 1700 h on the day of their capture and the CD Walkman was switched on to initiate sound in the Sound treatment. Subsequently every 6 h the behaviour of the megalopae were observed, at this time counts were made of the
number of individuals that had settled to the substrate and metamorphosed into the first instar benthic juvenile stage. When settlement and metamorphosis occurred in the group containers, first instar juveniles were removed to prevent cannibalism. The period of observation lasted no more than 20 min for both treatment and control. In the current study ‘settlement’ is defined as a behavioural process which involves movement out of the water column to a benthic substrate, and ‘metamorphosis’ as a physiological process which includes loss of larval characteristics retained in the megalopa and the transformation to the adult reptant body form (Hadfield, 2000; Forward et al., 2001). During each 6 hourly observational period descriptions of behaviour of each megalopa was categorized in the following manner. 1 – ‘Normal’ pre-settlement swimming behaviour, i.e., Highly active swimming, low number of downward swimming events , no exploratory crawling behaviour; 2 - Medium swimming activity, medium number of downward swimming events, small amount of exploratory crawling behaviour; 3 – Low swimming activity, high number of downward swimming events, extensive exploratory crawling behaviour; 4 – Complete settlement and metamorphosis, i.e., no swimming activity.

The experiment was terminated when all experimental megalopae in both treatment and control had metamorphosed. The settled juvenile crabs were kept for 10 d following the experiment in flowing seawater and fed, and were monitored for post-experimental mortality.
Figure 2.1: Schematic diagram of a side view of one experimental replicates showing the layout of the water bath, speaker, container holding an individual megalopa, container holding a group of megalopae and water levels. Not to scale.
2.2.3 Sound source and recording

Recordings of typical reef noise in the vicinity of the study locations were collected using a calibrated Sonatech BM 216 omnidirectional hydrophone (10 Hz to 60 kHz flat response) connected to an automated recording system contained in an underwater housing (Figure 2.2). The hydrophone was calibrated by recording a NetMark 1000 acoustic pinger (specifications: source level 130 dB re 1 µPa at 1 m, 10 kHz signal, 300 ms pulse length, 4 s repetition rate). Digital recordings were transferred to a PC and the spectral composition analyzed using MATLAB software with codes specifically written for these recordings. A typical 4 min sequence of each recording was randomly selected and transferred to a CD for playback in the Sound treatment of the settlement experiments.

For the experiments using the three temperate crab species a recording of North Reef in north-eastern New Zealand (36º 15’ S, 174º 47’ E) during the summer at dusk on a new moon was used. A calibrated hydrophone (High Tech, Inc., HTI–96–MIN) attached to a digital recorder (Sound Devices 722 solid state recorder (48 kHz; 24-bit)) (Figure 2.3) was used to adjust the sound level produced by the Sony speakers in each experimental Sound treatment tank to 114 dB re 1µPa, which was within the typical range of ambient sound level for evening chorus at reefs such as North Reef in New Zealand’s coastal waters (Tolimieri et al., 2000; Radford et al., 2008b). There was a peak in the spectra around 1.2 kHz, which is thought to be produced by feeding of the sea urchin, *Evechinus chloroticus*, while the higher frequency pulses were the snaps of snapping shrimp (Tait, 1962; Castle, 1974; Radford et al., 2008a).

The two tropical crab species were exposed to a recording of the reef immediately offshore of Coconut Beach at Lizard Island on the Great Barrier Reef (GBR) (14º 39.5’ S, 145º 26’ E) during the summer at dusk on a new moon. A calibrated hydrophone (High Tech, Inc., HTI–96–MIN) was used to adjust the sound level produced by the Sony speakers in each experimental Sound treatment tank to 109 dB re 1µPa, which is within the typical range of ambient sound level of evening chorus on the GBR in Australia (Cato & McCauley, 2002). This recording consisted of a chorus of pops made by nocturnal fishes together with a higher frequency crackle produced by snapping shrimp, as well as other feeding and calling sounds typical of a coral reef (Cato & McCauley, 2002; Simpson et al., 2004).
Figure 2.2: Remote hydrophone system used for recording natural reef sounds.

Figure 2.3: Hydrophone system used to adjust sound levels in the experimental tank.
2.2.4 Data analyses

For the experiments for each species non-parametric statistical methods were used to test for a difference in median TTM within the replicates the Sound treatment because the data was not continuous (Zar, 1999). The following analytical steps were repeated for the experiment for each crab species. Firstly, the Kruskal-Wallis one-way analysis of variance on rank was used to test for a difference in the median TTMs among the replicate group-housed megalopae within the same treatment (i.e., Sound and Silent analysed separately). If this test found no difference the data from the replicates were pooled. Secondly, the Mann-Whitney $U$ test was used to test for a difference in median TTM between the individually and group-housed megalopae within the same treatment (i.e., Sound and Silent analysed separately). If this test found no difference in the median TTMs the individual and group settlement data were also pooled for each treatment. Lastly, the Mann-Whitney $U$ test was used to compare the median TTMs for megalopae in the Sound treatment versus the Silent control. For all statistical tests, $P$ values $\leq 0.05$ were considered to be significant. A metamorphosis rate for each treatment within each species was also calculated with the Sen’s Slope Analysis for the data points between the last sample prior to the first megalopa metamorphosing and the sampling event when the last megalopa to metamorphose for each treatment. All analyses were performed using the software Sigma Stat 4.0 (Systat Software, Inc.) and Minitab 16.1.0. (Minitab, Pty.).

2.3 RESULTS

2.3.1 Sound analysis

In the experiments for both temperate and tropical crab species the broadcast sound within the experimental tanks had similar overall spectral composition and sound level to the source signals recorded from the natural habitats (Figure 2.4a & b). Hydrophone recordings taken in the Silent control confirmed the absence of sound transfer from the Sound treatment or other external sources. The flat response at approximately 35 dB re 1 $\mu$Pa is the lower recording limit of the recording equipment (Figure 2.4c).

2.3.2 Pooling

In all five crab species there were no significant differences in the median TTMs among the group replicates for both the treatment and control (Kruskal-Wallis test) (Table 2.1).
Therefore, for each crab species the settlement data for group-housed megalopae from the replicate tanks within each treatment were pooled and compared with corresponding data for individually-housed megalopae for each treatment. There were also no significant differences in the median TTM between individually and group-housed megalopae in either the treatment or the control for all species examined (Mann-Whitney U test, Table 2.1) (Figure 2.5), indicating there were no interactive effects on TTM between individuals in the group experiments. Therefore, for all crab species the group and individual data were pooled within treatment and control to test for the overall treatment effect, i.e., Sound versus Silent.

2.3.3 Behavioural observations

A general description of changes in behaviour was compiled using a set pre-set scale. Prior to metamorphosis, swimming activity of larval crabs was present in both the dark and light periods. However, swimming activity appeared to be greater during the dark periods than during the light periods. The behaviour of megalopae that were approaching metamorphosis changed noticeably including reduced swimming activity, more frequent downward swimming to the settlement substrate followed by “exploratory crawling” whereby the megalopae would slowly crawl around on the artificial settlement substrate (Figure 2.6). This behaviour was consistent with pre-settlement behaviour previously observed in other crab species (Forward et al., 2001) and was consistent among the megalopae of all five species and preceded settlement and metamorphosis. There were no observed differences in the settlement behaviour of megalopae between the Sound treatment and Silent control except for timing of the onset of this behaviour. In the Sound treatment this behaviour was consistently observed well in advance of the Silent control for every species examined (Figure 2.6).

Across all treatments and species the megalopae did not settle and metamorphose to the first instar benthic juvenile until at least 24 h after the start of the experiment, with some taking up to 114 h. Mortality was absent during these experiments and all first instar crabs that were kept for up to 10 days after the conclusion of the experiment survived.
Figure 2.4: Spectral composition of underwater sound when recorded at coastal habitats and when replayed in experimental tanks. a) Coconut Beach Reef, Lizard Island – tropical waters, b) North Reef, north-eastern New Zealand – temperate waters. Black lines represent natural sound in situ, grey lines represent replayed sound in experimental Sound treatment. c) Silent control.
Table 2.1: Summary of comparisons among median TTM among the group replicates and between the group and individually housed megalopae for the treatment and control (i.e., Sound versus Silent) for five crab species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>$P$ – value Group replicates</th>
<th>H – statistic</th>
<th>$P$ – value Group vs. indiv.</th>
<th>U – value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hemigrapsus sexdentatus</em></td>
<td>Sound</td>
<td>0.658$^a$</td>
<td>0.836</td>
<td>0.275$^b$</td>
<td>2.000</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.337$^a$</td>
<td>2.174</td>
<td>0.822$^b$</td>
<td>4.000</td>
</tr>
<tr>
<td><em>Cyclograpsus lavauxi</em></td>
<td>Sound</td>
<td>0.984$^a$</td>
<td>0.032</td>
<td>0.817$^b$</td>
<td>4.000</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.453$^a$</td>
<td>1.584</td>
<td>0.184$^b$</td>
<td>1.500</td>
</tr>
<tr>
<td><em>Macrophthalmus hirtipes</em></td>
<td>Sound</td>
<td>0.706$^a$</td>
<td>0.697</td>
<td>0.050$^b$</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.455$^a$</td>
<td>1.576</td>
<td>0.822$^b$</td>
<td>4.000</td>
</tr>
<tr>
<td>Grapsidae sp. one</td>
<td>Sound</td>
<td>0.639$^a$</td>
<td>0.895</td>
<td>0.102$^b$</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.722$^a$</td>
<td>0.651</td>
<td>0.121$^b$</td>
<td>1.000</td>
</tr>
<tr>
<td>Grapsidae sp. two</td>
<td>Sound</td>
<td>0.860$^a$</td>
<td>0.303</td>
<td>0.121$^b$</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.784$^a$</td>
<td>0.486</td>
<td>0.121$^b$</td>
<td>1.000</td>
</tr>
</tbody>
</table>

$^a$Kruskal – Wallis test showing no significance (>0.05)

$^b$Mann-Whitney U test showing no significance (>0.05)
Figure 2.5: Median TTM in group and individual replicates. a) Hemigrapsus sexdentatus, b) Cyclograpsus lavauxi, c) Macrophthalmus hirtipes, d) Grapsidae sp. one and e) Grapsidae sp. two. Grey bars represent Sound treatment, white bars represent Silent control.
Chapter 2 – Induction of Settlement

Proportion of behaviour occurring

Hemigrapsus sexdentatus

Cyclograpsus lavauxi

Macrophthalmus hirtipes

One
Two
Three
Four

Time (h)
Figure 2.6: Proportions of categorized behaviours during the course of the experiment for the temperate and tropical species *Hemigrapsus sexdentatus*, *Cyclograpsus lavauxi*, *Macrophthalmus hirtipes*, Grapsidae sp. one and Grapsidae sp. two. a) Sound treatment and b) Silent control.
2.3.4 Sound effect on TTM

In all five species of crabs the megalopae in the Sound treatment had a significantly shorter median TTM than those in the Silent control (Figure 2.7).

The tropical species Grapsidae sp. two had the largest difference in median TTMs between the treatment and control, with a median TTM of 69 h in the Sound treatment and 114 h in the Silent control, i.e. a 75 h difference (Mann – Whitney U test, \( P = 0.004 \); Table 2.2). This was followed by Grapsidae sp. one with a median TTM of 72 h in the Sound treatment and 123 h in the Silent control, i.e., 51 h difference (Mann – Whitney U test, \( P = 0.004 \); Table 2.2).

The same trend was also present in the crab species from temperate waters, where megalopae of *M. hirtipes*, *C. lavauxi* and *H. sexdentatus* in the Sound treatment had a significantly shorter median TTM than those in the Silent control, 72 h and 108 h (48 h difference) (Mann-Whitney U test, \( P = 0.004 \); Table 2.2), 30 h and 75 h (45 h difference) (Mann-Whitney U test, \( P = 0.005 \); Table 2.2) and 63 h and 96 h (33 h difference) for each species respectively (Mann-Whitney U test, \( P = 0.005 \); Table 2.2).

Difference in time to the first metamorphosis between the treatment and control varied substantially among the five species (Table 2.3), with *C. lavauxi* exhibiting the smallest difference of 12 h and Grapsidae sp. two exhibiting the greatest difference of 84 h. Grapsidae sp. two also had the greatest difference in time to completed metamorphosis between the treatment and control, with all the megalopae completing metamorphosis in 108 h in the Sound treatment and 168 h in the Silent control (Table 2.3). *H. sexdentatus* had the smallest difference in time to complete metamorphosis between the two (24 h).

2.3.5 Rates of metamorphosis

The metamorphosis rate of the Silent control was 1.5 times faster than that of the Sound treatment in *H. sexdentatus* (13.7 and 9 respectively). However, both *C. lavauxi* and *M. hirtipes* had faster metamorphosis rates in the Sound treatment, 1.8 times and 1.3 times faster respectively (Table 2.3).

The metamorphosis rates of the Silent control were faster than the Sound treatment in both tropical species. Grapsidae sp. one had a metamorphosis rate in the Silent control (8.3) that was 1.3 times faster than in the Sound treatment (6.3). Grapsidae sp. two had a
metamorphosis rate 1.6 times faster in the Silent control (9.2) compared to the Sound treatment (5.5) (Table 2.3).

**Hemigrapsus sexdentatus**

**Cyclograpsus lavauxi**

**Macrophthalmus hirtipes**

---

50
Figure 2.7: Percentage of total number of megalopae metamorphosed against time (h). a) *Hemigrapsus sexdentatus*, b) *Cyclograpsus lavauxi*, c) *Macrophthalmus hirtipes*, d) Grapsidae sp. one and e) Grapsidae sp. two. Solid blue line represents the Sound treatment and the green solid line represents the Silent control.
Table 2.2: Summary of comparisons among median TTM s and settlement rates for each treatment (i.e., Sound versus Silent) for five crab species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total number of individuals (n)</th>
<th>Treatment</th>
<th>Median TTM (h)</th>
<th>Difference in median TTM (h)</th>
<th>$P$ - value</th>
<th>$U$ value</th>
<th>Metamorphosis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hemigrapsus sexdentatus</em></td>
<td>12</td>
<td>Sound</td>
<td>63</td>
<td>33</td>
<td>*** (0.005)</td>
<td>0.500</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Silent</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
<td>13.7</td>
</tr>
<tr>
<td><em>Cyclograpsus lavauxi</em></td>
<td>15</td>
<td>Sound</td>
<td>30</td>
<td>45</td>
<td>*** (0.005)</td>
<td>0.500</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Silent</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td>8.3</td>
</tr>
<tr>
<td><em>Macrophthalmus hirtipes</em></td>
<td>14</td>
<td>Sound</td>
<td>72</td>
<td>48</td>
<td>*** (0.004)</td>
<td>0.000</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Silent</td>
<td>108</td>
<td></td>
<td></td>
<td></td>
<td>8.4</td>
</tr>
<tr>
<td><em>Grapsidae sp. one</em></td>
<td>15</td>
<td>Sound</td>
<td>72</td>
<td>51</td>
<td>*** (0.004)</td>
<td>0.000</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Silent</td>
<td>123</td>
<td></td>
<td></td>
<td></td>
<td>8.3</td>
</tr>
<tr>
<td><em>Grapsidae sp. two</em></td>
<td>15</td>
<td>Sound</td>
<td>69</td>
<td>75</td>
<td>*** (0.004)</td>
<td>0.000</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Silent</td>
<td>144</td>
<td></td>
<td></td>
<td></td>
<td>9.2</td>
</tr>
</tbody>
</table>

*** indicates a significant difference in TTM s between treatments (significant at $p < 0.05$, Mann-Whitney $U$ test). Test statistic rounded to 3 dp.
Table 2.3: Summary of comparisons among first metamorphosis, completed metamorphosis and the difference between the treatment and control (i.e., Sound versus Silent) for five crab species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>First metamorphosis (h)</th>
<th>Difference (h)</th>
<th>Completed metamorphosis (h)</th>
<th>Difference (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hemigrapsus sexdentatus</em></td>
<td>Sound</td>
<td>42</td>
<td>30</td>
<td>90</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
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<td></td>
<td>114</td>
<td></td>
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<tr>
<td><em>Cyclograpsus lavauxi</em></td>
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<td>12</td>
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<tr>
<td></td>
<td>Silent</td>
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<td></td>
<td>108</td>
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<tr>
<td><em>Macrophthalmus hirtipes</em></td>
<td>Sound</td>
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<td>24</td>
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<td>63</td>
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<tr>
<td></td>
<td>Silent</td>
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<td></td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Grapsidae sp. one</td>
<td>Sound</td>
<td>30</td>
<td>78</td>
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</tr>
<tr>
<td></td>
<td>Silent</td>
<td>108</td>
<td></td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>Grapsidae sp. two</td>
<td>Sound</td>
<td>42</td>
<td>84</td>
<td>132</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>126</td>
<td></td>
<td>174</td>
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</table>
2.4 DISCUSSION

There have been a number of laboratory and field studies in the last decade investigating specific physical and chemical cues such as temperature, salinity and adult odour that shorten or lengthen the TTM in the megalopae of crabs (O'Connor, 1991; Harvey & Colasurdo, 1993; Lim, 1997; O'Connor & Gregg, 1998; Gebauer et al., 2002; 2004). Cues that were found to shorten the TTM were most often chemical cues associated with sources from settlement habitats, adult habitats or nursery areas, (i.e., adult habitat substrate, conspecific odour, related species odour and estuarine water) (Forward et al., 2001). For example, the effect of water-soluble conspecific odour on metamorphosis was tested by exposing megalopae to water in which adults had been held. It was found that the TTM for megalopae of the Atlantic mud crab, Panopeus herbstii, decreased with higher concentrations of adult water, and was not affected by odours from fishes and other crab species (Rodriguez & Epifanio, 2000).

This current study provides the first evidence that underwater sound consistently shortens the TTM in a range of tropical and temperate crab megalopae, by between 34 and 60%. The response was also found to be consistent whether the experimental megalopae were housed individually or in groups. In contrast, some previous studies have found that increased TTM can be the result of decreased experimental density (Fernandez et al., 1994; Forward et al., 1996). For the blue crab, Callinectes sapidus, reduced TTM was due to physical interactions between the megalopae and mortalities due to vulnerability to cannibalism during metamorphosis (Fernandez et al., 1994).

Recent research has shown that the pelagic stages of some marine organisms such as decapod crustaceans and fish can orientate and actively swim toward underwater reef sound (Tolimieri et al., 2002; Tolimieri et al., 2004; Jeffs et al., 2005; Leis & Lockett, 2005; Simpson et al., 2008a). For example, five species of coastal crab megalopae consistently orientated and swam towards an artificial source of underwater reef sound (Radford et al., 2007). The results of these previous studies indicate that underwater reef sound may be used as a long distance orientation cue for locating coastal settlement habitat in some crab species, including two of the species examined in the current study. Despite the increasing evidence for the use of ambient underwater sound as an orientation cue for pelagic stages of coastal crustaceans, no studies to date have examined the role of underwater sound as a short distance settlement cue for these stages. The current study provides the first evidence of ambient underwater sound decreasing the TTM of megalopae of both temperate and tropical coastal crab species, by as much as a half in some species such as, Cyclograpsus lavauxi and
Grapsidae spp. two. This reduction of time in the water column is likely to reduce the risk of predation, which is often very high during the pelagic megalopal phase of the lifecycle and improve the ability to settle into suitable habitats for the reptant phase of the lifecycle (O’Connor, 1991; O’Connor & Gregg, 1998). Early settlement can also improve initial growth or survival following metamorphosis (O’Connor & Gregg, 1998).

Crab megalopae are thought to possess a temporal threshold beyond which metamorphosis occurs even in the absence of suitable settlement cues (Weber & Epifanio, 1996). From our results it would appear that there is perhaps a longer temporal threshold in the two tropical Australian species, or that sound is a more dominant cue for initiating metamorphosis in these species. Compared to the temperate New Zealand crab species, the initiation of metamorphosis among megalopae of the two tropical Australian Grapsid species was delayed for much longer in the absence of a sound cue (108 and 126 h for Grapsidae sp. one and two respectively). However, once metamorphosis was initiated in the Silent control it was rapid, with the rate of metamorphosis being higher in the Silent control than the Sound treatment for both species.

Overall, there was no consistent pattern in the rates of metamorphosis between treatments and the controls among all five species even though it might be expected that megalopae that were consistently responding to a sound cue would also produce a higher metamorphosis rate. The variability in metamorphosis rates may be due to the absence of secondary metamorphosis cues which are known to be important in some crab species (Gebauer et al., 2004) or may be related to the delayed metamorphosis culminating in a spontaneous metamorphosis once a threshold has been reached (Gebauer et al., 2003).

The results of the current study appear to greatly extend the role that ambient underwater sound may play in triggering behavioural and physiological changes in pre-settlement animals looking for suitable settlement habitats. The consistent behavioural response to acoustic cues found in five crab species from both temperate and tropical waters suggests the phenomenon has the potential to be widespread geographically and among crab species. If this is the case, then underwater acoustic cues may play a very significant role in the settlement and recruitment processes of many important coastal crab species.
Chapter Three:
Unique habitat acoustic signatures

3.1 INTRODUCTION

A number of experimental studies have concluded that ambient underwater sound emanating from coastal habitats may act in guiding some settlement stage decapods and fishes to suitable settlement habitats on coastal reefs (Stobutzki & Bellwood, 1998; Tolimieri et al., 2000; Jeffs et al., 2003; Jeffs et al., 2005; Leis & Lockett, 2005; Simpson et al., 2005a; Montgomery et al., 2006; Radford et al., 2007; Simpson et al., 2008a; Simpson et al., 2008b). For example, it was demonstrated that settlement stage and juvenile reef fishes orientate with respect to the surrounding soundscape, especially the high-frequency (570 – 2000 Hz) component of reef sound (Simpson et al., 2008b). Although the extent to which underwater reef sound influences the settlement and metamorphosis of reef organisms is unknown, there is evidence that reef sound can decrease the time to metamorphosis (TTM) in a number of temperate and tropical crab species (Stanley et al., 2010). It is therefore possible that spatial variation in ambient underwater sound could contribute to the large amount of spatial variability that is typically seen in the settlement of larval reef organisms (Doherty & Fowler, 1994; Caselle & Warner, 1996). If larval settlers possess the sensory abilities to detect and utilize ambient underwater sound, as demonstrated by both electrophysiological and behavioural experiments (Leis & Lockett, 2005; Wright et al., 2005; 2008), it would be beneficial for these settlers to remotely detect and react to differences in underwater sound if it is reliably representing differences in the potential settlement habitat from where it is being produced.

