Fish and Ships:

Impacts of Boat Noise on the Singing Fish, *Porichthys notatus*

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Abstract

As anthropogenic ocean noise rises, research into its impacts on marine life is intensifying. Recent studies show concerning effects of noise on a variety of taxa, including fish. However currently lacking are *in situ* studies: the majority of fish studies have been lab-based, which lack the natural conditions and interconnections that put results in context. Further, the dearth of baseline information on natural fish sounds, communication and behaviours, limits predictions of noise impacts. Here I investigated the highly vocal plainfin midshipman (*Porichthys notatus*) in its natural habitat to determine the effects of boat noise on wild fish. *Porichthys notatus* uses sound to communicate during courtship and aggression, and depends on paternal care to safeguard nests in intertidal zones over several months. I first described acoustic communication features of *P. notatus in situ* by quantifying its vocalizations from longterm audio recordings gathered via hydrophones near a nesting site. I then characterized behaviours associated with acoustic signals by analyzing audio and video data of nest-guarding *P. notatus*. Finally, I determined the response of *P. notatus* to live motor-boat noise by examining behavioural and vocal activity of *P. notatus* in boat noise, ambient and control conditions. In addition to the manual analysis, I used an automated approach to determine overall movement of *P. notatus* under boat noise, ambient and control conditions. Findings reveal that when exposed to boat noise, fewer *P. notatus* predators were documented in the vicinity of *P. notatus* nests, while *P. notatus* increased overall time spent moving inside nests. Thus, noise benefits *P. notatus* indirectly by decreasing predator pressure, yet has direct negative impacts on *P. notatus* by increasing stress and metabolic costs. Such results reveal fitness consequences at both species and ecosystem scales, and indicate the importance of accounting for ecological relationships when predicting noise effects.

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Declaration

All data included in this thesis were collected by me, and all interpretation and writing is my own, with the exceptions listed below.

Parts of Chapter 1 are included in the following book chapter: Cullis-Suzuki, S. (2015). Singing fish in an ocean of noise: effects of boat noise on the Plainfin midshipman (*Porichthys notatus*) in a natural ecosystem. In: Popper AN, Hawkins AD, editors. *The Effects of Noise on Aquatic Life II*. Springer Science+Business Media, New York. In press.

In Chapter 2, the TOAD was built by Glen Dennison of TRIUMF, University of British Columbia, to suit my design specifications. Technical details in the Materials and methods section under 'TOAD' were written by Glen before being edited by me. This chapter has been accepted with revisions by the journal Ecology and Evolution.

Chapter 4 received input and advice on statistics from Rob Williams of Oceans Initiative (all statistical figures are my own). Figure 5 was created in Matlab by Stan Dosso of the University of Victoria, BC, using my data.

Chapter 5 represents a collaboration with Nancy Wang and Alexandra Branzan Albu of the University of Victoria, BC. Nancy Wang created the algorithm and produced the results. Alexandra Branzan Albu provided direction and guidance in the creation of the algorithm, and obtained funding for Nancy Wang. Appendices 5.1 and 5.2 are the output of their work. The data were collected and provided by me. The manual analysis upon which the algorithm was based was completed by me, as was the interpretation of results, written text, and Appendix 5.3.

This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

Chapter 1: Introduction

1.1 THE RISE OF OCEAN NOISE

Contrary to popular belief, beneath the surface, the ocean is not a silent world (Johnson, Everest, & Young, 1947; Popper & Hastings, 2009b). Natural sources of noise include the crashing of coastal waves, geologic activity, rainfall, icebergs cracking, moving ice, marine mammal calls, fish scraping, and the snapping of invertebrates like shrimp (Johnson, Everest, & Young, 1947; National Research Council, 2003; Weilgart, 2007; Hildebrand, 2009; Andrew, Howe, & Mercer, 2011; Boyd *et al.*, 2011). Indeed before commercial whaling exterminated the vast majority of great whales, it is thought that the oceans were much 'louder' than today, as whales produce some of the loudest sounds made by any animal (Stocker & Reuterdahl, 2012).

While natural sources of noise have always been present, nowadays the oceans are full of new noise. 'Anthropogenic noise' includes sounds associated with industrial activity such as oil exploration and drilling, pile driving, wind farms, gear related to fishing or aquaculture, sonar, explosives, and shipping (Hildebrand, 2009). Also known as ‘acoustic pollution’ (Weilgart, 2007), anthropogenic noise is estimated to have increased both in background levels and in peak intensities (Hildebrand, 2009). In particular, low frequency noise has risen, having swelled by about 20 dB since the mid-1900s (for comparison, the difference between a small outboard engine at 20 knots and an air-gun array is about 100 dB; Hildebrand, 2009; see Box 1.1 for definitions of intensity and frequency). This rise in ocean noise follows the expansion of shipping, today a global phenomenon: over ninety percent of international commerce is now carried by ship (Hastings, 2008). Noise resulting from traffic is indeed so globally widespread it is likely the largest source of anthropogenic noise both on land (Barber, Crooks, & Fristrup, 2010) and in the ocean (Hastings, 2008; Brumm, 2010), and is expected to increase further as population and trade grow (Brumm, 2010; Boyd *et al.*, 2011).

1.2 EFFECTS OF NOISE ON MARINE LIFE

Many forms of marine life – from cetaceans to crustaceans to coral – depend on sound to communicate, find prey, orient themselves, and understand the aquatic world around them (Southall *et al.*, 2007; Hastings, 2008; Popper & Hastings, 2009b; Vermeij *et al.*, 2010; Boyd *et al.*, 2011). Unlike the more easily studied and somewhat better understood effects of noise on land animals (Kasumyan, 2009; for reviews see Warren *et al.*, 2006 and Barber, Crooks, & Fristrup, 2010), including monkeys (e.g., Duarte *et al.*, 2011), birds (e.g., Slabbekoorn, Peet & Grier, 2003), anurans (e.g., Parris, Velik-Lord, & North, 2009), insects (e.g., Lampe *et al.*, 2012), and bats (e.g., Schaub, Ostwald, & Siemers, 2008; Siemers & Schaub, 2011), the effects of noise on marine ecosystems and organisms are less well known. This lack of information is worrying, as marine animals are potentially more vulnerable to anthropogenic noise than land animals, given the efficiency of underwater sound propagation (Tyack *et al.*, 2011). Water transmits sound over four times more effectively than air, and with low sound attenuation, underwater noise produces “larger footprints” (Hatch & Fristrup, 2009). Indeed, as low light and turbidity underwater lead to poor visibility (Stocker, 2002), hearing is often a more important sense than sight for aquatic life over terrestrial (Popper & Hastings, 2009b).

Box 1.1 Intensity versus frequency

A sound's 'loudness' refers to its intensity, which represents energy through area/time and its direction. Intensity is measured in decibels (dB). Underwater sound is measured in reference to 1 microPascal (1 μPa) as opposed to air, which is referenced to 20 μPa. Thus, a crude rule (as sound perception is biased to the individual's hearing capacity) is the higher the intensity, the 'louder' the sound. Frequency (cycles/second) is measured in Hertz (Hz) and is another important measure of underwater sound. As every auditory species has a particular frequency range, frequencies determine what we can hear, and help to categorize noise sources (Fig. 3.1).

With the rise of anthropogenic noise has come interest in determining its effects on marine life. To date, the best-studied group is that of cetaceans (Southall *et al.*, 2007; Slabbekoorn *et al.*, 2010; Radford, Kerridge, & Simpson, 2014). While such research still remains limited (Hastings, 2008; Popper & Hastings, 2009a), accumulating evidence suggests marine mammals can suffer serious disturbance and damage from acute noise, including changing diving behaviours and vocalizations (Tyack *et al.*, 2006; Nowacek *et al.*, 2007; Goldbogen *et al.*, 2013), while chronic noise can result in increased stress hormones and higher call intensities in whales (Parks *et al.*, 2011; Rolland *et al.*, 2012). These results are not surprising, given the primary role of sound in the lives of whales and its profound significance in their social and biological realms (Weilgart, 2007). What is surprising perhaps is the breadth of negative impacts recently discovered across other taxonomic groups: massive acoustic trauma and death in cephalopods exposed to low frequency sound (André *et al.*, 2011); changes to natural behaviours and physiology of crustaceans after exposure to boat noise (Chan *et al.*, 2010; Wale, Simpson, & Radford, 2013a, 2013b); as well as internal bruising in crabs and deformities and developmental delays in bivalves exposed to seismic noise (DFO, 2004; de Soto *et al.*, 2013). These findings raise the possibility that lesser understood and smaller animals like fish could be even more affected by noise than whales, especially as the relatively small size of fish compared with acoustic wavelengths offer only a limited ability for their bodies' to respond to oscillations, which can then lead to increased physical trauma (Hastings, 2008).

My thesis seeks to address this substantial knowledge gap on effects of anthropogenic noise on fish by focusing on boat noise. Boat noise is the most widespread and chronic source of anthropogenic noise in the ocean, and likely of greatest impact to fish (Halpern *et al.*, 2008; Slabbekoorn *et al.*, 2010). Unlike other sources of high frequency acoustic pollution (e.g., sonar or pile driving; Popper & Hastings, 2009b), shipping traffic generally emits frequencies “below several hundred Hertz” (Popper, 2003), which transmit further (Halfwerk *et al.*, 2011). In addition, the frequency of shipping noise often overlaps with those of marine animal sounds (Fig. 3.1), making persistent shipping noise potentially the most problematic form of acoustic pollution for life in the sea (Malakoff, 2010; Slabbekoorn *et al.*, 2010).

In the following sections, I will explain the importance of sound in the lives of fish, how fish detect sound, and what is currently known about effects of anthropogenic noise on fish. I will finish by outlining my study, which focuses on a highly vocal species of fish *in situ*, and describe implications of ecosystem-based research.

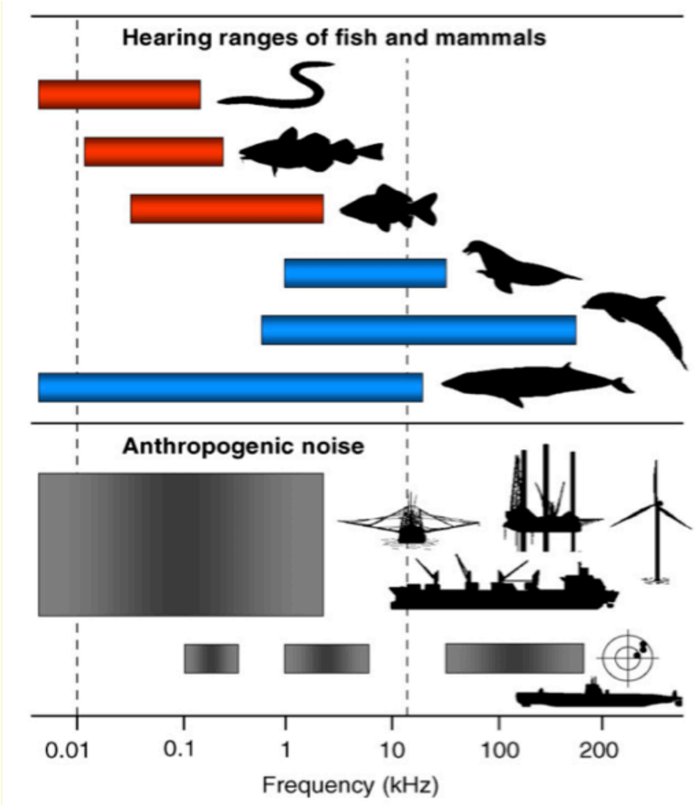

Figure 1.1 Examples of hearing ranges of marine life and sources of anthropogenic noise and intensities. From Slabbekoorn *et al.*, 2010.

1.2.1 Fish and noise

Fish contribute to natural ocean noise. Whether by grazers scraping on coral reefs (Munger *et al.*, 2011), mating ceremonies of drum-fish in temperate waters (Ramcharitar, Gannon, & Popper, 2006), or antagonistic grunts from tropical fish (Kasumyan, 2009), "The sounds produced by fish form the basis of the natural acoustic background in water bodies" (Kasumyan, 2009). Freshwater fish also make noise, including piranhas (Millot, Vandewalle, & Parmentier, 2011), sturgeon (Johnston & Phillips, 2003) and bullheads (Ladich, 1990). Indeed, at least 1,000 species of fish are known to be capable of making sound (Kasumyan, 2009), and all species studied to date are able to hear (Slabbekoorn *et al.*, 2010).

Box 1.2 Fish hearing

Fish can ‘hear’ through a few different mechanisms. All fish sense water particle motion produced by underwater sounds through structures within their heads, which consist of otoliths, membranes and hair cells (Webb, Fay, & Popper, 2008). These organs make up the auditory system, or fish ‘ears’ (note their inner ear structure is much the same as ours; Webb, Fay, & Popper, 2008) and detect sound from otoliths moving in the inner ear (Zeddies *et al.*, 2010). Sound pressure in contrast is perceived by some fish through pressure changes via internal gas-filled structures (e.g., swim bladders): a change in surrounding pressure causes these gas sacs to move, and this sensation is then transferred to the ear where sound interpretation takes place (Weeg, Fay, & Bass, 2002; Webb, Fay, & Popper, 2008; Zeddies *et al.*, 2010). The lateral line- found along the fish's body surface- is yet another organ associated with fish hearing. It detects mechanical or pressure changes in the surrounding water via neuromasts, essentially structures consisting of sensory haired receptors. This organ plays an important role in orientation during schooling (Webb, Fay, & Popper, 2008) and predator-prey interactions (Abboud & Coombs, 2000).

Based on their anatomy, in the past, fish species have been lumped into two groups: hearing ‘specialists’ and hearing ‘generalists’ (Webb, Fay, & Popper, 2008). Recently, however, such potentially confusing and possibly inaccurate distinctions have been abandoned (Popper & Fay, 2011), and the ‘auditory detection continuum’, which indicates a scale of hearing ability, is probably more appropriate (Slabbekoorn *et al.*, 2010; Fay & Popper, 2012).

Further to anatomical structures, sound and fish hearing are complicated by other, outside considerations: “Sound propagation can be affected by many factors, the most influential of which are: (i) frequency of the sound; (ii) water depth; and (iii) density differences within the water column, which vary primarily with temperature and pressure (Urick, 1983)” (cited in Nowacek *et al.*, 2007). Thus noise, particularly in lower frequencies, is affected by other variables, including temperature and weather (Halfwerk *et al.*, 2011).

Recent studies reveal the significance of sound in the lives of fish (McCauley, Fewtrell, & Popper, 2003; Anderson *et al.*, 2011), including its importance in early life stages (Simpson *et al.*, 2004; Wright *et al.*, 2008; Radford *et al.*, 2011). Like many other marine animals, fish use sound for orientation and environmental recognition (Simpson *et al.*, 2008), communication (Bass & McKibben, 2003; Maruska & Mensinger, 2009), and foraging (Slabbekoorn & Bouton, 2008).

While we have known for millennia that fish can produce sounds (Aristotle remarked on this phenomenon back in the fourth century BC; Tower, 1908; Bass & Ladich, 2008), it is only recently - within the last 100 years or so - that studies investigating fish hearing and sound generation really commenced (Kasumyan, 2009). More recently still - within the last decade – there has been a shift in focus from species-specific neurophysiological fish studies (e.g., Bass & McKibben, 2003) towards broader, more applied studies discussing ecological ramifications of anthropogenic noise on fish, and potential policy implications (e.g., Holles *et al.*, 2013; Radford, Kerridge, & Simpson, 2014). Thus, given this new field of research, and certainly relative to marine mammal studies, information on noise effects on fish is limited (Popper & Hastings, 2009a). Recently however, several reviews have summarized what is known about impacts of noise on fish (see Popper & Hastings, 2009a; Slabbekoorn *et al.*, 2010; Radford, Kerridge, & Simpson, 2014). Considering the enormous number of fish species (over 30,000 described to date; www.fishbase.org) and physiological variety, generalizations about effects of noise on fish are difficult. Yet new examples on a range of fish species show harmful direct and indirect consequences of anthropogenic noise. Directly, studies show noise can physically harm fish through distress, injury, hearing loss, or even death (Amoser & Ladich, 2003; McCauley, Fewtrell, & Popper, 2003; Wysocki, Dittami, & Ladich, 2006; Popper & Hastings, 2009a). Indirectly, noise can affect their ability to sense their environment (Slabbekoorn & Bouton, 2008) and to use natural acoustic cues, including via masking, whereby the frequency of anthropogenic noise overlaps that of natural noise and blocks important auditory information (Slabbekoorn *et al.*, 2010); Vasconcelos, Amorim, and Ladich (2007) document such effects in the lab, and suggest potential consequences of their results for fish in the wild (e.g., decreases in species abundance; see Brumm, 2010). Noise can also increase stress levels in fish (Anderson *et al.*, 2011), which can alter their spatial distribution (Pearson, Skalski, & Malme, 1992; Slotte *et al.*, 2004) and group formations (e.g., schooling behaviours; Pearson, Skalski, & Malme, 1992), and impact reproductive success (Sarà *et al.*, 2007).

While these experiments present insightful results, it must be noted that for the most part they refer only to lab-based scenarios. Hence, our ability to extrapolate from their conclusions to real-life situations remains limited (Popper & Hastings, 2009b). One known example of a study on noise effects on fish was conducted in a protected area (see Picciulin *et al.*, 2010), while a handful of others were conducted in altered or semi-wild conditions (e.g., Amorim *et al.*, 2010; Vasconcelos *et al.*, 2010; Holles *et al.*, 2013; see also Simpson, Purser, & Radford, 2015, which included an open-water component); these however remain exceptions. A great urgency for field-based studies persists (Popper & Hastings, 2009b; Slabbekoorn *et al.*, 2010).

To address this disparity I performed my study exclusively in the wild, in an intertidal ecosystem off the Pacific Northeast coast. By choosing to focus on the plainfin midshipman *Porichthys notatus*, a territorial species of fish accessible at low tides, I was able to set up experiments around their natural nesting sites without the use of nets, pens or cages. This allowed for unrestricted movement or migration of the fish at any time, more closely mirroring natural circumstances. Fish were never handled or moved to a lab, factors that can influence their behaviour (Hassel *et al.*, 2004; Popper & Hastings, 2009b). Further, this is one of the first studies to evaluate effects of boat noise on fish at both a species and ecosystem level. By monitoring fish in their natural habitats, I was able to record ecosystem-influenced behaviours and interspecific associations, which allow for a more realistic representation of the impacts of noise on fish in dynamic environments.

Below I further elaborate on *P. notatus*, introduce the Pacific Northeast coast, and explain the rationale for choosing a study site in this location.

1.3 CASE STUDY: THE NORTH PACIFIC AND THE SINGING FISH

Along the Northeast Pacific, people have probably known for millennia that marine animals like whales and fish make noise. This is evidenced by old folklore and oral cultures of indigenous coastal First Nations peoples from long ago which recall natural noises, including coming from the intertidal (R. Bouchard, pers. comm.). In contrast, scientific animal acoustic research in this region is recent, and has focused mainly on marine mammals, including pinnipeds (Kastak *et al.*, 2005; Mulsow *et al.*, 2011) but predominantly, whales (e.g., Barrett-Lennard, Ford, & Heise, 1996; Ford, Ellis, & Balcomb, 2000). Some studies have described the potential impact of human-generated noise on whales from sources such as acoustic harassment devices set to deter seal predation on caged salmon (Morton & Symonds, 2002), and from vessels (Lusseau *et al.*, 2009). Just as on land it has been shown that noisier neighbourhoods can cause birds to sing louder (Slabbekoorn, Peet, & Grier, 2003; Parks *et al.*, 2011), research in the Pacific Northeast has found that boat noise causes killer whales *Orcinus orca* to call louder (Holt *et al.*, 2009). Documentation of sounds and hearing capacity of fish in the North Pacific include studies on Pacific herring *Clupea pallasii* (Wilson, Batty, & Dill, 2004), rockfish *Sebastes* spp. (Nichols, 2005; Sirovic *et al.*, 2009), walleye pollock *Theragra chalcogramma* (Mann *et al.*, 2009), salmon *Oncorhynchus kisutch and Oncorhynchus tschawytscha* (Boyes, 1982) and an intertidal fish called the plainfin midshipman *Porichthys notatus* (Sisneros, 2007).

1.3.1 The plainfin midshipman

Porichthys notatus is one of 69 species of toadfish (Batrachoididae) and 14 species of midshipman (www.fishbase.org). During the winter months in the Northeast Pacific, *P. notatus* is a deep-dwelling fish, living at depths of several hundred meters, while during summer months it appears in intertidal waters to procreate (McCosker, 1986). *Porichthys notatus* detects sound via its internal ears (Sisneros, 2007), and possibly lateral line (Weeg & Bass, 2002; Zeddies *et al.*, 2012) and swim bladder (Popper & Fay, 2011). Scientific studies describing in-depth characteristics and acoustic behaviours of *P. notatus* are relatively recent, with one of the most detailed and informative occurring late in the 20th century (see Brantley & Bass, 1994). However sounds of *P. notatus* have been noted for centuries (likely longer) by various indigenous groups inhabiting the Northeast Pacific coast (R. Bouchard, pers. comm.). The vocal tendencies of *P. notatus* have given rise to such descriptive names as talkative fish, singing fish, and canary fish (Kasumyan, 2009).

Porichthys notatus has two adult male morphs, Type I (alpha male) and Type II (sneaker male). These morphs vary in size, courtship and parenting behavior, reproductive strategy, and vocal ability (Bass, 1996). Type I males produce several agonistic sounds, including the grunt (which is also made by females), the grunt train (a sequence of short, repetitive grunts), and the growl (Bass, Bodnar, & Marchaterre, 1999). They also produce a vocalization known to be associated with reproduction, and is of particular interest to researchers: the hum (Bass, 1996; Sisneros & Bass, 2005), which attracts females during the mating season (Sisneros, 2009b). This hum can last uninterrupted for up to an hour (Bass, 1996) and is produced by sonic muscles that vibrate against the fish's gas-filled swim bladder (Sisneros, 2009a).

Almost all recent studies to date on *P. notatus* have focused on neurophysiological aspects of sound production and detection and have been based in the lab (McKibben & Bass, 1999; Sisneros, 2012; Zeddies *et al.*, 2012; Petersen *et al.*, 2013). While some studies have investigated the role of sound and noise on other species of toadfish (e.g., pure tone playbacks mimicking longer conspecific calls were found to elevate stress hormones in the Gulf toadfish *Opsanus beta*: Remage-Healey & Bass, 2005; auditory threshholds of Lusitanian toadfish *Halobatrachus didactylus* were found to increase in ship noise conditions: Vasconcelos, Amorim, & Ladich, 2007), to date no studies have directly evaluated impacts of boat noise on *P. notatus*.

1.3.2 Ship noise and the North Pacific

Ocean noise in the North Pacific is a concern. Scientists estimate that human activity has contributed around 3 dB of ambient noise to this region every decade since the mid 1900s (Andrew *et al.*, 2002; McDonald, Hildebrand, & Wiggins, 2006), which can be linked to the increase in commercial shipping. This is especially concerning given that marine organisms on this coast already face an uncertain future due to climate change (Hazen *et al.*, 2012) and ineffective marine protection (Robb *et al.*, 2011). Noise could decrease resiliency of organisms when faced with other pressures (Barber, Crooks, & Fristrup, 2010; Boyd *et al.*, 2011; for example, on its own increasing temperatures from climate change can be tolerated by mussels, but with the added pressure of increased predation due to limited space (caused by warming waters) together exacerbate mussel mortality; Harley,

2011) and could result in unexpected outcomes (e.g., through cascading effects; see Francis, Ortega, & Cruz, 2009). Despite these other challenges, and while the Northeast Pacific in particular is highly impacted by multiple sources of human-use, shipping remains a primary concern (Ban, Alidina, & Ardron, 2010). Indeed, "Shipping is probably the most extensive source of noise in the oceans, especially along major shipping channels (e.g., from Alaska to California for supertankers carrying oil)"; Popper, 2003. Increased shipping in this area is pending due to a recent surge in development proposals, mainly focused on transporting oil (Heise & Alidina, 2012).