Ambient underwater sound in the sea has been found to be composed of a relatively wide range of frequencies from a number of acoustic sources, both abiotic and biotic (Cato, 1992; Acosta et al., 1997). Abiotic sources are usually produced by the effect of the wind and waves, and typically produce sound in the frequency band of 100 – 1000 Hz. Biotic sounds are produced by a range of activities by marine life including reproductive displays, territorial defence, feeding, and echolocation (Hawkins & Myrberg, 1983). These sounds can cover a very large frequency range (50 – 130,000 Hz) (Bayoumi, 1970; Au & Banks, 1998; Onuki &
Temporal patterns of ambient underwater sound have been observed at several locations in New Zealand (Tait, 1962; Castle, 1974; Radford et al., 2008a), and several locations in tropical and temperate Australia (Cato, 1976; 1978; 1980; 1992; Cato & Tavener, 1997). Predictable temporal variability (time of the day, daily, lunar, seasonal) in ambient underwater sound has also been observed. Dawn and dusk choruses have been found which consist of a distinct increase in biotic sound and is associated with the emergence and increased activity of crepuscular organisms (Tait, 1962; Cato, 1978; 1992; Cato & McCauley, 2002; Radford et al., 2008b). A study investigating the temporal periodicity of ambient sound levels from a coastal location in north–eastern New Zealand showed that there was a maximum in urchin and snapping shrimp sounds during the new moon and a minimum during the full moon, while there were little or no differences in overall sound level and frequency composition between the two quarter moons and day-to-day within each moon phase (Radford et al., 2008b). They found that during all seasons and during all moon phases there was an increase in the frequency 700 – 2000 Hz bandwidth during dusk compared with noon due to the dusk chorus.

It is known that a major component of reef sound is typically produced by the animals inhabiting the reef concerned (Cato, 1992; Cato & Tavener, 1997; Cato & McCauley, 2002). For these reasons it is logical to expect that differences in the presence and abundance of resident noise-producing animals could potentially result in different coastal habitats generating sounds of different overall intensity and frequency composition. There have been few previous studies investigating the spatial differences in ambient underwater sounds in shallow coastal waters (Wilson et al., 1985; Deane, 1999). However, no studies have specifically addressed whether the different shallow water coastal habitats encountered over the spatial scale of larval settlers (kilometres) are producing different sounds due to habitat related differences in sound producing animals such as urchins, snapping shrimp and fishes (Cato, 1992; Radford et al., 2008a). Therefore, the aim of the current study was to investigate differences in overall character (intensity level, spectral composition, and temporal variation) of the ambient underwater sound generated from three distinct habitat types (macroalgae dominated rocky reef, urchin dominated rocky reef, and sandy beach) found relatively close to one another (<10 km apart) within a 20 km section of north-eastern New Zealand coastline.
3.2 METHODS

3.2.1 Study sites

To evaluate the differences in ambient underwater sound among three habitat types, underwater recordings of ambient sound were taken during the first and last quarter moon phases during October 2008 at six locations on the coast around Leigh, north-eastern New Zealand (Figure 3.1). These moon phases were chosen because a previous study had shown there were no differences in the sound produced by urchin and snapping shrimp during these two moon phases (Radford et al., 2008b). These locations were selected to represent three different types of benthic habitat according to a habitat map created by (Leleu et al., 2006) with two sampling sites selected for each type of habitat: macroalgae dominated rocky reef; Waterfall Reef (M1) and One Spot Reef (M2), urchin dominated rocky reef; Rusty Ladder Reef (U1) and Nordic Reef (U2), and sandy beach; Pakiri Beach North (SB1) and Pakiri Beach South (SB2). At each of these sites, recordings were taken during two periods of the day, 1200 – 1300 h (noon) and 1700 – 1800 h (dusk) to identify any differences between noon and the dusk chorus (Radford et al., 2008b).
Figure 3.1: Map of study sites; (M1) Waterfall Reef, (M2) One Spot Reef, (U1) Rusty Ladder, (U2) Nordic Reef, (SB1) Pakiri Beach North, (SB2) Pakiri Beach South.
3.2.2 Recording system

3.2.2.1 Boat deployed floating hydrophone

Habitat recordings were made using a floating hydrophone system to reduce extraneous noise associated with recording directly from a floating vessel. The recording system consisted of a calibrated HTI-96-MIN wideband omnidirectional hydrophone (High Tech, Inc., flat frequency response over the range of 10 Hz – 24,000 Hz) that was weighted down vertically to 10 m water depth from the outside of a sealed floating barrel which contained a Sound Devices, LLC. – 722 solid state recorder (48 kHz; 24-bit) (Figure 3.2). Several 5 min recordings were taken at each site in approximately 15 – 20 m of water at each habitat site at about 20 m from the margin of the coastal fringing reef at the reef sites and 100 m from the shoreline at the sandy beach sites during 1200 – 1300 h (noon) and 1700 – 1800 h (dusk). No anthropogenic sources of noise, such as large ships or power boats, were present in the vicinity at the time of recording. All recordings were conducted in near calm conditions (<0.5 m wave height & <2.6 ms\(^{-1}\) wind speed) (Climate Station, Leigh Marine Laboratory).

Digital recordings from the recorder were transferred to a PC and analysed using MATLAB 2008a software with codes specifically written for the recordings.

Figure 3.2: Floating hydrophone set-up which is deployed and recovered from a boat.
3.2.1 Data analysis

3.2.1.1 Wind speeds and sea swell

Variability in wind speed among sites may influence the production of ambient underwater sound in coastal environments through the noise produced by water motion at the sea surface and the oscillation of bubble clouds generated by breaking waves (Wilson et al., 1985; Kolaini & Crum, 1994; Cato & Tavener, 1997; Cato & McCauley, 2002). Therefore, average hourly wind speeds (average speed recorded every 2 s during the hour) were simultaneously recorded at the weather station at the University of Auckland’s Leigh Marine Laboratory (Climate Station Database, Leigh Marine Laboratory). These data were compared using a Two-Way ANOVA to determine if there were differences in wind conditions between sampling Site (M1, M2, U1, U2, SB1 & SB2) and Period (time of day of recording – noon or dusk).

Sea swell conditions could also have an effect on the ambient underwater sound recordings, however, all recordings were taken during conditions of less than 0.5 m swell to minimise this effect (www.swellmap.com & www.marineweather.co.nz).

3.2.1.2 Spectral levels and proportions

Acoustic power (sound intensity) spectra plots were generated to compare dusk and noon choruses among sites. The acoustic power spectra for each site were generated using Fast Fourier Transformation (FFT) analyses of five 10 s samples, which were randomly selected from the 5 min underwater recording taken during noon and dusk and smoothed using a triangular window. Data were high pass filtered to 100 Hz to remove any 50 Hz interference and any effect of sea surface waves. Total sound intensity ($P_{RMS}^2$) was calculated for each of the five randomly selected sub-samples. The sub-samples were then band pass filtered into four frequency bins: 100 – 800 Hz (predominantly composed of wind dependent noise, sound of small waves breaking at the sea surface, some fish species, and other low frequency sounds from distant shipping and storms (Cato, 1978; 1992; Cato & McCauley, 2002)), 801 – 2500 Hz (frequency range dominated by urchin feeding sound (Tait, 1962; Radford et al., 2007; Radford et al., 2008a)), 2501 – 20000 Hz (frequency range dominated by snapping shrimp sounds (Au & Banks, 1998)) and 20001 – 24000. The overall mean proportion of total sound intensity was calculated for each frequency bin. For each period (noon or dusk) the proportion of total sound intensity was arcsine transformed and analysed.
using a general linear mixed model, with Frequency bins, Habitat type (macroalgae dominated rocky reef, urchin dominated rocky reef, and sandy beach) and Period (time of day of recording – noon or dusk) as fixed factors. Habitat and Frequency bin were also nested within the random effect of Site. Significant differences between individual pairs of means were determined using Tukey’s tests once the general linear model had determined an overall significant difference among means.

3.2.1.3 Snapping shrimp

For each 5 min sound recording, five random 10 s sub-samples were selected and the numbers of snaps produced by snapping shrimp were estimated. This was achieved by setting a threshold level on the raw data where any transient spike that was < 0.2 s in duration and above a preset threshold level (adjusted to the overall intensity of each recording) was counted as a shrimp snap. Initial manual analysis verified that this automated method provided a precise and accurate count of number of snaps.

The mean number of snaps for each site was compared using a general linear mixed model, which enabled comparisons to be made among Habitat type and Period (fixed effects). Period and Habitat type was also nested within Site (random effect) (Littell et al., 1996; McCullagh & Searle, 2001). Tukey’s post-hoc tests were used to identify differences between individual pairs of sample means. All data were analysed using SPSS and SAS, and results from sampling presented as the statistical mean ± the standard error of the mean.
3.3 RESULTS

3.3.1 Wind speeds and sea swell

There was no statistical significance among mean hourly wind speeds for Site, Period, and interaction term Site × Period for which the ambient underwater sound recordings were taken (Table 1), with an overall average wind speed over all sampling periods of $3.41 \pm 0.03$ m s$^{-1}$. Consequently, wind could not be the cause of any differences in the intensity levels of the recorded ambient underwater sound.

Sea swell conditions were unlikely to have affected site recordings because all recording events occurred in conditions of less than 0.5 m of swell, with an overall average sea swell of $0.38 \pm 0.03$ m over all sampling periods.

### Table 3.1: Results of the Two – Way ANOVA for wind speeds.

<table>
<thead>
<tr>
<th>Level</th>
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<th>F-Value</th>
<th>P-Value</th>
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<tr>
<td>Site</td>
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<td>0.47</td>
<td>0.8</td>
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<tr>
<td>Period</td>
<td>1,84</td>
<td>0.41</td>
<td>0.5</td>
</tr>
<tr>
<td>Site × Period</td>
<td>5,84</td>
<td>0.01</td>
<td>1.0</td>
</tr>
</tbody>
</table>

### Table 3.2: Mean hourly wind speeds (m s$^{-1}$) ± std error for Site and Period during each recording period.

<table>
<thead>
<tr>
<th>Site</th>
<th>Noon</th>
<th>±SE</th>
<th>Dusk</th>
<th>±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>3.43</td>
<td>0.22</td>
<td>3.53</td>
<td>0.23</td>
</tr>
<tr>
<td>M2</td>
<td>3.43</td>
<td>0.22</td>
<td>3.53</td>
<td>0.23</td>
</tr>
<tr>
<td>U1</td>
<td>3.43</td>
<td>0.22</td>
<td>3.53</td>
<td>0.23</td>
</tr>
<tr>
<td>U2</td>
<td>3.43</td>
<td>0.22</td>
<td>3.53</td>
<td>0.23</td>
</tr>
<tr>
<td>SB1</td>
<td>3.25</td>
<td>0.11</td>
<td>3.29</td>
<td>0.32</td>
</tr>
<tr>
<td>SB2</td>
<td>3.25</td>
<td>0.11</td>
<td>3.29</td>
<td>0.32</td>
</tr>
</tbody>
</table>

3.3.2 Spectral levels proportion of total sound intensity

Among habitats the sound spectra were different in overall appearance (Figure 3.3), which suggests that the acoustic characteristics can vary over relatively small spatial scales, among differing habitats, and between periods (noon and dusk). The reef habitat sites had
higher spectrum levels in the frequency band 800 – 2500 Hz (largely dominated by urchins) with a peak around 900 – 1200 Hz and a slightly smaller peak in the 2500 – 20000 Hz frequency band (largely dominated by snapping shrimp), with a broad peak at 5000 Hz. However, at the sandy beach habitat sites (SB1 & SB2) these peaks in these two frequency bands (Figure 3.3e, f) were much smaller, with very low levels of urchin and snapping shrimp noise which are most likely due to attenuated sound which has travelled from distant reefs. The sandy beach habitat sites (Figure 3.3e, f) had predominantly higher sound levels in the lower frequencies (100 – 400 Hz) when compared with all of the four reef sites.

At noon the proportion of total sound intensity in the four frequency bands (Figure 3.4b, d, f) were significantly different for Frequency ($F_{3,9} = 90.85$, $P < 0.0001$) and the interaction between Habitat × Frequency ($F_{6,9} = 26.45$, $P < 0.0001$), but was similar among Habitat ($F_{2,3} = 0.02$, $P = 0.98$). All sites had similar proportions of total sound intensity in the frequency band 801 – 2500 Hz. At noon the mean proportions of total sound intensity in the frequency band 2501 – 20000 Hz were similar in all four of the reef habitat sites (M1, M2, U1 & U2) (62.6 and 53.8 $P_{RMS}^2$ respectively) but both were significantly greater than the sandy beach habitat sites (SB1 & SB2) (17.06 $P_{RMS}^2$). The mean proportions of total sound intensity were significantly higher for SB1 and SB2 in the frequency band 100 – 800 Hz. The 20001 – 24000 Hz frequency band was similar for all habitats at noon (M vs. SB; $q = 0.6675$, $P = 1.0$, M vs. U; $q = 0.9743$, $q = 1.0$, SB vs. U; $q = 0.6908$, $P = 1.0$).

At dusk the mean proportions of sound intensity in the four frequency bands (Figure 3.4a, c, e) were significantly different for Frequency ($F_{3,9} = 52.08$, $P < 0.0001$) and the interaction between Habitat × Frequency ($F_{6,9} = 38.52$, $P < 0.0001$), while the mean proportion of sound intensity was similar among Habitat ($F_{2,3} = 0.0$, $P = 1.0$). The urchin dominated habitat sites (U1 & U2) had a significantly higher mean proportion of total sound intensity in the frequency band 801 – 2500 Hz at dusk compared with the two other habitat types; macroalgae dominated rocky reef and sandy beach. At dusk the macroalgae dominated habitat sites (M1 & M2) had a significantly higher ($P > 0.005$) proportion of total sound intensity in the 2501 – 20000 Hz frequency band than the urchin dominated ($P = 0.005$) and sandy beach habitat sites ($P < 0.001$). At both noon and dusk the sandy beach habitat sites (SB1 & SB2) had a significantly higher mean proportion of total sound intensity in the 100 – 800 Hz frequency band than both the other two habitat types. All of the habitats had a similar proportion of total sound intensity in the frequency band 20001 – 25000 Hz at both noon and dusk ($P > 0.05$).
At dusk the urchin dominated rocky reef sites (U1 & U2) had a significantly higher proportion of total sound intensity in the 801 – 2500 Hz frequency band than compared with noon ($P < 0.005$). In contrast, at noon at these same sites (U1 & U2) there was a significantly higher proportion of total sound intensity in the 2501 – 20000 Hz frequency band ($P = 0.05$).

The macroalgae dominated rocky reef sites (M1 & M2) had a significant rise in the proportion of total sound intensity in the 801 – 2500 Hz frequency band at dusk compared with noon ($P = 0.001$), with all other frequency bands being similar between dusk and noon. The total sound intensity for all frequency bands was similar between dusk and noon at both the sandy beach habitat sites (SB1 & SB2).
Chapter 3 – Habitat Signatures

Spectrum level (dB re 1 µPa²/Hz)

Frequency (Hz)
Figure 3.3: Noon and dusk spectra of ambient underwater sound recordings at sites in north-eastern New Zealand. a) Rusty Ladder (U1), b) Nordic Reef (U2), c) One Spot Reef (M2), d) Waterfall Reef (M1), e) Pakiri Beach North (SB1), f) Pakiri Beach South (SB2). Blue lines represent dusk and red lines represent noon.
Chapter 3 – Habitat Signatures

**Dusk**

- Proportion of total sound intensity ($P_{RMS}^2$) (%)
- Frequency band (Hz)

**Noon**

- Proportion of total sound intensity ($P_{RMS}^2$) (%)
- Frequency band (Hz)

**Dusk**

- Proportion of total sound intensity ($P_{RMS}^2$) (%)
- Frequency band (Hz)
Figure 3.4: Proportion of total sound intensity ($P_{\text{RMS}}^2$) occurring in four frequency bands at dusk and noon for the three different types of habitats. a) Dusk at urchin dominated rocky reef sites (U1 & U2), b) Noon at urchin dominated rocky reef sites (U1 & U2), c) Dusk at macroalgae dominated rocky reef sites (M1 & M2), d) Noon at macroalgae dominated rocky reef sites (M1 & M2), e) Dusk at sandy beach sites (SB1 & SB2), and f) Noon at sandy beach sites (SB1 & SB2).
3.3.3 Snapping shrimp

The number of snaps produced by snapping shrimp showed significant differences for Habitat ($F_{2,3} = 17.17, P = 0.02$), Period ($F_{1,3} = 43.55, P = 0.007$) and the interaction term Time × Habitat ($F_{2,3} = 14.62, P = 0.028$) (Figure 3.5). Overall, the Nordic Reef site (U2) had more snaps produced by snapping shrimp than any other site at dusk (dusk = $755 \pm 68$, noon = $99 \pm 6$), whilst Pakiri Beach South (SB2) site had the least (dusk = $138 \pm 39$, noon = $59 \pm 5$).

Both the urchin dominated rocky reef sites (U1 & U2) had a similar number of snapping shrimp snaps (U1 dusk = $617 \pm 18$, U1 noon = $112 \pm 23$, U2 dusk = $755 \pm 68$, U2 noon = $99 \pm 6$), but had significantly more snaps at dusk than the macroalgae dominated rocky reef sites (M1 & M2) (M1 dusk $348 \pm 25$, M2 dusk = $339 \pm 38$) and sandy beach habitat sites (SB1 & SB2) (SB1 dusk = $170 \pm 46$, SB2 dusk = $138 \pm 39$).

There were a significantly higher number of snaps produced by the snapping shrimp at dusk compared with noon at every site. The number of snaps produced at dusk was different among each habitat type but similar between the sites within each habitat type. The number of snaps produced at noon was similar among all sites except for SB2 which had significantly fewer mean number of snaps.

![Figure 3.5: Mean number ± S.E. of snapping shrimp snaps per 10 s for six sites recorded at noon and dusk.](image-url)
3.4 DISCUSSION

The present study investigated differences in spectral composition and intensity of ambient underwater sound emanating from three distinct habitat types within a 20 km section of the north-eastern coast of the temperate waters of New Zealand (macroalgae dominated rocky reef, urchin dominated rocky reef and sandy beach). This is thought to be the first study to identify distinct differences in spectral composition of underwater sound from localised types of benthic habitat over such a small geographical scale.

A previous study in this region of the coast (north-eastern New Zealand) found that sea urchins are one of the major producers of noise on this coast (Castle, 1974). It was found that the feeding behaviour of the urchin *Evechinus chloroticus* produces a resonance frequency between 800 – 2800 Hz (Castle, 1974; Radford *et al*., 2008a). Therefore, the most pronounced differences in spectral composition from different habitats in the current study were expected to be found in the frequency band associated with grazing urchin (800 – 2500 Hz). The sound in this frequency range was expected to be more intense at the urchin dominated rocky reef habitat sites (U1 & U2) than in the macroalgae dominated rocky reef habitat sites (M1 & M2) and sandy beach habitat sites (SB1 & SB2) by as much as 20 dB re 1 µPa. This was confirmed when analysing the proportion of total sound intensity in the 800 – 2500 Hz frequency band, the urchin dominated rocky reef sites (U1 & U2) had significantly greater proportions of total sound intensity than either of the macroalgae dominated rocky reef sites (M1 & M2) and sandy beach habitat sites (SB1 & SB2). In contrast to the differences among habitat types, the recordings taken at each of the two sites within each of the three habitat types (M1 & M2; U1 & U2; SB1 & SB2) were similar in terms of their spectral composition and temporal changes in their spectra. The frequency band (800 – 2500 Hz) also produced most of the variation in sound intensity among the habitats at dusk. The presence of large numbers of urchins is an important characteristic of New Zealand temperate coastal reefs and their grazing behaviour maintains areas of barren rocky reef habitat with small amounts of macroalgae growth and correspondingly higher abundance of crustose coralline algae (Shears & Babcock, 2002).

Another source of variation among the recordings from the three types of benthic habitat was the sound generated by snapping shrimp species (*Alpheus* sp. & *Synalpheus* sp.) in the 2500 – 20000 Hz frequency band. Snapping shrimp are among the major sources of biological sound in shallow bays, harbours and inlets, in both temperate and tropical waters (Fish, 1964; Au & Banks, 1998; Cato & McCauley, 2002) and can occur in high densities...
with burrows spaced at 5 – 50 cm apart (Obermeier & Schmitz, 2003). Peak to peak source levels from one individual shrimp can vary from 115 dB to 189 dB re 1 µPa at 1 m (Au & Banks, 1998) and over a relatively small area they can increase the sound levels by 20 – 30 dB (Cato & Tavener, 1997; Cato & McCauley, 2002). Intensity of the sound produced is dependent upon the claw size and body length, species, density of the shrimp bed, and time of day (Johnson et al., 1947; Fish, 1964; Au & Banks, 1998; Radford et al., 2008b). Assuming that the number of snaps estimated from the sound recordings at dusk roughly indicated the number of snapping shrimp present in the vicinity of the reef at dusk, then urchin dominated rocky reef habitat sites (U1 & U2) had significantly more shrimp than macroalgae dominated rocky reef habitat sites (M1 & M2). This suggests that habitat type has a large influence on the number of snapping shrimp residing in an area. Previous studies have demonstrated a nocturnal increase in intensity of snapping shrimp sound with an increase of 2 – 5 dB during dusk compared with daytime (Knowlton & Moulton, 1963), whereas the current study found a 4 – 10 dB increase at dusk compared with noon depending on habitat and site. The habit of snapping is associated with defensive and offensive behaviour; consequently, some animals other than snapping shrimp increase their foraging activity and may produce an increase in sound levels at this time (dusk chorus) by causing the shrimp to increase its snapping (Tait, 1962; Knowlton & Moulton, 1963; Cato, 1992; Lowry & Suthers, 1998; Radford et al., 2008b).

An acoustic cue that carries information about the type of benthic habitat at its source, in addition directional information, would be of considerable value to pelagic settlement stage reef animals which are attempting to remotely identify and locate a suitable settlement habitat. The sound produced by the reef habitat sites (M1 & M2, U1 & U2) was significantly higher in sound intensity at dusk than at noon. This result is similar to findings from a number of studies of ambient underwater sound in temperate and tropical waters around Australia which have shown a consistent presence of dusk choruses (Cato, 1978; McCauley & Cato, 2000). In shallow coastal waters these distinct dusk choruses commonly lasted for a few hours just after sunset (Cato, 1992). Dusk is also known to be a time when nocturnal settlers of coastal crustaceans and reef fishes have most frequently been observed to become active in the water column (Devries et al., 1994b; Fisher & Bellwood, 2003; Forward et al., 2004). Therefore, if larval settlers are using sound for orientation, dusk is a suitable time to become active in the water column as sound levels are at their highest. At this time animals that provide habitat information within these acoustic cues will be making their greatest acoustic contribution to ambient noise. Therefore at dusk, reef sound is at its optimal conditions for remotely
distinguishing among habitats and this is the time where larvae should be utilizing the acoustic cues.

Previous research has found that sound emanating from reefs provides an important orientation and settlement cue for a number of pelagic settlement stage crustaceans and reef fishes (Stobutzki & Bellwood, 1998; Tolimieri et al., 2000; Jeffs et al., 2003; Simpson et al., 2004; Tolimieri et al., 2004; Jeffs et al., 2005; Leis & Lockett, 2005; Radford et al., 2007; Simpson et al., 2008a; Stanley et al., 2010), as it is able to be transmitted long distances through water with very little attenuation and is highly directional (Rodgers & Cox, 1988; Richardson et al., 1995). An ability to detect underwater acoustic cues together with strong swimming capabilities provides the potential for pelagic settlement stages to reliably orient and swim toward suitable settlement habitats (Shanks, 1995; Leis & Carson-Ewart, 1999; Jeffs & Holland, 2000). The two types of rocky reef habitat used in the current study both produced ambient sound of sufficient intensity and in frequency ranges that were likely to be transmitted beyond the habitat and aid in the orientation and settlement behaviour of settling larvae (Radford et al., 2005). Larval reef fish on the Great Barrier Reef, Australia, have been shown to be able to detect the position of reefs from a distance of more than 1 km and use this information to orientate their swimming (Leis, 2006). It has also been demonstrated that certain species of reef fish larvae can not only hear reef sound but are able to also determine direction of the source, and are clearly attracted to it (Tolimieri et al., 2004). Settling reef fishes also use sounds to orientate toward and select reefs (Simpson et al., 2005a; Simpson et al., 2008b). Furthermore, the taxonomic composition of reef fishes settling on experimental patch reefs varied depending upon the frequency components (low < 570 Hz & high > 570 Hz) of artificially broadcast sources of modified underwater reef sound (Simpson et al., 2008b). Fish species from four families were found to preferentially settle on reefs broadcasting the higher frequency component of reef sound, which is mostly produced by marine invertebrates, such as snapping shrimp. Invertebrate fauna are often found to live on complex reef structures which provide refuge against predators and rely on this for survival. These habitats are also important for the survival of newly settled reef species, as it provides protection and a source of prey. Therefore, the presence of other invertebrate species provides a potentially reliable indication of habitat quality and potential prey.

Settlement and metamorphosis was advanced in the megalopae of several species of coastal brachyuran crabs when they were exposed to ambient levels of underwater reef sound (Stanley et al., 2010). When considering this previous study it is possible to suppose that
differences in the sound emanating from different habitat types may result in different settlement responses to these sounds as well as being used in guiding orientation of pelagic stages to the appropriate settlement habitat. Overall, the results reviewed above suggest that settlement stages of fishes and crabs use available reef sound as orientation, settlement and metamorphosis cues but may also be discriminating spectral difference within these cues which could relate to possible differences in habitat.

In the current study, habitat type greatly affected the number of snaps produced by snapping shrimp and sound produced by the grazing urchin *E. Chloroticus*; the presence of invertebrates and associated sounds emitted from these habitats therefore provide a reliable indicator of habitat type and quality. This shows a parallel with terrestrial habitats, as they can also be distinguished remotely to evaluate differences between terrestrial communities occupying different areas or changes in time using acoustic signatures (Sueur *et al.*, 2008). Acoustic recordings have also been used to describe temporal and spatial structure of tropical forest communities, i.e., insects and multispecies, in both the canopy of rainforest in Borneo and in South American forests (Riede, 1997; Hammer & Barrett, 2001). Riede (1997) concluded that on the temporal scale nocturnal, diurnal and dusk communities could be differentiated and dominant species identified. The maximum differences seen in acoustic production in the current study, especially in the reef habitats, were observed in the dusk recordings. This suggests that in order to determine differences among habitat types, underwater sound recordings should be conducted times of intensity maximum, i.e. dusk.

The findings of the current study coupled with previous findings highlight that differences in underwater sound output may play a highly significant role in the recruitment and settlement processes of larval reef fishes and crustaceans. The statistically significant differences in underwater sound occurring over relatively short distances could provide a basis for habitat selection by settlement stage organisms. Future work should focus on establishing the broader application of these results, determining the sensory thresholds of larval reef species and investigating in more depth the habitat discrimination ability of settling stage larvae of reef fishes and crustaceans.
Chapter Four: Settlement response to ambient underwater sounds associated with different habitat types

4.1 INTRODUCTION

In most marine ecosystems many organisms, such as crustaceans, fishes and molluscs, have a complex life cycle which includes a pelagic larval phase capable of great dispersal distances. The settlement period at the end of the larval phase, when the organism selects and settles to a benthic habitat, is a critical period in the life cycle (Werner, 1988; Lecchini & Poignonec, 2009). Mortality is high during, and directly after settlement, and for this reason there is strong selection pressure to find and select a habitat that supports growth and survival (Lecchini & Galzin, 2003). Many post-settlement individuals are often found in greater numbers in structurally complex habitats that provide refuge against predation (e.g., fish and crustaceans in macroalgae or coral heads or rubble (Shulman, 1984; Carr, 1994)). A wide range of settlement and metamorphosis cues have been identified that can be involved in the selection of suitable settlement habitat by late-stage brachyuran crab larvae. These cues include salinity, depth, substrate rugosity, as well as a range of chemical cues associated with conspecifics, settlement substrates, aquatic vegetation and potential prey (Forward et al., 2001). Although, settlement habitat choice is a well described phenomenon, the present study identifies the potential of a novel habitat-specific settlement cue – underwater sound.

As many reef dwelling larvae are known to be capable of preferentially arriving at settlement habitats, a number of studies have investigated what cues are used by late-stage larvae to locate these preferred settlement habitats. Many potential orientation cues exist such as the oceanic currents and swells, celestial (Smith & Smith, 1998), visual and polarised light (Kobayashi, 1989; Leis & Carson-Ewart, 1999), chemical (Kingsford et al., 2002; Lecchini et al., 2005), magnetic (Boles & Lohmann, 2003), electric fields and underwater sound (Tolimieri et al., 2000; Jeffs et al., 2003; Leis & Lockett, 2005; Simpson et al., 2005a; Montgomery et al., 2006; Radford et al., 2007; Simpson et al., 2008b). However, to date,
there is only clear empirical evidence to support the operation of underwater sound (Simpson et al., 2005a), oceanic forces and to a certain extent visual and chemical cues.

Ambient underwater sound has long been regarded as one of the most robust candidates for guiding onshore orientation by pelagic larvae. It is able to be conducted over large distances, is directional, can carry significant biological information about the habitat of origin and is current independent, unlike olfactory cues (Hawkins & Myrberg, 1983; Kingsford et al., 2002). In a recent study it was found that within a relatively small section of a coast (< 20 km) it is possible to find differences in spectral and temporal composition of ambient underwater sound that is associated with different types of coastal habitat (Chapter Three). It would be a great advantage to late-stage larvae if they were able to use the signals being emitted from a reef to remotely identify suitable settlement habitats. A number of studies have demonstrated that ambient underwater sound emanating from coastal habitats attracts the settlement stages of a broad range of families of reef fishes, crustaceans and coral (Stobutzki & Bellwood, 1998; Tolimieri et al., 2000; Jeffs et al., 2003; Jeffs et al., 2005; Leis & Lockett, 2005; Simpson et al., 2005a; Montgomery et al., 2006; Radford et al., 2007; Vermeij et al., 2010). Also recent study observed that settlement and subsequent metamorphosis was advanced in the megalopae of several species of coastal brachyuran crab when exposed to ambient levels of underwater reef sound (Stanley et al., 2010). What remains unclear is whether late-stage larvae have the capability to discriminate between different habitats based on differences in the underwater sound generated by these different habitats.

Therefore, the aim of this present research was to determine the potential for settling stages of coastal brachyuran crab species to discriminate among different settlement habitats based on habitat-specific differences in underwater sound. Experiments were conducted on settlement stage coastal crab species in temperate and tropical waters to examine if the results were consistent over a wide range of marine environments. It also extends earlier investigations to uncover any possible artefacts brought about by laboratory conditions, by running parallel experiments in the laboratory and in the field with higher levels of replication to more robustly test the phenomenon.
4.2 METHODS

The study was undertaken during November 2009 to April 2010 in temperate waters near the Leigh Marine Laboratory in north-eastern New Zealand, and also in tropical waters near the Lizard Island Research Station on the Great Barrier Reef in north-eastern Australia.

Figure 4.1: Maps showing in-field experimental habitat sites in New Zealand: a) Pakiri Beach, b) Waterfall Reef, c) Whangateau Estuary and recording sites for laboratory treatments: 1) North Reef, 2) Mahurangi Harbour and 3) Pakiri Beach.
4.2.1 Source of megalopae

Light traps were used to capture pelagic megalopae for behavioural assays (Hickford & Schiel, 1999; Meekan et al., 2001; Stanley et al., 2010). Up to eight light traps were deployed at night within 500 m of the shoreline, at least 15 m apart and submerged 2 m in water of 5 – 7 m depth. The traps were recovered within 2 h of sunrise the following morning. When large planktivorous fishes were found in a light trap, megalopae were not used in the experiments as they may have altered behaviour due to stress from being in the presence of a predator (Forward & Rittschof, 2000). The megalopae were transported in seawater to a nearby laboratory where they were counted, sorted into settlement stage and identified to lowest taxonomic level possible given the available taxonomic descriptions (Wear & Fielder, 1985; McLay, 1988; Jones & Morgan, 2002). Only intermoult pre-settlement (i.e., natant and active swimming) megalopae of similar size and age were selected for use in the experiments. The
megalopae were held in a flowing filtered (40 µm) seawater system with natural light period and ambient temperature (14 – 31.0 °C, dependant on timing and location) until experiments began the following evening. Five species of brachyuran megalopae were selected for use in the assays. The two temperate species, *Hemigrapsus sexdentatus* and *Cyclograpsus lavauxi*, both from the family Grapsidae, are common species known to be associated with nearshore subtidal and intertidal habitats, under boulders, amongst macroalgae and on the rocky shores. The three tropical species, *Cymo andreossyi*, *Schizophrys aspera* and *Grapsus tenuicrustatus* were identified to be members of the Xanthidae, Majidae and Grapsidae families respectively; and are known to be associated with hard coral and on coral shore habitats.

### 4.2.2 Laboratory-based behavioural assays

Each laboratory-based experiment consisted of four sound treatments (three distinct habitat sound types and one silent (control)), and within each treatment there were three replicate water baths used to maintain a constant water temperature for megalopae throughout the experiment. The baths were acoustically isolated using rubber mats to prevent any transfer of acoustic energy from the surrounding environments into the experimental treatment. The absence of any significant acoustic signal in the Silent control tanks was confirmed by recording with a calibrated hydrophone (High Tech, Inc., HTI – 96 – MIN).

![Figure 4.3: Schematic diagram of a side view of one of the experimental replicates showing the layout of the water bath, speaker and settlement vials.](image)

Each replicate water bath contained 5 – 10 plastic vials (depending on the number of animals caught) (250 ml) with a sealed lid housing a single randomly selected megalopa in
filtered (1 µm) and UV treated seawater. The vials had a roughened base acting as a chemically inert settlement surface for settling megalopae. All replicates for both the sound treatments and Silent control had a weighted Sony loudspeaker inside a watertight plastic bag which was submerged in the water bath (Figure 4.3). For the sound replicates only, a Sony CD Walkman D – EJ815 was connected to the speaker and used to continually play a 4 min loop of recorded ambient underwater sound into the water bath and through the acoustically transparent plastic containers holding the crabs (Gerber, 1978).

When on a single night sufficient (> 120) megalopae of the same species were collected from the light traps to conduct the experiments they were randomly allocated to an experimental treatment and replicate. All megalopae in each treatment were kept under natural light period and ambient water temperature (14 – 31.0° C, depending on local ambient temperature) for the duration of the experiment. In the absence of an anechoic chamber, all laboratory-based experiments were conducted in a quiet concrete floored and walled laboratory.

The megalopae were added to the experiment at 1700 h on the day of their capture and the CD Walkman was switched on to initiate sound in the sound treatments. Subsequently every 6 h an observational period occurred, at which time counts were made of the number of megalopae that had settled onto the base of the vials and metamorphosed into the first instar benthic juvenile stage. Each period of observation lasted no more than 40 min for all treatments. The time from establishing the experiment to the first observational period when a megalopa was observed to have settled and proceeded through metamorphosis was termed time to metamorphosis (TTM). When the observational period occurred at night, red light was used to observe behaviour because prior testing demonstrated there was little or no visual response by megalopae under red lighting (Cronin, 1986). In the current study ‘settlement’ is defined as a behavioural process which involves movement out of the water column to a benthic substrate, and ‘metamorphosis’ as a physiological process which includes loss of larval characteristics retained in the megalopa and the transformation to the adult reptant body form (Hadfield et al., 2001).

During each 6 hourly observational period descriptions of behaviour of each megalopa was categorized in the following manner. 1 – ‘Normal’ pre-settlement swimming behaviour, i.e., Highly active swimming, low number of downward swimming events , no exploratory crawling behaviour; 2 - Medium swimming activity, medium number of downward swimming events, small amount of exploratory crawling behaviour; 3 – Low swimming
activity, high number of downward swimming events, extensive exploratory crawling behaviour; 4 – Complete settlement and metamorphosis, i.e., no swimming activity.

The experiment was terminated when all experimental megalopae in all treatments had metamorphosed. The settled juvenile crabs were kept for 5 – 10 d following the experiment in flowing seawater, fed and were monitored for post-experimental mortality.

4.2.2.1 Habitat sound recordings for laboratory-based experiments

Recordings of typical ambient underwater sound were made at the three different habitats selected for the laboratory-based sound treatments. For temperate waters, sound treatments were recorded from north-eastern New Zealand during the summer at dusk on a new moon; North Reef (36° 15’58 S, 174° 47’37 E), a macroalgae dominated rocky reef habitat, Mahurangi Harbour (36° 20’51 S, 174° 45’54 E), a harbour with extensive sandy/broken shell seafloor habitat, Pakiri Beach (36° 13’31 S, 174° 42’34 E), an open sandy beach habitat (Figure 4.1 – 1, 2 and 3). For tropical waters, sound treatments were recorded from waters near the Lizard Island Research Station, north-eastern Australia on the Great Barrier Reef (GBR) during the summer at dusk on a new moon; Coconut Reef (14° 40’51 S, 145° 28’17 E), a continuous frontal fringing coral reef habitat, Horseshoe Reef (14° 41’13 S, 145° 26’37 E), an isolated coral back reef habitat interrupted with areas of sand and coral rubble, Lagoon (14° 41’26 S, 145° 27’28 E), a lagoon habitat with extensive sandy seafloor in the centre of the Lizard Island Group, distant (~400 m) from fringing reefs (Fig. 4.2 – 1, 2 and 3). In situ habitat sounds were recorded using a remote recording system which consisted of a calibrated HTI 96-MIN hydrophone connected to an automated recording system and a digital recorder Roland Edirol R09HR, contained in an underwater housing (Figure 4.4). The recorder was installed in approximately 15 m of water (depending on the tide) for the temperate treatments, excluding the Mahurangi Harbour which was in 6 m and approximately 7 m of water for the tropical treatments excluding the Lagoon which was in 12 m. The hydrophone was calibrated by recording a NetMark 1000 acoustic pinger (specifications: source level 130 dB re 1 µPa at 1 m, 10 kHz signal, 300 ms pulse length, 4 s repetition rate). Digital recordings were transferred to a PC and the spectral composition analysed using MATLAB software with codes specifically written for these recordings. Ten typical 4 min sequences from each habitat recording were selected and from those one was randomly selected and transferred to a CD for playback in the sound treatments of the laboratory-based experiments.
A calibrated hydrophone and recorder (High Tech, Inc., Mississippi, USA HTI – 96 – MIN, Sound Devices, Wisconsin, USA 722 recorder) was used to adjust the sound level produced by the Sony speakers in each experimental sound treatment tank to 100 dB re 1µPa, 80 dB re 1µPa and 70 dB re 1µPa RMS level in the 100 – 24000 range for North Reef, Pakiri Beach and Mahurangi Harbour respectively (Figure 4.5), which was within the typical range of ambient sound level for evening chorus at such habitats in New Zealand’s coastal waters (Tolimieri et al., 2000; Radford et al., 2008b) and to 90 dB re 1µPa, 75 dB re 1µPa and 60 dB re 1µPa RMS level in the 100 – 24000 range for Coconut Reef, Horseshoe Reef and Lagoon respectively (Figure 4.6), which was within the typical range of ambient sound level of evening chorus at other similar habitats on the GBR in Australia (Cato & McCauley, 2002)

Recordings of the habitat sounds in the experimental tanks were recorded with a calibrated hydrophone (High Tech, Inc., HTI – 96 – MIN) for comparison with the source signals recorded from the natural habitats and the spectral composition analysed using MATLAB software with codes specifically written for these recordings.

### 4.2.3 Field-based behavioural assays

For the three temperate species, field-based experiments consisted of three distinct habitat sites (treatments); Waterfall Reef (36° 16’05 S, 174° 48’06 E) a macroalgae
dominated rocky reef, Pakiri Beach (36° 13’31 S, 174° 42’34 E) an open sandy beach, and Whangateau Estuary (36° 18’55 S, 174° 45’24 E) an estuary with an extensive area of sandy/broken shell seafloor (Figure 4.1 – a, b and c). For the three tropical species, field-based experiments consisted of two habitat sites (treatments); Horseshoe Reef (14° 41’13 S, 145° 26’37 E) an isolated coral back reef habitat interrupted with areas of sand and coral rubble and Loomis Beach lagoon (14° 40’59 S, 145° 27’13 E) which had an extensive area of sandy seafloor that was approximately 1km from fringing reefs (Figure 4.2 – a and b). Each habitat site consisted of three replicates, each replicate with 5 – 10 individually housed megalopa (number determined by light trap catch rates) in a 250 ml plastic vial containing filtered (1 µm) and UV treated seawater with a sealed lid, identical to those used in laboratory-based experiments. Vials were held in a vertical position approximately 400 mm from the sea floor in a frame. The replicates were 1 m apart, tethered to the seafloor on sand 2 m from the reef at reef sites in 5 – 8 m of water depending on the habitat.

The megalopae were added to the habitat sites by divers at approximately 1700 h on the day of their capture. At dawn (0800 h) and dusk (1700 h) divers visited the habitat sites at which time counts were made of the number of individuals that had settled to the base of the vials and metamorphosed into the first instar benthic juvenile stage. Settlement and metamorphosis definitions were unchanged from the laboratory-based methods. The experiment was terminated when all experimental megalopae in all treatments had metamorphosed or poor weather conditions no longer permitted safe diving at the habitat sites.

4.2.3.1 Habitat site sound recording

Recordings of the ambient underwater sound in the vicinity of the experimental habitat sites were collected during the experiment using the remote recording system previously described, (Figure 4.1 a, b and c and 4.2 a and b). The recorder was installed in approximately 7 m of water (depending on the tide). Recordings at noon and dusk were taken and the spectral composition analysed using MATLAB software with codes specifically written for these recordings to confirm the habitats were acoustically distinct.
4.2.4 Data analyses

For both laboratory and field experiments for each species non-parametric statistical methods were used to test for differences in median TTM within each sound treatment as the data were not continuous (Zar, 1999).

Kruskal-Wallis comparison of ranks or Mann-Whitney U tests were used to test for a difference in the median TTMs among the replicates within the same treatment (i.e. each treatment analysed separately). If this test found no difference among the three replicates the data from the replicates were pooled for an experiment-wide analysis. The Kruskal-Wallis test was then used to compare the median TTMs for megalopae among the treatments. For all statistical tests, \( P \) values \( \leq 0.05 \) were considered to be significant. To isolate difference among individual treatments a Dunn’s pairwise multiple comparison procedure was used. A metamorphosis rate for each treatment within each species was also calculated with a Sen’s slope analysis for the data points between the last sampling event prior to the first megalopa metamorphosing and the sampling event when the last megalopa metamorphosed. All analyses were performed using the software Sigma Stat 4.0 and Minitab 16.1.0.