More ships could be problematic for species such as the plainfin midshipman that inhabits the same coastal range as these tanker routes. On land, it is known that traffic noise interferes with low-frequency bird communication, resulting in changes to site selection and impaired male-female communication (Goodwin & Shriver, 2011; Halfwerk *et al.*, 2011). Low-frequency shipping noise could have similar unintended negative consequences for the plainfin midshipman, as it overlaps with a major part of *P. notatus'* call-frequency band associated with reproduction (~100 Hz; Brantley & Bass, 1994; Sisneros, 2009c; Fig. 3.1), and could thus potentially impact their reproductive success (Sisneros, 2009a).

1.4 RESEARCH OBJECTIVES

Here, for the first time, I evaluate the effects of boat noise on wild fish at a species and ecosystem level. The chapters that follow address this research goal in several different ways.

Appropriate and cost-efficient technology did not exist to collect the data required for this study; thus, in order to undertake field research I had to help construct it. Chapter 2 therefore describes the equipment I designed and had created specifically for this research. It details the effectiveness of this tool for my specific purposes, and potential contribution to future studies. This chapter has been accepted with major revisions to Ecology and Evolution.

In Chapter 3 I use passive acoustics to determine the acoustic footprint of wild plainfin midshipmen near a nesting ground. Vocalizations from field recordings reveal diel patterns relating to call types and call durations. By conducting a behavioural study in the field, I also evaluate the effectiveness of the 'grunge', a grunt and lunge behaviour enacted by nest-guarding male plainfin midshipmen in response to heterospecific intruders and predators. These studies provide information on natural call characteristics of *P. notatus*, along with its behaviour during agonistic acoustic communication, and present findings in an ecosystem context.

Chapter 4 describes an experiment in which I expose nest-guarding plainfin midshipman males to boat noise. In addition to boat noise, here I evaluate the behaviour of the plainfin midshipman under two other conditions: ambient (boat no engine) and control (no boat, no engine). In this chapter I take into consideration ecosystem effects of boat noise by looking at its impacts on potential plainfin midshipman predators, and discuss implications of anthropogenic noise on predator-prey interactions.

Finally, in Chapter 5, I explain and trial a new, automated approach to analyzing movement patterns of the plainfin midshipman. A mechanized detection system is described, whereby a computer algorithm, based on a template of my manual annotations, calculates total amount of movement by the plainfin midshipman in a given sample (i.e., video file). Movement patterns under three conditions- boat noise, ambient and control (same experimental set-up as in Chapter 4)- are included in the analysis. I then explore the wider applicability of the method and its implications for future research studies that depend on visual interpretation of long video data sets.

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Chapter 2: The TOAD advantage: Teamed Optic-Acoustic Device yields novel information on species communication in wild marine ecosystems

2.1 PREFACE

In order to conduct research on the effects of noise on marine organisms *in situ*, the right equipment is required. Currently lacking are affordable and adaptable tools with longterm recording capacities to collect data via both visual and acoustic means; this considerably limits the ability of researchers to collect relevant information on natural behaviours of organisms in the wild (Rountree *et al.*, 2006). Here, I showcase the Teamed Optic-Acoustic Device (TOAD) in a trial study in an intertidal ecosystem. The TOAD proves to be an effective tool for collecting behavioural data on wild nesting plainfin midshipmen fish, and produced data presented in chapters 3 and 4.

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Title: The TOAD advantage: Teamed Optic-Acoustic Device yields novel information on species communication in wild marine ecosystems

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Abstract With the rise of anthropogenic noise in the sea, understanding the role of sound in the lives of marine organisms has gained urgency. Yet the tools to collect meaningful data on this topic from long-term field-based examples are lacking. Multimodal recording techniques that incorporate optic and acoustic capacities can provide fundamental insights into organism interactions and behaviours not obtainable through single-stream techniques like video alone, and *in situ* studies offer more accurate representations of natural ecosystems, describing scenarios most closely replicating real-life. Here, we address knowledge gaps by describing the construction and implementation of the Teamed Optic-Acoustic Device (TOAD), a novel and cost-effective monitoring system (<$1,000 USD) which can be used in most marine ecosystems and in depths of up to 244 meters. We explain trialing the TOAD in a shallow-water ecosystem off the West coast of Canada, and focus on its ability to document wild vocalizations and associated behaviours of the plainfin midshipman (*Porichthys notatus*), a soniferous fish. In 30 hours of recording, the TOAD documented 838 individual organism encounters comprising 30 different species. In addition, previously unknown interspecific vocal communication between *P. notatus*, crab, and other fish species was captured. These results underscore the TOAD's successful performance, and the potential of this system in future marine studies.

Key-words: Vocalization, plainfin midshipman, *Porichthys notatus*, *in situ*, predator-prey, video, audio, acoustic communication.

Introduction

Most of what we know about sound and marine life centers on marine mammals (Popper 2003), but the lives of many other organisms are influenced by sound. For example, of the 30,000 known fish species, all those studied can hear, and so far 800 are known to make sounds (Slabbekoorn *et al.* 2010). Sound influences habitat selection in juvenile coral (Vermeij *et al.* 2010), and is used by crustaceans for orientation (Simpson *et al.* 2011). What is more, a growing number of studies suggest animals beyond cetaceans can be detrimentally affected by anthropogenic noise: impacts include physiological changes and irreparable hearing loss in fish (McCauley, Fewtrell, & Popper 2003; Anderson *et al.* 2011), massive acoustic trauma and death in cephalopods (André *et al.* 2011), internal bruising in crustaceans (DFO 2004), and deformities and developmental delays in bivalves (de Soto *et al.* 2013). Despite these concerns, the role of sound within individual species and marine ecosystems is still poorly understood. With global anthropogenic noise on the rise (Ross 2005; Hildebrand 2009), these uncertainties take on greater urgency (Slabbekoorn *et al.* 2010; Boyd *et al.* 2011).

There are many benefits of obtaining acoustic data. Primarily, they can yield valuable information about marine animal behavior, communication, and ecosystem dynamics (Rountree *et al.* 2006). By identifying individual vocalizations on a species-specific level, new information about a population can be discovered (Sirovic *et al.* 2009; Colleye & Parmentier 2012). Further, by obtaining baseline soundscape information, particularly in pristine places, acoustic insight into ecosystems can help to inform future use and management (Krause 2012). Finally, passive acoustics, a non-invasive observational technology, can further allow for the tracking and monitoring of marine stocks over a long period, and can thus be useful in fisheries science to estimate difficult parameters such as stock location, abundance, and spawning grounds, without harming populations (Rountree *et al.* 2006).

In spite of these benefits, very few behavioural studies on wild marine animals beyond cetaceans have sought acoustic data. The majority of research to date has centered on obtaining visual information, either directly through dive or snorkel studies, or passively through the use of cameras (for a full review of underwater cameras see Favaro *et al.* 2012. Note that while many video cameras have an audio capacity under water, such audio recordings are not as accurate as those achieved through hydrophones, and can fail to capture important sound characteristics; Mosharo & Lobel 2012). With only a singular data stream (e.g., visual or audio), a limited and incomplete snapshot of the world is received. On their own, data achieved through a singular mode of perception can lead to biases, and even faulty conclusions. For example, with purely visual information, a silent picture is obtained, and important cues- e.g., ships passing, fish communicating, dolphins foraging- are all omitted, and physical responses to noise or behaviours associated with sound are lost. Likewise, unsupported or partially supported audio results in sounds that cannot be identified (Wall *et al.* 2014), and are sometimes even misidentified (Sprague & Luczkovich 2001). Multisensory observation tools allow for a fuller and more accurate observation of organism behaviour (Rypstra *et al.* 2009).

In order to understand the impacts of noise on marine life, and to make any relevant conclusions, a basic knowledge of organisms' natural behaviours, including communication habits, is necessary. It is only then, with such a baseline, that predictions on potential impacts can be projected (Popper & Hastings 2009). To date there lacks readily available, affordable technology for the seamless and simultaneous capture of quality video and acoustic data for the purposes of documenting acoustic communication of marine organisms and their associated behaviours in their natural habitat, despite the expressed necessity for obtaining such data, especially as they relate to anthropogenic noise (Popper & Hastings 2009; Slabbekoorn *et al.* 2010; Radford, Kerridge, & Simpson 2014). Thus, to address this considerable research gap, here we describe the TOAD: Teamed Optic-Acoustic Device, a low cost, highly effective audio/video system for long-term monitoring of marine life. While greatly informative, multisensory studies on wild fish are exceptionally rare (Lugli *et al.* 2004; Rountree *et al.* 2006; Kasumyan 2009); thus, for this trial study, we conducted the assessment in an intertidal ecosystem in the Pacific Northeast, and focused on a highly vocal species of fish, the plainfin midshipman, *Porichthys notatus* Girard 1854.

Trial study

From May to September, and from California to Alaska, *P. notatus* migrates up from depths of several hundred meters into the intertidal to lay eggs and reproduce (Arora 1948; Sisneros 2012). During these summer months, it is one of the largest- up to 38 cm long (www.fishbase.org)- and noisiest organisms in the intertidal zone (McCosker 1986). Like other species of toadfish, *P. notatus* takes up residence in burrowed out nests under rocks (Brantley & Bass 1994; Fine & Thorson 2008). Thus, at low tides during the summer in Canada and the United States, their nests are easily accessible; along with a fierce territoriality exhibited by alpha males, this exposure makes *P. notatus* an extremely useful species for behavioural studies (Brantley & Bass 1994; McKibben & Bass 1998).

Alpha male *P. notatus* morphs (as opposed to females or ‘sneaker males’ of the same species) produce three main vocalizations: hums, grunts and growls (Bass, Bodnar, & Marchaterre 1999). All three calls are emitted at around 100 Hz and can be heard from above water (S. Cullis-Suzuki, pers. obs.). While hums are known to be associated with reproduction (Bass 1996; Sisneros & Bass 2005), grunts and growls are less understood, but are generally thought to correspond with conspecific agonistic interactions (Brantley & Bass 1994). For this trial study, we attempt to use the TOAD to investigate the purpose and function of these antagonistic calls.

Materials and methods

LICENSES AND PERMITS

All field-work procedures were reviewed first by the University of York, United Kingdom, which authorized risk assessments and gave ethical approval. A scientific collection license from the Department of Fisheries and Oceans Canada (DFO) was also obtained for the duration of the field season in BC, as was a certificate of approval from the Canadian Council on Animal Care.

TOAD CONSTRUCTION

All TOAD construction and assembly took place at the Engineering Physics lab at the University of British Columbia. The pressure housing was designed out of 7.6 cm plastic acrylonitrile butadiene styrene (ABS) tubing and O-ring seals, and all clear windows (i.e., lens, light covers) were turned out of 1.9 cm Plexiglas. Three pod light-emitting diodes (LEDs) were inserted into two ABS capsules on either side of the lens (LEDs>1000 lumens; light output 3 x 260 lm per pod, total 1560 lm), and red light filters were added to decrease fish's sensitivity to light (McKibben & Bass, 1998; Cappo, Speare, & De'ath 2004; Widder *et al.* 2005); Fig. 2.1.

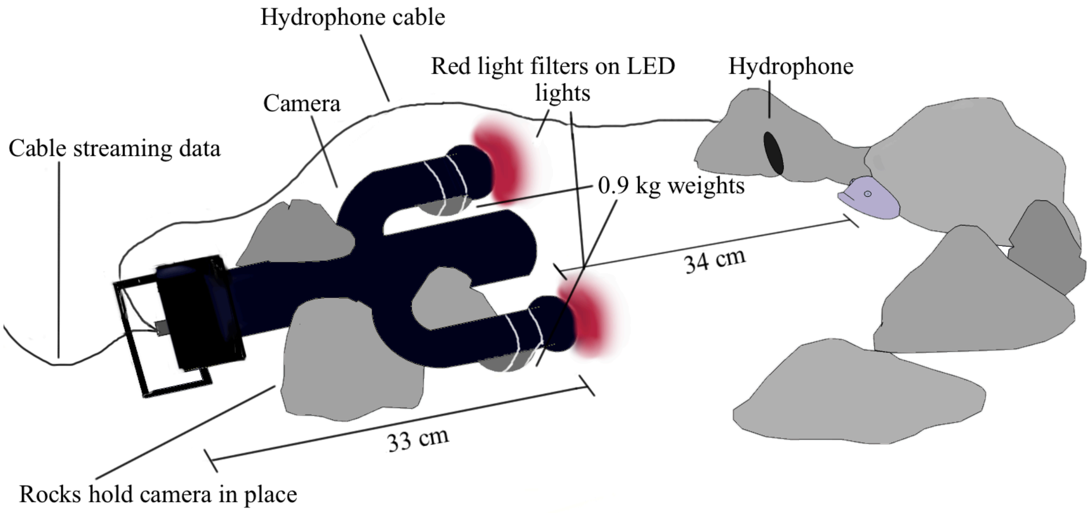

Fig. 2.1 Schematic of the TOAD assembled for trial in front of typical *P. notatus* nest.

The camera itself was a low cost CTV analog video camera with NTSC format output (> 350 lines resolution) with auto iris (stripped down printed circuit board size: 4.2 cm square less lens). Cooling for the LED lights occurred through housing walls from two round custom aluminum heat sinks. Pressure testing was performed in the ocean prior to trial, where the camera housing was established to withstand depths of 244 m. Total size of the enclosed camera component was L 33 cm x H 7.6 cm x W 40.6 cm (W = edge to edge of outer light capsules). For our trial, two 0.9 kg weights were attached to the bottom of the TOAD (one on each LED capsule) to increase camera stability. The entire camera system was created for under $500 USD.

To add an audio capacity to the system we inserted a hydrophone with an 8 m cable (HTI-96-min by High Tech Inc, $299.95 USD from Wildlife Acoustics, Massachusetts, USA; max. depth: 3,048 m). The acoustic data, converted into electrical impulses by the hydrophone, then fed into the FM audio input and modulated to the corresponding VHF video channel; both audio and video were demodulated and recorded simultaneously, ensuring proper time synchronization between the two. The 8 m hydrophone cable emerging from the end of the TOAD was freely maneuverable, allowing for close and precise placement of the hydrophone near *P. notatus* nests. The TOAD required a topside external power supply of 9-12 VDC, 1.5 W (note: for a longer cable more voltage is required, e.g., 3,048 m = 40 VDC). Live audio and video data were streamed through a weighted 91.4 m Belden waterproof RG59 cable (75 ohm) back to a temporary research station overlooking the field site (e.g., Wardle *et al.* 2001); Fig. 2.2. Real-time images and audio were available on laptops and speakers, respectively, set up in the research station. These data were then recorded onto VHS tapes and digitized through a MacBook Pro via Pinnacle Dazzle Recorder Plus (as in Amorim *et al.* 2013) and recorded as video files onto Seagate 2TB external hard drives.

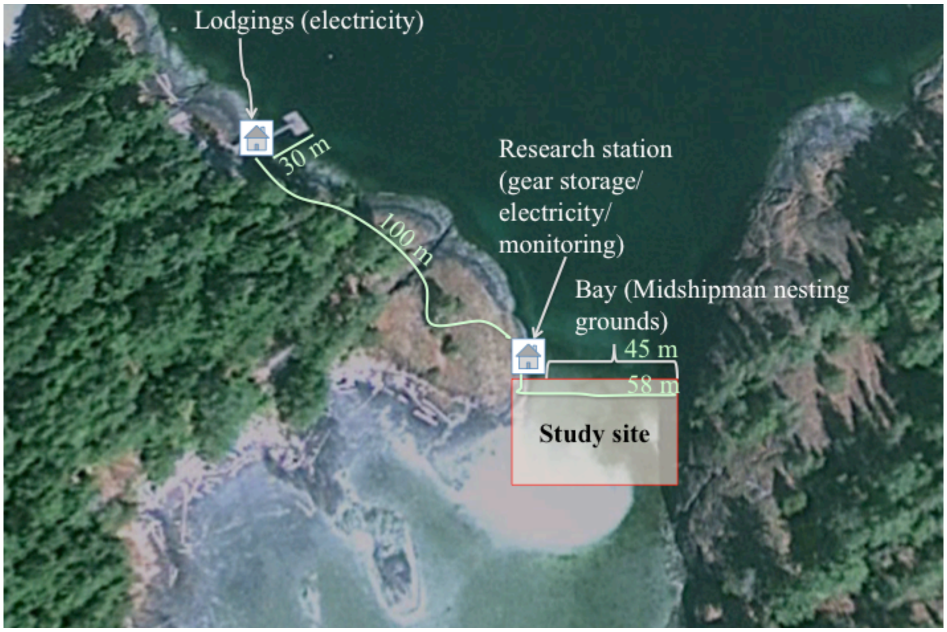

Fig. 2.2 Field site and set-up for trial study, Quadra Island, BC. Map data: Google, GeoEye. 2012.

TRIAL SET-UP

Between June 7th- 27th, 2013, fifteen distinct *P. notatus* nests were selected in a small intertidal bay on Quadra Island, BC (lat/lon: 50.11159, -125.21757). Nests were chosen during the day at the lowest tides, and selected based on presence of a nest-guarding alpha male *P. notatus* as well as presence of eggs, and site accessibility. Presence of *P. notatus* and eggs were determined through direct observation or use of an underwater pipe camera to ensure rocks shielding nests were never overturned (for effects of such disturbance on ecosystem see Cogliati, Neff, & Balshine 2013). After nest selection the TOAD was set up in front of the chosen site: the TOAD's camera distance from nest entrance was dictated by substrate and maximization of field of view, averaging 34 cm and never more than 44.5 cm, while the TOAD's hydrophone was secured under a rock and positioned near the nest entrance (Fig. 2.1). The substrate at our location was rocky bay and sand. At high tides at our site the TOAD was never deeper than 9.1 m.

DATA ANALYSIS

We collected data from the TOAD over the course of fifteen days (approximately 18 hours/day). For this present analysis, 30 hours of video and audio data were analyzed: two hours of data per site were reviewed, one during daylight (approximately 6 pm; note daylight recording times were restricted to tides that covered nests) and one after dark (approximately 10 pm). Each site represented a different nest, and a different alpha nest-guarding *P. notatus* male; thus, fifteen individuals were evaluated in the present analysis (n = 15). Footage was reviewed on a 55.9 cm Samsung monitor by one consistent investigator. When species identification was uncertain, appropriate experts were contacted for verification. Some organisms were potentially recounted as identifying individuals was not always possible (Favaro *et al.* 2012); however while increasing the field of view of the camera (i.e., setting it further back from nest entrance) might have helped decrease this effect, it would also have decreased accuracy of species identification, so was not applied.

Results

OBSERVATIONS

The TOAD

The TOAD worked well in this trial. Visibility inevitably depended on ocean conditions, but for all 30 samples assessed here, was adequate. In each sample, the principal *P. notatus* under investigation was visible. Although identification of other organisms to the species-level was not always possible, they were usually identified to family (smaller species were lumped into higher taxa, i.e., jellyfish, worms and amphipods; Table 2.1). The TOAD proved to be very stable despite currents, waves and rough conditions, and even withstood large predators pushing into frame (e.g., harbour seals *Phoca vitulina* and river otters *Lontra canadensis* searching for prey). There was a low level of electronic interference perceptible in audio but did not obstruct recordings. Night video was slightly more difficult to analyze due to low light conditions and black and white images and may have affected organism detection: a total of 61 unidentifiable species were counted, and 64% occurred during night samples; results however were not significant (day sightings: mean 1.47 ± 0.52 SE, $n = 15$; night sightings: mean 2.6 ± 1.03 SE, $n = 15$; paired t-test, $t = 0.97$, $P > 0.05$). Disparities could also be explained by nocturnal versus diurnal species and differences in species recognizability.

Table 2.1 Species identified[*] in 30 hours of footage (two hours at each of the fifteen sites).

#	Common name	Scientific name	# of sightings	# of nests w/ sightings	Known predator (of E= eggs; A= adults)
1	Hermit crab	*Paguroidea (Superfamily)*	179	9	
2	Red rock crab	*Cancer productus*	162	10	E (pers. obs.[**])
3	Gunnel	*Pholidae (Family)*	96	15	E (pers. obs.[**])
4	Shiner perch	*Cymatogaster aggregata*	93	6	
5	Whitespotted greenling	*Hexagrammos stelleri*	78	10	E (pers. obs.[**])
6	Coon striped shrimp	*Pandalus danae*	55	10	
7	Sculpin	*Cottidae spp.*	50	11	
8	Shore crab	*Hemigrapsus spp.*	33	6	E (pers. obs.[**])
9	Shrimp	*Caridea (Family)*	21	1	
10	Ochre star	*Pisaster ochraceus*	12	7	
11	Jellyfish (other spp.)	*Medusozoa (Subphylum)*	7	3	
12	Plainfin midshipman[***]	*Porichthys notatus*	6	2	E (pers. obs.[**])
13	Leather seastar	*Dermasterias imbricata*	5	4	
14	Sunflower star	*Pycnopodia helianthoides*	5	3	
15	Pacific snake prickleback	*Lumpenus sagitta*	5	1	
16	Amphipod	*Amphipoda (Order)*	4	1	
17	Spiny dogfish	*Squalus acanthias*	4	2	A (Robinson, Lapi, & Carter 1982)
18	Whelk	*Muricidae (Family)*	4	3	
19	Helmet crab	*Telmessus cheiragonus*	2	2	
20	Isopod	*Idotea sp.*	2	1	
21	Pacific staghorn sculpin	*Leptocottus armatus*	2	1	
22	Worm	*Polychaeta (Class)*	4	2	
23	Crangon shrimp	*Crangon spp.*	2	2	
24	Northern kelp crab	*Pugettia producta*	1	1	
25	Dungeness crab	*Metacarcinus magister*	1	1	
26	Moon jellyfish	*Aurelia labiata*	1	1	
27	Water jellyfish	*Aequorea sp.*	1	1	
28	Pile perch	*Rhacochilus vacca*	1	1	
29	Six-rayed seastar	*Leptasterias spp.*	1	1	
30	Harbour seal	*Phoca vitulina*	1	1	A (Luxa & Acevedo-Gutiérrez, 2013)
		Total	838		

[*] Not included in this list were organisms that I was unable to identify, which occurred 61 times in the 30 hours of footage. These organisms were barely visible or too fleeting for accurate species identification.

[**] S. Cullis-Suzuki, pers. obs.

[***] In addition to nest-guarding alpha male *P. notatus* under investigation.

Predators

A striking observation from the recordings was the number and diversity of heterospecifics that visited *P. notatus* nests or appeared within the camera's field of view, most of them potential predators to developing *P. notatus* eggs with the occasional predator to adult *P. notatus*. In 30 hours of recording, 838 organisms were identified comprising 30 different species (Table 2.1), seven of which are established predators to *P. notatus* in either adult, juvenile or egg form (Robinson, Lapi, & Carter 1982; Luxa & Acevedo-Gutiérrez 2013; S. Cullis-Suzuki, pers. obs.), and many more likely to be egg predators (e.g., seastars, hermit crabs, sculpin). As expected, predator composition and abundance differed between night and day (Fig. 2.3): individually, certain species were more likely to be seen either during night or during the day (e.g., Coon striped shrimp *Pandalus danae*, which was only observed during night; day mean 0 ± 0 SE, n = 15; night mean 3.40 ± 1.55 SE, n = 15; paired t-test, t = 2.20, $P < 0.05$). Differences in total species detection during day and night respectively however were not significant (day mean 27.73 ± 4.76 SE, n = 15; night mean

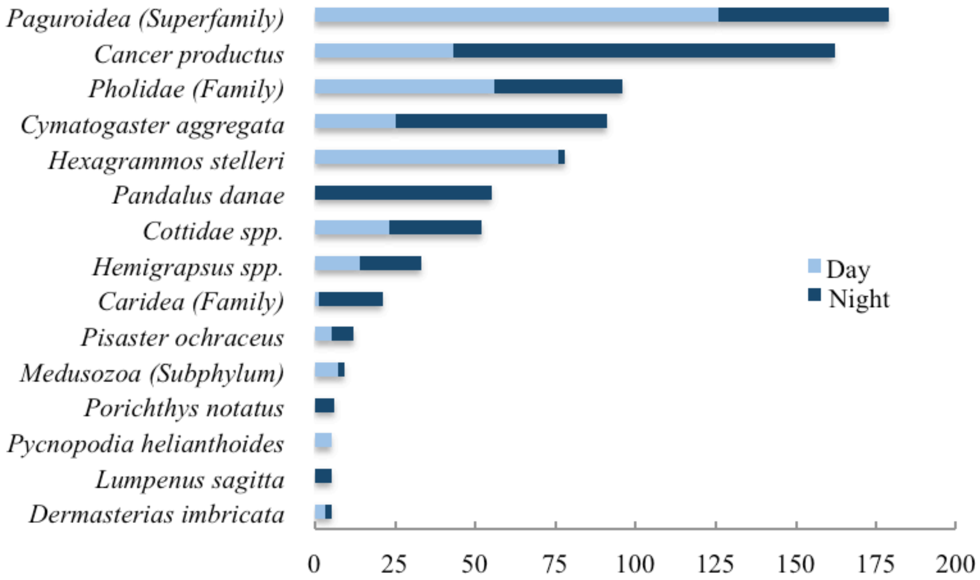

Fig. 2.3 Day versus night sightings of species (>5).