4.3 RESULTS

4.3.1 Laboratory-based experiments

4.3.1.1 Sound analyses

In the laboratory experiments for both temperate and tropical crab species, the played back sound within the experimental tanks had a similar overall spectral composition and sound level to the source signals recorded from the natural habitat sound in situ (Figure 4.5 & 4.6). The Silent control had no sound transfer from any other external sources. The flat response at approximately 35 dB re 1 \( \mu \)Pa is the lower recording limit of the recording equipment (Figure 4.5d & 4.6d).

In the recording from North Reef there was a peak in the spectra around 700 – 1200 Hz, which is produced by feeding of the sea urchin, *Evechinus chloroticus*, whereas the higher frequency pulses were the snaps of snapping shrimp (Figure 4.5a). The peak in the spectra in the Mahurangi Harbour treatment recording was around 2500 – 15000 Hz from the snaps of snapping shrimp, however, these were approximately 20 dB re 1\( \mu \)Pa Hz\(^{-1/2}\) lower than the
level for this frequency band and less frequent than in the North Reef recording (Figure 4.5b). The Pakiri Beach recording had its highest levels of sound in the low frequency range of 100 – 500 Hz which is due to waves breaking on the shore (surf beat). There were also very low levels of sea urchin and snapping shrimp noise probably derived from distant reefs (Figure 4.5c). The spectra for the different habitats were different in overall appearance indicating that the acoustic characteristics varied markedly among the habitats (Figure 4.5).

Figure 4.5: Spectral composition of underwater sound when recorded at coastal habitats in north-eastern New Zealand and when replayed in experimental tanks. a) North Reef, b) Mahurangi Harbour and c) Pakiri Beach. Black lines represent natural sound in situ and grey lines represent replayed sounds in experimental sound treatments. d) Spectral composition in experimental Silent control.
The recording of the Coconut Reef treatment consisted of a chorus of pops made by fishes as well as other feeding and calling sounds typical of a healthy and extensive coral reef. The peak in the spectra from this site was around 2500 – 7000 Hz which was a high frequency crackle produced by snapping shrimp (Figure 4.6a). The recording of Horseshoe Reef was approximately 15 dB re 1µPa Hz$^{-1/2}$ less intense than Coconut Reef and there was no pronounced peak in the spectra in the higher frequencies, indicating a fewer number snaps from snapping shrimp (Figure 4.6b). There were far fewer snaps from snapping shrimp in the recording of Lagoon (Figure 4.6c) and at much lower levels than both Coconut Reef and Horseshoe Reef. The highest levels of sound occurred in the lower frequency range (100 – 1500 Hz). The spectra for the different habitats were different in overall appearance indicating that the acoustical characteristics varied among the habitats (Figure 4.6).

Figure 4.6: Spectral composition of underwater sound when recorded at coastal habitats at Lizard Island, north-eastern Australia and when replayed in experimental tanks. a) Coconut Reef, b) Horseshoe Reef, c) Lagoon. Black lines represent natural sound in situ and grey lines represent replayed sounds in experimental sound treatments. d) Spectral composition in experimental Silent control.
4.3.1.2 Laboratory-based behavioural assays

A general description of changes in behaviour was compiled using a set pre-set scale. Prior to metamorphosis, swimming activity of larval crabs was present in both the dark and light periods. However, swimming activity was higher during the dark periods than during the light periods. When megalopae were approaching metamorphosis their behaviour changed noticeably, including reduced swimming, more frequent downward swimming to the settlement substrate (vertical migration) followed by “exploratory crawling” whereby the megalopae would slowly crawl around on the artificial settlement substrate (Figure 4.3). This behaviour is consistent with pre-settlement behaviour previously observed in other crab species (Forward et al., 2001) and was consistent among the megalopae of all five species in the current study and preceded settlement and metamorphosis. Although the swimming and settlement behaviour was consistent among all the treatments and replicates, the timing for the onset of this behaviour differed. In the temperate experiments the onset of settlement behaviour was consistently observed sooner in the North Reef treatments compared to the Pakiri Beach treatment and Silent control. Likewise, in the tropical experiments the onset of this behaviour occurred well in advance in the Horseshoe Reef treatments compared to the Lagoon and Silent treatments.

In the laboratory-based experiments, all five crab species there was no significant difference in the median TTM among the replicates within each of the four treatments ($P > 0.05$; Table 4.1). Therefore, for each crab species the TTM data for the replicates within each treatment were pooled to test for an overall treatment effect.

Median TTM differed significantly among treatments for the megalopae of all crab species tested (Kruskal-Wallis test, $P < 0.05$; Table 4.2 & Figure 4.7). Both temperate species, *H. sexdentatus* and *C. lavauxi*, had significantly different median TTM between all paired combinations of treatments (Tukey’s test $P < 0.05$) except between Pakiri Beach and Silent control ($P > 0.05$). North Reef had the smallest median TTM (i.e. fastest settlement) in both *H. sexdentatus* and *C. lavauxi* of 58 h and 39 h respectively, followed by Mahurangi Harbour of 74 h and 48 h respectively, then Pakiri Beach of 105 h and 69 h respectively, and finally Silent, of 105 h and 72 h. *Hemigrapsus sexdentatus* had the largest difference in median TTM between North Reef (58 h) and Silent (150 h) for the temperate species, a difference of 47 h ($P < 0.05$).
In the tropical crab species *C. andreossyi* and *G. tenuicrustatus*, the median TTM between all paired combinations of treatments were significantly different (Tukey’s test *P* < 0.05), except between Lagoon and Silent. *Schizophrys aspera* had significantly different median TTM between Coconut Reef vs. Silent, Horseshoe Reef vs. Silent, Coconut Reef vs. Lagoon and Horseshoe Reef vs. Lagoon (*P* < 0.05), but similar median TTM between Coconut Reef vs. Horseshoe Reef and Silent vs. Lagoon (*P* > 0.05). Coconut Reef had the shortest median TTM in all *C. andreossyi*, *G. tenuicrustatus* and *Schizophrys aspera* of 66 h, 36 h and 54 h respectively, followed by Horseshoe Reef of 78 h, 43 h and 72 h respectively, then Lagoon of 96 h, 54 h and 96 h and lastly Silent of 99 h, 54 h and 102 h. *Grapsus tenuicrustatus* had the largest difference in median TTM between Coconut Reef (54 h) and Silent (102 h) for the tropical species, a difference of 48 h (*P* < 0.05).

The difference in time to the first megalopae to metamorphose varied substantially among the treatments (Table 4.3). Both species *H. sexdentatus* and *G. tenuicrustatus* exhibited the greatest difference between the fastest and slowest treatment, both being 36 h. The temperate species *H. sexdentatus* had the greatest difference of 42 h to completed metamorphosis (whereby all megalopae had metamorphosed) (Table 4.3).
Table 4.1: Comparisons of median TTM among the replicates within each treatment in laboratory-based experiments for five crab species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment (habitat)</th>
<th>H – statistic</th>
<th>P – value among replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hemigrapsus sexdentatus</em></td>
<td>North Reef</td>
<td>2.83</td>
<td>0.24</td>
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<tr>
<td></td>
<td>Mahurangi Harbour</td>
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<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Pakiri Beach</td>
<td>0.78</td>
<td>0.51</td>
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<td></td>
<td>Silent</td>
<td>0.93</td>
<td>0.15</td>
</tr>
<tr>
<td><em>Cyclograpsus lavauxi</em></td>
<td>North Reef</td>
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<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Mahurangi Harbour</td>
<td>2.85</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Pakiri Beach</td>
<td>0.47</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>2.65</td>
<td>0.27</td>
</tr>
<tr>
<td><em>Cymo andreossyi</em></td>
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<td>0.83</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Horseshoe Reef</td>
<td>1.28</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Lagoon</td>
<td>0.10</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.85</td>
<td>0.65</td>
</tr>
<tr>
<td><em>Schizophrys aspera</em></td>
<td>Coconut Reef</td>
<td>0.75</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Horseshoe Reef</td>
<td>0.36</td>
<td>0.83</td>
</tr>
<tr>
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<td>Lagoon</td>
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<td>0.89</td>
</tr>
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<td>Silent</td>
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<td>0.32</td>
</tr>
<tr>
<td><em>Grapsus tenuicrustatus</em></td>
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<td>0.69</td>
</tr>
<tr>
<td></td>
<td>Horseshoe Reef</td>
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<td>0.16</td>
</tr>
<tr>
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<td>Lagoon</td>
<td>0.85</td>
<td>0.65</td>
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<tr>
<td></td>
<td>Silent</td>
<td>2.29</td>
<td>0.32</td>
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</table>
## Table 4.2: Comparisons among median TTM and metamorphosis rates for each treatment in laboratory-based experiments for five crab species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total number of individuals (n)</th>
<th>Treatment (habitat)</th>
<th>Median TTM (h)</th>
<th>H – statistic</th>
<th>P – value</th>
<th>Metamorphosis rate</th>
</tr>
</thead>
<tbody>
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<td><em>Hemigrapsus sexdentatus</em></td>
<td>24</td>
<td>North Reef</td>
<td>58</td>
<td></td>
<td></td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>24</td>
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<td>74</td>
<td>34.0</td>
<td>&lt;0.001***</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Pakiri Beach</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>24</td>
<td>Silent</td>
<td>105</td>
<td></td>
<td></td>
<td>6.8</td>
</tr>
<tr>
<td><em>Cyclograpsus lavauxi</em></td>
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<td>39</td>
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<td>Silent</td>
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<td>Silent</td>
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<tr>
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<td>14.7</td>
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<td>47.9</td>
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<td>Silent</td>
<td>102</td>
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</table>

*** Asterisk indicates a significant difference in TTM between treatments (P < 0.05, Kruskal-Wallis test).
Table 4.3: Summary of comparisons among first metamorphosis, completed metamorphosis and the difference between each the fastest and the slowest treatment in laboratory-based experiments for five crab species

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment (habitat)</th>
<th>First Metamorphosis (h)</th>
<th>Difference (h)</th>
<th>Completed metamorphosis (h)</th>
<th>Difference (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hemigrapsus sexdentatus</em></td>
<td>North Reef</td>
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<tr>
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<td>Mahurangi Harbour</td>
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<td>36</td>
<td>120</td>
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<tr>
<td></td>
<td>Pakiri Beach</td>
<td>60</td>
<td></td>
<td>138</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>66</td>
<td></td>
<td>138</td>
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<td><em>Cyclograpsus lavauxi</em></td>
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</tr>
<tr>
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<td>Mahurangi Harbour</td>
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<td>18</td>
<td>84</td>
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</tr>
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<td>Pakiri Beach</td>
<td>36</td>
<td></td>
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<td></td>
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<td>Silent</td>
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<td></td>
<td>96</td>
<td></td>
</tr>
<tr>
<td><em>Cymo andreossyi</em></td>
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<td>102</td>
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<td>114</td>
<td>36</td>
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<td></td>
<td>Lagoon</td>
<td>60</td>
<td></td>
<td>138</td>
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<td></td>
<td>Silent</td>
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<td></td>
<td>138</td>
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<tr>
<td><em>Schizophrys aspera</em></td>
<td>Coconut Reef</td>
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</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>Silent</td>
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<td>84</td>
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<tr>
<td><em>Grapsus tenuicrustatus</em></td>
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</tr>
<tr>
<td></td>
<td>Silent</td>
<td>66</td>
<td></td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>
4.3.1.3 Laboratory-based rates of metamorphosis

Both species of temperate crabs, *H. sexdentatus* and *C. lavauxi* had a faster metamorphosis rate in the North Reef treatment than in the Silent control (1.2 times faster) (Table 4.2). In the tropical species, both *C. andreossyi* and *S. aspera* had faster metamorphosis rates in the Coconut Reef treatment than the Silent control, 1.1 times and 1.8 times faster respectively (Table 4.2). However, *G. tenuicrustatus* had a faster metamorphosis rate in the Silent control when compared to the Coconut Reef treatment (1.1 times faster).
Figure 4.7: Percentage of total number of megalopae metamorphosed over time (h) in laboratory-based experiments. a) Hemigrapsus sexdentatus, b) Cyclograpsus lavauxi, c) Cymo andreossyi, d) Schizophrys aspera, and e) Grapsus tenuicrustatus.
4.3.2 Field-based experiments

4.3.2.1 Habitat site sound recording

The spectra from the underwater sound recorded from the habitat sites for the temperate experiments show differences in the spectral composition, temporal variation and overall levels among sites (Figure 4.8a, b and c). Waterfall Reef showed the highest overall sound levels among the temperate study sites with only small differences in sound level through a wide range of frequencies (300 – 10000 Hz) (Figure 4.8a). The dominant sound sources in the recordings were snapping shrimp and grazing sea urchin (800 – 15000) and some fish species (50 – 4000 Hz). Whangateau Estuary had very low levels of low frequency sound due to the absence of waves breaking on the surface and sound-producing fish species (Figure 4.8b). Levels of sound increased in the higher frequencies (2000 – 15000 Hz) due to the snaps of snapping shrimp, however, there were far less numbers of snaps when compared to waterfall Reef. Pakiri Beach had the highest sound levels in the lower frequencies (100 – 1000 Hz) (Figure 4.8c) which is due to the sound of waves breaking along the nearby shoreline.

The spectra graphs for the tropical experimental habitat sites also showed differences in spectral composition, temporal variation and overall level. Horseshoe Reef had higher levels of sound in the 2000 – 20000 Hz frequency range when compared to Loomis Beach lagoon which is predominately due to the presence of snapping shrimp and also high levels in the 100 – 800 Hz frequency range due to the presence of many sound-producing fish species (Figure 4.8d). Loomis Beach lagoon had much lower levels of both low and high frequency sound due to the lower abundance of sound-producing organisms (Figure 4.8e), assuming that the number of snaps estimated from the sound recordings roughly indicate the number of snapping shrimp present in the vicinity at that given time.
Figure 4.8: Spectral composition of underwater sound recorded at the experimental habitat sites at Leigh, north-eastern New Zealand and Lizard Island, north-eastern Australia showing differences in overall level, spectral composition and temporal variation. a) Waterfall Reef, b) Whangateau Estuary c) Pakiri Beach, d) Horseshoe Reef and e) Loomis Beach lagoon. Blue lines represent dusk and black lines represent noon.
4.3.2.2 Field-based behavioural assays

In the field-based experiments for all five crab species there was no significant difference in the median TTM among the replicates for all of the habitats ($P > 0.05$; Table 4.4). Therefore, for each crab species the TTM data for the replicates within each habitat were pooled to test for an overall treatment effect.

Median TTM differed significantly among treatments for the megalopae of all crab species ($P < 0.05$; Table 4.5 & Figure 4.9). In the temperate crab species, the megalopae at the Waterfall Reef habitat had a significantly shorter median TTM than those at the Whangateau Estuary. *Cyclograpsus lavauxi* had a median TTM of 63 and 96 h (33 h difference) and *H. sexdentatus* had a median TTM of 63 and 87 h (24 h difference) respectively ($P < 0.001$; Table 4.5). Due to poor weather the Pakiri Beach habitat could not be sampled past 120 h for both *H. sexdentatus* and *C. lavauxi* and therefore could not be included in analysis. However, in the first 120 h there was a visible lag in the TTM at the Pakiri Beach habitat when compared with the other two habitats (Figure 4.9 a & b).

The tropical species *C. andreossyi* had the largest difference in median TTMs between the two habitats, with a median TTM of 114 h at the Horseshoe Reef habitat and 144 h at the Loomis Beach lagoon habitat, this is a 30 h difference ($P < 0.05$; Table 4.5). This was followed by *G. tenuicrustatus* with a median TTM of 63 h at the Horseshoe Reef habitat and 92 at the Loomis Beach lagoon habitat, a 29 h difference ($P < 0.005$; Table 4.5). *Schizophrys aspera* had a 29 h difference, with a median TTM of 72 h and 96 h at the Horseshoe Reef and Loomis Beach lagoon habitats respectively ($P < 0.001$; Table 4.5).

The difference in time to the first megalopae to metamorphose varied among the treatments (Table 4.6). The tropical species *C. andreossyi* exhibited the greatest difference between the two treatments with the Horseshoe Reef treatment starting metamorphosis 39 h ahead of Loomis Beach lagoon. The tropical species *Grapsus tenuicrustatus* had the greatest difference to completed metamorphosis, with a 57 h difference (Table 4.6).
Table 4.4: Comparisons of median TTM among the replicates within each habitat site in field-based experiments for five crab species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment (habitat)</th>
<th>H – statistic</th>
<th>P – value among replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hemigrapsus sexdentatus</em></td>
<td>Waterfall Reef</td>
<td>0.45</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Whangateau Estuary</td>
<td>2.22</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Pakiri Beach</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td><em>Cyclograpsus lavauxi</em></td>
<td>Waterfall Reef</td>
<td>0.30</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Whangateau Estuary</td>
<td>0.15</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Pakiri Beach</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td><em>Cymo andreossyi</em></td>
<td>Horseshoe Reef</td>
<td>0.93</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Loomis Beach lagoon</td>
<td>1.32</td>
<td>0.52</td>
</tr>
<tr>
<td><em>Schizophrys aspera</em></td>
<td>Horseshoe Reef</td>
<td>2.47</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Loomis Beach lagoon</td>
<td>0.69</td>
<td>0.74</td>
</tr>
<tr>
<td><em>Grapsus tenuicrustatus</em></td>
<td>Horseshoe Reef</td>
<td>0.54</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Loomis Beach lagoon</td>
<td>1.72</td>
<td>0.46</td>
</tr>
</tbody>
</table>
Table 4.5: Comparisons among median TTM and metamorphosis rates for each habitat site in field-based experiments for five crab species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total number of individuals (n)</th>
<th>Treatment (habitat)</th>
<th>Median TTM (h)</th>
<th>U - statistic</th>
<th>P - value</th>
<th>Metamorphosis rate</th>
</tr>
</thead>
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<td><em>Hemigrapsus sexdentatus</em></td>
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<td>30</td>
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<td>87</td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td><em>Cyclograpsus lavauxi</em></td>
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<td>Waterfall Reef</td>
<td>63</td>
<td>175.5</td>
<td>&lt;0.001***</td>
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<tr>
<td><em>Cymo andreossyi</em></td>
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<tr>
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<tr>
<td></td>
<td>15</td>
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<td>96</td>
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<td></td>
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<td>0.002***</td>
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<td>Loomis Beach lagoon</td>
<td>92</td>
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</table>

***Asterisk indicates a significant difference in TTM between habitat sites (P < 0.05, Mann-Whitney U test).
Table 4.6: Summary of comparisons among first metamorphosis, completed metamorphosis and the difference between each the fastest and the slowest treatment in field-based experiments for five crab species

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment (habitat)</th>
<th>First Metamorphosis (h)</th>
<th>Difference (h)</th>
<th>Completed metamorphosis (h)</th>
<th>Difference (h)</th>
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<td></td>
<td>Whangateau Estuary</td>
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<td>33</td>
<td>144</td>
<td>24</td>
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<tr>
<td></td>
<td>Pakiri Beach</td>
<td>72</td>
<td>n/a</td>
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<tr>
<td><em>Cyclograpsus lavauxi</em></td>
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<tr>
<td><em>Cymo andreossyi</em></td>
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</table>
4.3.2.3 Field-based rates of metamorphosis

All species, both temperate and tropical, had faster metamorphosis rates at Waterfall Reef and Horseshoe Reef habitats respectively, compared with Whangateau Estuary, Pakiri Beach or Loomis Beach lagoon habitats. The tropical species *G. tenuicrustatus* had the largest difference in metamorphosis rates; megalopae at the Horseshoe Reef were 1.8 times faster than those at the Loomis Beach lagoon (24 and 13 respectively). Followed by *S. aspera* which had a 1.3 times faster metamorphosis rate at Horseshoe Reef. Both temperate species *H. sexdentatus* and *C. lavauxi* had 1.2 times faster metamorphosis rate at Waterfall Reef than at Whangateau Estuary. The tropical species, *C. andreossyi* had the smallest difference in metamorphosis rate, 1.1 times faster at Horseshoe Reef compared with Loomis Beach lagoon. The settlement rate in the Pakiri Beach starts with a similar overall rate trend to both the other two temperate habitat sites for *C. lavauxi*, however, in *H. sexdentatus* the rate is much lower (4.4 compared to 14 and 11).
Figure 4.9: Percentage of total number of megalopae metamorphosed over time (h) in field-based experiments. a) Hemigrapsus sexdentatus, b) Cyclograpsus lavauxi, c) Cymo andreossyi, d) Schizophrys aspera, and e) Grapsus tenuicrustatus.
4.4 DISCUSSION

The current study is the first to demonstrate the ability of settlement stage marine organisms to discriminate among suitable settlement habitats on the basis of natural ambient underwater sound. The phenomenon appears to be common as it was present in all five species tested from several families of coastal brachyuran crabs, and it was present both in temperate and in tropical waters. In all species of crab tested in both laboratory-based and field-based experiments, the settlement and metamorphic responses observed were significantly faster in habitat types that specifically matched the benthic stage habitat types for these species, i.e., habitats associated with rocky or coral reefs. In contrast, habitats where association is uncommon or adverse (i.e., harbour, lagoon or sandy bottomed beach and silent), the time to settlement and metamorphosis was delayed. For example, when comparing most favourable to least favourable settlement habitat type in the laboratory-based experiments TTM was shortened by 33 – 47% and in the field-based experiments by 21 – 34%. The results of the current study corroborate the preferential response to an ambient reef sound treatment over a silent treatment in the same species of settlement stage crabs (Stanley et al., 2010). This current study also eliminates the suggestion of any potential artefacts created in the laboratory affecting TTM results and also greatly extends the knowledge on crab settlement behaviour and their ability to discriminate among suitable settlement habitats by using acoustic information alone.

This result is parallel with what might be expected given the differences in the distribution of juvenile and adult crab among these habitats. Both the temperate species *H. sexdentatus* and *C. lavauxi* showed the strongest response to the macroalgae dominated rocky reef habitat type, these species tend to be associated with this kind of intertidal and subtidal habitat as settled juveniles and adults (Wear & Fielder, 1985; McLay, 1988). The habitat type to show the next strongest response was the sandy/broken shell bottomed harbour, both the sound recording used in the laboratory-based experiment and the field habitat used in the field-based experiments displayed some degree of acoustic similarities to the macroalgae dominated rocky reef treatments. Although the sound level was lower, there were similar biotic signals in the 2500 – 5000 Hz frequency band, indicating the presence of snapping shrimp but not in the same densities (Cato, 1992; Radford et al., 2008b). All three of the tropical species tested, *C. andreossyi, S. aspera* and *G. tenuicrustatus* showed the strongest response to the coral reef habitat type as opposed to the lagoon habitat, this is also to be expected as adults are known to be strongly associated with coral reef shores and use their
complexity for protection and prey capture, and they are also found living in symbiosis with hard corals (Jones & Morgan, 2002).

As larvae are competent to actively select a suitable habitat in which to settle, live and survive in, there is certain sensory information they must encounter to guide them to the particular habitats. Many reef dwelling larvae appear to use visual and chemosensory/olfactory cues when settling. Conspecific odour was found to reduce time to metamorphosis in the crab species Chasmagnathus granulate, Uca pugilator, Panopeus herbstii and Rhithropanopeus harrisii (Forward et al., 2001). Migrating galaxiid species are seen to preferentially select water that contains adult odour (Baker & Montgomery, 2001). However, both chemical and visual cues have their limitations. As the chemical stimuli are in a water medium the ocean currents carry the stimuli causing it to only be effective either downstream of the source or at very small spatial scales before it becomes substantially diluted (Montgomery et al., 2001). Visual cues allow fine scale habitat selection, however, this only appears useful at reasonably small spatial scales (e.g., 5 – 10 m in coral trout larvae, Plectropomus leopardus (Leis & Carson-Ewart, 1999)), as increased turbidity will obstruct view to distant habitats. It has been observed in both the current and previous studies (Forward et al., 2001), that when megalopae are approaching metamorphosis their behaviour changes noticeably with reduced swimming activity, more frequent vertical migration, followed by ‘exploratory crawling’ on the settlement substrate. This early grasping response could perhaps help to keep the larvae within a once encountered and suitable sound field.

There have been numerous studies in the last decade investigating physical settlement cues and habitat selection methods in many species of pelagic larvae, many of these also give inadvertent evidence of the use of ambient underwater sound (O'Connor, 1991; Doherty et al., 1996; O'Connor & Gregg, 1998; Forward et al., 2001; Montgomery et al., 2001; Gebauer et al., 2004; Lecchini, 2005; Leis & Lockett, 2005; Stanley et al., 2010). For example, observations of habitat selection by rockfish (Genus Sebastes) recruits found that substratum type, relief, algal type and abundance had a significant influence on the settlement habitat selection (Carr, 1991). Similarly, 95% of megalopae and newly moulted first benthic instar of the crab Carcinus maenas L were found in structurally complex habitats and clearly avoided settlement on the open sand habitat (Hedvall et al., 1998). It has also observed that the availability of reef-related resources, specifically food and shelter, play a significant role in determining density and distribution of many common temperate reef fish recruits (Levin, 1994). A study on a small labrid fish, Pseudolabrus celidotus or ‘spotty’, in New Zealand...
waters showed recruitment differs markedly between habitats, with highest recruitment in habitats dominated by large macrophytic brown algae (Jones, 1984). Habitats dominated by large macrophytic algae have been found to have a distinct acoustic signature from other kinds of habitats, i.e., urchin barren and shell bottomed harbours, not only in their overall spectral composition but also in sound level (Chapter 3), these differences are often due to the acoustic activity of species abundant and primarily occupying the certain habitat type. For example, an urchin barren habitat in north-eastern New Zealand is characterised by a peak in the spectrum in the frequency range 800 – 2500 Hz which is predominantly due to the grazing behaviour of the sea urchin (Evechinus chloroticus) (Tait, 1962; Castle, 1974; Radford et al., 2008a; Radford et al., 2010).

Many habitats known to attract high numbers of larval and juvenile marine organisms have similar characteristics, they provide shelter from predators with complex structures such as rocks and algae or coral heads or rubble (Sale, 1968; Levin, 1994) and are rich in food sources (Jones, 1984; Levin, 1994). These habitats are often biologically complex and can emit sounds within specific frequency bands that are associated with the variety of sound-producing animal residents that characterise the habitat, such as snapping shrimp, sea urchins and fishes (Cato, 1976; 1978; 1992; Au & Banks, 1998; Radford et al., 2010). These optimal settlement habitats for many species of pelagic larvae are therefore potentially emitting valuable acoustic cues that can be detected and used in conjunction with other available sources of information as a reliable indicator of habitat type.

In the past decapod crustaceans have been considered to have a limited ability to detect acoustic stimuli due to the fact they are a similar density to sea water, aside from their exoskeleton, and do not possess any gas filled spaces similar to those associated with pressure detection hearing in fishes (Breithaupt & Tautz, 1990; Popper & Fay, 1999; Popper et al., 2001b). Decapod crustaceans have a wide variety of sensory structures that have been studied in relation to hearing ability. Some receptors have been identified that have the ability to respond to certain aspects of underwater sound such as particle motion, hydrodynamic flows and potentially pressure changes (Fraser & Macdonald, 1994; Popper et al., 2001a). In particular, many decapod crustaceans have several types of receptors capable of responding to the particle motion component of underwater sound that would be capable of providing directional information on a sound source (Popper et al., 2001b; Montgomery et al., 2006); these include mechanoreceptors, such as setae, chordotonal organs and internal statocyst receptor systems. Now several studies focusing on decapod crustaceans have elicited clear
behavioural responses to underwater sound (Jeffs et al., 2003; Radford et al., 2007; Stanley et al., 2010). There have also been a number of electrophysiological investigations on both adult and presettlement larvae that have provided evidence of their hearing competence, demonstrating that fish and crustaceans have the sensory abilities to use ambient reef sound (Budelmann, 1992b; Popper & Fay, 1993; Popper et al., 2001b; Simpson et al., 2005b; Wright et al., 2005; Wright et al., 2010).

In the current study the preferential habitat type of settling stages of five species of crabs were clearly associated with a reduction in time to settlement and metamorphosis, i.e., the macroalgae dominated rocky reef in temperate species and the dense coral reef in the tropical species. These habitats had the highest levels of high frequency sound 800 – 15000 Hz compared with the other habitats, which is predominantly due to presence and abundance of resident soniferous marine organisms, such as the sea urchin and snapping shrimp. A possible explanation for this preferential response by the crabs to this habitat type and possibly high frequency sound is the fact that invertebrate fauna commonly live on complex rocky reef, coral reef or rocky shore structures and rely on this for survival. This habitat is also essential for all life stages of the species tested in the current study and their primary adult habitat (Wear & Fielder, 1985; McLay, 1988; Jones & Morgan, 2002), therefore, the presence and abundance of other invertebrate species provides a reliable indication of habitat type and quality. Also, fish vocalisations are known to be in the lower frequencies and fish pose a direct predatory threat to crab megalopae and therefore may not provide a reliable indicator of the suitability of the habitat (Bayoumi, 1970; Connaughton, 2004; Onuki & Somiya, 2004). Further investigation into the effects of sound level and the frequencies within underwater sound which trigger these responses should be carried out to gain more insight into the use of underwater sound as a settlement and orientation cue (explored in Chapter Five).

Existing evidence suggests that ambient underwater sound effects the distribution and orientation of settlement stage reef organisms (Budelmann, 1992a; Simpson et al., 2004; Leis & Lockett, 2005; Montgomery et al., 2006; Radford et al., 2007). The results of the current study provide evidence that crab megalopae can discriminate among habitat types by the acoustic underwater sound signature they emanate and actively select to settle and metamorphose at the preferred environment. The ability to remotely identify a suitable habitat in which to settle by using readily available acoustic cues would be a major advantage for settlement stage reef organisms at both close range and at some distance offshore.
Chapter Five: Behavioural response thresholds in New Zealand crab megalopae

5.1 INTRODUCTION

There is great variability in the audible sound frequency ranges among different fish species and their larvae (Chapman & Hawkins, 1973; Enger et al., 1993; Higgs et al., 2004; Egner & Mann, 2005; Fay, 2009; Wysocki et al., 2009). Studies into the hearing abilities of pre-settlement larvae of coral reef fish have been primarily to ascertain the potential of ambient underwater sound as an orientation cue (Kenyon, 1996; Kenyon et al., 1998; Leis et al., 2002; Wright et al., 2005; 2007; 2008; Wright et al., 2010). Many of these investigations have been performed using auditory brainstem response (ABR) and other electrophysiological methods which can be used to produce an audiogram, which presents the lowest level of sound that individuals of the species at different developmental stages can hear as a function of frequency (Kenyon, 1996; Kenyon et al., 1998; Wright et al., 2005; 2008; Wright et al., 2010). However, the minimum sound level that individuals of a species will show a behavioural response to (behavioural response threshold) may not be consistent with the auditory response threshold determined with electrophysiological methods (Kenyon et al., 1998). Therefore, a combination of electrophysiological and behavioural data is needed to determine the auditory abilities of the animal, and to completely understand the ecological and behavioural relevance of these abilities (Popper et al., 2001a; Montgomery et al., 2006).

Both electrophysiological and behavioural approaches have their advantages; electrophysiological tests have the advantage of not requiring behavioural training or housing animals under controlled experimental conditions, which in the case of larvae can be difficult (Wright et al., 2010). Behavioural tests of auditory thresholds are often more sensitive than electrophysiological methods by 10 – 30 dB (Gorga et al., 1988; Kenyon et al., 1998; Kojima et al., 2005) and electrophysiological methodologies vary among studies, sometimes making comparisons precarious (Higgs, 2002).

Compared to the abundance of knowledge concerning the visual, tactile and chemosensory systems in decapod crustaceans, the acoustic sensory systems remain relatively
unknown (Popper et al., 2001a). The majority of studies on hearing in decapod crustaceans have involved the adults of various species of lobster (Popper et al., 2001a). These have provided clear evidence that these animals use a variety of different receptors to detect water borne sound and vibrations (Cohen & Dijkgraaf, 1961; Budelmann, 1992a; Popper et al., 2001a). For example, the freshwater crayfish, *Orconectes limosus*, was found to have a threshold of about 0.1 µm peak-to-peak displacement of an isolated statocyst, with a broad maximum frequency from 150 to 2350 Hz (Breithaupt & Tautz, 1988). This earlier work has been supported by subsequent anatomical and physiological evidence, with the identification of the sensory structures and electrophysiological evidence of sound reception in the prawn, *Palaemon serratus* (Lovell et al., 2005). Many of these studies have involved investigation of the physiological responses of the various receptors through placing recording electrodes directly into the receptors or innervating neurons, while a much smaller number of studies have involved investigation into behavioural responses (Popper et al., 2001a). A set of distinct postural motor responses elicited by the Norway lobster, *Nephrops norvegicus* (L), when exposed to sound frequencies of 20 – 180 Hz have been characterised (Goodall et al., 1990). The authors then used these responses during free field experiments in a Scottish sea loch to establish the acoustic response threshold. *Nephrops norvegicus* were found to be sensitive to particle displacement rather than sound pressure with a response threshold of 0.888 µm, which was independent of frequency within the range 20 – 200 Hz (Goodall et al., 1990).

Ambient underwater sound has been long regarded as one of the most probable cues for guiding onshore orientation by pelagic larvae (Montgomery et al., 2006). Previous authors have claimed that the settling stages of coastal crab species respond to underwater sounds by using it to orientate their swimming toward the coast, and that as a consequence underwater sound could be of considerable ecological importance in influencing the settlement success of coastal crustaceans (Jeffs et al., 2003; Radford et al., 2007). In addition, this current study has found evidence that underwater sound from reefs can initiate settlement behaviour and reduce the time to metamorphosis in settlement stage crab larvae. However, there have been no investigations into the auditory thresholds or behavioural thresholds of the settlement stages of coastal crab species to levels of underwater sound. Therefore, the spatial scale at which underwater sound could be used as an effective behavioural cue and in turn the potential ecological significance of the observed behaviour remains uncertain.

By gaining behavioural response thresholds for the settlement and metamorphosis responses to underwater sound stimuli, it enables the estimation of the spatial scale at which
an acoustic settlement and metamorphosis cue could reliably work for the late-stage larvae of crabs. Previously studied settlement cues in crabs, such as chemical and tactile cues are thought to operate at multiple scales including molecular, microhabitat and ecosystem (Rittschof et al., 1998). At very fine spatial scales surface energy (polar dispersive forces as measured by wettability) is important as it can affect the effectiveness of adhesives used in attachments. Also it has been suggested that low frequency vibrations may be related to the permanence and thickness of a surface. Permanence defines whether the surface will last sufficiently long for growth, maturation and reproduction in an organism (Rittschof et al., 1998). These fine scale cues may provide information on the nature and stability of a settlement substrate for a settling organism. At intermediate spatial scales, odours from the habitat, conspecifics and prey species can affect settlement and metamorphosis, and can also result in the arrival of larvae to microhabitats (Harvey & Colasurdo, 1993; Forward et al., 1996; O’Connor & Gregg, 1998; Rodriguez & Epifanio, 2000; Forward et al., 2001). At larger spatial scales, the presence of waterborne odours can signal to a larva its arrival in environment or gross habitat type, such as an estuary (Rittschof et al., 1998). However, exact estimates of the spatial scales at which many settlement and metamorphosis cues operate has not been determined because many of the studies into detecting cues in crabs are tested in a laboratory setting in order to be able to control other experimental variables.

Therefore, the aim of the current research was to determine the settlement and metamorphosis behavioural response thresholds of four species of New Zealand crab megalopae by experimentally exposing them to different levels of broadcast reef sound recorded from their preferred settlement habitat. In addition, experiments were also conducted on two of these crab species to determine the effect on settlement and metamorphosis of exposure to varying levels of sound recorded from an unfavourable settlement habitat for these species. The aim of these additional experiments was to determine whether the observed settlement and metamorphosis response thresholds were specific to preferred habitat type.
5.2 METHODS

The study was undertaken during October 2010 to December 2010 in temperate waters near the Leigh Marine Laboratory in north-eastern New Zealand.

Figure 5.1: Maps showing recording sites for ambient underwater sounds that were used in laboratory behavioural assays: 1) North Reef – macroalgal dominated rocky reef habitat, 2) Pakiri Beach – open sandy beach habitat.

5.2.1 Source of megalopae

Light traps were used to capture pelagic megalopae for the behavioural threshold experiments (Hickford & Schiel, 1999; Meekan et al., 2001). Up to four light traps were deployed on dusk within 500 m of the shoreline, 7 – 30 m apart, dependant on the deployment location, and submerged 2 m from the surface in water of 5 – 10 m depth. The traps were recovered within 2 hours of sunrise the following morning. When large planktivorous fishes were found in a light trap, megalopae were not used in experiment as they may have altered behaviour due to stress from being in the presence of a predator (Forward & Rittschof, 2000).
The megalopae were transported in seawater to the nearby Leigh Marine Laboratory where they were counted, sorted into settlement stage and identified to lowest taxonomic level possible, given the available taxonomic descriptions (Wear & Fielder, 1985; McLay, 1988). Only intermoult pre-settlement (i.e., natant and active swimming) megalopae of a similar size and age were selected for use in the experiments. The megalopae were held in a flowing filtered (40 µm) seawater system with natural light period and ambient temperature (15 – 22°C, dependant on timing) until experiments began the following evening. Four species of temperate brachyuran megalopae were used. *Hemigrapsus sexdentatus*, *Cyclograpsus lavauxi* and *Leptograpsus variegatus* are all common coastal species of crabs that are from the family Grapsidae. The adults of these species are known to be associated with nearshore subtidal and intertidal habitats, under boulders, amongst macroalgae and on rocky shores (Wear & Fielder, 1985). *Austrohelice crassa* (formally *Helice crassa*) is also from the family Grapsidae, however, adults are known to be associated with enclosed beaches, sheltered harbours, lagoons, estuaries, and mangrove swamps (Wear & Fielder, 1985). Individuals of this species will usually construct a burrow in well drained, firm benthic sediment (McLay, 1988).

### 5.2.2 Sound recordings for threshold experiments

Recordings of the typical ambient underwater sound were made at two different habitat types (i.e., open sandy beach and a macroalgae dominated rocky reef) and used for the behavioural threshold experiments. Sound treatments were recorded from north-eastern New Zealand during the summer at dusk on a new moon; North Reef (36º 15’54.14” S, 174º 47’37.47” E) a macroalgae dominated rocky reef and Pakiri Beach (36º 13’33. 85” S, 174º 42’31. 96” E) an open sandy beach (Figure 5.1 – 1 and 2). *In situ* habitat sounds were recorded using a hydrophone hanging beneath a float to reduce extraneous noise associated with recording directly from a floating vessel. The recording system consisted of a calibrated HTI-96-MIN wideband omnidirectional hydrophone (High Tech, Inc., flat frequency response over the range of 10–24,000 Hz) that was weighed down vertically to 10 m water depth from the outside of a sealed floating barrel which contained a Sound Devices 722 solid state recorder (48 kHz; 24-bit). Several 5 min recordings were taken at 1700 – 1800 h (dusk) in approximately 15 – 20 m of water at each habitat site at about 20 m from the margin of the coastal fringing reef at the reef site and 100 m from the shoreline at the sandy beach site. No anthropogenic sources of noise, such as large ships or power boats, were present in the vicinity at the time of recording. All recordings were conducted in near calm conditions (<0.5
m wave height and <2.6 ms\(^{-1}\) wind speed) (Climate Station, Leigh Marine Laboratory).

Digital recordings from the recorder were transferred to a PC and analysed using MATLAB 2008 software with codes specifically written for the recordings to calculate sound levels and produce power spectra.

### 5.2.3 Laboratory-based threshold experiments

Each laboratory-based experiment consisted of five sound treatments (four distinct sound levels and one silent), and within each treatment there were three replicate water baths used to maintain a constant water temperature for megalopae throughout the experiment. The baths were acoustically isolated using rubber mats to prevent any transfer of acoustic energy from the surrounding environment into the experimental treatments. The absence of any significant acoustic signal in the Silent treatment tanks was confirmed by recording with a calibrated hydrophone (High Tech, In. HTI – 96 – MIN).

![Diagram](image)

**Figure 5.2**: Schematic diagram of a side view of one of the experimental replicates showing the layout of the water bath, speaker and settlement vials.

Each replicate water bath contained 5 – 10 plastic vials (250 ml) with a sealed lid housing a single randomly selected megalopa in filtered (1 µm) and UV treated seawater. The vials had a roughened base acting as a chemically inert settlement surface for settling megalopae. All replicates for both the sound treatments and Silent treatment had a weighted Philips loudspeaker (4 Ω, 5 watts) inside a watertight plastic bag which was submerged in the water bath (Figure 5.2). For the sound replicates only, a Sony CD Walkman D – EJ815 was
connected to the speaker and used to continually play a 4 min loop of recorded ambient underwater reef sound into the water bath and through the acoustically transparent plastic containers holding the crabs (Gerber, 1978).

When on a single night when sufficient (> 150) megalopae of the same species were collected from the light traps to conduct the experiments, they were randomly allocated to an experimental treatment and replicate. All megalopae in each treatment were kept under natural light period and ambient water temperature (15 – 22° C, depending on local ambient temperature) for the duration of the experiment. In the absence of an anechoic chamber, all laboratory-based experiments were conducted in a quiet concrete floored and walled laboratory.

The megalopae were added to the experiment at 1700 h on the day of their capture and the CD Walkman was switched on to initiate sound in the sound treatments. Subsequently every 6 h an observational period occurred, at which time counts were made of the number of megalopae that had settled onto the base of the vials and metamorphosed into the first instar benthic juvenile stage. The time from establishing the experiment to the first observational period when a megalopa was observed to have settled and completed metamorphosis was termed time to metamorphosis (TTM). Each period of observation lasted no more than 40 min for all treatments. When the observational period occurred at night, red light was used to observe behaviour because prior testing demonstrated there was little or no visual response by megalopae to the red lighting (Cronin, 1986). In the current study ‘settlement’ is defined as a behavioural process which involves movement out of the water column to a benthic substrate, and ‘metamorphosis’ as a physiological process which includes loss of larval characteristics retained in the megalopa and the completion of the moult to the adult reptant body form (Hadfield et al., 2001). A behavioural threshold was determined by the lowest sound level for which TTM was significantly lower than the TTM for the Silent treatment.

The experiment was terminated when all experimental megalopae in all treatments had metamorphosed. The settled juvenile crabs were kept for 5 – 10 d following the experiment in flowing seawater, fed and monitored for post-experimental mortality.

### 5.2.3.1 Tank set-up for North Reef and Pakiri Beach experiments

A calibrated hydrophone and recorder (High Tech, Inc., Mississippi, USA HTI – 96 – MIN, Sound Devices, Wisconsin, USA 722 recorder) was used to adjust the sound level produced by the Philips speakers in each experimental sound treatment tank. Separate
experiments were run for the two different habitat sounds, to determine the sound level at which crab larvae demonstrated reduced TTM compared to the Silent treatment, i.e., the behavioural threshold. For the experiments using recorded sound from North Reef the following sound level treatments were used; 135 dB re 1µPa (High), 126 dB re 1µPa (Ambient level – as determined from field recording), 100 dB re 1µPa (Low), 90 dB re 1µPa (Lowest) RMS level in the 100 – 24000 Hz range and Silent treatment (no replayed sound).