32.20 ± 6.13 SE, n = 15; paired t-test, t = 0.75, P > 0.05).

Plainfin midshipman

Video recordings revealed *P. notatus'* time was spent cleaning its nest, checking and aerating its eggs (e.g., with its mouth or by moving water with pectoral and tail fins) and keeping predators out. *P. notatus* was observed picking up shells and sticks with its mouth and expelling them outside its nest. It did this also with seastars (Ochre star *Pisaster ochraceus* and Leather star *Dermasterias imbricata*), ripping them off the ceiling or floor of their nests and spitting them out beyond. Sometimes *P. notatus* appeared to be resting (no perceptible movement). During the 30 hours of recordings, *P. notatus* was never observed to eat. Only once did we observe a *P. notatus* leave its nest, and it returned an hour later. None of the fifteen *P. notatus* observed were seen producing hums, but all produced grunts. Growls were

Fig. 2.4 Sequence of new behaviour, the 'grunge': a) *P. notatus* guards nest; b) *Cancer productus* crab advances; c) *P. notatus* lunges and grunts simultaneously at crab; crab departs.

detected through the hydrophone, although the source was usually unclear (i.e., they could have been from the individual under investigation, or from another individual close by; Bass, Bodnar, & Marchaterre 1999). Grunts were almost always associated with heterospecific encounters (never with conspecific encounters) and occasionally during egg care. The source of the grunt was easily detected due to associated body movements (sudden quivers) of *P. notatus* during grunt emission.

A new behaviour was discovered while using the TOAD in shallow waters: in response to approaching heterospecific predators, alpha male *P. notatus* reacted with a coupled grunt and lunge (Fig. 2.4). We term this response the ‘grunge'. The grunge was enacted on multiple species, including gunnel species (Pholidae family), whitespotted greenling *Hexagrammos stelleri* and red rock crab *Cancer productus* (Fig. 2.4). The grunge appeared to be a clear defense mechanism exercised by nest-guarding *P. notatus* to deter potential predators from entering the nest and eating developing eggs.

Discussion

The TOAD was proven to be a reliable, cost-effective and non-invasive mode of acquiring long-term audio and video data on natural marine ecosystems. In our trial, the audio component of the TOAD was able to capture vocalizations from *P. notatus* while the video component put those calls in context, shedding light on cause of call emission, and allowing for a fuller interpretation of *P. notatus*’ interactions and behaviours with other marine organisms. By combining audio and video, and allowing for the identification of animal calls, the TOAD provides a potential solution to challenges associated with 'matching sounds with behaviours' (Rountree *et al.* 2006).

Along with the aforementioned benefits of a multisensory capacity, another big advantage of the TOAD was its ability to collect long-term continuous data. Traditionally, passive audio and video techniques have been highly effective in obtaining information about the marine world in a non-destructive way (e.g., Ramcharitar *et al.* 2006; Rountree *et al.* 2006; Harvey *et al.* 2007; Luczkovich *et al.* 2008; Sirovic *et al.* 2009; Favaro *et al.* 2012). Stationed video cameras can be less intrusive than other techniques, such as tagging studies (Jones *et al.* 2013), dive surveys (Lobel 2005), Remotely Operated Vehicles (Rountree & Juanes 2010), or BRUVs (Baited Remote Underwater Video Surveys; see Cappo *et al.* 2004). However, even small, stationed cameras (including the newer, more affordable GoPros) face the significant drawback of being restricted to time-limits imposed by memory cards or batteries (Cappo *et al.* 2004; Thomson & Heithaus 2014). Being a cabled system, i.e., tethered at all times to an external power supply, the TOAD bypasses challenges associated with recording limits, as uninterrupted capture of video data is instead constrained only by the capacity of hard drives; this allows observational studies to go beyond incomplete snapshots of wild interactions and behaviours (e.g., Bortone, Martin, & Bundrick 1991; Mosharo & Lobel 2012) and instead document ecosystem dynamics and natural patterns within the context of long timeframes (e.g., Barnes *et al.* 2013). This cabled-system also allows for real-time observations, as live data from the TOAD can be streamed to an external monitor on land (as described in this study); observations can thus be noted as they occur, and any necessary adjustments can be made immediately, e.g., changing camera position, etc. Finally, it should be noted that while for our trial we chose a cable length of 91.4 m, other studies could consider shorter/longer lengths to suit their site: for example, in shallow depths, the TOAD's power cable can reach a maximum length of 292 m.

The TOAD worked well in the intertidal environment trialed here. In particular, the system was easy to assemble near fish nests at low tide. However the TOAD is not limited to intertidal zones: indeed as the maximum depth for the camera component is 244 m and the maximum depth of the hydrophone is 3,048 m, such a system could be effective in other habitats. On its own, the camera can be used as a 'dropcamera', essentially suspended above a desired habitat or scene, or even slowly moving across it. (However, when the hydrophone is employed, cables moving through the water would result in drag noise; thus for best results when using both video and audio components of the TOAD, its recommended use is anchored to substrate.) Future trials will have the added benefit of viewing dimensions through the camera: since this trial, four laser diodes have been added to the system to assist in bottom measurements.

There were however various unavoidable field-based challenges that presented themselves throughout the course of this research. For example: waves, decreased visibility, objects such as seaweed getting caught on the camera lens, organisms like burrowing crabs obstructing view of a *P. notatus* nest, etc.; however, immediate viewing of live-streamed data through a land-based monitor allowed for instant obstruction identification, and usually resulted in the quick clearing of impediments from the camera lens. While bad weather conditions were not common in our location in June, they still occurred, and, due to time-constraints regarding *P. notatus* egg development (like other fish species; see

Lissaker & Kvarnemo, 2006), our trial thus spanned calm and rougher days. Another challenge was land-based predators such as northwestern crows *Corvus caurinus* and western gulls *Larus occidentalis* who were regularly attempting to dislodge territorial *P. notatus* from their nests at low tides. Further, for this research we were dependent on low tides for positioning the TOAD and setting up our study; in future sites that aren't exposed at low tides or are not as easily accessible, other options like SCUBA diving could eliminate dependence on ideal tides. Finally, while the TOAD was arranged in as minimally invasive a manner as possible, it must be noted that by positioning the TOAD in front of nests, the habitat of *P. notatus* was inevitably altered and could have affected *P. notatus*' vulnerability to predators, as well as the vulnerability of their nests (Cooke *et al.* 2008).

To date, the majority of research on *P. notatus* relates to neurophysiology, and almost all recent studies have been performed in the lab (e.g., Weeg, Fay, & Bass 2002; Bass & Ladich 2008; Sisneros 2009; Suk *et al.* 2009; Zeddies *et al.* 2010; Alderks & Sisneros 2011); in contrast, little information exists on *P. notatus'* natural interactions and behaviour in the wild. Through the TOAD and its associated *in situ* arrangement, the grunge, a novel behaviour of a vocal intertidal fish in its natural habitat, was recognized and recorded. Further, we provide first evidence that grunts are used frequently in antagonistic heterospecific encounters by male *P. notatus* while defending nests against a diversity of potential predators. This study shows the benefit of longterm coupled optic-acoustic tools and the effectiveness of the TOAD, which allowed for unforeseen novel insights into predator-prey communication, a topic that warrants particular attention as marine acoustic environments change (Slabbekoorn *et al.* 2010). On a much larger-scale, cabled observatories are expected to 'transform' ocean sciences owing to the huge amount of data they amass (Barnes *et al.* 2013); here, we provide a small-scale affordable alternative for localized, *in-situ* studies.

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Data accessibility

All TOAD data used in this study (i.e., audio/video files) are archived on a web server available at Ocean Networks Canada (www.oceannetworks.ca).

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Chapter 3: Sounds fishy: Quantifying and interpreting fish sounds in the sea

3.1 INTRODUCTION

Soundscapes, defined as “the relationship between a landscape and the composition of its sound” (Pijanowski *et al*., 2011), are fundamental components of environments. They comprise all types of noise, including natural sources from animals (‘biophony’), wind and geologic forces (‘geophony’), and those from man-made origins (‘anthrophony’; Pijanowski *et al.*, 2011; Krause, 2012). Soundscapes act as ‘acoustic daylight’, providing important information to inhabitants by guiding orientation and conveying information about their surroundings (Slabbekoorn & Bouton, 2008; Fay, 2009).

Under water soundscapes exist as well (Fay, 2009). Contrary to old assumptions (Johnson, Everest, & Young, 1947; Popper & Hastings, 2009), healthy marine ecosystems are far from quiet: from whales (Wall *et al.*, 2014) to sea urchin and shrimp (Radford *et al.*, 2008) to fish (McCosker, 1986; McCauley & Cato, 2000) animals dominate natural soundscapes.

Interpreting biophony in ocean ecosystems and quantifying species’ contributions is difficult: identifying animal calls can be a tedious process (Chesmore & Ohya, 2004; Mellinger, 2004), even for large species such as whales; for example, the mysterious ‘bioduck’ sound was revealed to be the Antarctic minke whale *Balaenoptera bonaerensis* after over half a century of investigation (Risch *et al.*, 2014). Much of the ocean’s biophony has not yet been identified, and it is thus unknown how much individual species contribute to these soundscapes (Rountree *et al.*, 2006). For fish, while over 800 species are known to vocalize, “the number of unidentified underwater sounds attributed to fishes is far greater than those that can be positively identified” (Rountree *et al.*, 2006).

Of those sounds that have been identified, the purpose or context of the calls is not always known. Although the majority of fish sounds are currently attributed to reproductive behaviour (Johnston & Phillips, 2003; Locascio & Mann, 2005; Gannon, 2008; Luczkovich *et al.*, 2008; Webb, Fay, & Popper, 2008; Kasumyan, 2009) studies quantifying specific behaviours are rare (Ripley & Lobel, 2004; Tricas & Boyle, 2014). Indeed, "it is important to realize, that the behavioural significance of only a small fraction of fish sounds is known" (Ladich, 1997a). Information on vocalizations produced during defensive or agonistic encounters, as well as during nest care and adventitious circumstances, is particularly lacking (Lobel, Kaatz, & Rice, 2010). Historically, such sounds were discovered through direct manipulation of subjects (e.g., Ladich, 1997b; for a review see Table 1 of Ladich, 1997a), thus yielding results unrepresentative of typical fish behaviours performed in natural habitats (Tavolga, 1977; Gannon, 2008). Less intrusive studies reveal agonistic vocal communication between fish and other fish species, though its function remains somewhat speculative (Mann & Lobel, 1998; Rollo & Higgs, 2008; Dunlap, DiBenedictis, & Banever, 2010). Today, what we know about aggressive vocal responses of fish remains predominantly from studies on conspecifics (Myrberg & Thresher, 1974; e.g., Mckibben & Bass, 1998; Amorim & Neves, 2008; Vasconcelos *et al.*, 2010; Verzijden *et al.*, 2010; Estramil *et al.*, 2014; Pereira *et al.*, 2014).

As noise pollution from boats and other anthropogenic sources change ocean soundscapes around the globe (Boyd *et al.*, 2011), information on biophony, including identification, classification and quantification, becomes more urgent if noise impacts are to be fully understood (Radford, Kerridge, & Simpson, 2014). A large-scale, systematic effort to categorize fish sounds, such as Fish and Mowbray's (1970) contribution in the mid 1900s in the Northwest Atlantic (Fish & Mowbray, 1970), would further this field markedly. In the meantime, identification of sounds at smaller scales would be more practical, with *in situ* studies offering ecologically relevant information (Mann, Hawkins, & Jech, 2008; for an example see recent study by Tricas & Boyle, 2014).

Here I focus on a coastal ecosystem in the Northeast Pacific, and examine the highly vocal fish the plainfin midshipman (*Porichthys notatus*), by describing its acoustic footprint in a wild, intertidal habitat. To do this I applied a two-part approach: first, I used passive acoustics to capture *P. notatus*' soundscape and establish its vocal repertoire *in situ*. Then, after identifying the *grunt* as its dominant vocalization through manual audio detection, I conducted a behavioural study in the field to determine context, purpose and effectiveness of this call in the wild, illustrating acoustic communication with heterospecifics.

3.2 MATERIALS AND METHODS

3.2.1 Passive acoustics: Identifying vocalizations

Field collection

From April 22nd to August 16th, 2012, long-term acoustic data were recorded passively from a HTI-96-MIN hydrophone (Wildlife Acoustics, MA, USA) attached to the seabed at 1 m below chart datum under a private dock in Heriot Bay on the east coast of Quadra Island, British Columbia (lat/lon: 50.11159, -125.21757; see Fig. 2.2, Chapter 2).

These data were recorded onto a Song Meter SM2$^+$ Weatherproof Passive Recorder (Wildlife Acoustics, MA, USA) 16-bit with a sampling rate of 44.1 kHz. The recorder was fastened to the dock approximately 100 m from a *P. notatus* nesting ground (Fig. 2.2, Chapter 2). Data were recorded as .wav files onto four internal 32 GB memory cards. The recorder relied on four D-batteries which necessitated replacement approximately every nine days, at which time data from memory cards were downloaded and memory cards cleared and replaced.

Manual analysis

My analysis focused on the month of June, as it is the peak calling period for *P. notatus* (Arora, 1948). Audio data across five dates in June were manually screened for vocalizations (June 1st, 7th, 15th, 22nd and 30th) by taking the first five minutes from each hour of each 24-hour cycle and conducting visual and audio analysis of spectrograms (as in Wall *et al.*, 2014) in Audacity 2.0.6 (Hamming window, FFT 4096, overlap 50%). This resulted in ten hours of manually annotated audio. As each five-minute segment of audio could take up to 45 minutes to analyze manually, and data on each hour of the day per date were necessary to explore diel patterns in call activity, time constraints restricted analysis to five dates only. AKG Reference Headphones K550 were used during audio analysis to capture faint sounds and increase accuracy of vocalization detection. In Audacity, vocalizations were highlighted and labeled as Grunts, Growls, Hums, Grunt trains or

Groans; labeling automatically recorded start and stop times of vocalizations, and their duration (see Fig. 3.1). Other sounds (waves, bubbles, boats, etc.) were noted. Labels and times of vocalizations were then exported as text files into Excel 2011 for organization, and finally into PRISM 6.0 for statistical analysis.

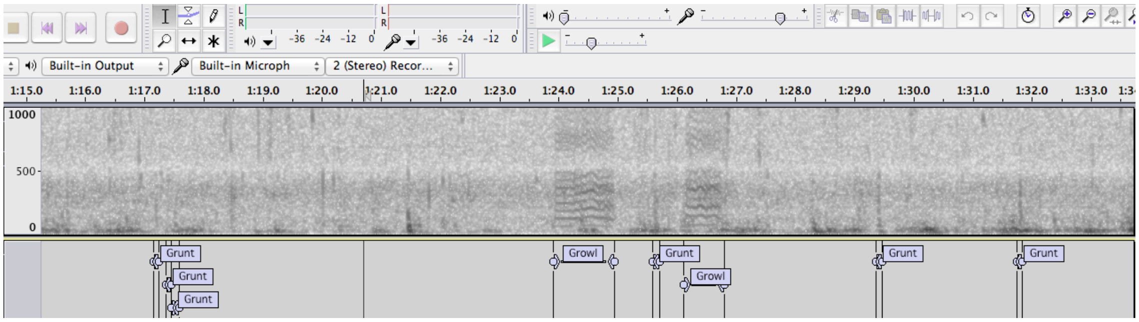

Figure 3.1 Example of spectrogram analysis and manual annotation of vocalizations in Audacity (data represented here are from June 22nd, 2012, at 5pm).

3.2.2 Behavioural study: Determining function of primary vocalization

The next step of this research was to identify the function of the grunt, which, through spectrogram analysis of passive acoustic recordings, was newly determined to be the primary vocalization of *P. notatus* ('primary' defined here as the highest number of calls). To do this I conducted a behavioural study, implementing the Teamed Optic-Acoustic Device (TOAD) to capture continuous video and audio data of nesting plainfin midshipmen in an intertidal bay (for full experimental-set up, device and site details, see Chapter 2). I used the TOAD and a separate HTI-96-MIN hydrophone to record 15 different nests with 15 individual plainfin midshipmen over the course of 15 different days in June of 2013. To reduce the potential for discrepancies in *P. notatus* behaviour associated with offspring development (e.g., decreased parental nest-guarding as eggs mature; Lissåker & Kvarnemo, 2006), I included only nests containing eggs in similar early development stages. Nests spanned an area of roughly 50 m x 20 m. Dominant substrate was rocky bottom and sand, and the site was adjacent to a nearby eelgrass bed.

TOAD recordings began at 6pm and ran until the following morning. From these, sixty hours were analyzed by selecting four-hour chunks between 10pm and 2am for each of the fifteen nests; this timeframe yielded the most consistent, uninterrupted recording block across all videos. Camera lights were fitted with red light filters to reduce interference with fish behaviour (Cappo, Speare, & De'ath, 2004; Widder *et al.,* 2005). Video footage, which included audio, was reviewed on a MacBook Pro 15" computer using VLC Media Player (ver 2.0.4) at a playback speed of 4.0. When an organism was sighted, video speed was reduced to real-time or slower for species identification and to detail *P. notatus* response/behaviour. I defined predators as heterospecific animals capable of consuming *P. notatus* in egg, juvenile or adult form (for a list of all potential *P. notatus* predators, see Chapter 2). Unlike in Chapter 2, whereby all species observed in the camera's view were presented including non-predator species such as worms and jellyfish, here, I include only potential heterospecific predator species, as they were revealed to be associated with grunts (Chapter 2). Appearances of other *P. notatus* were not included, nor were organisms that appeared multiple times in quick succession at the periphery of the screen (e.g., shiner perch on June 12th; sculpin on June 27th), as they were assumed to be the same organism.

All vocalizations and predator encounters were documented in an Excel spreadsheet, detailing time, predator species, predator size (for red rock crab *Cancer productus*), grunt emission, effectiveness of grunt at predator (if relevant) and additional notes. Grunts were defined as bursts of sound emitted from the mouth of *P. notatus*, lasting between less than one second and several seconds in length, and emitted in any context (e.g., as a byproduct of nest cleaning/aerating eggs, or directly by interacting with predators). All predator encounters were then further reviewed to assign grunt intention, and isolate grunt scenarios. Grunt events were then imported as video files into Final Cut Pro X 10.1.4 to isolate audio data and were then exported as .wav files. These files were then imported into Audacity 2.0.6 to analyze signals and calculate call length. Call information was then organized in Excel 2011, and imported into PRISM 6.0 for statistical analysis.

3.3 RESULTS

3.3.1 Passive acoustics- Manual analysis

The manual analysis of acoustic data revealed all four vocalizations of *P. notatus* were detected in the field during the month of June: the *hum*, *growl*, *grunt* and *grunt-train* (calls previously described; Brantley & Bass, 1994; Bass, Bodnar & Marchaterre, 1999; Bass & Clark, 2003; Sisneros, 2009). Another sound, the *groan*, was documented as well: spectrally and audibly, it is most similar to the growl (Fig. 3.2) and was therefore grouped with growls for the purpose of this study.

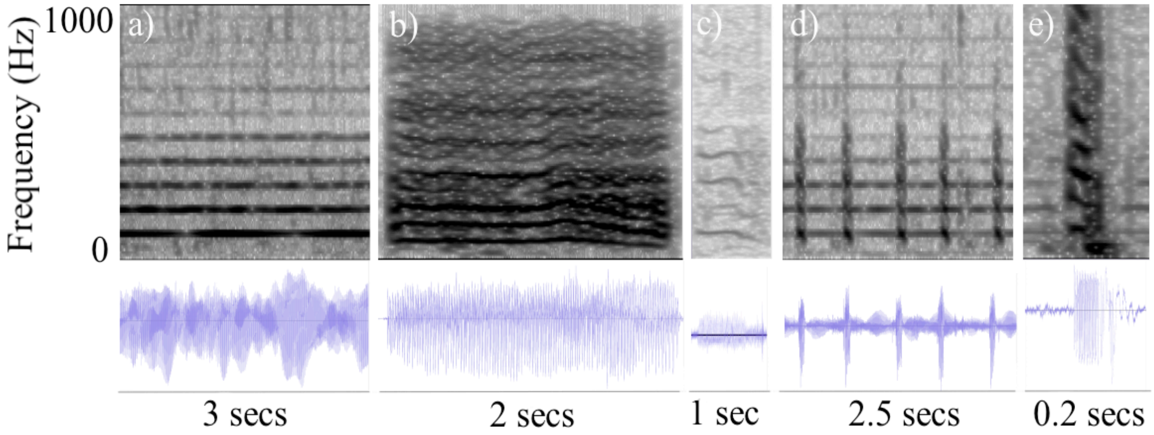

Figure 3.2 Spectrograms (above) and oscillograms (below) of different *P. notatus* calls: a) hum; b) growl; c) groan; d) grunt train; e) grunt.

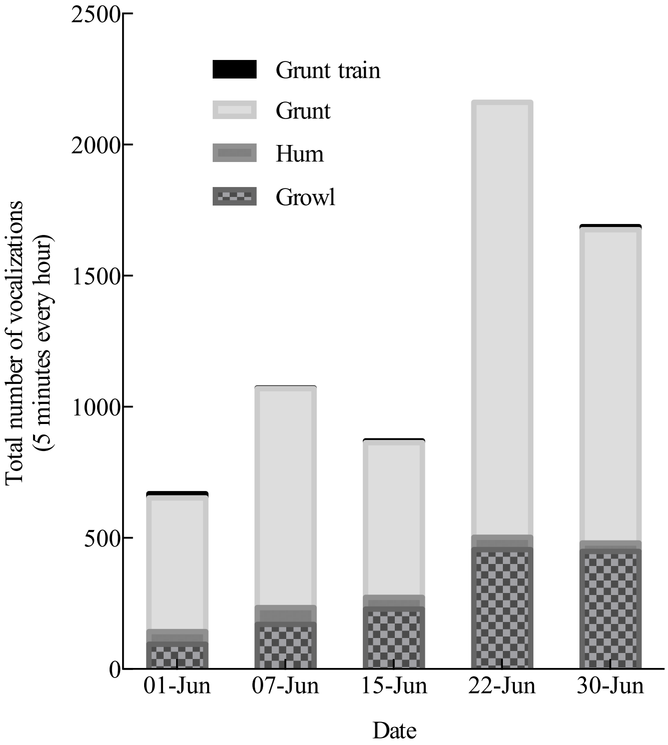

Figure 3.3 Number of vocalizations from five-minute samples from each hour, across all five dates in June.