For the experiments using recorded sound from Pakiri Beach the following sound level treatments were used; 125 dB re 1µPa (High), 103 dB re 1µPa (Ambient level – as determined from field recording), 90 dB re 1µPa (Low) RMS level in the 100 - 24000 Hz range and Silent (no replayed sound). There was also an additional treatment included in this experiment; 126 dB re 1µPa (Ambient Reef sound– as determined from field recordings at North Reef). This extra sound treatment was included to provide a direct comparison for a sound cue from a preferred settlement habitat, i.e., reef habitat. It was not appropriate to make direct comparisons of median TTM values between the North Reef and Pakiri Beach experiments because the experiments were conducted with different sets of wild-caught larvae that could be at different stages of development. The replayed sounds in the experimental tanks were recorded with a calibrated hydrophone (High Tech, Inc., HTI – 96 – MIN) for comparison with the source signals recorded from the natural habitats and the spectral composition analysed using MATLAB software with codes specifically written for these recordings.

The digital recordings of the typical ambient underwater sound in both habitat types were either left at ambient level (Ambient treatment), amplified (High treatment) or faded (Low and Lowest treatments) to reach the level set for each treatment using Adobe Audition software (Adobe Systems, Inc.).

5.2.4 Data analyses

For the experiments for each species, non-parametric statistical methods were used to test for a difference in median TTM within the replicates for each sound treatment because the data were not continuous (Zar, 1999). Kruskal-Wallis comparison of ranks was used to test for a difference in the median TTMs among the replicates within the same treatment (i.e., each treatment analysed separately). If this test found no difference among the three replicates the data from the replicates were pooled for each treatment and then used in an experiment-wide comparison of treatments using the Kruskal-Wallis test to compare the median TTMs. For all statistical tests, P values ≤ 0.05 were considered to be significant. To isolate
differences among individual treatments a Dunn’s pairwise multiple comparison procedure was used to test for differences among each treatment combination because not all sample sizes were equal. A metamorphosis rate for each treatment within each species was also calculated with a Sen’s slope analysis for the data points between the last sampling event prior to the first megalopa metamorphosing and the sampling event when the last megalopa metamorphosed. A one way analysis of variance (ANOVA) was used to test for a difference in metamorphosis rate among treatments using rates calculated for each replicate within treatments. To isolate differences among treatments a Tukey’s test was used, this tests for differences among every treatment combination. All analyses were performed using the software Sigma Stat 4.0 (Systat Software, Inc.) and Minitab 16.1.0 (Minitab, Pty.).

5.2.5 Estimates of transmission range of acoustic settlement cue

The observed threshold levels seen in the different crabs species tested were used in conjunction with theoretical acoustic transmission loss models (spherical and cylindrical spreading from a point source) to estimate at what distance from the source (settlement habitat) the acoustic cue would be detectable by megalopae given the measured behavioural response thresholds (Urick, 1983). For the recordings taken at 20 m from the reef an additional 13 dB was added to match the estimated source level at the reef calculated based on cylindrical spreading from the reef source (pers. comm. C. Radford). For the purposes of comparison it was assumed that megalopae would show a behavioural response to the sound once they were sufficiently close to the sources habitat that the ambient sound level was the same as the threshold level (TL) for the crab. This assumption leads to the following equations for spherical and cylindrical spreading from measured level (ML) that were then used to calculate the range (R) at which the megalopae were theoretically likely to be able to respond to the underwater reef sound. Attenuation was not accounted for in the model as underwater sounds below 10 kHz lose less than 1 dB km\(^{-1}\) due to absorption by the medium (Mann, 2006).

\[
\begin{align*}
\text{Spherical spreading} & : & \text{ML} - A + 13 &= 20\log (R) \\
\text{Cylindrical spreading} & : & \text{ML} - A + 13 &= 10\log (R)
\end{align*}
\]
5.3 RESULTS

5.3.1 Sound analysis for North Reef experiments

The broadcast sound within the experimental tanks had a similar overall spectral composition to the source signals recorded from the natural habitat. In the original field recordings (North Reef) there was a peak in the spectra around 700 – 1200 Hz, which is produced by the feeding of the sea urchin, *Evechinus chloroticus*, whereas the higher frequency pulses were the snaps of snapping shrimp (Figure 5.3). The power spectra of the experimental tanks showed that the frequency composition of the replayed tank was reasonably consistent with the original field recording, with a small reduction in sound level in the higher frequencies (10000 – 24000 Hz) for the Ambient and High treatments (Figure 5.3). The Silent treatment had no sound transfer from any external sources. The flat response at approximately 35 dB re 1 µPa is the lower recording limit of the recording equipment (Figure 5.3 e).

5.3.2 Laboratory-based threshold experiments in North Reef experiments

In all four crab species that were tested there was no significant difference in the median TTM among the replicates within each of the five sound treatments (*P* > 0.05) (Table 5.1). Therefore, for each species the TTM data for the replicates were pooled for each treatment to then test for an overall treatment effect.

Median TTM differed significantly among the sound treatments for the megalopae of all three rocky reef species tested (Kruskal-Wallis test *P* < 0.05, Table 5.2 & Figure 5.4a, b and c) with High and Ambient sound treatments consistently producing the shortest TTM. Using pairwise multiple comparisons, *Hemigrapsus sexdentatus* had the most separation among the sound treatments, with significant differences in median TTM identified between six of the ten pairs of treatment comparisons (Figure 5.5a). *Cyclograpsus lavauxi* had significant differences in median TTM between eight of the ten treatment comparisons (Figure 5.5b). *Leptograpsus variegatus* had significant differences in median TTM between four of the ten treatment comparisons (Figure 5.5c). Among all three of these species the Silent treatment consistently had the longest median TTM and Ambient sound treatment consistently had the shortest median TTM (Figure 5.5). *Leptograpsus variegatus* possessed the lowest behavioural response threshold of 90 dB, followed by *C. lavauxi* with a threshold of 100 dB, and lastly *H. sexdentatus* with 126 dB re 1 µPa. Median TTM did not differ significantly among the
treatments of different sound levels of unfavourable settlement habitat (North Reef) for the tunnelling mud crab, *Austrohelice crassa* (H = 6.131, P = 0.177).

Figure 5.3: Spectral composition and sound level of underwater sound when recorded at North Reef in north-eastern New Zealand and when replayed in the North Reef experimental treatment tanks. a) High – 135 dB re 1µPa, b) Ambient – 126 dB re 1µPa, c) Low – 100 dB re 1µPa, d) Lowest – 90 dB re 1µPa RMS level in the 100 – 24000 Hz range. Blue lines represent natural sound recorded and either amplified or faded and Black lines represent replayed sounds in experimental sound treatments. e) Silent treatment.
Table 5.1: Statistical comparisons of median TTMs among the replicates within each treatment in North Reef experiments for four crab species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment (sound level dB)</th>
<th>H - statistic</th>
<th>P – value among replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemigrapsus sexdentatus</strong></td>
<td>High (135)</td>
<td>0.75</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Ambient (126)</td>
<td>0.43</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Low (100)</td>
<td>0.81</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Lowest (90)</td>
<td>0.61</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.55</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Cyclograpsus lavauxi</strong></td>
<td>High (135)</td>
<td>0.18</td>
<td>3.34</td>
</tr>
<tr>
<td></td>
<td>Ambient (126)</td>
<td>0.19</td>
<td>3.33</td>
</tr>
<tr>
<td></td>
<td>Low (100)</td>
<td>0.68</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Lowest (90)</td>
<td>0.90</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.69</td>
<td>0.74</td>
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<tr>
<td><strong>Leptograpsus variegatus</strong></td>
<td>High (135)</td>
<td>0.97</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Ambient (126)</td>
<td>0.40</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>Low (100)</td>
<td>0.44</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Lowest (90)</td>
<td>0.69</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.93</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Austrohelice crassa</strong></td>
<td>High (135)</td>
<td>0.40</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>Ambient (126)</td>
<td>0.80</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Low (100)</td>
<td>0.45</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>Lowest (90)</td>
<td>0.47</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.73</td>
<td>0.62</td>
</tr>
</tbody>
</table>

### 5.3.3 Rates of metamorphosis in North Reef experiments

In the North Reef experiments two of the rocky reef species, *H. sexdentatus* and *C. lavauxi* both had significantly higher metamorphosis rates in the High and Ambient treatments than the Low, Lowest and Silent treatments (ANOVA, F = 5.8 & 11.2 respectively, *P* < 0.05, Tukey’s test *P* > 0.05, Table 5.2). The metamorphosis rate in *Cyclograpsus lavauxi* was 1.6 faster in the High treatment than in the Silent treatment (Table 5.2). *Leptograpsus variegatus and A. crassa* did not have increasing metamorphosis rates with increasing sound level (Table 5.2).
### Table 5.2: Comparisons among median TTM s and metamorphosis rates for the North Reef experiments in four crab species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total number of individuals (n)</th>
<th>Treatment (sound level dB)</th>
<th>Median TTM (h)</th>
<th>H – statistic</th>
<th>P – value</th>
<th>Metamorphosis rate</th>
<th>F - statistic</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemigrapsus sexdentatus</strong></td>
<td>24</td>
<td>High (135)</td>
<td>48</td>
<td></td>
<td></td>
<td>9.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Ambient (126)</td>
<td>48</td>
<td></td>
<td></td>
<td>10.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Low (100)</td>
<td>75</td>
<td>53.9</td>
<td>***&lt; 0.001</td>
<td>8.3</td>
<td>5.8</td>
<td>***0.002</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Lowest (90)</td>
<td>78</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Silent</td>
<td>84</td>
<td></td>
<td></td>
<td>5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cyclograpsus lavauxii</strong></td>
<td>30</td>
<td>High (135)</td>
<td>68</td>
<td></td>
<td></td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Ambient (126)</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Low (100)</td>
<td>84</td>
<td>25.8</td>
<td>***&lt; 0.001</td>
<td>3.7</td>
<td>11.2</td>
<td>***0.001</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Lowest (90)</td>
<td>108</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>Silent</td>
<td>114</td>
<td></td>
<td></td>
<td>3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Leptograpsus variegatus</strong></td>
<td>15</td>
<td>High (135)</td>
<td>96</td>
<td></td>
<td></td>
<td>4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Ambient (126)</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Low (100)</td>
<td>108</td>
<td>23.8</td>
<td>***&lt; 0.001</td>
<td>4.3</td>
<td>0.5</td>
<td>0.742</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Lowest (90)</td>
<td>108</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Silent</td>
<td>150</td>
<td></td>
<td></td>
<td>3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Austrohelice crassa</strong></td>
<td>30</td>
<td>High (135)</td>
<td>57</td>
<td></td>
<td></td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Ambient (126)</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Low (100)</td>
<td>73</td>
<td>6.1</td>
<td>0.177</td>
<td>6.9</td>
<td>0.6</td>
<td>0.813</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Lowest (90)</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Silent</td>
<td>71</td>
<td></td>
<td></td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

***Asterisk indicates a significant difference in median TTM s between treatments (P < 0.05, Kruskal-Wallis test) and significant difference in metamorphosis rate (P < 0.05, ANOVA).
Figure 5.4: Percentage of total number of megalopae metamorphosed over time (h) in experiments replaying North Reef sound at various sound levels. a) *Hemigrapsus sexdentatus*, b) *Cyclograpsus lavauxi*, c) *Leptograpsus variegatus*, and d) *Austrohelice crassa*.
Figure 5.5: Treatment groupings according to similarity of median TTM in North Reef experiments. Treatments linked by a horizontal line do not differ in median TTM from other treatments along the same line. Separate lines indicate significant differences ($P < 0.05$, Dunn’s test). *Austrohelice crassa* was not included as the test returned an insignificant result.
5.3.4 Estimates of transmission range of acoustic settlement cue

Using the measured sound levels recorded from North Reef during summer, at dusk, over a new moon, it was estimated that megalopae of *L. variegatus* could be expected to show a settlement behavioural response from the reef out to a distance of 199 m assuming spherical spreading, and out to 39811 m from the reef assuming cylindrical spreading (Table 5.3). This distance was considerably shorter for *H. sexdentatus* as it had a much higher behavioural response threshold, out to 5 m and 20 m assuming spherical and cylindrical spreading respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Threshold level</th>
<th>Cylindrical</th>
<th>Spherical</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leptograpsus variegatus</em></td>
<td>90 dB</td>
<td>39811</td>
<td>199</td>
</tr>
<tr>
<td><em>Cyclograpsus lavauxi</em></td>
<td>100 dB</td>
<td>7943</td>
<td>89</td>
</tr>
<tr>
<td><em>Hemigrapsus sexdentatus</em></td>
<td>126 dB</td>
<td>20</td>
<td>5</td>
</tr>
</tbody>
</table>

5.3.5 Sound analysis for Pakiri Beach experiments

In the field recordings at Pakiri Beach the low frequencies in the range of 100 – 800 Hz were dominant, which is mostly likely due to abiotic noise sources (i.e., wind and waves). There were also low levels of higher frequency sound present, probably derived from distant reefs (Figure 5.6a, b, c). The power spectra from the experimental playback tanks showed that the sound had a similar overall spectral composition to the original field recordings except for slightly reduced levels in the lower frequencies (100 – 300 Hz) for the Pakiri Beach High (125 dB re 1 µPa), Ambient (103 dB re 1 µPa) and Low (90 dB re 1 µPa) treatments. This reduction in sound level in the lower frequencies is due to some limitations of the frequency reproduction capabilities of the speakers used in the experiments. However, the composition
of the higher frequencies (301 – 20000 Hz) remained fairly constant with that of the field recording, but with some slight variations due to the effects of replaying sound in small tanks (Figure 5.6). In the Ambient Reef treatment there was a peak in the spectra around 700 – 1200 Hz, and higher frequency pulses from 200 – 10000 Hz (Figure 5.6d).

The Silent treatment had no sound transfer from any external sources. The flat response at approximately 34 dB represents the lower recording limit of the sound recording equipment (Figure 5.6e).

5.3.6 Laboratory-based threshold experiments in Pakiri Beach experiments

In both crab species that were tested, *H. sexdentatus* and *L. variegatus*, there was no significant difference in the median TTM among the replicates within each of the five sound treatments (*P* > 0.05) (Table 5.4). Therefore, for each species the TTM data for the replicates were pooled within each treatment to then test for an overall treatment effect.

Median TTM differed significantly among the sound treatments for the megalopa of both species tested (Kruskal-Wallis test, *P* < 0.05, Table 5.5 & Figure 5.7) with the Ambient Reef sound treatment consistently producing the shortest TTM when compared with the Pakiri Beach sound treatments at three sound levels and a Silent treatment. Using a Dunn’s pairwise multiple comparisons there was shown to be no significant difference in median TTM among the three Pakiri Beach sound level treatments (High 125 dB, Ambient 103 dB and Low 90 dB re 1 µPa) and the Silent treatment for both *H. sexdentatus* and *L. variegatus* (*P* > 0.05) (Figure 5.8). However, there was a significant difference between each Pakiri Beach sound level and the Ambient Reef sound treatment.
Figure 5.6: Spectral composition and sound level of underwater sound when recorded at Pakiri Beach in north-eastern New Zealand and when replayed in Pakiri Beach experimental treatment tanks. a) High – 125 dB re 1µPa, b) Ambient – 103 dB re 1µPa, c) Low – 90 dB re 1µPa, d) Ambient Reef sound – 126 dB re 1µPa RMS level in the 100 – 24000 Hz range. Black lines represent natural sound recorded and either amplified or faded and blue lines represent replayed sounds in experimental sound treatments. e) Silent treatment.
5.3.7 Rates of metamorphosis in Pakiri Beach experiments

In the Pakiri Beach experiments both species (*H. sexdentatus* and *L. variegatus*) had a significantly faster metamorphosis rate in the Ambient Reef sound treatment when compared to all of the other sound treatments (Pakiri Beach High, Ambient, Low and Silent) (ANOVA, F = 32.3 & 38.3 respectively, $P < 0.05$, Tukey’s test $P < 0.05$, Table 5.5). Metamorphosis rates were 1.6 times faster for *H. sexdentatus* and 1.7 times faster for *L. variegatus* in the Ambient Reef sound treatment than for the other treatments.

---

**Table 5.4: Statistical comparisons of median TTM among the replicates within each treatment in Pakiri Beach experiments for two crab species.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>H - statistic</th>
<th>$P$ – value among replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hemigrapsus sexdentatus</em></td>
<td>High (125)</td>
<td>3.46</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Ambient (103)</td>
<td>1.83</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Low (90)</td>
<td>1.39</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>0.92</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Ambient Reef (126)</td>
<td>1.04</td>
<td>0.60</td>
</tr>
<tr>
<td><em>Leptograpsus variegatus</em></td>
<td>High (125)</td>
<td>2.51</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Ambient (103)</td>
<td>1.36</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Low (90)</td>
<td>1.30</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Silent</td>
<td>1.56</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>Ambient Reef (126)</td>
<td>4.17</td>
<td>0.12</td>
</tr>
</tbody>
</table>
Table 5.5: Statistical comparisons among median TTMs and metamorphosis rates in Pakiri Beach experiments for four crab species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total number of individuals (n)</th>
<th>Treatment (sound level dB)</th>
<th>Median TTM (h)</th>
<th>H – statistic</th>
<th>P – value</th>
<th>Metamorphosis rate</th>
<th>F - value</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hemigrapsus sexdentatus</em></td>
<td>15</td>
<td>High (125)</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Ambient (103)</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Low (90)</td>
<td>54</td>
<td>26.8</td>
<td>***&lt;0.001</td>
<td></td>
<td>32.9</td>
<td>***&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Silent</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Ambient Reef (126)</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Leptograpsus variegatus</em></td>
<td>15</td>
<td>High (125)</td>
<td>84</td>
<td></td>
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<tr>
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<td>15</td>
<td>Ambient (103)</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Low (90)</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>84</td>
<td>12.8</td>
<td>***0.012</td>
<td></td>
<td>38.3</td>
<td>***&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Ambient Reef (126)</td>
<td>66</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

***Asterisk indicates a significant difference in TTMs among treatments (P < 0.05, Kruskal-Wallis test) and significant difference in metamorphosis rate (P < 0.05, ANOVA).
Figure 5.7: Percentage of total number of megalopae metamorphosed over time (h) in Pakiri Beach experiments. a) *Hemigrapsus sexdentatus*, b) *Leptograpsus variegatus*.
### a) *Hemigrapsus sexdentatus*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Silent</th>
<th>Low</th>
<th>Ambient</th>
<th>High</th>
<th>Ambient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>90 dB</td>
<td>103 dB</td>
<td>125 dB</td>
<td>Reef 126 dB</td>
</tr>
</tbody>
</table>

### b) *Leptograpsus variegatus*

<table>
<thead>
<tr>
<th>Treatment (dB)</th>
<th>Silent</th>
<th>Low</th>
<th>Ambient</th>
<th>High</th>
<th>Ambient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reef</td>
</tr>
</tbody>
</table>

Figure 5.8: Treatment groupings according to similarity of median TTM in Pakiri Beach experiments. Treatments linked by a horizontal line do not differ in median TTM from other treatments along the same line. Separate lines indicate significant differences ($P < 0.05$, Dunn’s test).
5.4 DISCUSSION

Previously, the studies on auditory capabilities and behavioural response thresholds in marine animals have been focused on fishes and mammals (Higgs et al., 2004; Egner & Mann, 2005; Houser & Finneran, 2006; Horodysky et al., 2008; Fay, 2009; Mulsow & Reichmuth, 2010). There are only a handful of investigations on the hearing abilities and behavioural response thresholds of larval fishes and no published results that specifically examine the behavioural response thresholds of crustacean larvae (Kenyon, 1996; Wright et al., 2005; 2008). However, there are a small number of studies which have demonstrated that settlement stages of coastal crabs show an attraction and orientation response to underwater reef sound, although the ecological importance or spatial scale over which these behaviours operate have not been identified (Jeffs et al., 2003; Radford et al., 2007).

Previous studies investigating the auditory capabilities of crustaceans have focused on electrophysiological methods (Breithaupt & Tautz, 1988; 1990; Popper et al., 2001a; Lovell et al., 2005). For example, a study by Lovell et al. (2005) described both the anatomy of the sensory structures of the statocyst while also providing electrophysiological evidence of sound reception in the adult prawn, *P. serratus*. The statocyst was shown to be sensitive to the motion of water particles displaced by low frequency sounds ranging from 100 – 3000 Hz (Lovell et al., 2005). However, some previous behavioural measurements of hearing ability in fishes have shown experimental animals to be more sensitive than observed using the ABR methods, although the results of the two experimental approached are not always consistent (Kenyon et al., 1998; Higgs, 2002).

5.4.1 Behavioural response threshold levels to North Reef habitat recordings

The experiments replaying North Reef sound to three reef-dwelling crab species, found that *H. sexdentatus* exhibited the highest acoustic response threshold (lowest sensitivity) to underwater sound; there was a significant reduction in TTM in sound treatments with sound levels of 125 dB re 1 µPa and above when compared with the Silent treatment. *Leptograpsus variegatus* showed the lowest acoustic response threshold (highest sensitivity) to underwater sound, and there was a significant reduction in time to metamorphosis (TTM) in treatments 90 dB re 1 µPa and above (100, 126 and 135 dB) when compared with the Silent treatment. It is possible that the behavioural response threshold in this species could be lower than that measured as there were no intermediate sound level treatments set between the Lowest sound
treatment (90 dB) and the Silent treatment. A greater range of experimental treatment sound levels would provide better resolution in determining behavioural thresholds in future studies.

Once the response threshold had been met (showing a significant reduction in TTM compared to the Silent treatment), *C. lavauxi* showed a graded response with decreasing TTM to sound levels exceeding this threshold level. This suggests that proximity to the sound source, or settlement habitat, is important in inducing a faster settlement and metamorphosis. For example, *C. lavauxi* showed the greatest reduction in TTM in the 126 dB and 135 dB sound treatments (60 and 68 h respectively), an intermediate response in the 100 dB sound treatment (84 h) and no response in the 90 dB sound treatment (108 h) when compared to the Silent treatment (114 h). The graded response could be reflecting the speed at which metamorphosis should occur at a certain distance from a settlement habitat, it would be detrimental for the larvae to detect a settlement cue and metamorphose at an accelerated rate thereby completing metamorphosis before it reached its desired settlement destination. The results also suggest that underwater sound as a settlement and metamorphosis cue does not simply trigger a behavioural response but is more likely to moderate the behavioural and physiological process by continuous exposure.

The identification of these acoustic behavioural thresholds also help to predict the spatial scale at which these acoustic settlement and metamorphosis cues are operating. For example, when considering the thresholds of all species tested, it would appear that the settlement cues have the potential to elicit a behavioural response at some distance from the settlement habitat. *Leptograpsus variegatus* exhibited the lowest response threshold (90 dB) to replayed North Reef sound which his equated to an estimated maximum settlement response distance of approximately 199 km and 39.8 km assuming cylindrical spreading and spherical spreading of sound from the reef source, respectively (Table 5.3). These distances were substantially greater that for *H. sexdentatus*, however, the large sound level (25 dB) gap between treatments may fail to detect the exact threshold between these, therefore, potentially reducing the range over which the settlement response is likely to be elicited by the sound.

If the acoustic source (the reef) is assumed to be a point source it is possible to calculate the loss of reef noise using a theoretical model of either spherical or cylindrical spreading. In shallow water, where sound can be reflected off the surface and bottom, the spreading may approach cylindrical, where sound cannot propagate uniformly, in all directions. Intensity is decreasing linearly with distance to the sound source. However, in deep water spreading will approach spherical, this ignores reflection of sound at surface and at the bottom, the sound
intensity generated by the source is propagated uniformly in all directions, in a boundless medium (Urick, 1983). In many shallow water environments the amount of spreading loss will usually be somewhere between the spherical and cylindrical spreading cases (Urick, 1983). These detection estimates are conservative as a reef is not necessarily a point source but more a linearly extended area with multiple random point sources and field measurements clearly showed a zone surrounding the reef where there is little loss of sound (Radford, pers. comm.). This highlights the limits of using sound propagation models and the need to collect thorough experimental data rather than relying on simplified models.

The findings of the current study indicate that underwater sound as a settlement and metamorphosis cue may be species specific. For example, the tunnelling mud crab, *Austrohelice crassa*, showed no response to any sound levels of replayed reef sound, whereas all the reef associated species that were tested showed a significant decrease in TTM once their behavioural threshold had been reached. This demonstrates that *A. crassa* is not sensitive to the reef acoustic cue. As the reef is not an essential habitat during any part of the life cycle of *A. crassa* (Wear & Fielder, 1985; McLay, 1988) it would be detrimental for this crab to be responding to a settlement cue from a habitat that it would not live in. However, it is possible that this species of crab may respond to an acoustic cue produced at an estuarine habitat because as an adult it typically inhabits burrows dug in soft sediments on the fringes of estuaries or mangrove swamps (Wear & Fielder, 1985). It is also possible that this species does not exhibit a settlement response to acoustic cues.

The differences in response thresholds seen among the reef associated species may be due to species specific requirements. For example, *L. variegatus* is thought to primarily prey upon sessile organisms or slow moving macro-invertebrates, whereas both *H. sexdentatus* and *C. lavauxi* are both known to eat a large variety of seaweed as their predominant food source (McLay, 1988). It is possible that specific acoustic, chemical or other cues associated with more subtle differences in settlement habitat, such as an algal chemical cue, may further mediate the settlement response with some species.

### 5.4.2 Behavioural response threshold levels to Pakiri Beach habitat recordings

The Pakiri Beach experiments revealed that sound level alone does not explain the settlement and metamorphosis response observed in settlement stage crab larvae exposed to ambient underwater reef sound. Both *H. sexdentatus* and *L. variegatus* showed no significant response to varying levels of open sandy beach sound, even when the sound level was at a
similar level (less 1 dB) to the ambient sound at their preferred settlement habitat, rocky reef. There was no significant reduction in TTM in any species tested in the treatments broadcasting Pakiri Beach recordings at 90 dB, 103 dB, 125 dB re 1 µPa, or a Silent treatment, while there was a significant reduction in TTM in the Ambient Reef sound treatment. These results demonstrated that it is the frequency and temporal composition of underwater sound rather than the sound level per se that is an important characteristic for the mediation of settlement and metamorphosis in settlement stage crab larvae.

5.4.3 Conclusions

The current study has determined the settlement and metamorphosis behavioural response thresholds to levels of underwater reef sound in a number of species of New Zealand crab larvae. It also provides further evidence of settlement stage crabs discriminating among suitable settlement habitats on the basis of the sound it produces. The results also confirm that the frequency and temporal composition of underwater sound rather than the sound level are the important characteristics for an acoustic settlement cue. The behavioural response thresholds to ambient underwater reef sound determined during the current study enable estimation of the spatial range that the settlement and metamorphosis cue could be operating, which potentially extends to many kilometres in the species with low behavioural threshold (high sensitivity) to preferred habitat, i.e., *L. variegatus*. However, the settlement stage larvae of some crab species showed a graded response with increasing reductions in TTM with increasing sound level, which would equate with increasing proximity to the target settlement reef, i.e., *C. lavauxi*. Overall, these results greatly extend the knowledge and ecological context of sound acting as a settlement and metamorphosis cue to the late-stage larvae of coastal crab species. Future work should focus on gaining greater resolution of the behavioural response threshold sound levels of these species and also use electrophysiological methods to produce an audiogram on hearing capabilities in a number of species so we can better define the spatial scale over which this important behavioural response operates, and its relative importance in ensuring the successful settlement and recruitment of important coastal crab species.

During the current study the potential ranges of the acoustic cue used in settlement and metamorphosis in larval crabs was estimated. The current study identified behavioural response thresholds in the nearfield, and these thresholds were then used to estimate potential detection distances in the farfield. However, any differential abilities by megalopae to detect
particle motion versus the pressure component of underwater sound will influence the veracity of these detection distance estimates. Therefore, there is a need to determine whether crab larvae are responding to the particle velocity, the pressure component of underwater sound or both, as at this point in time it is not known.
Chapter Six: General Discussion

6.1 OVERVIEW

The research presented in this thesis examined aspects of the role that ambient underwater sound plays for late-stage marine larvae, especially crab megalopae, attempting to find a suitable settlement habitat on the coast. The research addressed four key aims to better understand the acoustic environment, the settlement responses to reef sound and the behavioural response thresholds of nine species of commonly occurring crab megalopae. The four aims were: 1) Investigate the effect ambient underwater sound has on settlement stage crab larvae in terms of settlement and metamorphosis; 2) Investigate the differences in the acoustic character of three distinct habitat types in north-eastern New Zealand; 3) Determine the potential of settlement stage crab larvae to discriminate among different settlement habitats based on differences in habitat-specific underwater sound alone; and 4) Determine the behavioural response thresholds in a number of New Zealand crab species to different underwater habitat sounds.

6.2 AMBIENT UNDERWATER SOUND AS A NOVEL SETTLEMENT AND METAMORPHOSIS CUE

The pelagic larval phase in the lifecycle of many coastal organisms typically ends with settlement, where the larvae will select a suitable habitat in which to settle (O'Connor & Gregg, 1998). The location and the selection of settlement sites by larvae is frequently modulated by settlement cues, a variety of which have been identified in many different species (Forward et al., 2001; Montgomery et al., 2001; Lecchini, 2004; 2005; Lecchini et al., 2005). A number of laboratory- and field-based studies have identified specific physical and chemical cues that shorten or lengthen the time to metamorphosis (TTM) in the megalopae (late-stage larvae) of crabs (O'Connor, 1991; Harvey & Colasurdo, 1993; Lim, 1997; O'Connor & Gregg, 1998; Gebauer et al., 2002; 2004). The settlement and metamorphosis cues that have been identified have most often been chemical cues originating from settlement
or adult habitats, (e.g., estuarine water, aquatic vegetation and biofilms) (Forward et al., 2001). For example, the TTM of the megalopae of the Zuiderzee crab, *Rhithropanopeus harrisii*, was significantly shorter when exposed to estuarine water than when exposed to offshore water (Fitzgerald et al., 1998). Underwater sound is now recognised as being perhaps the strongest candidate for use as a long distance orientation cue for larval stages of reef fish and decapod crustacean (Simpson et al., 2005a; Montgomery et al., 2006). Many authors have demonstrated that both larval fishes and decapods will actively swim towards reef sound, both naturally occurring sound and natural sound that is artificially replayed in the field (Tolimieri et al., 2000; Jeffs et al., 2003; Leis et al., 2003; Simpson et al., 2004; Tolimieri et al., 2004; Jeffs et al., 2005; Leis & Lockett, 2005; Simpson et al., 2005a; Radford et al., 2007; Simpson et al., 2008a; Simpson et al., 2008b). However, the role ambient underwater sound may play as a settlement and metamorphosis cue has not been previously examined. Research presented in Chapter two of this thesis provided the first evidence that underwater sound consistently and significantly shortened the TTM in five species of crab megalopae by up to 60% in temperate species and 52% in tropical species. Between the two tropical species used in the experiment, *Grapsidae* sp. two had the largest difference in median TTM between the two treatments, with 69 h in the Sound treatment and 114 h in the Silent treatment. Among the three temperate species, *Cyclograpsus lavauxi*, had the largest difference in median TTM between the two treatments, with 30 h in the Sound treatment and 75 h in the Silent treatment. For all reef associated crab species that were tested, settlement behaviour occurred substantially earlier and median TTM was significantly shorter in megalopae exposed to ambient levels of reef sound compared to a Silent treatment (Chapter Two).

When comparing the reduction in TTM seen in the current research to that of other proven settlement cues some caution is necessary. Due to limited space and the facilities needed for rearing larvae in the laboratory megalopae in the current study were collected directly from the plankton using light traps and were carefully visually segregated by species and by moult stage for experimentation. A number of previous studies investigating settlement and metamorphosis cue have been carried out on megalopae which were reared in the laboratory, as a result specific ages (e.g., time of moult to the megalopal stage) are known. Therefore, direct comparisons on the reduction in TTM with studies using laboratory raised megalopae are not advised due to unknown differences in age of the megalopae; instead, relative percentage reductions can be compared. During the current research ambient reef sound decreased the median TTM in megalopae by between 21 to 60% in the nine crab species that were examined. This appears to be consistent, with a greater upper limit, with the
reduction of TTM observed using other known settlement cues using both laboratory reared and plankton caught crab megalopae (Forward et al., 2001). However, in many of these previous studies the experiments have been carried out in the laboratory and have almost certainly not have controlled for the possible effect of sound on settlement and metamorphosis (Forward et al., 1996; Weber & Epifanio, 1996; O'Connor & Gregg, 1998; Rodriguez & Epifanio, 2000; Forward et al., 2003; O'Connor, 2005). This sound can come from many sources in the lab such as noisy laboratory machinery (e.g. fume hood), electrical noise, air conditioning units or ceiling fans, or even oxygen supply to water in the housing tanks. In the current study all extraneous noise was controlled for.

Chemical cues associated with settlement and metamorphose are very diverse, and can be supplied from a multitude of sources (e.g., odour of conspecifics, aquatic vegetation, biofilms, humic acids, adult substrates, related crab species, and potential prey) (Forward et al., 2001). Some chemical cues have been found to be species specific, such as particular chemicals leached from certain macroalgae species induce settlement in the blue crab, Callinectes sapidus (Forward et al., 1996). However, other chemical cues, such as chemicals associated with conspecific adults, consistently produce a settlement response in most species of crab that have been tested (Forward et al., 1996; Welch et al., 1997; Gebauer et al., 1998; Forward et al., 2003; Gebauer et al., 2004).

The results of the current study demonstrated consistent responses to ambient underwater reef sound, with a reduction in TTM of all temperate and tropical reef species of megalopae that were tested. The results greatly extended the role that ambient underwater sound plays in the settlement processes in late-stage crab larvae given that sound has not previously been associated with triggering the behavioural and physiological changes involved in settlement and metamorphosis. The findings of the research presented in Chapter Two of this thesis led to the formation of the aims in Chapter Three and Chapter Four respectively, i.e., whether there is spectral and/or temporal difference in ambient underwater sound originating from different habitat types and whether settlement stage crab larvae can discriminate and respond to the spectral difference in underwater sound associated with habitat type.


6.3 **UNIQUE ACOUSTIC SIGNATURES**

Due to the dispersive ability of many coastal marine organisms, settlement stage larvae are often faced with a choice among multiple settlement habitats. Ambient underwater sound may be a useful mechanism for identifying an appropriate settlement site for these larval settlers. However, a major barrier in determining the ecological role of ambient underwater sound in the settlement stage of marine larvae is the lack of understanding of the spatial variation of underwater sound in shallow coastal environments, i.e., “the soundscape” (Simpson *et al.*, 2008b). As a result, one of the main aims of the present research was to investigate differences in overall character (intensity level, spectral composition, and temporal variation) of the ambient underwater sound generated at distinct habitat types over the spatial scale of larval settlers (kilometres) on the coast of north-eastern New Zealand (Chapter Three).

The results of this research revealed marked differences in the acoustic characteristics of three distinct coastal habitat types: macroalgae dominated rocky reef, urchin dominated rocky reef, and open sandy beach. The reef habitat sites (macroalgae dominated rock reef and urchin dominated rocky reef) produced sound that was significantly more intense in the frequency bands dominated by biotic sounds (800 – 2500 and 2500 – 20000 Hz) compared to the beach habitat sites, where the abiotic frequency band (100 – 799 Hz) (e.g., wind and wave noise) dominated (Radford *et al.*, 2008b; 2008a). This suggests that habitat type greatly affects overall character (frequency composition, spectral level and temporal variation) of the ambient underwater sound being emitted. Also, many of the differences in the sound produced by the reef habitats became more evident at dusk compared with noon. There was an increase in biological sound in the dusk recordings compared with the noon recordings for the reef habitat types, especially the urchin dominated rocky reefs. The 800 – 2500 Hz frequency band caused most of the variation among the habitats at dusk. Sound in this frequency band is attributed to the feeding behaviour of the grazing sea urchin (*Evechinus chloroticus*) which cause the hemispherical tests of these organisms to resonate at frequencies within this frequency band depending on their size (Radford *et al.*, 2008a). This increase in sound intensity at certain times will extend the range of transmission of the signal from the reef. Interestingly, these periods of highest sound intensity are concurrent with temporal variation in larval settlement of many species in both temperate and tropical reef systems, with greatest settlement generally occurring around the early evening on a new moon during the summer (Victor, 1991; Hobbs & Botsford, 1992; Tricklebank *et al.*, 1992; Caselle & Warner, 1996;
Kingsford et al., 2002; Lozano & Zapata, 2003; McIlwain, 2003). These results, along with the results from Chapter Two, highlight that differences in underwater sound may play a significant role in the recruitment processes by providing a reliable indicator of habitat type and quality to settlement stage larvae capable of using ambient underwater sound as a settlement cue. An acoustic cue that communicates information on habitat type and quality and direction to habitat would be of enormous value to late-stage larval marine organisms attempting to remotely identify suitable settlement habitats.

Diurnal and/or circatidal rhythms have been observed in metamorphosis, swimming activity and vertical migration in the late-stage larvae of a range of crab species (Forward et al., 2001). Diurnal rhythms have been well described and are believed to be related to predator avoidance during the day, and feeding activities during the night (Hobbs & Botsford, 1992; Fernandez et al., 1993; Young, 1995). The time of metamorphosis is thought to be influenced by both rhythms, although, there has been very little consistency among species studied thus far (Zeng et al., 1997; Garrison, 1999; Zeng et al., 1999; Forward et al., 2001). The Dungeness crab, *Metacarcinus magister*, (formally known as *Cancer magister*) possesses a diurnal metamorphosis rhythm in which most of the megalopae will moult at night time (Fernandez et al., 1994). This diurnal metamorphosis is thought to reduce the risk from visual predators such as fishes which are active during the day (Fernandez et al., 1994). In contrast, the predation argument is not supported by studies with *C. sapidus* (blue crab) megalopae which metamorphose during the day (Forward et al., 1996). This timing of metamorphosis is consistent with observations of transport into estuaries, as megalopae actively enter the water column during a flood time during the night but are absent during the day because they are thought to have moved onto or near the seafloor (DeVries et al., 1994a; Tankersley et al., 1995). This pattern of behaviour is thought to occur as a result of light inhibition of swimming during the day, thus during the day megalopae are not active in the water column (Forward & Rittschof, 1994).

Metamorphosis occurs when swimming activity is minimal, as it is virtually impossible to maintain muscle contractions during this time when the new carapace is forming (Forward et al., 1996). During the research presented in Chapter Two and Chapter Four the megalopae of *C. lavauxi*, *Hemigrapsus sexdentatus* and *C. andreossyi* were showing higher amounts of settlement behaviour (downward swimming to the substrate, exploratory crawling and grasping) and subsequent metamorphosis during the evening hours than in the daylight hours, particularly in the field-based experiments. *Schizophrys aspera* and *Grapsus tenuicrustatus*
showed less of a preference for evening metamorphosis, however, there was still a larger proportion of metamorphosis in the evening hours compared to day-time hours (Chapter Four). When considering the diurnal variation seen in ambient reef sound the evening preference for settlement and metamorphosis in these species could also be attributed to the utilisation of an optimal acoustic window. During dusk, acoustic cues are at their greatest in terms of transmission range and when biological contribution to the acoustic cue is at its highest, providing the greatest amount of information on the source habitat (Tait, 1962; Cato, 1978; 1992; Radford et al., 2008b). At this time the megalopae may be receptive to the acoustic cues and metamorphose in the subsequent hours. However, as the replayed sound cue in the laboratory-based experiments were continuous, it remains unclear if the larvae are physiologically prepared to respond to a settlement cue (competent to metamorphose), and when, and for how long, do the larvae need to be exposed to the acoustic cue (in this case underwater sound) for it to induce settlement. This question warrants further research.

There are very few published experiments specifically designed to determine the earliest moment in megalopae development (metamorphic competence) and the length of time of contact with a cue is required to initiate a behavioural response in larvae (Gebauer et al., 2003). In most studies contact with the settlement cue occurs at the beginning of the megalopae stage, following the moult from the prior larval instar (Gebauer et al., 2003). The suitable moment and length of time during which the larva is in contact with the cue have been demonstrated for the crab species Sesarma curacaoence and Chasmagnathus granulate, where they were experimentally placed into contact with the cue (conspecific adult odour) for different periods of time and at different times in their development. The megalopae of S. curacaoence had to come into contact with the chemical cue (conspecific adult odours) when approximately 65% of their moult cycle had elapsed and the cue had to be present for about one day (Gebauer, unpublished data). In C. granulate, the exposure event had to occur at approximately 32 – 53% of the megalopae development and for about five days (Gebauer et al., 2003). Any later in development or shorter contact with the cue resulted in no effect on the timing of metamorphosis for both species. To further understand the operation of sound as a settlement and metamorphosis cue, additional research into the window of receptivity should be pursued to determine whether it is necessary to have continuous exposure or if a narrower window exists and at which part during the megalopae phase the larvae are receptive to the cue.
6.4 DISTINGUISHING AMONG SETTLEMENT HABITATS

The larvae of some species of brachyuran crabs remain in nearshore environments, while others are transported offshore until they make the return journey as megalopae and then settle and metamorphose in a suitable habitat where they live as adults (Paula et al., 2001; Daly & Konar, 2008). Crab larvae were once thought to disperse with the currents with little to no control over their route until they fortuitously encountered suitable settlement habitat (Frank et al., 1993). This concept has changed, however, with the knowledge that late-stage crab larvae have impressive swimming abilities, sufficient to overcome ambient currents (Luckenbach & Orth, 1992; Shanks, 1995; Valero et al., 1999). The well-developed swimming and sensory abilities of crab megalopae enable them to detect multiple stimuli (e.g., chemicals, visual, water movement, underwater sound, temperature, salinity, pressure, rugosity, gravity and polarized light), and respond to these stimuli by significantly altering their spatial distribution and therefore potentially their settlement habitat (Kingsford et al., 2002).

Several field studies have reported that the late-stage larvae of coastal crabs are attracted to artificial sources of coastal reef sound. For example, the late-stage larvae of five species of coastal crabs showed an orientation response inside an in situ choice chamber towards a sound source playing underwater reef sound (Radford et al., 2007). Also the zoea and megalopae of both Brachyuran and Anomuran crabs were found in significantly higher numbers from samples taken from light traps deployed in conjunction with reef sound than those without sound (Jeffs et al., 2003). A study on the Great Barrier Reef in Australia provided contrasting results, here there was no significant differences in the number of brachyuran megalopae caught depending on the sound treatment, however, brachyuran zoea were caught in significantly higher numbers in the traps playing reef noise than the silent control traps (Simpson et al., 2011). The apparent impartial behaviour towards reef noise in the megalopal stages could be explained by the settlement response induced by the reef noise (Simpson et al., 2011). Similar to the responses seen in the current study (Chapter Two, Four and Five), megalopae could have been responding to the broadcast reef sound with downward swimming to the substrate, moving away from the traps with the reef sound playback. These previous studies proposed that the late-stage larvae of many coastal crab species may use underwater sound to orientate to the coast and could be of considerable ecological importance in influencing the settlement success of coastal crustaceans. If late-stage crab larvae are capable of using ambient underwater sound in long distance orientation, as concluded in the
studies above, it would be logical to consider the use of underwater sound in fine scale selection of a suitable settlement habitat, as a settlement and metamorphosis cue, which is proven in Chapter Four of this thesis.