Grunts, growls and hums were detected on each date examined (Fig. 3.3), at every hour of the day (see, for example, grunts in Fig. 3.4). (Note, these data represent two-hour samples taken from the first five minutes of each hour, over 24 hours.) Calls increased in number around 4am, peaked between 5-7am (31% of all vocalizations occurred during this period), and diminished throughout the day with lowest numbers between 10pm-3am (Fig. 3.4). Total vocalizations (again, from the 24 five-minute samples) ranged from 668 on June 1st to 2,153 on June 22nd. Grunt trains were detected on each date except June 22nd. Grunts were the most common vocalization detected with 4,789 events, followed by growls (1,397 events), hums (236 events) and grunt trains (36) for a total of 6,458 distinct vocalizations (Fig. 3.3; for full data table, see Appendix 3.1). Grunts and growls peaked at 6am, while hums were heard most often at night, and grunt trains appeared to occur randomly.

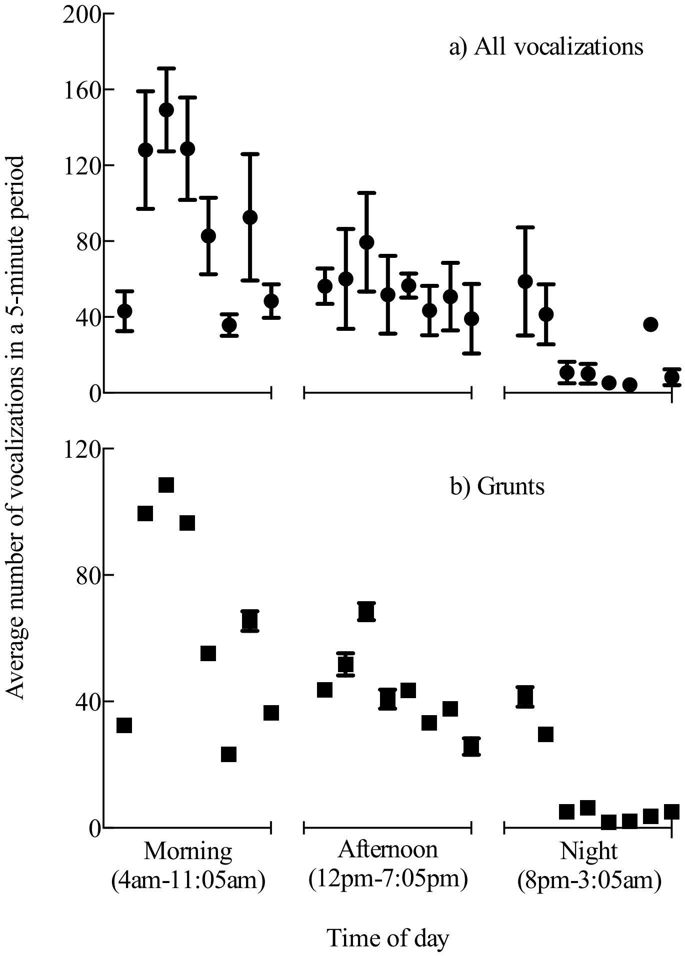

Figure 3.4 Average number of vocalizations in the first 5-minutes of each hour of the day across all five dates in June for a) All vocalizations and b) Grunts; bars represent standard error. 24-hour day is broken into three categories: Morning, Afternoon and Night.

Call length varied by vocalization type (Fig. 3.5). Hums averaged 171.4 seconds long (SE ± 7.3, n = 236), growls 1.4 seconds (SE ± 0.0, n = 1,397), grunt trains 37.3 seconds (SE ± 6.2, n = 36), and grunts 0.1 seconds (SE ± 0.0, n = 4,789). Figure 3.6 depicts circadian fluctuations in call duration, with longest calls occurring at night.

3.3.2 Behavioural study – Function of the grunt

Between 10pm-2am, in 60 hours of recording across 15 nests and 15 dates, I documented 602 predators (Fig. 3.7; mean ± SE: 40.1 ± 8.8, n = 15; Table 3.1). I also recorded 56 grunts, eight of which were made *unintentionally*, or as a byproduct of another activity (Kasumyan, 2008; Barber, Crooks, & Fristrup, 2010), here observed during nest care behaviours. Another seven grunts were *unspecified*, referring to grunts elicited for unknown reasons: in cases here, possible sources of incitement were out of sight.

The remaining 41 grunts were associated with *grunges*: here for the first time I describe the grunge, a grunt coupled with a forward lunging motion towards a visible predator. Four predator taxa elicited grunge behaviour: red rock crab (31 grunge events), helmet crab *Telmessus cheiragonus* (1 grunge event), gunnel spp. of family Pholidae (3 grunge events), and sculpin spp. of family Cottidae (6 grunge events); Table 3.1. Note, of the 41 grunge events, there were six occasions where *P. notatus* grunged two to three times during the same predator encounter (Table 3.1). Grunge events occurred only for a small proportion of all predator visits: in total there were 249 predator visits from red rock crab, 118 from gunnel spp., 102 from sculpin spp., and 6 from helmet crabs. Other taxa that visited nest sites but did not elicit grunge behaviour here were: harbour seals *Phoca vitulina*, seastar spp. (sunflower star *Pycnopodia helianthoides*, ochre star *Pisaster ochraceus*, mottled star *Evasterias troscheli*, and leather star *Dermasterias imbricata*), coonstripe shrimp *Pandalus danae*, crangon shrimp *Crangon spp.*, shiner perch *Cymatogaster aggregata*, striped perch *Embiotoca lateralis*, whitespotted greenling *Hexagrammos stelleri*, black-clawed crab *Lophopanopeus bellus*, northern kelp crab *Pugettia producta*, shore crab *Hemigrapsus spp.*, hermit crab (superfamily Paguroidea), and Pacific snake prickleback *Lumpenus sagitta* (see Table 2.1, Chapter 2, for list of all potential predators). Thus, grunge responses from *P. notatus* occurred in 5.6% of all predator encounters observed, or in 7.2% of all encounters with predators documented to elicit grunge responses here.

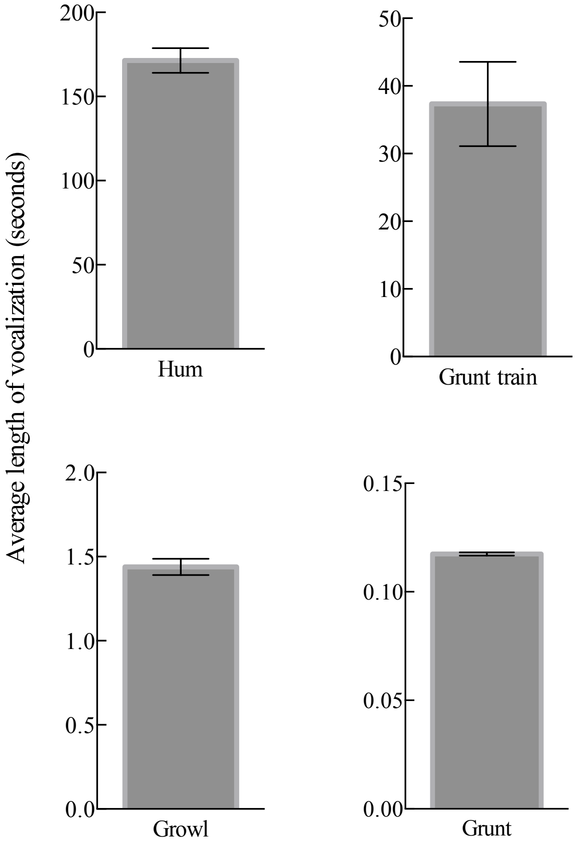

Figure 3.5 Average length of vocalization across all dates by call type: hum, grunt train, growl and grunt.

All documented grunge events were directed at heterospecifics: in the three events where other *P. notatus* visited nest sites, no vocalizations occurred. On average, over the 15 days analyzed here, *P. notatus* displayed 3.7 grunt events between 10pm and 2am (SE + 1.05, n = 15) and 2.7 grunges (SE + 0.88, n = 15); Table 3.1. Of the 41 grunge events, 39 ended with the predator leaving the *nest area*- defined as within the camera's field of view- immediately, i.e., within seconds. In 32 events, the predator fled after one grunge, while it took two grunges on four occasions for the predator to flee, and a further three grunges on two other occasions to repel the predator. In the last two cases, where grunges did not prove immediately successful, predators did eventually leave the nest area without ever penetrating nests. Therefore, grunges were *effective*, defined as causing a predator to flee the nest area immediately, 94% of the time noting that six events involved multiple grunges (SE + 4.10%, n = 34), or in 76% of all grunge events including encounters with predators eliciting multiple grunges (SE + 6.79%, n = 41; Table 3.1).

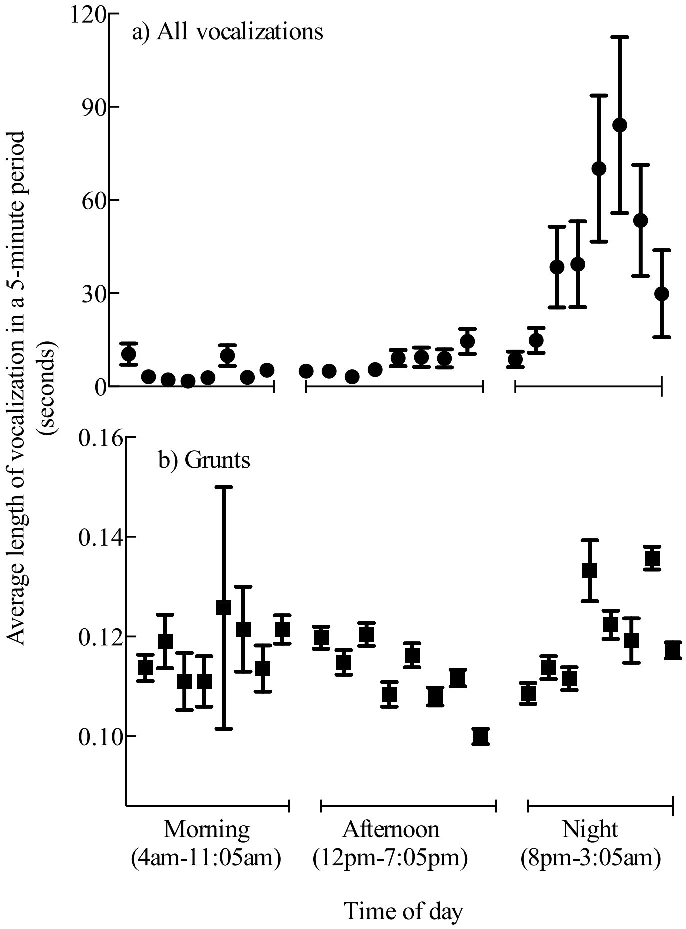

Figure 3.6 Average length of vocalizations in the first 5-minutes of each hour of the day across all five dates in June for a) All vocalizations and b) Grunts; bars represent standard error. 24-hour day is broken into three categories: Morning, Afternoon and Night.

Across all videos, only seven predators, all of different species, were observed to successfully enter *P. notatus* nests, while a further seven predators retreated into a narrow

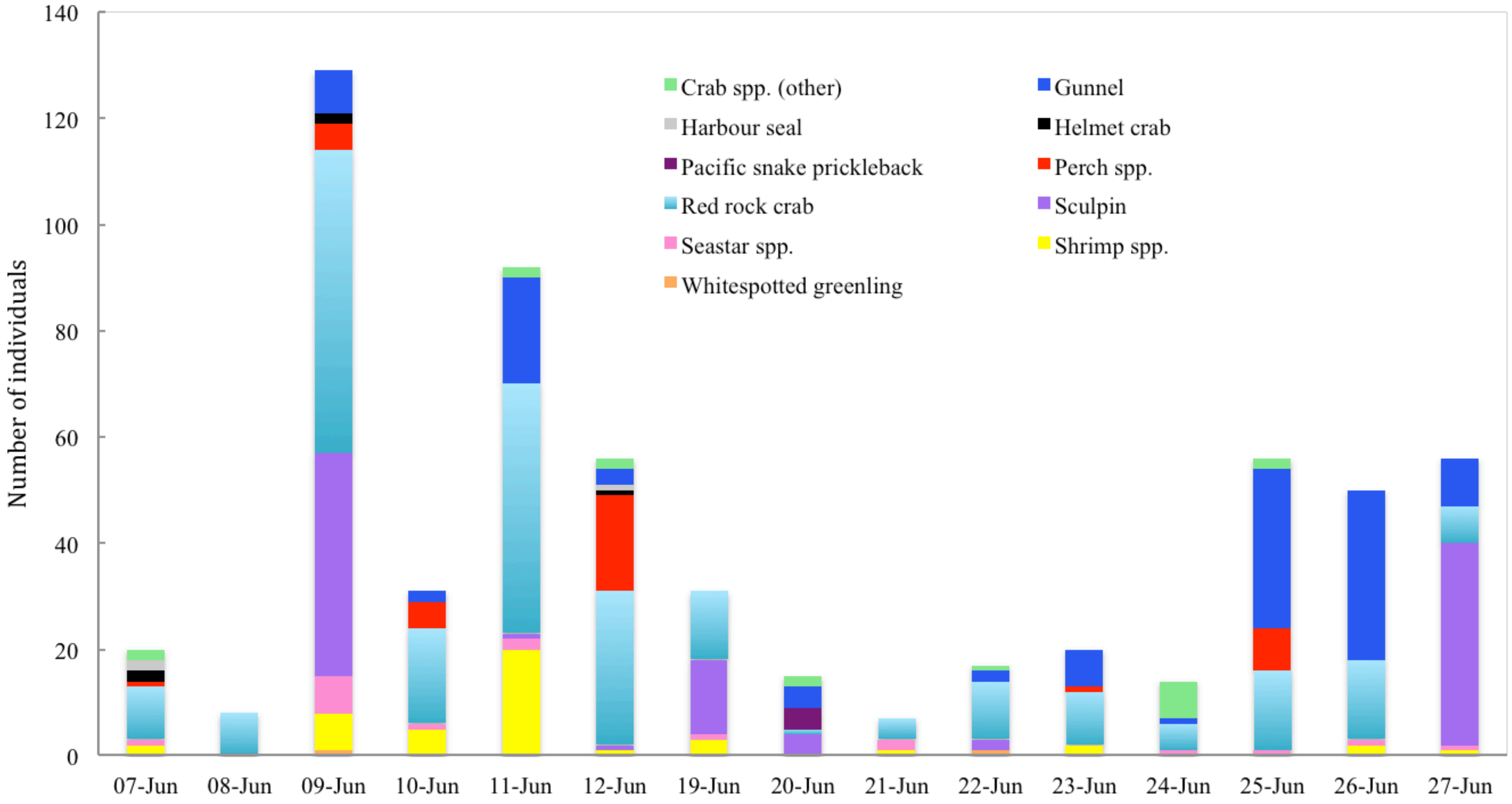

Figure 3.7 Abundance of all predator groups observed over the 15 dates of investigation. Perch spp. include shiner perch, striped perch, and unidentifiable perch spp.; Seastar spp. include mottled seastar, ochre star, sunstar, leather star, and unidentifiable seastar spp.; Shrimp spp. include crangon shrimp, coon stripe shrimp, and unidentifiable shrimp spp.; Crab spp. (other) include black-clawed crab, hermit crab, northern kelp crab, shore crab, and unidentifiable crab.

area of the nest inaccessible to *P. notatus* (Appendix 3.2). No grunges were elicited in any successful nest penetration events; however in four of the seven cases the predator eventually left the nest following other defensive behaviours employed by *P. notatus* (see Appendix 3.2).

Across predators, grunts emitted during grunge events lasted on average 0.55 seconds (SE ± 0.09, n = 41). Grunts were longest for red rock crab (mean ± SE: 0.68 ± 0.11, n = 31), followed by helmet crab (0.2, n = 1), then sculpin (0.18 ± 0.02, n = 6), with gunnels eliciting shortest average grunts (0.07 ± 0.02, n = 3); Appendix 3.3. Adventitious grunts emitted during nest care behaviours averaged 0.1 seconds (SE ± 0.03, n = 8); Figure 3.8. Crab species and fish species, respectively, were lumped together to increase power. A Fisher's exact test revealed crab were significantly more likely to elicit a grunge reaction than fish ($p < 0.01$, n = 516 total events; Fig. 3.9). A Kruskal-Wallis test showed a significant difference in grunt duration between Crab, Fish and Adventitious groups (H = 22.8, $p < 0.0001$), and a Dunn's post-hoc test revealed significant differences between Crab and Fish as well as Crab and Adventitious groups ($p < 0.001$; Fig. 3.8). A two-tailed Mann Whitney test showed significance between Crab and Fish groups, as well as between Crab and Adventitious groups ($p < 0.0001$); Crab versus Fish results are represented in Fig. 3.10.

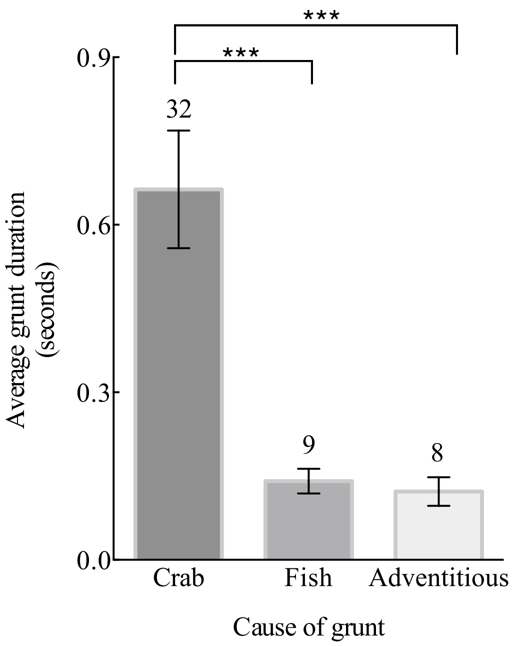

Figure 3.8 Average duration of grunt by provoking predator group (Crab = red rock crab + helmet crab; Fish = sculpin spp. + gunnel spp.; Adventitious = unintentional grunts emitted during nest care). Numbers above columns denote N, black bars represent SE, and asterisks represent significance (*** = $p < 0.001$).

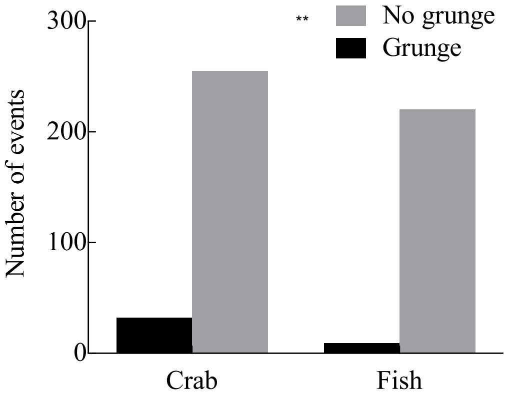

Fig. 3.9 Proportion of predator encounters eliciting grunge events. Asterisks denote significance of Fisher's exact test (** = p < 0.01).

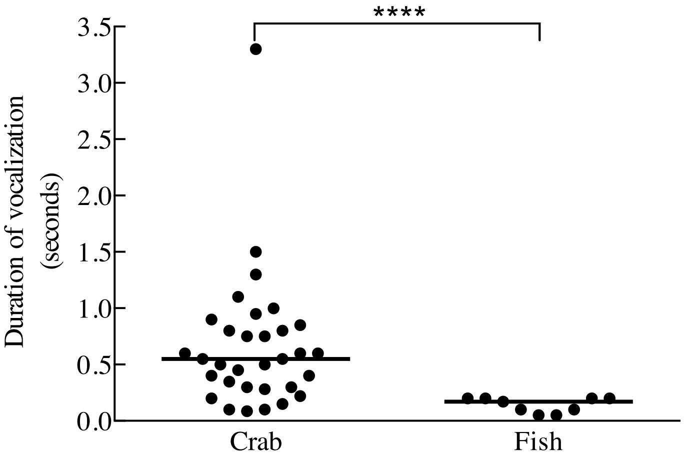

Figure 3.10 Scatterplot showing calls are significantly longer for Crab (red rock crab and helmet crab) than for Fish (gunnel spp. and sculpin spp.), Mann Whitney test, $p < 0.0001$ (significance represented by asterisks).

Table 3.1 Grunge events observed over 15 dates, where Total predators = total number of predators in camera's field of view observed between 10pm-2am; #, species of predators = number of each species of predator to elicit grunge. Shaded cells denote occasions with two or three grunge events directed at a single predator.

Date	Total predators	Grunts	Grunges	Effective grunges	#, species of predator
07-Jun	20	10	3	2	3 red rock crab
08-Jun	8	1	0	0	0
09-Jun	129	7	2	2	1 gunnel, 1 red rock crab
10-Jun	31	0	0	0	0
11-Jun	92	4	4	4	4 red rock crab
12-Jun	56	9	7	7	6 red rock crab, 1 helmet crab
19-Jun	31	0	0	0	0
20-Jun	15	0	0	0	0
21-Jun	7	1	1	1	1 red rock crab
22-Jun	17	2	2	1	1 red rock crab
23-Jun	20	12	12	6	6 red rock crab
24-Jun	14	0	0	0	0
25-Jun	56	1	1	1	1 red rock crab
26-Jun	50	3	3	3	2 gunnel, 1 red rock crab
27-Jun	56	6	6*	4	5 sculpin
Total	602	56	41	31	
Average	*40.13*	*3.73*	*2.73*	*0.94***	
SE	*8.84*	*1.05*	*0.88*	*0.04*	

*There was also one isolated event in which *P. notatus* lunged at a sculpin without grunting; in this event, the sculpin departs.

**This represents 32/34 grunge events.

3.4 DISCUSSION

3.4.1 Passive acoustics- Characterizing calls and observing patterns

This two-pronged study shed light on how *P. notatus* uses sound in its natural habitat. The analyses on longterm passive recordings revealed that fish contribute considerably to this coastal soundscape: in total, in 10 hours of audio across all five dates in June, close to 6,500 *P. notatus* vocalizations were detected, encompassing all four main calls (Fig. 3.3). *Porichthys notatus* was the primary source of biophony in all 120 audio files analyzed, detected at every hour of the day and night, thus disproving previous ideas that *P. notatus* vocalizations are purely nocturnal (Ibara *et al.*, 1983; Brantley & Bass, 1994). Even hums, previously thought to occur only in the evening (Bass, 1996) were here detected 24 hours a day, but with definite peaks at night (Fig. 3.6). Patterns in call numbers, i.e., peak and low times, coincided with the type of call detected during those periods: grunts were most abundant early in the morning, while hums were the dominant vocalization at night (Figs. 3.4, 3.6). Therefore, as grunts are shorter than hums (Sisneros & Bass, 2005), Figures 3.4 and 3.6 show an inverse relationship between the number and length of calls: as the average number of vocalizations increase, the average length decreases. This is because the longer calls - hums - peak during the night.

In the evening when many *P. notatus* hum concurrently, their hums form choruses and create *acoustic beats* (Bass, Bodnar, & Marchaterre, 1999; Webb, Fay, & Popper, 2008) which dominate the soundscape. In my recordings therefore, if other vocalizations were present during those peak chorusing times, they were likely masked by the hum, thus grunts or growls were very rarely detected here during peak humming periods. Further, it was also not possible through spectrogram analysis to detect where one hum began and another ended, or how many hums were present at one time. Contrary to all other calls therefore, the number of hums presented here (Figs. 3.3, 3.4) is not a meaningful measure. While *P. notatus* is undoubtedly dominating the local soundscape during these chorusing events, measuring humming output in ways beyond call counts would yield more informative data.

All four vocalizations- hum, growl, grunt, and grunt train- were present on the first date of recording (April 22nd, 2012) as well as the last (August 16th, 2012); as hums are associated with spawning (Brantley & Bass, 1994), this suggests *P. notatus* reproduces over at least five months, and likely longer. Along the coasts of California and Washington, the spawning period is estimated to last from late spring until late summer, though limited information exists on exact start and end times (DeMartini, 1990). Evidence for further call diversity was detected here with a new type of vocalization, the *groan*: audibly most similar to the growl but with unique spectral characteristics (Fig. 3.2), the groan has been alluded to anecdotally (Fish, 1948) but never previously characterized in the literature. The groan's presence suggests *P. notatus* calls are even more complex than previously thought (similar to Gulf toadfish; Thorson & Fine, 2002), and warrants further inspection, particularly as it relates to a specific behaviour (e.g., defense, alarm call, etc.). Here, for the purpose of this study, groans and growls were grouped together.

On June 1st, 15th and 30th of 2012, the tides were small, with very little variation between highs and lows. However on June 7th and 22nd, big tides were present: very high tides occurred early in the morning (~3am) and early evening (~6pm), and very low tides occurred late morning (~10:30am). From these data there did not appear to be a clear correlation between number of vocalizations and tidal cycle. For example, while a secondary peak in vocalizations occurred on June 22nd at 10am and a small peak on June 7th at 11am, secondary peaks were also seen at 10am on June 1st and 15th (i.e., dates with small tides). However it should be noted that some of the larger tides occurred in the evening, when chorusing obscured other calls and could have impacted call counts as discussed above.

Weather varied over recording dates and within each 24-hour period analyzed, with light rain detected in some recordings. Other identifiable sources of noise which could have obscured detection of *P. notatus* calls included waves, bubbles, boats, marine animals, and the dock under which the recordings were made. Dock creaks and slapping of water could have contributed to false detections, as these sounds sometimes shared spectral characteristics with the grunt. Nevertheless, typical *P. notatus* calls, especially hums, growls and grunt trains, were easily distinguishable from other sources of noise, and had obvious start and stop times (except for hums, for aforementioned reasons relating to chorusing).

Passive acoustic recordings were useful in establishing the number and type of vocalizations in this soundscape. However, individual callers were not isolated. Fish identification based on call characteristics has been achieved before in studies on Gulf toadfish: Thorson and Fine (2002) recorded calls of Gulf toadfish *Opsanus beta* from the field over three hours. While they did not have video to corroborate their findings, they assumed the amplitude of call harmonics was distinct and constant enough to assign ownership of calls to individual fish, and that individuals did not move. Observations from *P. notatus* in aquaria support this work, describing individual differentiability through calls

(Fish, 1948). Future work on individual recognition of *P. notatus* in passive acoustic recordings would add data on community size, conspecific call variation, and potentially, physical attributes of callers (e.g., body size; see Parmentier *et al.*, 2013).

Another area of suggested future research regarding the passive acoustic study is around quantifying detectability of *P. notatus* calls at this field site, an issue discussed in the passive acoustic study on Gulf toadfish (Thorson & Fine, 2002). At my field site, the substrate near the hydrophone was mostly sand bottom, with two rock islands approximately 10 m and 120 m away, respectively (Fig. 2.2, Chapter 2). Further investigations still might reveal how much other sources of ocean noise, such as waves, rain, and boats, could contribute to call masking. Additionally, it is unknown how many *P. notatus* were present in the area (although a day dive in July 2011 suggests high numbers: 15 *P. notatus* were viewed between the dock and the nesting site, on a line transect of approximately 100 m x 4 m), and over what range. Without underwater cameras, it was not possible to deduce with any certainty how many vocalizations were made from the same fish, although 'near' versus 'far' calls were indicators. An added SCUBA component could bolster this research by estimating through visual surveys the number and range of *P. notatus* in the area.

Passive acoustics offered an effective tool for the identification and characterization of *P. notatus* calls at this field site. They also set the backdrop for the behavioural component of this research: as grunts were here discovered to be the most abundant call, their context and purpose became the focus of the next step of this project, the behavioural study.

3.4.2 Behavioural study- The grunge and interspecific communication

Fish can send and receive visual, chemical, electric and acoustic signals (Sluijs *et al.*, 2010); using these same modalities, they can also communicate with heterospecifics. Most such examples involve the conveyance of information through visual systems, e.g.: groupers opening mouths to attract cleaner fish (Trivers, 1971), sunfish resting at the surface of the ocean to attract parasite-extracting albatross (Abe *et al.*, 2012), or sculpin avoiding trout based on their size (Chivers *et al.*, 2001). Examples of chemical communication across heterospecifics include prey fish releasing disturbance signals (summarized in Brown, Ferrari, & Chivers, 2011), detecting predators via olfactory cues (Dixson, Munday, & Jones, 2010) and sensing pheromones from feces (Brown, Chivers, & Smith, 1995). Lesser documented are examples of heterospecific electric communication, but evidence suggests fish change their behaviour in response to electric signals from other species (e.g., electric knifefish *Apteronotus* spp. increased chirp rates in the presence of other electric fish species; Dunlap, DiBenedictis, & Banever, 2010). Finally, fish are known to communicate vocally with heterospecifics in aggressive circumstances (Salmon, 1967; Mann & Lobel, 1998; Tricas & Boyle, 2014).

The plainfin midshipman has been previously documented to communicate visually with heterospecific predators: it assumes defensive threat postures to intimidate predators and deter them from entering their nests (Arora, 1948; *pers. obs.*). This posture includes open mouths, splayed fins, and direct confrontation (similar to the Lusitanian toadfish *Halobatrachus didactylus*; Vasconcelos *et al.*, 2010). They are also believed to camouflage themselves from predators by means of photophores which create counter-illumination when viewed from below (Harper & Case, 1999). To my knowledge, this is the first study to quantitatively examine vocal communication of *P. notatus* with heterospecific predators.

Through this *in situ* study I clarified the behavioural motive behind *P. notatus*' predominant call, the grunt. The majority of grunts captured on video were associated with the grunge, a coupled grunt and lunge response enacted to deter heterospecific predators from venturing too near *P. notatus* nests (Table 3.1). When implemented, the grunge was effective at causing the predator to flee immediately: 32 out of the 34 predators fled the nest area immediately post grunge, noting that six fled after more than one grunge.

Nevertheless, with over 600 predator visits documented in the vicinity of the 15 nests sampled, it could be *P. notatus* reserved the grunge only for urgent or high-risk encounters: compared with the number of predator visits, *P. notatus* rarely implemented the grunge. Given its effectiveness, and the limited number of times it was captured over 10 hours, it could be assumed that the grunge is energetically expensive: indeed, the production of underwater calls alone is costly (Ladich, 1990), and as alpha *P. notatus* do not generally eat over several months while nesting (Arora, 1948; Sisneros *et al.*, 2009; similar to other species, such as the European bullhead; Bisazza & Marconato, 1988), the effect could be further exacerbated, especially when considered alongside their daily nest cleaning activities. While sound production in the oyster toadfish *Opsanus tau* is less costly than previously assumed due to the small size of contracting sonic muscles and the short length of the call (Amorim, Mccracken, & Fine, 2002), *P. notatus* hums last much longer and are known to be energetically expensive (Amorim, Mccracken, & Fine, 2002; Sisneros *et al.*, 2009). Likewise, while grunts are shorter, territoriality against heterospecifics in general is energetically taxing (Myrberg & Thresher, 1974). Here, noting the average number of grunts per hour (4.1; Table 3.1) and the additional physical requirement of lunges, *P. notatus* grunges are quite likely to be energetically costly, and thus reserved only for more threatening encounters.

Such ‘higher threat’ encounters include events where predators physically crossed into nest territory: as in a study on three-spot damselfish *Eupomacentrus planifrons*, intruders here were seemingly ignored until they crossed nest boundaries (Myrberg & Thresher, 1974). Indeed, one reason grunge numbers were so low likely relates to the effectiveness of *P. notatus*’ presence: in the 60 hours of video reviewed here, only seven predators were observed to successfully enter the main part of *P. notatus*’ nest; Appendix 3.2. In one event, a black-clawed crab enters the nest after a harbour seal causes *P. notatus* to hide; once the harbour seal leaves, *P. notatus* resumes its defensive posture facing the crab, and the crab departs. In another event, *P. notatus* directs a defensive posture towards a gunnel, and the gunnel departs. In the two cases involving sea stars, while the mottled star succeeded in entering the nest uninterrupted and stays attached to the nest ceiling, the arm of an ochre star gets nipped by *P. notatus* and the ochre star immediately responds by leaving the nest area. Finally, when a red rock crab appears to successfully enter *P. notatus*’ nest, a piece of wood is blocking the nest entrance, which could have provided a barrier between the crab and *P. notatus*; the crab, which was missing a claw and some legs, eventually departs on its own. In the last three examples of successful nest penetration by intruders, *P. notatus*’ response was unclear due to poor camera view.

Porichthys notatus eggs usually number several hundred per nest but can reach over a thousand. Such large numbers of eggs suggest high predation rates (Crane, 1981), corroborated by this study showing on average 40 predator visits per nest over a period of four hours (Table 3.1). Given the well-documented consequences of unguarded nests in species with paternal care including *P. notatus* (Arora, 1948; Bisazza & Marconato, 1988), it is safe to assume that without the presence of a nest-guarding *P. notatus*, many more predators would have entered nests and consumed eggs. In a study on bluegills *Lepomis macrochirus*, a freshwater fish with paternal care, significantly more predators were caught from unguarded nests than from guarded nests, and larval survivorship was significantly lower in unguarded nests (Bain & Helfrich, 1983). Correspondingly, in an exploratory video analysis conducted in 2012 at the same field site on Quadra Island, a nest-guarding *P. notatus* is suddenly seen being seized from its nest by a foraging river otter *Lontra canadensis*; less than three minutes later a juvenile whitespotted greenling appears, who begins ripping eggs from the nest and consuming them. A few minutes later it is joined by another juvenile whitespotted greenling to share the feast. Later on, a red rock crab enters the nest and is observed pulling eggs off the nest ceiling with its claws; the next morning, all *P. notatus* eggs were gone. This anecdotal evidence underscores the importance of paternal care in nest protection and egg development. Taken together, my data show that the physical presence of *P. notatus*, which often included a defensive posture (e.g., 'displays'; Cooke *et al.*, 2008), was enough to deter potential predators in most circumstances; the grunge was reserved only for predators who directly attempted to cross nest boundaries. Similarly, Ongarato and Snucins (1993) revealed smallmouth bass *Micropterus dolomieui* are overwhelmingly more likely to show aggression towards predators at the entrance of their nest than towards predators at other distances.

It must be noted that other predator species not listed in this study likely elicit grunge reactions as well, including land-based predators. For example, during camera setup at low tide, northwestern crows *Corvus caurinus* were witnessed pecking at exposed nest-guarding *P. notatus*, and provoking grunts (it was not possible from where I was to determine lunge response). It is also known that fish, including *P. notatus*, emit grunts when handled/prodded by people (Arora, 1948; Brantley & Bass, 1994; Ladich, 1997b). Further, while this study suggests the majority of potential predator species documented here do not elicit responses from *P. notatus*, in other circumstances they might: in video collected at this same field site in 2012, juvenile whitespotted greenling were seen provoking numerous grunges from *P. notatus* while trying to steal eggs from its nest; here, these fish were never captured eliciting grunges. Therefore, grunt emission must be situation-dependent and determined by perceived threat, which would include predator proximity.

Predator proximity to *P. notatus* nests was not measured here. In this study, any potential intruder within camera view was counted, as it was difficult to approximate how close a predator must be to the nest to elicit a grunge response. Low grunge numbers could also therefore be explained by the large number of predator species included in this analysis, as opposed to only those close enough to nests to elicit responses. Consequently, all data were further reviewed to obtain a provisional estimate on predator detectability by *P. notatus*; this was done by visually interpreting the relative location of predator to the nest entrance while considering any obstructions (e.g., something that inhibits *P. notatus*' ability to detect it). These rough approximations revealed that approximately 40% of predator appearances listed here may not have been close enough to *P. notatus* or within its view to be in the range of grunge behaviour. In a study on three-spot damselfish, Myrberg and Thresher (1974) estimate maximal distances of aggression towards intruders; they found heterospecifics were allowed to get much closer to nests than conspecifics before being attacked. It is possible that *P. notatus* alters its aggressive response depending on predator species; indeed its response to marine mammals differs drastically from its response to other predators (more on this below).

In this study, various factors made simple approximations- e.g., 'within 10 cm'- difficult to employ: for example, a predator could be within 5 cm of the nest entrance but blocked from *P. notatus*' view by a natural obstruction (e.g., a rock), thus potentially interfering with *P. notatus*' ability to perceive it. Distance between camera lens and nest entrance was also particularly difficult to gauge (a topic further discussed in Chapter 5), meaning predators could have been closer or farther from the nest than they appeared. Individual *P. notatus* variation could also be a factor: while most *P. notatus*' kept the majority of their bodies inside the nest while lunging, one was observed to lunge so far out its entire body left the nest (June 23rd, 2013). It was for these reasons that all predators within the camera's field of view were documented as potential nest intruders.

Grunts were only ever directed at heterospecifics. While lab-based studies on *P. notatus* suggest grunts are used exclusively in aggressive encounters with conspecifics (Brantley & Bass, 1994), here in the field no such examples were observed (note: only three encounters with conspecifics were documented). Similarly, a study on soldierfish showed agonistic calls were emitted primarily towards heterospecific predators and less towards intruders of the Holocentridae family (Salmon, 1967). In their field study, Mann and Lobel (1998) describe the ability of damselfish to distinguish conspecifics from heterospecifics, as reflected by the number of aggressive sounds enacted towards intruders. Myrberg and Thresher (1974) also reports evidence of heterospecific recognition by damselfish as determined by proximity to nests before attacking occurred (note, all intruders were fish species).

I focused on temporal aspects of call differences, which are considered to be the biggest source of import in acoustic fish communication (Vasconcelos *et al.*, 2011). Findings here reveal grunts vary between predator taxa groups: crabs evoke more and longer grunts on average than fish (Figs. 3.9, 3.10), and elicit more call variation (specifically, red rock crab; Appendix 3.3). This indicates an ability of *P. notatus* to distinguish between heterospecific predators, and modify calls based on intruder. Further evidence of predator recognition relates to observations suggesting that agonistic calls might be suppressed around predators targeting adult forms of *P. notatus* as opposed to eggs, and when other tactics such as hiding are available. For example, as documented here and in previous exploratory research at same field site, when river otters or harbour seals neared nests, *P. notatus* was observed to retreat deeper into nests and remain still. What's more, *P. notatus* vocalizations were never documented in the presence of these large predators; instead, *P. notatus* hid and remained silent to avoid detection and consumption. This reaction differed markedly from encounters with other *P. notatus* predators who targeted eggs, in which case *P. notatus* would turn to face them directly. This effect was found in another species of toadfish: in response to low frequency foraging sounds emitted by predatory bottlenose dolphin *Tursiops truncatus*, Gulf toadfish stopped vocalizing by 50% (Remage-Healey, Nowacek, & Bass, 2006). Likewise, Pacific herring *Clupea pallasii* were found to change swimming behaviour in response to predatory Odontocetes, forming schools, increasing swimming speed and dropping in the water column (Wilson & Dill, 2002). The hiding response in *P. notatus* was also seen in reaction to oar paddle noise, which mimics the sound of otters and seals splashing at the water's surface (a phenomenon further discussed in Chapter 5).

It is possible that grunt variation could be an indicator of predator size. However, while crab predators are more likely to provoke grunges (Fig. 3.9) and elicit longer and more complex calls (Figs. 3.8, 3.10, Appendix 3.3) and are typically much larger than fish at this field site, preliminary observations indicate predator size within species might not affect grunge response. This conclusion was also reached in a field study on three-spot damselfish, which measured physical agonistic behaviours in response to heterospecifics (Myrberg & Thresher, 1974). Future studies on *P. notatus* that can quantify predator size could investigate the potential relationship further.

Beyond call length, call repetition carries important information about signaling events, such as context of emission (Ladich, 1997a). Here, in the six cases where more than one grunge was directed at a single predator, only one was directed at a sculpin while the other five targeted red rock crab; further, red rock crab was the only species ever documented to elicit more than two grunges at one individual. This suggests red rock crab might be a more threatening predator to *P. notatus* than fish, or that it is more difficult to deter (in line also with more calls being provoked by crab). Despite this trend, the difference between Crab and Fish groups regarding call repetition was not significant (Fisher's exact test, $p > 0.05$; crab and fish species were grouped, respectively, to increase statistical power). Documentation of more grunge events is therefore needed to determine any relationship between predator type and grunt repetition.

Call frequency was not investigated here as a source of grunt variation as unlike call duration (Fine, 1978), it varies with water temperature (Brantley & Bass, 1994; Bass, Bodnar & Marchaterre, 1999), a parameter not collected from each nest in this study. Call amplitude was also not explored, as the distance between the hydrophone and each *P.*

notatus nest during grunt emission could not be accurately estimated. Both these factors might offer useful information in future studies on how this species perceives and interprets complex calls (Vasconcelos *et al.*, 2011).

Of the 56 grunt events documented, eight fit the adventitious category, i.e., sounds emitted accidentally or as a consequence of other activities (Mann & Lobel, 1998; Kasumyan, 2008; Barber, Crooks, & Fristrup, 2010; Colleye & Parmentier, 2012; Appendix 3.3). Seven of the adventitious grunts were emitted here during egg care when *P. notatus* was mouthing its eggs, likely cleaning them (Keenleyside, 1991, cited in Green & McCormick, 2004; Lissåker & Kvarnemo, 2006). The last adventitious grunt was produced when *P. notatus* attempted to remove a large rock from its nest, an effect previously observed in damselfish in similar circumstances (Mann & Lobel, 1998; Colleye & Parmentier, 2012). In video footage from this field site but not included in the analysis, *P. notatus* was also observed to produce adventitious grunts while attempting to dislodge seastars; conversely, seastars never appeared to incur directed grunts, unlike crab or fish (Table 3.1).

Unlike adventitious grunts, the grunt produced during grunge events is almost certainly intentional: there was only one documented event across all individuals when *P. notatus* lunged without vocalizing, whereas all visible grunts directed at predators occurred simultaneously with lunging, though to various degrees. This corresponds with findings from a study on cichlid fish *Tramitichromis intermedius* that showed quivering during courtship rarely occurred without vocalizing, but vocalizing always occurred with quivering, suggesting sound emission was intentional (Ripley & Lobel, 2004). In addition, many grunts outlasted the lunge (i.e., the grunt continued after *P. notatus* stopped moving) and were not typical of adventitious sounds associated with nest cleaning (e.g., short burst signals, Mann & Lobel, 1998; Appendix 3.3). Adventitious grunts showed little signal variation: those emitted during egg care were always 0.1 seconds in duration (mean ± SE: 0.1, ± 0.0, n = 7), while the one emitted during rock removal was only slightly longer at 0.3 seconds. In contrast, significant variation in grunt duration across predator groups provides evidence that grunts are modified to suit intruders (Fig. 3.8).

Despite being characterized as adventitious, in some cases adventitious sounds are thought to have a signal function (Kasumyan, 2008). Future research with longer datasets could investigate further the connection between nest care and grunts, for example, and help to quantify the number of unintentional versus intentional (directed) grunts.

As the grunt and lunge occur simultaneously during grunges, both are likely required to deter predators effectively: it has been documented that acoustic signals (e.g., grunt) can strengthen physical acts of aggression (e.g., lunge) by relaying information about the signaler (such as body size; Connaughton, Taylor, & Fine, 2000; Lobel, Kaatz, & Rice, 2010). Such behaviour is advantageous, particularly when other methods of defense are limited (e.g., body protection; Patek, 2001). However in a study on damselfish, visual displays were determined to be more important than acoustic behaviours in deterring intruders (Myrberg, 1997), and acoustic signals may only work alongside other signals (e.g., visual, chemical, etc.; Estramil *et al.*, 2014). Nevertheless the presence of the fish itself coupled with an acoustic threat is likely what creates an effective predator deterrent (Pereira *et al.*, 2014). Future work separating visual and acoustic components of the grunge behaviour would help to elucidate the importance of vocal mediums in *P. notatus* communication.

This study describes the grunt as the vocal component of the grunge. However, Appendix 3.3 shows grunges are composed of sounds that vary in signal composition, and that two calls documented here are categorized as growls. How grunts and growls differ in an ecological context is not known: to date, both have been linked with agonistic scenarios (Brantley & Bass, 1994; Bass, Bodnar, & Marchaterre, 1999; Sisneros & Bass, 2005; Rice, Land, & Bass, 2011), but it is not known when growls might be produced over grunts, or vice versa. Appendix 3.3 shows evidence that both might be emitted in similar circumstances, i.e., to deter predators from entering nests, but that growls might be produced in response to more threatening predators: here, the two documented growls were both directed at red rock crab (Appendix 3.3). As grunt length varies between Crab and Fish groups, it could be that growls are essentially exaggerated grunts, implemented in events when a predator is deemed more threatening (or less easily deterred).

Finally, future experiments should investigate the possibility that *P. notatus* grunts might serve other functions beyond deterring predators; for example, fish calls could act as warning signals (Ladich, 1997a). Grunts could be used to alert conspecific neighbours of danger by triggering awareness (Myrberg, 1981; Petersen *et al.*, 2013), warning potential mates of predators (Tricas, Kajiura, & Kosaki, 2006). Noting the high paternity loss and turnover amongst nest-guarding males (Cogliati, Neff, & Balshine, 2013), such warning calls could further benefit the community by alerting other alpha males who might take over their nests and guard their eggs, thus increasing the likelihood of reproductive success.

3.5 CONCLUSION

Putting it all together

Few studies have examined the relationship between sound production and fish behaviour (Ripley & Lobel, 2004; Lobel, Kaatz, & Rice, 2010). Further, by investigating *P. notatus in situ*, observations on behavioural characteristics in response to natural events and common predators were made possible (Gannon, 2008). I found that the majority of *P. notatus* vocalizations - 74% - were grunts, calls most often related to heterospecific agonistic interactions. These findings challenge previous assumptions that most sound produced by fish occurs during courtship and spawning (Fay, Popper, & Webb, 2008) and suggests that vocal fish communication in antagonistic situations is much more important than previously thought. For example, on discussing fish disturbance calls (which signal alarm, fright, discomfort, irritation or aggression), Gannon (2008) states: “It is important to realize that although it is common for humans to hear these calls, most fish species produce disturbance calls only during moments of acute distress, so it is probably not common for any individual fish to produce them in nature.” Until now, the use of fish distress calls as predator intimidation tactic has only been speculation (Myrberg, 1981; Ladich, 1997b; Gannon, 2008; Lobel, Kaatz, & Rice, 2010).

Almost all research to date on *P. notatus* vocalizations has focused on the hum (e.g., Bass, 1996; Bodnar & Bass, 1997; Sisneros *et al.*, 2004, but see Mckibben & Bass, 1998). Here, I focused on the less-explored grunt, and my results suggest it is *P. notatus*’ primary vocalization. Further, *P. notatus* grunts peak at dawn and early morning. As revealed from the behavioural component of this research, the majority of *P. notatus* grunts are produced in agonistic contexts with heterospecifics, and less often, in adventitious scenarios. Taken together, these results suggest that predation pressure might be particularly high during dawn and early mornings. Such findings run contrary to other species whose calls are mostly associated with reproduction, and predominantly heard at night (Gannon, 2008; Radford *et al.*, 2008), though other fish groups are also known to have peak agonistic calls at dawn or dusk including squirrelfish, sculpin, cod, and knifejaws (Salmon, 1967; Ladich, 1997a; Luczkovich & Keusenkothen, 2007). Investigating diel foraging cycles of *P. notatus* predators could further clarify grunt patterns and call activity (e.g., Bosiger & McCormick, 2014).

Finally, organisms in their natural habitats behave differently than when placed in lab-based settings. In their study comparing vocalizations and associated behaviours of blacktail shiners *Cyprinella venusta* in lab versus field conditions, Holt and Johnston (2013) conclude that fish calls differed significantly between domains. By investigating *P. notatus* in its natural ecosystem, I was able to obtain relevant information on natural vocalizations and behaviour of a highly vocal fish.