Settlement stage crabs appear to be using the significant variation in the ambient underwater sound along the coast (Chapter Three) to discriminate among habitat types in locating and settling to an optimal settlement site. Research presented in Chapter Four of this thesis demonstrated the ability of late-stage crab larvae to discriminate among suitable settlement habitats based on their acoustic signatures. Five species of coastal brachyuran crab larvae, from both temperate and tropical waters, had significantly shorter median TTM when exposed to underwater sound typical of the habitat types utilised by their benthic stage compared with less favourable habitat types. In the temperate species the macroalgae dominated rocky reef induced the greatest increase in speed of settlement in both laboratory- and field-based experiments, followed by the sandy/broken shell harbour. *Cyclograpsus lavauxi* had the strongest response in both the laboratory- and field-based experiments, showing a 44% decrease in median TTM in the laboratory-based experiments and a 52% decrease in median TTM in the field-based experiments when comparing the most favourable habitat type to the least favourable habitat type. In the tropical species the frontal coral reef sound caused the strongest response in both laboratory- and field-based experiments, followed by the sound from an isolated coral back reef. *Grapsus tenuicrustatus* had the strongest response in both the laboratory- and field-based experiments, showing a 47% decrease and a 32% decrease in median TTM respectively, when comparing the most favourable habitat type to the least favourable habitat type. These results are to be expected when taking into consideration the association of adults of these crabs to these specific habitats. *Cyclograpsus lavauxi*, the smooth shore crab, inhabits the intertidal area, under stones and on boulder beaches as adults.

The distribution of this species in New Zealand ranges from open, wave exposed coasts to sheltered harbours. *Grapsus tenuicrustatus* or the tropical rock crab occurs in the tropical intertidal region from low- to high-tide mark, on coral and rocky reefs sheltering in crevices (Jones & Morgan, 2002). This species of crab uses the reef complexity for protection and sourcing prey. The underwater sound emanating from the preferred habitats of this crab species has the highest proportion of total sound intensity in the mid to high frequencies (800 – 20000 Hz), which indicates the presence of sound producing organisms, such as snapping shrimp and sea urchins. The presence of these organisms provides a reliable indicator of the
habitat type and quality because invertebrate fauna commonly settle and live on complex reef structures (Hedvall \textit{et al.}, 1998).

If an ecological sound signal is to be used for remotely locating a suitable habitat for settlement, it would be logical to expect that the sensitivity of the organism receiving the signal would be tuned to its specific needs. It could be detrimental to receive and respond to a signal that does not reliably provide an ecological benefit. Once the organism has received and interpreted the signal, it could then use other available sources of information to further qualify any decision to exhibit a behavioural response to the signal. The discovery that the dominant biological sound sources produced at temperate reef habitats are produced by the sea urchin (\textit{E. chloroticus}) and snapping shrimp (\textit{Alpheus} and \textit{Synalpheus} spp.) has significant ecological implications, especially if the sound of these organism are in the acoustically attractive frequency bands for settlement stage marine larvae (Radford \textit{et al.}, 2008b; 2008a).

For example, trophic cascades can severely affect the urchin populations within an area (Paine, 1980; Polis \textit{et al.}, 2000), which in turn could affect the intensity and spectral content of the ambient noise emanating from the habitat. This could reduce the detection of the affected area by late-stage larval settlers, thereby reducing the numbers of settlers attracted to, and settling in that area.

The larval stages of reef organisms are known to not only be able to detect and use reef sound but also to be able to discriminate potential biological significance and respond to different underwater sounds (Leis \textit{et al.}, 2002; Tolimieri \textit{et al.}, 2002; Jeffs \textit{et al.}, 2003; Leis & Lockett, 2005; Simpson \textit{et al.}, 2005a; Radford \textit{et al.}, 2007; Simpson \textit{et al.}, 2008a; Simpson \textit{et al.}, 2008b). The larvae of the black-axil damsel reef fish, \textit{Chromis atripectoralis}, has been shown to demonstrate differences in swimming speed and directional movements when presented with either broadcast natural reef sound or artificial pure tones (Leis \textit{et al.}, 2002). Several families of settlement stage larval reef fishes have been shown to be able to discriminate differences in underwater sound that indicate potential differences in habitat (Simpson \textit{et al.}, 2008b). Simpson \textit{et al.}, (2008) conducted an experiment by broadcasting natural reefs sounds filtered into high frequency (570 – 2000 Hz) and low frequency (< 570 Hz) in combination with light traps and compared these catches with those from silent traps. From the seven families of fish represented in these catches (with > 10 individuals from each), four families were caught in significantly higher numbers in the high frequency traps than in either the low frequency or silent traps. The results presented in the current study were consistent whether the experiment was field- or laboratory- based and in temperate or tropical
waters. It demonstrating a working and novel behavioural assay that can provide an effective tool for gaining a better understanding of the behavioural responses of late-stage crab larvae to many aspects of underwater sound.

6.5 **RATES OF METAMORPHOSIS**

Receiving and responding to available settlement cues suggests an ecological advantage for the settler, because delayed metamorphosis has been shown to have high costs in the form of a reduction in postmetamorphic fitness (Forward *et al.*, 2001). Survival and body size of the first juvenile instars of *Chasmagnathus granulate* were significantly reduced when a delay in metamorphosis occurred in the previous megalopal phase (Gebauer *et al.*, 1999). Some invertebrates can delay metamorphosis for months in the absence of sufficient settlement cues (Pechenik, 1990), however, the late-stage larvae of brachyuran crabs lack this ability and in all species studied to date, metamorphosis occurs within 20 days (Forward *et al.*, 2001). Therefore, brachyuran megalopae appear to possess a temporal threshold beyond which metamorphosis is initiated even in the absence of a suitable habitat to settle to (Weber & Epifanio, 1996). From the results in the current study (Chapter Two and Four) it would appear there is a longer temporal threshold in the tropical species (Grapsidae sp. one, Grapsidae sp. two, *Cymo andreossyi*) compared to the temperate species. Alternatively, sound could be a more important cue for the induction of metamorphosis in these tropical species. There was a 78 h and a 84 h difference in time to first metamorphosis of Grapsidae sp. one and Grapsidae sp. two respectively, this was a much longer delay when compared to the New Zealand species (30, 12 and 24 h difference) (Chapter Two). However, once metamorphosis began, it was fairly rapid, with a higher rate of metamorphosis in the Silent control than the Sound treatment for *H. sexdentatus*, Grapsidae sp. one and Grapsidae sp. two.

The variability among metamorphosis rates seen among treatments, species and between experimental methods could be attributed to species specific differences in temporal thresholds and the ability to delay metamorphosis (Gebauer *et al.*, 2003). There has been little investigation into the consequences of delayed metamorphosis in decapod crustaceans and no consistent patterns have been found (Hunt & Scheibling, 1997). The most significant cost identified thus far has been in the form of reduced growth rates of first instar juveniles, which increases the chance of mortality due to a reduction in size (Gebauer *et al.*, 2003). Juveniles originating from megalopae with delayed metamorphosis are seen to be more vulnerable to benthic predation, such as cannibalism by conspecifics (Gebauer *et al.*, 2003), which has been
previously observed in settlement experiments where megalopae are held in group containers (Forward et al., 2001, pers. obs.). Post-settlement predation is considered an important causal mechanism in regulating population densities and structure in benthic decapods (Fernandez et al., 1993; Lovrich & Sainte-Marie, 1997).

There was no consistent pattern in metamorphosis rates among the treatments in all seven crab species tested (Chapter Two and Four). However, metamorphosis rates were more similar among treatments during the laboratory-based experiments than they were in the field-based experiments in Chapter Four. The variability among metamorphosis rates could also be related to the absence of other settlement cues that would occur in the natural situation and are known to be important in many species. Larvae may use a hierarchy of sensory cues, and different kinds of stimuli may be used at more than one spatial scale (Kingsford et al., 2002). In the natural situation it is possible that there is a synergistic effect of sound cues with other potential settlement cues (visual, chemosensory and physical) and during the experiments, especially laboratory-based, these cues were actively excluded. Together they may act to produce even more rapid settlement and metamorphosis than either cue acting in isolation. This would be of great ecological value as it would suggest faster settlement and subsequent metamorphosis into habitats once encountered. For example, a synergistic effect was seen in the megalopae of C. granulata, the presence of conspecific adults in combination with a muddy substrate (typical adult sediment type) was found to induce metamorphosis faster than each stimulus in isolation (Gebauer et al., 2003). Similar results have been reported for the megalopae of Uca pugilator (O’Connor, 1991). The suggested explanation for this result was that an accumulation of secreted substances by the adults onto the substratum produced a higher concentration of the cue and subsequently a greater effect of the cue on metamorphosis (Gebauer et al., 2003).

6.6 BEHAVIOURAL THRESHOLDS

Little is known in regards to the details of the auditory abilities of late-stage larval crabs, however, crustaceans have the highest capabilities in both sound production and sound reception among all marine invertebrates (Budelmann, 1992a). Excluding insects, crustaceans appear to not only produce and receive sound but also interact via acoustic signals. Several authors, including the current study, have demonstrated the ability of crustaceans to receive and respond to acoustic signals (Jeffs et al., 2003; Radford et al., 2007; Simpson et al., 2011).
Chapter Five of this thesis was aimed at investigating the behavioural response thresholds in a number of species of New Zealand crab megalopae and to also investigate the effects of sound pressure level, irrespective of frequency composition on settlement and metamorphosis. The results demonstrated that *Leptograpsus variegatus* exhibited the lowest behavioural response threshold (highest sensitivity) to underwater reef sound, with a significant reduction in TTM when exposed to replayed reef sound with an average of 90 dB re 1 µPa and greater, and containing reef sound in the frequency range 100 – 24000 Hz. *Hemigrapsus lavauxi* exhibited the highest behavioural response threshold (lowest sensitivity) to reef sound, with a significant reduction in TTM when exposed to reef sound with an average of 126 dB re 1 µPa and above. One estuarine species was examined during the current research as a control, *Austrohelice crassa*, the tunnelling mud crab. *Austrohelice crassa* exhibited no response to any of the underwater reef sound levels, however, the lack of response in this species to reef sound may be due to this species settling in different habitats because as an adult it typically inhabits burrows dug in soft sediments on the fringes of estuaries or mangrove swamps (Wear & Fielder, 1985). These results suggest that underwater reef sound as a settlement and metamorphosis cue is species specific (e.g., reef associated species), and highlights the importance of further investigation into the settlement responses of non-reef dwelling species to underwater reef sound. However, it is possible that this species may respond to an acoustic cue created at their optimal settlement habitat, an estuary.

During the research in Chapter Four of this thesis it was reported that the Whangateau Estuary has a distinct acoustic output compared to that of a rocky reef (Waterfall Reef). The estuary had the highest sound levels in the frequency band 2500 – 15000 Hz, while the highest sound levels at the rocky reef habitat occurred in the 700 – 1200 Hz frequency band. These differences are due to habitat related differences in the presence and abundance of soniferous organisms. For example, the sea urchin *E. chloroticus* is responsible for a high proportion of the sound in the 700 – 1200 Hz frequency band and is a dominant figure on the rocky reef, but is absent in the Whangateau Estuary. These results indicate that there may be species specific behavioural thresholds whereby below a certain sound level the signal may no longer function as a settlement cue. The differences in observed thresholds could be due to the sound level reaching the lower end of the auditory capabilities of the larvae or the signal is past a point of being a reliable indicator of an optimal settlement habitat and is therefore ignored.
Also, as underwater sound travels great distances it allows long range transmission of information underwater (Rodgers & Cox, 1988), potentially triggering settlement and metamorphosis in individual great distances from a suitable settlement habitat. However, the graded settlement response to sound level observed in Chapter Five suggests that proximity to the sound source (habitat) is important in inducing faster settlement and metamorphosis. These results suggest a continuous or repeated cue is needed in order to continue to decrease TTM and to continuously monitor location of the source (settlement site). If the cue merely triggering a mechanism, similar to those seen in other metamorphosis cues (Forward et al., 2001), then the larva may be advected away from the settlement site while still going through the process of settlement and metamorphosis.

The identification of these behavioural thresholds may also help to estimate the spatial scale at which these acoustic settlement and metamorphosis cues are operating. This threshold level can be used in conjunction with reef propagation models (Urick, 1983) to determine at what distance this acoustic settlement cue could operate. For example, in *L. variegatus*, TTM significantly increased once the acoustic signal fell below 90 dB and was estimated to theoretically be able to detect the ambient levels of reef noise emitted from North Reef (northeastern New Zealand) at 39811 m and 199 m assuming cylindrical and spherical spreading respectively. Species of New Zealand crab larvae have been observed to possess maximum sustained swimming speeds (MSSS) of 2.06 to 10.96 cm s\(^{-1}\) and for all species examined (*Hemigrapsus* sp., *Austrohelice crassa*, *Macrophthalmus hirtipes*, *Cyclograpsus* sp., *Ovalipes catharus*) MSSS exceeded trial current velocities for at least three hours of each tidal cycle (up to 25 cm s\(^{-1}\)) (Meder, unpublished data). It was also observed that two species were able to swim for a maximum of 36 hours, with one species covering a distance of almost 7 km (Meder, unpublished data). These sustained swimming abilities along with the estimated acoustic detection distances for certain species of crab suggest that an acoustic reef derived settlement cue can be operating at some distance from the source.

The importance of sound level was also investigated in the current study (Chapter Five) as it could be questioned whether it was the frequency composition of ambient reef sound or the elevated sound level producing the settlement response observed in the crab megalopae (Chapter Two and Four). Here, the megalopae of *H. sexdentatus* and *L. variegatus* exposed to an ambient reef sound control (most favourable habitat type) (126 dB re 1 µPa average) showed a reduction in TTM by at least 44% and 26% respectively when compared to the sound level treatments (124 dB, 99 dB, 90 dB re 1 µPa average) of an open sandy beach.
(unfavourable habitat type). These results indicate that in reef associated crab species it is the frequency composition of temporal variation within the acoustic signal rather than sheer sound level of the signal that the megalopae respond to with settlement behaviour and metamorphosis.

Many questions still remain concerning the reception mechanisms, auditory range (sound level and frequency range) and attractive frequencies of sound to the settlement stages of larval crabs. Pilot scale experiments have indicated a preferential settlement and metamorphosis response to the ‘high’ frequency (>799 – 24000 Hz) component of filtered underwater reef sound when compared to the ‘low’ frequency (<799 Hz) and a Silent control in the megalopae of *H. sexdentatus*, *C. lavauxi* and *M. hirtipes* (Stanley, unpublished data). These questions need to be further addressed to more clearly understand the ecological importance of sound in the settlement and recruitment processes, and to determine what components of underwater sound are responsible for inducing the behavioural responses in crab megalopae

6.7 **ANTHROPOGENIC NOISE IMPACTS**

The current research has shown that natural reef sound has a significant influence on the settlement and metamorphosis of late-stage larval crabs. Therefore, it would seem likely that anthropogenic underwater noise, especially of a continuous nature, has the potential to interfere with critical settlement and recruitment processes of crabs using ambient underwater to locate and settle into suitable habitats. In addition to natural biotic and abiotic sounds in the ocean there is also human generated noise from shipping traffic, oil and gas exploration and coastal construction etc. Due to the demand of renewable energy sources there has also been an increase in the construction of offshore wind farms and there are plans for them in nearshore areas in the near future (Nedwell & Howell, 2004; Madsen *et al.*, 2006). Research on existing wind farms and associated activities have shown that the operation of these have added to the ambient background noise in frequencies up to 2000 Hz and peak pressures of >200 dB re 1μPa at 1 m (Nedwell & Howell, 2004; Thomsen *et al.*, 2006). Anthropogenic noise in coastal environments is occurring at biologically relevant frequencies seen in the current study which have the potential to act as false orientation and settlement cues (Wahlberg & Westerberg, 2005). Also, some of these sources of anthropogenic noise have large peak pressure levels that may mask natural levels of ambient underwater reef sound used as a settlement cue, increase the auditory threshold or even damage sound reception
structures (Cato, 1992; Scholik & Hong, 2001; McCauley et al., 2003; Popper et al., 2004; Radford et al., 2008b).

Auditory masking occurs when biologically irrelevant sounds prevent an animal from hearing biologically relevant sound (Popper et al., 2003). A possible example of this was the observation of the capture of more than 5000 specimens of the post-larval stage (puerulus) of the spiny lobster, Jasus edwardsi, in a sea water intake of the New Plymouth power station in New Zealand compared with zero catches on collectors set along the coast nearby (Booth, 1989). A possible explanation for this aggregation was that the larvae were responding to the low frequency vibrational cues associated with the power station and using it as an orientation and settlement cue, as the other biological relevant sounds within the area were being ignored. Future research needs to determine the role of anthropogenic noise in interfering with the critical settlement and recruitment processes of settlement stage marine larvae.

6.8 CONCLUSIONS AND FUTURE DIRECTIONS

The current study was a detailed investigation into the responses of late-stage larval crabs, from both temperate and tropical regions, to ambient underwater sound. Collectively, the research results presented in this thesis shows that the ambient underwater sound originating from coastal habitats mediates the settlement and metamorphosis behaviour of the megalopae of many common coastal crab species.

Ambient underwater sound has been shown to have significant differences in frequency composition, intensity level and diurnal outputs among habitat types, and this forms the basis of a remote settlement cue which provides information on the habitat type, quality and suitability of its source location to receivers, such a crab megalopae, with the ability to detect and interpret the signal. Ambient underwater reef sounds were shown to significantly decrease TTM in several crab species when compared to other habitat types (e.g., estuary, sand beach or lagoon). The differences in the sound produced at different habitats were shown to induce marked differences in settlement and metamorphosis behaviour of the megalopae, depending on the crab species and the habitat sound source. For all species, the megalopae exposed to reef habitat sound treatments (macroalgae dominated rocky reef and fringing coral reef) exhibited the shortest times to metamorphosis, indicating a positive response to an optimal settlement habitat. Together these results have important ecological implications in the settlement processes of late-stage larval organisms which are attempting to find suitable
settlement habitat. The search, and subsequent selection, of suitable settlement habitat by crab megalopae will ultimately affect their survival and reproductive success. The consistent behavioural responses seen in nine brachyuran species from temperate and tropical waters suggest the phenomenon has the potential to be widespread geographically and among reef species.

These results also raise the possibility that anthropogenic noise could interfere with recruitment processes by disrupting or masking these important natural acoustic settlement cues and then leading to premature or reduced settlement.

The findings of the research presented in this thesis only focus on the specific responses seen in reef associated crab species, with the exception of one estuarine species as a control. The experiments were specifically designed to examine the effects of reef associated species to the possible acoustic environment they would encounter when preparing for settlement. It is likely that there is a different set of responses seen in estuary associated or pelagic species, or among other invertebrate taxa to natural acoustic cues, such as the soft shore species *A. crassa* observed during the current study. There have been a small number of examples of other invertebrates demonstrating behavioural responses towards sound. For example, the free-living larvae of a species of tropical coral were attracted to ambient reef sounds in an *in situ* choice chamber (Vermeij *et al.*, 2010). In the presence of low frequency sound waves (30 Hz) less than 1% of very young (0 days old) cyprids of the barnacle *Balanus amphitrite* settled (Branscomb & Rittschof, 1984). It also substantially reduced the percentage of settled in cyprids up to 13 days old. These results suggest the potential of low frequency sound waves as a means of inhibiting barnacle settlement.

Most recently it was demonstrated that the pelagic taxa Copepoda and Hyperiidae and the nocturnally emergent taxa Caridea, Gammaridea, Mysidae and Ostracoda actively avoided broadcast reef sound, with statistically higher numbers of the taxa found in a silent control trap to the trap playing back reef sound (Simpson *et al.*, 2011). This suggests there is a far greater range of invertebrate taxa, living in a range of habitat types, capable of responding to acoustic cues. It also provided the first experimental evidence in any marine organisms, that taxa found in the vicinity of reef, but do not settle to, actively avoid reef noise. These taxa are said to benefit from this behaviour as reefs are inhabited by a range of predators that feed there day and night (Simpson *et al.*, 2011). These results further highlight the role of ambient underwater sound as an important source of information for settlement and orientation for the early stages and adults in both invertebrates and vertebrates. While also highlighting the
possible uses in commercial application, for example, reducing settlement in biofouling species.

The research results presented in this thesis has greatly extended our previously limited knowledge of the influence that ambient underwater reef sound has on the settlement and metamorphosis of late-stage larval crabs. However, there are remaining questions and potential problems that highlight the need to pursue further research into many areas including:

- The role of acoustic settlement cues for estuarine or pelagic crab species.
- The potential to use sound as a deterrent for settlement in commercially important species, e.g., biofoulers, or to attract species of interest to settle on depleted reefs.
- Determine whether the settlement of other reef associated organisms, such as coral and fishes, is expedited by acoustic cues in the same manner as has been identified for crab megalopae.
- Determine whether there is a window of receptivity for acoustic settlement and metamorphosis cues in crab megalopae.
- Identify if there is a synergistic effect between acoustic and other settlement and metamorphosis cues, such as chemical cues.
- Determine auditory thresholds for coastal species of crab megalopae using auditory evoked potential (AEP) methods and compare these to behavioural threshold data.
- Examine whether crab megalopae have an ability to determine directionality of acoustic cues.
- Investigate the effect of anthropogenic noise on the settlement responses of late-stage larvae of crabs and other coastal organisms which show a response to natural acoustic cues.

In conducting the current research there were also several major difficulties encountered in working with underwater sound and settlement stage larvae in the field and in the laboratory. Specimen collection was a major obstacle during the current study, when
collection larvae in light traps larval numbers and species available varied substantially throughout the year, month and with varying weather conditions. The species used in the current study reflected what was caught in numbers great enough to run the experiments, additional species were caught but were released as not enough were captured. A way of dealing with this problem would be to rear larvae in the laboratory (Forward et al., 2001). This would also negate any problems with identification and sorting into settlement stage as the adult species and date of hatching would be known.

When running laboratory-based behavioural assays, sound frequency and level were difficult to control in small tanks due to the effects of reverberation and resonance. There has been a protocol created to minimise these distortions while continuing to used laboratory tanks (Akamatsu et al., 2002). The future use of some of these suggested approaches may help to overcome some of the practical difficulties encountered in undertaking this current study.
List of References


References


References


Thomsen, F., Ludemann, K., Kafemann, R., Piper, W. 2006. Effects of offshore wind farm noise on marine mammals and fish, biola, Hamberg, Germany on behalf of COWRIE Ltd