3.6 REFERENCES

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Chapter 4: Impacts of boat noise on the plainfin midshipman and its predators

4.1 INTRODUCTION

4.1.1 Noise and fish

The rise of anthropogenic noise and its effects on marine life are becoming a global concern (Boyd *et al.*, 2011). Research to date describes significant consequences of anthropogenic noise: from cetaceans to bivalves to cephalopods, acute noise can cause disturbance, structural damage, or death (Tyack *et al.*, 2006; André *et al.*, 2011; de Soto *et al.*, 2013; Goldbogen *et al.*, 2013). A growing body of literature also suggests there are unknown and possibly serious effects of low frequency, chronic sources of noise, such as boats, which are less known and of perhaps greater consequence given their global scale, omnipresence, and projected increase (Popper, 2003; Halpern *et al.*, 2008; Slabbekoorn *et al.*, 2010; Boyd *et al.*, 2011; Ellison *et al.*, 2012; Frisk, 2012). New studies reveal sea life such as whales (Rolland *et al.*, 2012), crabs (Wale, Simpson, & Radford, 2013) and even sea slugs (Nedelec *et al.*, 2014) can be negatively effected by boat noise.

Evidence that boat noise impacts fish is also accruing. Recently, fish studies have increased and expanded from investigating only acute causes (e.g., sonar, air guns and pile driving; Popper & Hastings, 2009b) to include those more persistent sources like boats (Amoser, Wysocki, & Ladich, 2004; Vasconcelos, Amorim, & Ladich, 2007; Holles *et al.*, 2013) and wind turbines (Wahlberg & Westerberg, 2005; Thomsen *et al.*, 2006). To date, known impacts of boat noise on fish range from masked communication (Vasconcelos, Amorim, & Ladich, 2007; Codarin *et al.*, 2009) to avoidance behaviour (Schwarz & Greer, 1984; Hjellvik, Handegard, & Ona, 2008), altered orientation (Holles *et al.*, 2013), foraging (Bracciali *et al.*, 2012) and schooling patterns (Sarà *et al.*, 2007). Physiologically, effects include reduced hearing capacity (Scholik & Yan, 2002), increased heart rate (Graham & Cooke, 2008), ventilation rates and oxygen usage (Simpson, Purser, &

Radford, 2015), and increased levels of stress hormones (Wysocki, Dittami, & Ladich, 2006).

Research on the ecological impacts of marine noise pollution has begun to inform discussions on noise management and regulation (Heise & Alidina, 2012). Yet what is noticeably lacking are field studies on wild fish in natural environments and exposed to real-life conditions (Graham & Cooke, 2008; Popper & Hastings, 2009a; Slabbekoorn *et al.*, 2010). Very little is known about the effects of anthropogenic noise on fish in the wild, though these data could yield the most informative predictions regarding consequences of increasing ocean noise. To date, almost all studies on noise and fish have been conducted in the lab, with conclusions drawn from fish in confined enclosures (Popper & Hastings, 2009a), or, more rarely, in altered or semi-wild conditions (Amorim *et al.*, 2010; Vasconcelos *et al.*, 2010; Vasconcelos *et al.*, 2011; Holles *et al.*, 2013; but see Picciulin *et al.*, 2010, which was conducted in a protected area).

This study set-out to assess two things: the impact of boat noise on the plainfin midshipman, *Porichthys notatus*, and the impact of boat noise on *P. notatus* predators. This was accomplished by conducting experiments on wild, nesting fish in their natural habitat. To my knowledge this is the first field-based study on fish to investigate effects of boat noise on both predators and prey concurrently.

4.1.2 The plainfin midshipman (*Porichthys notatus)*

The plainfin midshipman is a highly vocal species of fish found along the Northeast Pacific coast (Arora, 1948). For most of its life it inhabits deep waters (up to several hundred meters; Arora, 1948), but from May-August, these fish can be found underneath rocks in the intertidal zone laying eggs and guarding their young (Brantley & Bass, 1994).

There are three *P. notatus* morphs: females, alpha males, and sneaker males. Only the alpha males produce all three main vocalizations for which this fish is known: hums, grunts and growls (Bass, Bodnar, & Marchaterre, 1999). Hums are emitted by alpha males to attract females to mate (Bass, 1996; Sisneros & Bass, 2005), while the function of grunts and growls is not well understood but thought to be associated with conspecific agonistic encounters (Brantley & Bass, 1994). All three calls are generally quite loud (~125 dB re: 1 μPa; Weeg & Bass, 2002) and emitted at a low frequency of ~100 Hz at 16°C (Sisneros, 2009; Chagnaud, Baker, & Bass, 2011).

It is not known how *P. notatus* responds to boat noise in its natural environment. Almost all experiments on *P. notatus* to date have been conducted in lab conditions with the vast majority focused on neurophysiology (see Bass, Marchaterre, & Baker, 1994; Weeg & Bass, 2002; Weeg, Land, & Bass, 2005; Sisneros, 2007; Suk *et al.*, 2009; Chagnaud, Baker, & Bass, 2011, Petersen *et al.*, 2013; but with the exception of DeMartini, 1988; Brantley & Bass, 1994; Cogliati, Neff, & Balshine, 2013). What is known is that *P. notatus* is best able to detect low frequencies (Sisneros, 2007; Alderks & Sisneros, 2011), making it particularly vulnerable to masking by marine vessels, which tend to emit lower frequency noise (Popper, 2003; Picciulin *et al.*, 2010). Further, noting its shallow proximity during summer months (a period that generally coincides with increased pleasure boating), *P. notatus* becomes more exposed to boat noise than those fish in deeper waters (although it is thought that noise can impact fish far from the noise source due to long distance transmission and low levels of attenuation underwater; Fay & Popper, 2012).

Box 4.1 How the plainfin midshipman detects sound The primary mechanism of auditory detection in *P. notatus* occurs in the inner ear. Here, *P. notatus* detects both particle motion and sound pressure (McKibben & Bass, 1999), and the swim bladder is the organ that conveys changes in pressure (Popper & Fay, 2011; Coffin *et al.*, 2014). The lateral line system, though less understood, is thought to perceive sound in the near field (i.e., "within a few body lengths from the source"), and might be used in antagonistic conspecific encounters during the mating season (Weeg & Bass, 2002).

The first part of this study aims to fill a significant knowledge gap by assessing behaviours of wild fish in response to live boat noise in a natural ecosystem.

4.1.3 Noise and predator-prey dynamics

It is well-known that predator-prey interactions can structure marine ecosystems (Werner, *et al.*, 1983; Estes & Duggins, 1995; Hixon & Carr, 1997; Worm & Myers, 2003; Creel & Christianson, 2008; Heithaus *et al.*, 2008; Hunsicker *et al.*, 2011). For fish, predation is a primary cause of mortality and energy redistribution in natural marine systems (Frank *et al.*, 2005; Hunsicker *et al.*, 2011). As a result, ecosystem-based fishery models have attempted to incorporate such dynamics into their assessments (Christensen & Pauly, 1992); however, many single-species models remain, which can present oversimplified scenarios (Pauly *et al.*, 2002; Walters *et al.*, 2005). This failure to implement relevant parameters into fisheries assessments is in part due to the relatively poorly understood community relationships, and the inherent complexity of marine ecosystems (Hunsicker *et al.*, 2011). To understand trophic relationships and predator-prey dynamics, field-based research is necessary; yet to date very few predator-prey studies have been conducted in natural habitats (Hunsicker *et al.*, 2011).

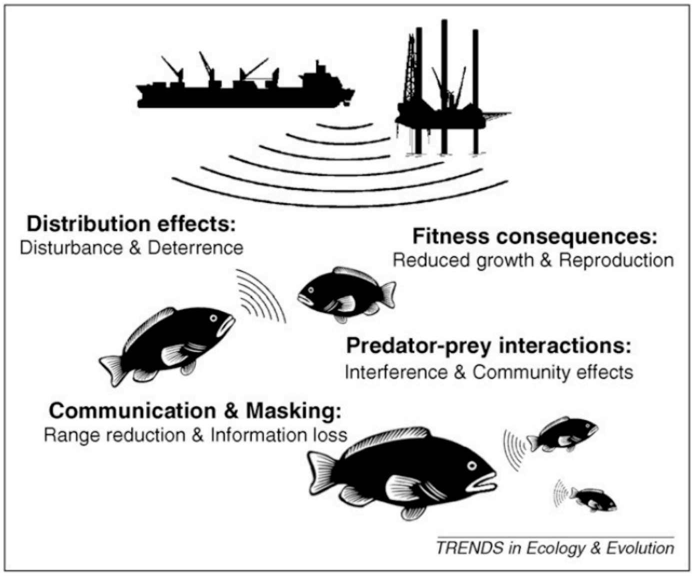

Figure 4.1 Four priority areas in need of future research regarding anthropogenic noise on fish, including predator-prey interactions. From Slabbekoorn *et al.* (2010).

When it comes to the effects of noise on predator-prey dynamics, our understanding is in its nascence. Indeed, though thought to be extensive, such effects reflect a topic generally overlooked, and "data are completely lacking in fish" (Slabbekoorn *et al.*, 2010; see Fig. 4.1). Measurement of single-species responses to noise continues to dominate research (Voellmy *et al.*, 2014a). Two recent studies looked at the effects of predators on fish: in one, a bird model simulated a predator while reactions of two tank-held fish (three-spined stickleback *Gasterosteus aculeatus*, and the European minnow *Phoxinus phoxinus*) were assessed under boat noise conditions; results showed responses to predators differed between the two species, with sticklebacks moving quicker and minnows showing no change (Voellmy *et al.*, 2014b). In the other, using lab-based and open water tests, European eels *Anguilla anguilla* were found to be more vulnerable to predators under boat noise scenarios (Simpson, Purser, & Radford, 2015). In both these studies, the effect of noise on predators, however, remained uninvestigated and is thus unknown. Predator data can be difficult to obtain from lab-based studies, as interactions depend on additional organisms and/or species, which are oftentimes unknown (M. Marchaterre, pers. comm.). Until now, research into noise effects on fish has typically been done on a simplistic, single-species and single-trophic level. Untangling how stressors affect multiple trophic levels is essential, as such complexities can lead to unforeseen consequences (e.g., impacts on one level could be counteracted by those on another; Francis, Ortega, & Cruz, 2009; Ferrari *et al.*, 2011).

This research investigates impacts of boat noise on both single and multi-trophic levels: by focusing on *P. notatus*, a highly vocal species of intertidal fish, and conducting my assessment in a natural ecosystem, I am able to identify individual species effects on wild *P. notatus*, as well as their predators.

4.2 MATERIALS AND METHODS

4.2.1 Ethics statement

The University of York, United Kingdom, reviewed all field-work procedures, authorized risk assessments and granted ethical approval for experiments. A certificate of research approval from the Canadian Council on Animal Care was granted (Application Number: A12-0133), along with a scientific collection license from the Department of Fisheries and Oceans Canada (DFO, License Number: XR 101 2013) for the duration of the field season in British Columbia, Canada.

4.2.2 Field set-up

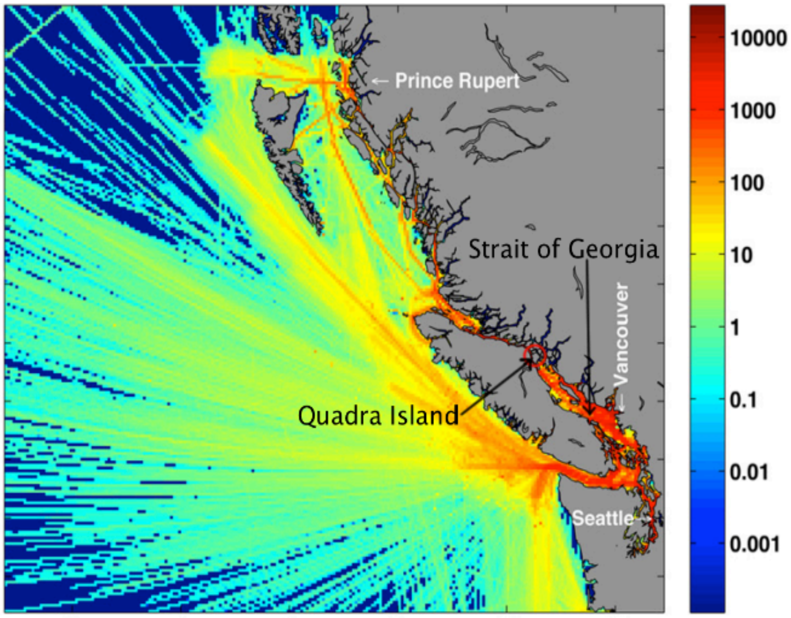

Figure 4.2 Map of field site and total hours of shipping traffic in 2008. From Erbe, Macgillivray, & Williams, 2012, with name additions of Quadra Island and Strait of Georgia.

The Strait of Georgia is an area highly impacted by ship traffic, with plans for further expansion (Ban, Alidina, & Ardron, 2010; Heise & Alidina, 2012; see Fig. 4.2). My field site however was located in a bay on the eastern side of Quadra Island (lat/lon; eg: 14°40’ S, 145°28’ E; Fig. 4.2) which was protected by small islands and thus relatively quiet. In 2013, experiments were executed over 15 days in June, *P. notatus*' most reproductive month (Arora, 1948). Typical boat traffic around this area in June includes commercial fishing boats and a nearby ferry, but is dominated by recreational boats (both fishing and pleasure).

Fifteen separate *P. notatus* nests were selected based on the presence of alpha males, egg clutches, and accessibility. A Teamed Optic-Acoustic Device (or TOAD; see Chapter 2) was positioned in front of each nest at low tide. The TOAD consisted of a custom-built underwater camera fitted with red light filters (to limit visual disturbance of fish to light; McKibben & Bass, 1998; Cappo, Speare, & De’ath, 2004; Widder *et al.,* 2005) and a coupled hydrophone (HTI- 96-min, High Tech Inc., Long Beach, MS). A second, additional hydrophone (also HTI-96 min) was arranged at the field site for backup (see Fig. 4.3). Data from experiments were recorded simultaneously on all devices. One nest (and thus one alpha male) was recorded per day (n = 15) and all experiments began at 5pm following equipment set up at low tides (except for two, which were slightly delayed due to tides and weather, respectively; see DeMartini, 1988). This proved to be the most consistent time of day for experiments in June, factoring in daylight, nest set-up and accessibility, and water depth for live boat treatments.

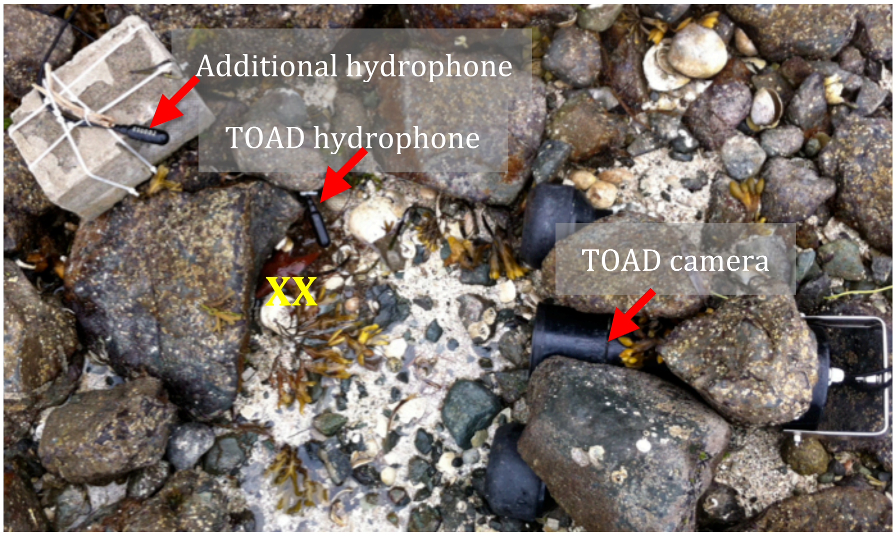

Figure 4.3 Experimental set-up at low tide. Yellow 'XX' denotes *P. notatus*' main nest entry.

4.2.3 Treatments

Three treatments were employed in this experiment: boat noise (boat, engine on), ambient noise (boat, engine off), and control (no boat, no engine; see Holles *et al.*, 2013). The boat noise was provided in real-time by a research volunteer driving a 4.3 meter aluminum motorboat with a 9.9 horsepower engine circling between 1-30 meters from the fish nest (see Holles *et al.*, 2013, for similar experimental design; note the boat was never directly on top of nests). Boat speed (slow, medium, fast; Holles *et al.*, 2013) depended on wind and wave conditions but was generally consistent at moderate to high speeds, simulating typical local boat traffic near the nest site. The ambient treatment was included to control for effects caused by boat presence, representing boat presence without noise and referring to the same 4.3 meter boat on site but with the engine turned off.

The order of treatments was randomized, and there were no gaps between treatments, i.e., when one treatment ended the next began immediately. Each treatment lasted approximately 15 minutes, thus each day's experiment was completed within an hour. In order to determine any longer-term effects from treatments, audio and video recording continued post-experiments (typically from 6pm until 8am the following morning; Picciulin *et al.*, 2010).

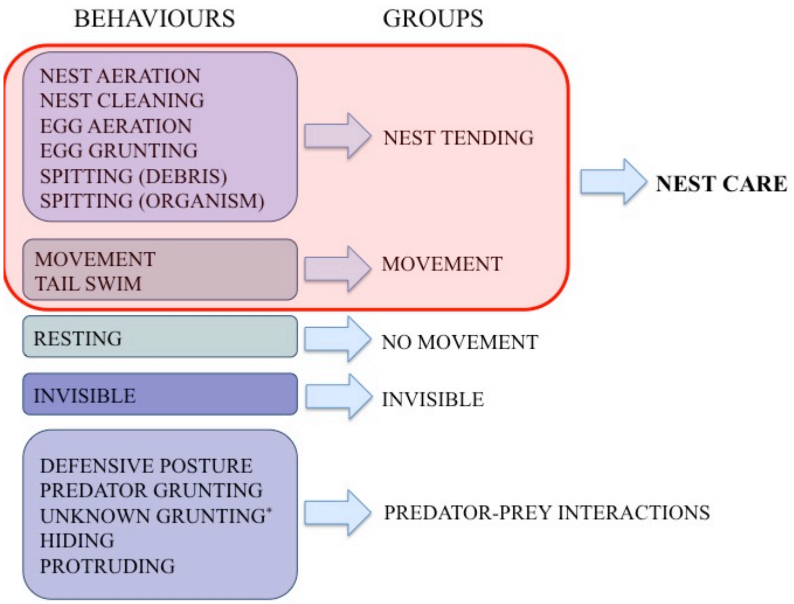

Figure 4.4 All 15 *P. notatus* behaviours observed in videos, and their respective groupings. Nest Care, made up of eight behaviours, was a larger grouping which was also analyzed. *Included in this behaviour are grunts associated with unknown causes (e.g., predators out of sight of camera).

Playbacks were not used in this research to decrease technical limitations associated with such studies, including the use of speakers (Slabbekoorn & Bouton, 2008; Fonseca & Alves, 2012) and to better replicate real-life conditions of boat noise effects (A. Rice, pers. comm.). As previously mentioned, experiments took place in a relatively quiet bay, so boats unrelated to this study did not interfere with experiments. Due to gear and time constraints such as limited data collection periods, particle motion was not included in this study.

4.2.4 Environmental conditions

My time-frame to conduct these experiments was limited: because alpha males care for egg clutches from multiple female partners throughout the summer, stage of egg development inside a nest can be varied (Arora, 1948; McKibben & Bass, 1998). As assessing response of male *P. notatus* to noise was this study's primary objective, and paternal care might differ depending on the stage of egg development (Cogliati *et al.*, 2013), nests chosen for experiments all possessed eggs in similar stages of development (in mid-late June, eggs are generally considered to be in early stages; Arora, 1948). Over the course of this study, eggs continued to mature, thus nests at the end of experiments contained slightly more developed eggs than those at the beginning. Waiting for ideal or steady weather conditions was therefore not possible if egg development across experiments was to stay relatively consistent; thus, experiments were conducted over 20 days on the first 15 days with low enough tides to access nests. Luckily, it never rained on experiment days, so rain noise was not a confounding factor, and most days were calm, categorized as a three or below on the Beaufort Wind Scale (i.e., under 10.6 knots).

4.2.5 Video analysis

All 15 videos were analyzed manually in real-time using VLC Media Player. To determine effects of treatments on *P. notatus* behaviour, 15 different *P. notatus* behaviours were identified from video samples and then catalogued; these behaviours were then clustered into five groups (Fig. 4.4). For each video, behaviour events and durations were recorded. To determine effects of treatments on *P. notatus* predators, all species observed in videos were recorded, noting duration of presence and interactions with *P. notatus* or nest (e.g., attempts to enter nest, etc.). Statistics were carried out in Prism version 6.0f.

4.3. RESULTS

4.3.1 Effects of boat noise on *P. notatus*

Figure 4.5 shows the signal to noise ratio (SNR) of boat and ambient treatments along with *P. notatus* vocalizations, using the control treatment as a baseline. This figure represents the potential for boat noise to mask *P. notatus* calls. Boat, ambient and control recordings were averaged over six samples, each thirty-seconds long and taken from six different dates (as in Holles *et al.*, 2013). Growl samples of *P. notatus* were shorter (as duration of calls were brief: <2 seconds) and were averaged over two recordings. Growls are multi-harmonic and have fundamental frequencies of 59-116 Hz, but note all three *P. notatus* vocalizations- growl, grunt and hum- are ~100 Hz (Sisneros & Bass, 2005). Overlap of boat noise with *P. notatus* calls is apparent at lower frequencies (e.g., 75-800 Hz; Fig. 4.5). Ambient noise does not overlap with *P. notatus* calls.

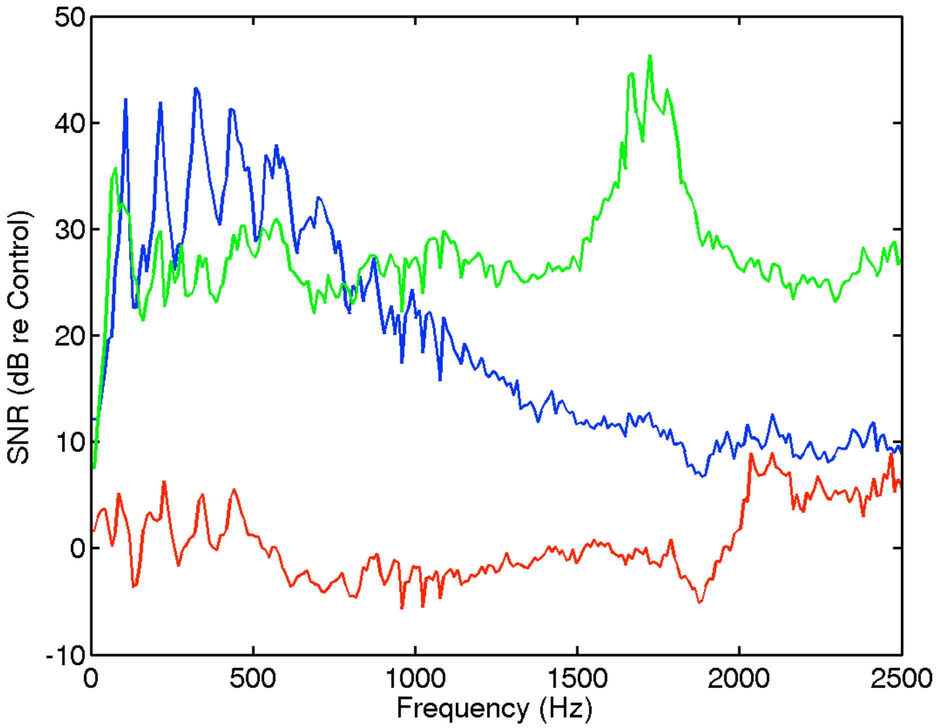

Figure 4.5 SNR power spectra created in MatLab of boat noise (green line), *P. notatus* growls (blue line), and ambient noise (red line) relative to control conditions. Plot parameters: Fast Fourier Transform (FFT) length 4096, averaging with 50% overlap using Hamming window (as in Deng *et al.*, 2014). Note: graph was clipped at 2.5 kHz to focus on lower frequency sounds (boat noise extended beyond 20 kHz).

An exploratory analysis using non-metric multidimensional scaling (n-MDS) in PRIMER-6 (PRIMER-E) was first conducted to determine relationships and patterns of the 15 behaviours with each treatment (Fig. 4.6; before applying n-MDS, data were 4th-root transformed and a Bray Curtis similarity index was used to create a resemblance matrix). For example, I evaluated whether certain behaviours were more closely associated with boat, ambient or control conditions. While obvious treatment-driven clusters did not emerge, individual patterns of behaviour did: for example, individual fish tended to exhibit similar behaviours across treatments (note general clustering by date, i.e., individual fish, Fig. 4.6).

Regarding individual behaviours (as identified in Fig. 4.4), there was no significant difference between treatments (Friedman tests, $p > 0.05$, $n = 15$). Due to small sample sizes, behaviours were then grouped; Nest Tending and Movement categories were further combined to describe Nest Care (as Movement generally corresponded with behaviours

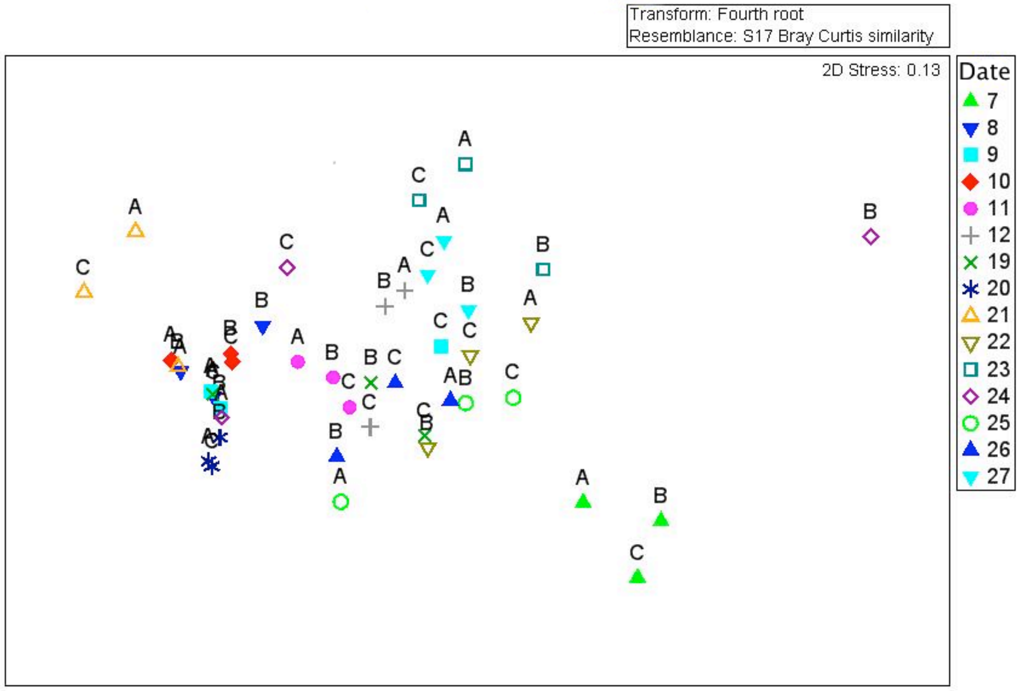

Figure 4.6 Two-dimensional MDS-plot showing clustering of *P. notatus* behaviours in relation to the three treatment groups (A = ambient, B = boat, C = control). 'Date' in legend refers to dates in June, and each date represents one individual fish (n = 15).

associated with nest care, e.g., nest cleaning). Nest Care data from all three treatments were not normally distributed (Shapiro-Wilk test, $p < 0.05$), and a Friedman test revealed no significance (repeated measures data, $p > 0.05$, n = 15; Fig. 4.7). Wilcoxon matched-pairs tests were also performed on each of the three treatment pairs to further investigate potential relationships (due to small sample sizes); while results were not significant between any pairs, a potential trend did appear between boat and control treatments (p = 0.1, n = 15).

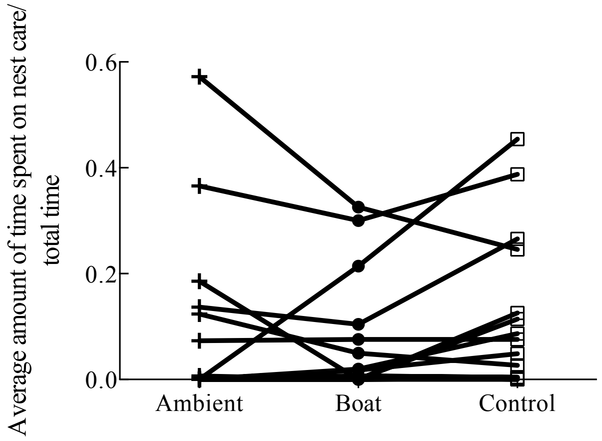

Figure 4.7 Nest Care behaviour of *P. notatus* under boat, ambient and control conditions, defined here as the average amount of time *P. notatus* spends on Nest Care divided by the total sample time. Friedman test, n = 15, $p > 0.05$.

Figure 4.8 View from the TOAD: examples of *P. notatus*, nest, and various predators from sample recordings: a) Classic *P. notatus* defensive position while guarding nest; b) Gunnel (Family *Pholidae)* and Ochre sea star (*Pisaster ochraceus*), both predators of *P. notatus* eggs, inching towards *P. notatus* nest; c) Two red rock crabs (*Cancer productus*), predators of *P. notatus* eggs, fight at entrance of *P. notatus* nest; d) River otter (*Lontra canadensis*), predator of adult *P. notatus*, roots out *P. notatus* with mouth.

Table 4.1 All species observed during video recordings across all treatments. Highlighted species are fish that predate on either egg or adult form of *P. notatus*, and constitute the predators group.

Common name	**Scientific name**
Gunnel	*Pholidae (Family)*
Kelp greenling	*Hexagrammos decagrammus*
Pacific staghorn sculpin	*Leptocottus armatus*
Pile perch	*Rhacochilus vacca*
Rockfish	*Sebastes spp.*
Sculpin (other)	*Cottidae spp.*
Shiner perch	*Cymatogaster aggregata*
Spiny dogfish	*Squalus acanthias*
Unknown	---
Whitespotted greenling	*Hexagrammos stelleri*
Plainfin midshipman	*Porichthys notatus*
Helmet crab	*Telmessus cheiragonus*
Hermit crab	*Paguroidea (Superfamily)*
Jellyfish	*Medusozoa (Subphylum)*
Leather sea star	*Dermasterias imbricata*
Ochre sea star	*Pisaster ochraceus*
Red rock crab	*Cancer productus*
Shore crab	*Hemigrapsus spp.*
Shrimp	*Caridea (Family)*
Sunflower sea star	*Pycnopodia helianthoides*

4.3.2 Effects of boat noise on *P. notatus* predators

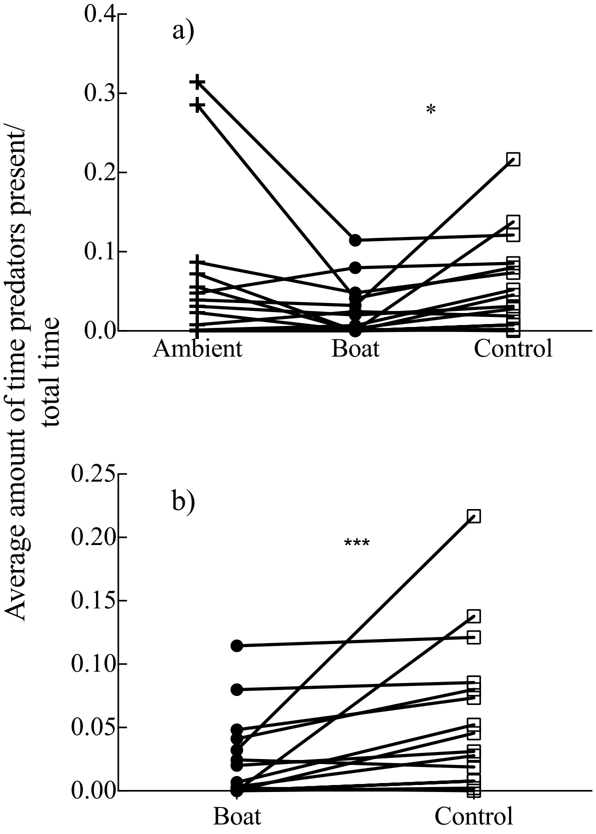

Figure 4.9 Presence of fish predators defined here as the average amount of time predators were present divided by the total sample time in a) all three treatments (Friedman test, $p < 0.05$, n = 15); b) boat and control conditions (Wilcoxon matched-pairs test, n = 15, p = 0.0005). Asterisks denote significance (* = $p < 0.05$, *** p = 0.0005).

For this part of the study, I was interested in the presence of *P. notatus* predators under all three conditions. Figure 4.8 shows examples of a typical *P. notatus* guarding nest, and predator interactions observed through the TOAD camera; note though that marine mammals were only observed outside of treatment times, possibly due to boat avoidance or preference for deeper waters (tides generally ebbed before experiments, thus water depth was relatively shallow during experiments). Presence here refers to the total amount of time predators were observed in frame during each video, divided by that video's total length. All species observed during recorded videos were documented (Table 4.1), along with length of appearance, behaviours, and any interactions with *P. notatus*. Occurrence of individual predators did not vary significantly between the three treatments (Friedman test, $p > 0.05$, n = 14). When analyzed in isolation however, there was a significant difference between boat and control conditions seen in red rock crab (Wilcoxon matched-pairs test, $p < 0.05$, n = 14). Further, when fish predators were grouped together (Table 4.1), significantly fewer predators were observed during boat noise conditions than during control: a Shapiro-Wilk normality test revealed data were not normal ($p < 0.05$) and accordingly, a Friedman test was applied, which detected significance (repeated measures data, $p < 0.05$, n = 15; Fig.

4.9a). A Dunn's post-hoc test revealed a significant difference between boat and control conditions ($p < 0.05$, $n = 15$). Wilcoxon matched-pairs tests were also conducted on all three treatment pairs, and showed significance between boat and control conditions ($p < 0.05$, $n = 15$; Fig. 4.9b). Note: another way to describe predator presence is the number of predator counts in a video; this was also compared across treatments and results proved to be statistically significant, showing fewer total fish predator numbers in boat noise over control conditions. However, as some predators might have been counted twice (for example, if they exited and then re-entered frame), this was not a preferred approach; thus total duration of predator presence was determined to be the better indicator of predator presence.

4.4 DISCUSSION

My results show that there are potential benefits of boat noise to *P. notatus*. Directly, boat noise did not significantly change the behaviour of *P. notatus*; indirectly however, it reduced the presence of *P. notatus* predators, which suggests a decreased vulnerability of *P. notatus* to predation, and thus a fitness advantage by reducing mortality.

4.4.1 Effects of boat noise on *P. notatus*

Nest care behaviour of *P. notatus* was not significantly affected by boat noise (Fig. 4.7), although the data suggest nest care may diminish under boat noise conditions (Wilcoxon matched pairs test between boat and control treatments, $p = 0.1$, $n = 15$); more data are needed to investigate this trend. Such results would parallel those from another study on damselfish (*Chromis chromis*) which showed a decrease in time spent on nest care when exposed to boat noise (Picciulin *et al.*, 2010). The role of paternal care in many fish species is widely known, including for *P. notatus* (Arora, 1948; Bass, 1996; Knapp, Wingfield, & Bass, 1999; Sisneros & Bass, 2003): without their constant egg fanning, nest oxygenation, and nest cleaning, courtship cues may be impeded (Meunier *et al.,* 2009; Wantola, 2013), and their eggs will perish (Arora, 1948). To keep its nest clean, *P. notatus* must work constantly to expel shells, sand, rocks, and even organisms that enter the nest (S. Cullis-Suzuki, pers. obs., and as seen also in the round goby, *Neogobius melanostomus*; Meunier *et al.*, 2009). Considering *P. notatus* does not eat for the duration of the spawning season, a period that can last up to four months (Arora, 1948; Cogliati, Neff, & Balshine, 2013), these activities have an energetic toll on *P. notatus* and can lead to high predation rates (Heithaus *et al.*, 2008). Coupled with their fierce territoriality, it is not surprising therefore that paternal mortality in *P. notatus* is very high; indeed, *P. notatus* has "the lowest documented levels of paternity in a species with obligate paternal care" as evidenced by DNA analyses comparing nest-guarding *P. notatus* with egg clutches within nests (Cogliati *et al.*, 2013). This reinforces the importance of *P. notatus* nest care behaviour, suggesting that without paternal sacrifice, reproductive success would decline.

According to Picciulin *et al.* (2010), the most frequently observed responses of fish to boat noise are: pausing of activities; flight reaction; alarm; and startle. In another study on how net-penned herring react to boat noise, avoidance was the primary response observed (Schwarz & Greer, 1984). Similarly, pomacentrids spent more time moving downward when exposed to moving boats than when exposed to moored boats (Bracciali *et al.*, 2012). In my study, none of these reactions were evident for *P. notatus* in response to boat noise which however parallels findings from other studies on gobiids, pomacentrids, labrids and the eagle ray, *Aetobatus narinari* (Lobel, 2009; Picciulin *et al.*, 2010). Picciulin *et al.*, (2010) explains that a lack of fish response does not equate to a lack of effect; indeed environmental factors such as risk and resources could influence reactions of fish, and therefore must be taken into account when attempting to determine fish responses to stressors.

In his study, Lobel (2009) notes that while fish continued to perform natural behaviours such as courting, spawning and cleaning under boat noise conditions, they would 'spook' from other sources of anthropogenic noise like scuba diving bubbles. The author concludes that the fish's failure to react to boat noise could be due to habituation. However, Schwarz and Greer (1984) argue that, in their study on net-penned herring, habituation to boat noise was not a statistically proven phenomenon. This supports observations from my research that suggest fish respond in a predictable way to natural threats (e.g., hiding from large predators), but not necessarily to unnatural or anthropogenic threats. Indeed, presenting wild fish with unnatural sounds can produce seemingly 'mixed' results: responses can be species-specific, showcasing the complexity of ecosystems (such as differences in alarm and startle responses; Pearson, Skalski, & Malme, 1992, blood parameters; Buscaino *et al.*, 2010, and foraging habits; Voellmy *et al.*, 2014a). Such mixed results have also been observed in predator reactions to boat noise (Voellmy *et al.*, 2014b). Thus, the lack of visible or predictable responses of fish to human-made noise does not necessarily mean no effect, or threat habituation, especially when the sound is not commonly heard.

4.4.2 Effects of boat noise on *P. notatus* predators

Implications of effects of predators

It is well-established that removing predators can decrease the diversity of ecosystems (Paine, 1966). However, such interactions are rarely taken into consideration in noise studies on animals (Chan *et al.*, 2010), despite the heavy influence of predation on prey survival and reproductive success (Voellmy *et al.*, 2014b). In the intertidal zone during the spawning season, *P. notatus* is heavily predated on by birds, marine mammals and other fish (DeMartini, 1988; Elliott, Struik, & Elliott, 2004; Love, 2011). In this study, an unanticipated effect of boat noise was a decline in *P. notatus* fish predators. As a group, fish predators retained certain commonalities. All species in this group (with the exception of the spiny dogfish *Squalus acanthias* which was only present on one occasion for three seconds during a control treatment and did not affect results) are of the class Actinopterygii, or ray-finned fishes. They possess lateral lines and inner ears (Slabbekoorn *et al.*, 2010), and share ancestral mechanisms for acoustic emission (Bass & Chagnaud, 2012). Reduced presence of fish predators during boat noise conditions could be attributable to the flight response, which has been documented in other fish species in reaction to noise (Boussard, 1981; Hassel *et al.*, 2004; Thomsen *et al.*, 2006; Picciulin *et al.*, 2010). This provokes further questions about *P. notatus* predators: how far do they flee? Where do they go? How long are they deterred? More information on each predator species would help to discern how each is responding, as reactions tend to be species-specific (Voellmy *et al.*, 2014b).

Nevertheless, while it is difficult to generalize across species, this grouping shows a definite indication that predator presence declines under boat noise conditions, which implies positive benefits to *P. notatus* through reduced predation encounters. Such unforeseen impacts have been observed in other ecosystems and with other species, as documented in Francis, Ortega, & Cruz, (2009) in respect to birds: "Contrary to expectations, noise indirectly facilitates reproductive success of individuals nesting in noisy areas as a result of the disruption of predator-prey interactions". Within an ecosystem therefore, in addition to individual effects, effects on the community must be taken into account to determine the full extent of stressor impacts on a species.

This however is difficult to achieve, as it relies on information specific to each species in that community. As mentioned previously the limited number of individual species' appearances in this study did not allow for an informative assessment on species-specific predator reactions to boat noise. For example there is reason to believe crabs are affected by boat noise (Chan *et al.*, 2010; Wale, Simpson, & Radford, 2013), especially given their ability to detect and respond to low frequency sounds (Hughes, Mann, & Kimbro, 2014). While a Friedman test revealed no significant difference in red rock crab presence between all treatments, red rock crabs did appear less frequently in boat conditions when measured solely against control conditions; the lack of significance in the Friedman test could be due to confounding factors related to this study's ambient treatment (more under Treatments section), thus this relationship should be further investigated. Specifically, to analyze species-specific responses, it would be useful for future studies to undertake longer treatment periods to increase the length of time *P. notatus* is exposed to predators and thus increase samples.

A decrease in fish predators infers a reproductive benefit to *P. notatus*, and may be the overriding consequence of boat noise on this species. However, in order to reach such a conclusion, more data on how boat noise impacts *P. notatus* at multiple ecosystem levels are needed, and would help rule out consequences that may not have been detected here (especially in light of this study's limited samples). In sum, the collective outcome from the two levels examined here- i.e., that of prey and predator- is difficult to predict, and extrapolating oversimplified results (e.g., single-species effects) to real-life situations can be dangerous. Both the costs and benefits of noise must be assessed, each of which depends on an understanding of effects at the individual and community levels (Read, Jones, & Radford, 2014).

4.4.3 Study benefits and drawbacks

Experimental set-up

The relatively limited time of 15 minutes for each treatment per fish led to a small sample size of behavioural data, and though trends emerged, is likely the reason no significant difference was found between conditions for any of the 15 individual *P. notatus* behaviours. For example, data suggest *P. notatus* spends more time defending its nest under boat noise conditions than during control: in total, across all 15 dates, *P. notatus* spent 110 seconds exhibiting defensive behaviours under boat conditions, compared with a total of 39 seconds during control conditions. However, more behavioural data are needed to determine significance. Grouping behaviours therefore helped to increase sample sizes and determine patterns. Another way to increase behavioural data could be achieved by using an additional camera: the Microcam, a small, endoscopic-like camera (see Chapter 5), could be positioned inside the nest to capture the majority of individual *P. notatus* behaviours (for example, the Microcam successfully captured behaviours of *P. notatus* 86% of the time as described in Chapter 5, compared here with 21% of the time using the TOAD), while the TOAD could continue documenting predator interactions outside of the nest.

While the wider angle and outside nest placement of the TOAD might have limited the ability to document *P. notatus* behaviours inside its nest, these same characteristics allowed the TOAD to effectively detect predator visits and behaviours: by ensuring the camera captured the entire nest entrance, any interactions predators had with *P. notatus* and/or its nest were documented (see examples in Fig. 4.8). Nevertheless, the number of predators viewed in a 15-minute recording varied: for example, fish predator appearances ranged from zero to seven in boat and control conditions, averaging 2.2 across recordings, while fish predator duration varied between 0% of a given sample to 31%, and averaging

5% across recordings. There were not enough predator encounters of individual species during a given sample to perform meaningful statistics; grouping predators, such as all fish predators, was thus performed.

Treatments

A potential confounding factor was introduced to the ambient treatment when paddles were used to orient the boat: *P. notatus* appeared to react to this paddle noise by hiding and ceasing movement. This also led however to the novel observation that *P. notatus* responds to paddle noise in the same way it responds to natural predators, like seals or otters. See Chapter 5 for a full discussion.

The live boat noise treatment in contrast seemed to work well. Beyond eliminating challenges and biases associated with playback recordings (Slabbekoorn & Bouton, 2008; Fonseca & Alves, 2012; A. Rice, pers. comm.), another chief benefit of using live boats for the treatments was the ability to pick up all sounds associated with boats, such as noise produced from waves. This source of noise from boats is rarely portrayed in playback studies, and more accurately reflects real-life conditions of that experiment day. However, it must be noted that only one boat was used for the boat treatment, which puts it at risk for issues surrounding pseudoreplication (for a full discussion, see Slabbekoorn & Bouton, 2008); future studies should incorporate multiple boats for the treatment (as in Bruintjes & Radford, 2014).

4.4.4 Emergent questions and future studies

One natural question that emerges following this boat noise experiment is how boat size might influence noise. Given that larger vessels produce greater sound pressure levels, and that the larger the vessel the further the noise's reach, it is generally thought that larger boats could be most harmful to marine life (Schwarz & Greer, 1984). Smaller boats are thought to emit higher frequency noise, owing to the particular properties of their propellers (e.g., size, position in water, blade speeds, etc.; Erbe, Macgillivray, & Williams, 2012). However, impacts of noise from small boats could be just as problematic: "it is believed that in coastal and inland areas, where there is an abundance of recreational boats with higher speed propellers and engines, the sound is actually louder than from larger vessels" (Graham & Cooke, 2008). Further, as Fig. 4.5 depicts, small boats generate noise at low frequencies as well. Indeed, as shown in my results, small boats elicit reactions from fish predators. A previous study which investigated impacts of boat noise on the fathead minnow *Pimephales promelas* employed playbacks of boat noise generated by a 55 horsepower engine over two hours; results showed significant increases in the fathead minnow's auditory thresholds (Scholik & Yan, 2002). In contrast, my study used a 9.9 horsepower engine with 15-minute noise treatments, indicating that even small engines emitting noise over a short amount of time can produce effects in fish (as seen here in fish predators). Thus, an obvious follow-up study would be to compare noise effects from boats of various sizes. Such a study however would not be suitable in the intertidal arena employed here if very large boats (e.g., tankers) were to be included; in this case a playback study might be more appropriate. Ultimately, how noise affects marine life will depend on the location of the boat emitting the noise, the quantity and type of the boats, and the ecosystem in which it is received (Holles *et al.*, 2013).

Another follow-up study to this research would be to monitor the effects of boat speeds on *P. notatus* and its predators. While in my study, the boat was driven at moderate to high speeds, there is evidence to suggest fish might respond more to slower boats: for example, Mueller (1980) cited in Graham and Cooke (2008) describes slow boats causing more disturbance to longear sunfish *Lepomis megalotis* during spawning than fast boats. This could be investigated relatively easily in future studies by breaking up the boat noise treatment into two: 'slow boat' and 'fast boat', and could be achieved by adding another treatment to the study.

An additional dimension that warrants further consideration when addressing impacts of noise is time of year. For example, recreational boating activity generally coincides with better weather, which occurs during summer months in Canada. This is also the reproductive period of *P. notatus*, meaning *P. notatus* is most at risk from boat noise during this time. Similarly, Kahl (1991) showed canvasback birds *Aythya valisineria* were more at risk to human disturbance during reproductive months. However, exactly where *P.*

notatus goes after spawning and after moving into deeper waters is not known, which means these fish could also be exposed to risk from boat noise between spawning seasons.

Finally, Fig. 4.5, which depicts spectral overlap of *P. notatus* and boat noise frequencies, raises another important question: is fish communication being masked by boat noise? Long-term studies investigating success of reproduction in noisy versus quiet conditions would be one way to address this question, as female *P. notatus* depend on the alpha male's hum to locate and select mates. A follow-up study to this research could be to determine the effectiveness of antagonistic vocalizations against predators (i.e., grunts or growls) in all three conditions. By increasing the duration of treatment time (and thus increasing the potential for predator/*P. notatus* interactions), this research could show if *P. notatus* intimidation calls were less effective against predators in noisy versus quiet conditions. This was not achievable in the present study due to a lack of predator-provoked vocalization events; future research would require longer samples- ideally over many hours, rather than the 15-minute samples employed here- to obtain enough events to yield more meaningful results.

4.5 CONCLUSION

In natural ecosystems, species do not exist in isolation or under consistent conditions. My results show the importance of field-based experiments, as impacts of stressors in natural ecosystems are complex and often unpredictable (Graham & Cooke, 2008; Francis, Ortega, & Cruz, 2009). This is the first study to look at effects of boat noise on wild prey and predators simultaneously *in situ*, and demonstrates the benefit of multi-trophic level investigations and the importance of understanding effects of stressors at the community-level. It also warns of the dangers from oversimplified conclusions based on single-species lab studies, which can often only yield one piece of a bigger picture. Finally, given the number of current human-caused threats to marine ecosystems (Halpern *et al.*, 2008), predicting how organisms will respond to multiple stressors concurrently will be of paramount importance when considering impacts to marine life (see for example, Chan *et al.*, 2010).

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Chapter 5: Detecting fish movement in response to boat noise: An automated approach

5.1 INTRODUCTION

Today a multitude of methods exist to collect acoustic, image or video data for biological research (Rountree *et al.*, 2006; André *et al.*, 2011; Rezzolla, Boldrocchi, & Storai, 2014; Ydesen *et al.*, 2014). Such datasets can be short, or they can span hours, months and even years (Wall *et al.*, 2014). Post-data collection, recordings must then be analyzed. This is usually done by researchers who filter through data manually (e.g., Witman, Etter, & Smith, 2004; Wall *et al.*, 2014), a process which can be extremely tedious, time-consuming, and prone to error: observer fatigue can cause inaccuracies (Bennett, Judd, & Adams, 2000; Walther, Edgington, & Koch, 2004), and observer bias can occur (a phenomenon exacerbated in cases with multiple observers; Thompson & Mapstone, 1997; Fitzpatrick *et al.*, 2009). Manual inspection of long datasets can lead to delays in analysis and in some cases, prevent analysis altogether (Gorsky *et al.*, 2010; Wall *et al.*, 2014).

Recently, with the rapid evolution of computer technology, the opportunity to expand computer-based tools to benefit other, previously unrelated fields, has emerged. Indeed, advancements in marine ecological research by 'machine processing' has already begun (Kane *et al.*, 2004). For example, movement tracking of deep-sea species has been accomplished through automated video analysis (Aguzzi *et al.*, 2009), identification of cetacea vocalizations has been facilitated by automated detection of acoustic events (Zaugg *et al.*, 2010), and classification of fish species and sizes- particularly useful when sorting commercial catches- has been achieved through image processing (White, Svellingen, & Strachan, 2006).

Computer processors can eliminate setbacks typically associated with manual analysis. Of perhaps greatest advantage is their ability to process large volumes of data quickly, an impossibility under former manual analysis (Noldus, Spink, & Tegelenbosch, 2001). For example, one trial using image processing techniques was able to correctly assign individuals to seven species of fish 99% of the time, with the potential to process as many as 30,000 fish in one hour (White, Svellingen, & Strachan, 2006). With this technology, events of interest can be picked-up quickly by the algorithm, while a final review of the trimmed-down data can then be undertaken by a human observer. Further, computer processors allow for the quantification of parameters like spatial measurements which cannot be achieved manually (e.g., calculation of distances, speeds, etc.; Noldus, Spink, & Tegelenbosch, 2001). While other challenges can emerge with the application of computer automation- e.g., the inability of machines to distinguish features or events as well as the human-eye- in many cases, accuracy of detections increases (Noldus, Spink, & Tegelenbosch, 2001; Delcourt *et al.*, 2009).

Such technologies are still very new, but current uses in fish research, despite their particular challenges with fishes' quick speed and directional changes (Delcourt *et al.*, 2009), already include tracking (MacIver & Nelson, 2000), species identification (White, Svellingen, & Strachan, 2006), size measurement (Harvey *et al.*, 2003), space utilization (Kane *et al.*, 2004), travel distance (Ylieff & Poncin, 2003), and swimming speed and direction (Pinkiewicz, Purser, & Williams, 2011). What's more, computer programs are not only limited to categorizing visual data: automated audio programs are also effective methods for sorting through long acoustic datasets, and have been valuable in detecting fish sounds and patterns (Mann & Jarvis, 2004; Locascio & Mann, 2005; Mann *et al.*, 2009).

Computer processing in biological research remains a rapidly evolving and valuable field. However, limitations exist. Such programs can be expensive (e.g., Noldus' EthoVision XT; www.noldus.com), and complicated: a good understanding of the software and programming is mandatory for correct execution (Rountree *et al.*, 2006). Further, the ability to detect characteristics of events accurately is still dependent on an initial template, usually done manually (e.g., Pinkiewicz, Purser, & Williams, 2011; Aguzzi *et al.*, 2012) or by creating a 3D model (MacIver & Nelson, 2000), meaning human error and biases can remain in the data. Finally, most tracking systems used in fish research today depend on controlled lab conditions, including fixed lighting and captive organisms, meaning environmental complexity and dynamism (i.e., real-life conditions) are not represented (Delcourt *et al.*, 2006).

For this study, an automated program was developed to detect motion in underwater videos of wild *P. notatus* inside its nest under experimental boat noise, ambient and quiet conditions. (For reviews on boat noise and effects on fish, including importance of this research, see Chapters 1 and 4.) The most frequent responses of fish to boat noise include moving away from noise, hiding, and startling (Picciulin *et al.*, 2010); therefore, automatic measurement of fish movement could yield information on type of fish's behavioural response, and ultimately, their energy expenditure (Cooke *et al.*, 2003). These data could potentially help to determine impacts of boat noise on fish fitness (e.g., Graham & Cooke, 2008). To our knowledge this is the first study to attempt automated tracking of individual intertidal fish in their natural habitat.

5.2 MATERIALS AND METHODS

The experimental set-up followed that described in Chapter 2, whereby 15 nests containing 15 different nest-guarding male plainfin midshipmen (*Porichthys notatus*) were selected in a sheltered bay on Quadra Island, British Columbia, over 15 days in June, 2013. These nests were then exposed to three noise treatments: ambient, boat and control. The boat employed for both ambient and boat treatments was a 4.3 meter aluminum motorboat with a 9.9 horsepower engine. During ambient treatments, the boat engine was turned off, whereas during boat treatments the boat was driven in real-time by a research volunteer. In both treatments the boat stayed within 30 meters of fish nests. (See Chapter 2 for full details on experimental and video set-up, along with ethics statement, and Chapter 4 for acoustic treatments.)

5.2.1 Video data

Whereas in Chapter 2, video data were recorded through a Teamed Optic-Acoustic Device (TOAD), here video data were obtained through a Microcam (MVC2120WP-LED, Micro

Video Products, www.microvideo.org). The Microcam, a small, durable underwater camera, could be forced into narrow enclosures inaccessible to most other cameras (including the TOAD) thus allowing for continuous insight into *P. notatus* activities and behaviours occurring inside nests. The Microcam focus was set to 0.3 meters to suit nest sites (nests were never larger than 0.3 meters in diameter and never smaller than 0.15 meters), and a red light filter was added to the lens to reduce fish disturbance (McKibben & Bass, 1998; Cappo, Speare, & De'ath 2004; Widder *et al.*, 2005). The Microcam proved to be particularly effective for monitoring alpha male *P. notatus*, as these morphs are highly territorial and spend the entire summer under rocks, calling to females and taking care of their young (as opposed to the other two morphs, females and sneaker-males, whose life-history characteristics differ; Brantley & Bass, 1994). Further, when threatened, alpha males generally dive deeper into their nests rather than fleeing (Arora, 1948; S. Cullis-Suzuki, pers. obs.; although manipulation of rocks covering their nests can cause nest abandonment (Cogliati, Neff, & Balshine, 2013; A. Bhandiwad, pers. comm.) and was therefore not conducted in this study).

By excavating surrounding rocks and/or digging into sand, the camera was positioned near *P. notatus* nests and held in place by rocks; any holes that were created by removing rocks were promptly filled in again once the Microcam had been positioned. The Microcam's custom 91.5 meter cable extended to reach the land-based research station where it was attached to an external power supply, and data were recorded onto external Seagate 2TB hard drives via a MacBook Pro laptop (see set-up for the TOAD and relevant Figures in Chapter 2).

The Microcam possessed a built-in audio component. However, an independent and highly sensitive hydrophone (HTI- 96-min, High Tech Inc., Long Beach, MS) was also established outside each nest to better document acoustic events and potential associations with *P. notatus*' physical behaviours (see Chapter 2 for details on hydrophone and set-up).

One nest was recorded per day over 15 days in June, 2013 (n = 15). Each nest was exposed to all three randomized and consecutive noise treatments. Forty-five videos were thus recorded in all: 15 under boat noise conditions, 15 ambient, and 15 control. After problem-video removal (see below), thirty-nine videos remained and were included in this analysis, representing equal numbers of boat, ambient and control scenarios (i.e., 13 of each). All videos lasted 15 minutes, except in two cases where recording issues necessitated video clipping: June 8th boat (shortened to 12 minutes 36 seconds) and June 24th ambient (shortened to 5 minutes 43 seconds).

5.2.2 Video analysis

The algorithm created to run the analysis for this research was trained from manual annotations performed by me. These annotations were completed for nine videos- three ambient, three boat, three control- and this template formed the basis of the algorithm from which the remaining 30 videos included in the analysis were computed. For the technical description of the computer program see Fig. 5.1 and Appendix 5.1. Note that the algorithm did not differentiate between movement duration and type (for example: 'cleaning', 'breathing', 'swimming', etc.); however, the manual annotations did. Once the algorithm was completed and resultant graphs depicting movement for each video created, the graphs for all 39 videos were reviewed against each video in real-time (see Fig. 5.1b but over the entire 15 minute duration of a typical video). Problems and discrepancies led to changes and strengthening of the algorithm, or video deletion (see below); in all other cases, they were included as sources of error, meaning the final scores factored in all inaccurate detections. Statistics were carried out in Prism version 6.0f.

5.3 RESULTS

5.3.1 Automated detections

Thirty-nine videos were included in this analysis, 13 under boat conditions, 13 under ambient, and 13 under control. The algorithm was successful at detecting fish movement (see Fig. 5.1) with an average precision rate of 85% (where precision equals the number of correct algorithm identifications divided by the sum of correct plus false algorithm identifications), a recall rate of 84% (where recall equals the number of correct algorithm

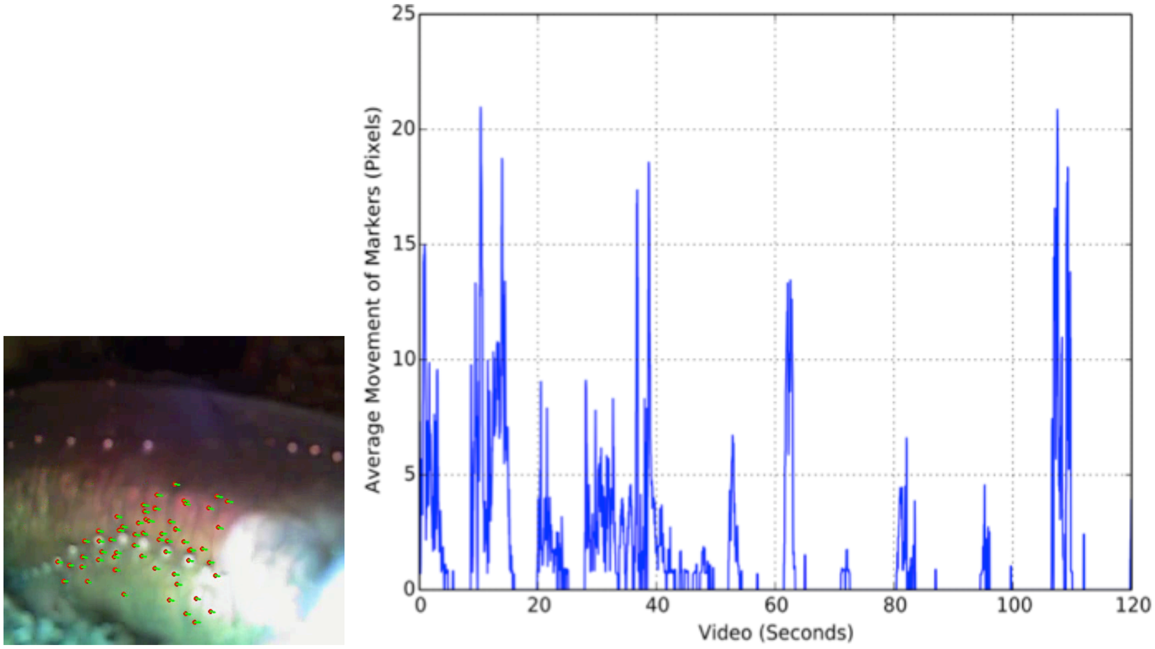

Figure 5.1 a) Filtered image. Example of movement markers picked-up on *P. notatus*' body in one frame (all non-movement markers have been removed). b) Example of algorithm's movement detection > 0 pixels over two minutes (Control conditions, June 20th, minutes 10-12).

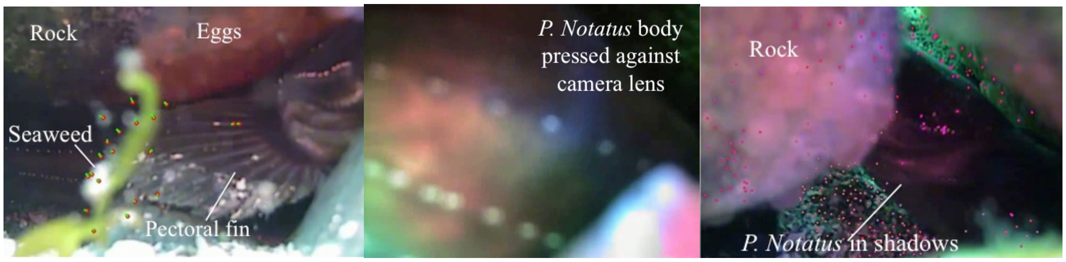

Figure 5.2 Sources of error include: a) Other moving objects leading to false detections (here, seaweed; b) *P. notatus* too close to camera lens and out of focus, leading to failed motion detection; c) Shadows inhibiting movement detection.

identifications divided by the number of actual correct identifications) and an F1 of 82% (where F1, also known as the 'harmonic mean' between precision and recall, equals precision times recall, multiplied by two, then divided by the sum of precision and recall); see Appendix 5.2 for breakdown of all scores. The majority of accuracy errors in these nine videos were due to two causes: current and aeration-related events.

Aeration-related events- rhythmic pectoral, tail or head bobbing movements- were often subtle and most errors occurred when shadows obscured the source of movement (e.g.,

Table 5.1 Primary sources of error (not including sources of video deletion, as shown in Appendix 5.3.)

Event	**No detection**	**False detection**
Shadow-based movement	√	
Movement too close to lens	√	
Movement too far from lens	√	
Movement too quick	√	
Movement extremely slow*	√	
Body part moving too small	√	
Breathing		√
Floating particles (other moving objects in frame, e.g., seaweed, plankton, sand, etc.)		√

* Down-sampling, described in Appendix 5.1, allowed slower movements to be picked up; however, extremely slow movements were not detected.

pectoral fin or tail), resulting in failed detections by algorithm. 'Breathing', consisting of gills opening and closing, was even more subtle and was not classified as movement. However, on some occasions gill movements were large enough to be detected, and were then classified as false positives.

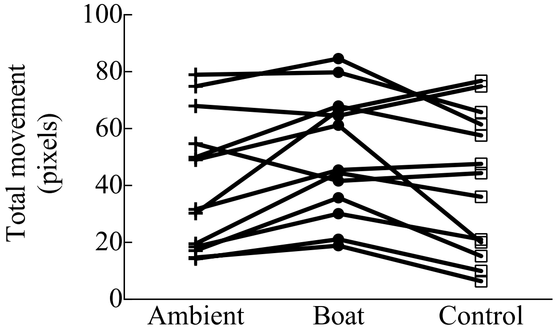

Figure 5.3 Total movement (pixels) of all three (paired) conditions.

Currents led to false algorithm detections by creating unintentional *P. notatus* movement: from an algorithm perspective the subtle differences between intended movement (i.e., induced by *P. notatus*) versus unintended movement (i.e., induced by current) was non-differentiable, and thus led to an overall decrease in accuracy scores. However it is important to note that while currents and shadows did contribute to error, their occurrence was generally consistent across matched treatments, as all videos taken on the same date were consecutive (taken 15 minutes apart) and exposed to similar environmental conditions. Figure 5.2 depicts common examples of algorithm error. The main sources of error are summarized in Table 5.1: here, 'extremely slow' generally describes movement events that are less than 0.5 pixels, while 'too quick' refers to anything over 40 pixels/frame. 'Too close to lens' describes events that are less than 2 features, meaning there were not enough features for detection, a limit also employed for movement events 'too far from lens' (but which were generally not encountered: when *P. notatus* was further from the lens other features from the surroundings were usually picked up). Video corruption was observed on six different dates across all three treatments, amounting to 38 events totaling two minutes and six seconds, and averaging three seconds in length. These events occurred in < 0.5% of footage across all 39 videos included in this analysis. *P. notatus* was out of frame on 50 different occasions, each event averaging 22 seconds in length, for a total of 18 minutes and 27 seconds, or 3% of total time across all 39 videos (i.e., not including June 21st videos; see Appendix 5.3). *Porichthys notatus* was the principal organism in frame for over 99% of footage across all 39 videos included in the analysis, and 93% of the time across all 45 original videos. See Appendix 5.2 for full breakdown of scores and Appendix 5.3 for all episodes of video deletion.

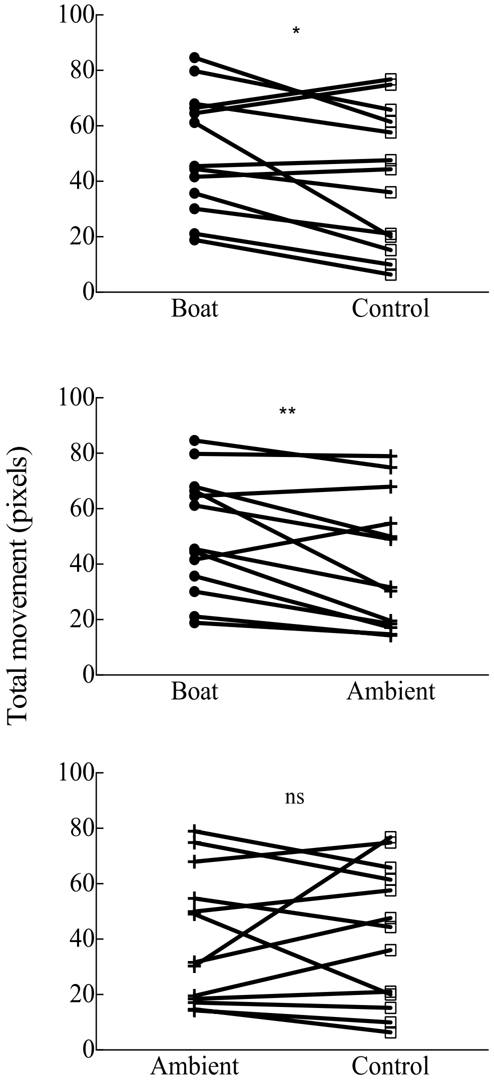

Figure 5.4 T-tests representing total movement (> 0 pixels) in all 13 videos for each treatment pair (boat-control, boat-ambient, ambient-control). Stars represent significance (* = $p < 0.05$, ** = $p < 0.01$, ns = not significant).

5.3.2 Boat noise and fish movement

Total movement

After data passed a normality test (Shapiro-Wilk normality test, $p > 0.05$, $n = 13$), a repeated measures one-way ANOVA was performed, showing results were significant ($p < 0.05$, $n = 13$); Fig. 5.3 shows relationship between (paired) treatments. A post-hoc Tukey test revealed a significant difference between boat and ambient treatments ($p < 0.05$, $n = 13$). In view of small sample sizes, t-tests were performed on all treatment pairs: results

Table 5.2 The difference in total *P. notatus* movement detected between boat-ambient and boat-control conditions on each of the 13 dates included in the analysis. Positive numbers indicate more movement in boat than ambient/control conditions; negative numbers indicate more movement in ambient/control than boat conditions.

Boat-ambient		Boat-control	
Date	Diff. total mvmt	Date	Diff. total mvmt
24-Jun	36.17	12-Jun	41.14
11-Jun	24.94	23-Jun	23.18
26-Jun	18.55	26-Jun	20.49
09-Jun	18.06	27-Jun	13.98
25-Jun	13.82	08-Jun	12.49
12-Jun	12.11	07-Jun	11.22
20-Jun	11.64	09-Jun	10.37
23-Jun	9.78	20-Jun	9.04
07-Jun	6.83	11-Jun	8.39
08-Jun	4.15	25-Jun	-2.18
27-Jun	0.84	10-Jun	-2.79
22-Jun	-3.43	24-Jun	-10.27
10-Jun	-13.13	22-Jun	-10.34

proved significant between boat and ambient ($t = 3.090$, $p < 0.01$, $n = 13$) and boat and control conditions ($t = 2.438$, $p < 0.05$, $n = 13$); Fig. 5.4. In both boat-ambient and boat-control conditions, *P. notatus* moved significantly more in boat noise conditions (paired t-tests, $n = 13$, $p < 0.05$; Fig. 5.4). Total movement refers to the total percentage of frames with movement >0 pixels; in other words, the number of frames showing any movement divided by the total number of frames in a video. Table 5.2 shows the differences in total movement between boat and ambient and boat and control conditions for each of the 13 dates included in the analysis. On eleven of the thirteen dates, total movement was greater in boat than in ambient conditions; likewise, on nine of the thirteen dates, total movement was greater in boat than control conditions. Negative numbers indicate the opposite was true (i.e., more movement was detected in ambient or control than boat conditions); note size of numbers indicates extent of movement difference.

5.4 DISCUSSION

5.4.1 Microcam

The Microcam proved to be an effective tool for recording behaviours of *P. notatus* inside its nest. In each of the fifteen nests, an alpha male was present suggesting that the positioning of the camera was not overly disruptive: when highly disturbed, *P. notatus* will abandon its nest (A. Bhandiwad, pers. comm.). Camera lights however, could have altered

P. notatus behaviour: while red light filters were added to the Microcam prior to deployment to minimize disruption (McKibben & Bass, 1998; Cappo, Speare, & De'ath 2004; Widder *et al.*, 2005), responses of fish to artificial light are species-specific (Marchesan *et al.*, 2005), so the presence of light might still have affected *P. notatus* (see for example, McKibben & Bass, 1998, and Volpato *et al.*, 2013). Still, lighting was consistent across treatments and videos, so any resulting effect should be seen in all samples.

As size did not differ much between nests (all nests were generally ~0.15 - 0.3 meters in diameter) and with a consistent 0.3 meter camera lens focus, a similar view into all nests was ensured. Further, whereas the TOAD camera in Chapter 2 provided a view of the nest entrance along with a snapshot of the surrounding ecosystem and is thus better suited to document events external to the nest (e.g., predator-prey interactions), the Microcam offered a view into the nest itself, where *P. notatus* spends the vast majority of its time. Indeed this 'internal' camera substantially increased observational time of *P. notatus* as compared with the TOAD: *P. notatus* was visible only 21% of the time with the TOAD, compared to 86% of the time with the Microcam across all original 45 videos and 97% of the time in footage from the 39 videos included in the analysis. Thus for this evaluation, which monitored behaviour of individual *P. notatus* under noise conditions, the Microcam's arrangement was ideal.

Finally, in contrast with the TOAD, other organisms were rarely seen in the Microcam videos as few visitors entered *P. notatus*' nests; indeed in the 39 videos included in the analysis, there were only four such occasions when a crab, hermit crab, or gunnel were visible inside the nest (for exact times and dates see Appendix 5.3). This helped to increase the accuracy of movement detection by the algorithm.

5.4.2 Algorithm

Causes of video deletion

Forty-five videos were recorded for this study, and six were omitted entirely from the analysis: three from June 19th and three from June 21st. On June 19th, a shell landed on the lens of the Microcam, completely obstructing the view into *P. notatus'* nest for all three treatments that day. Lens obstruction was a problem given the study's natural settings: the Microcam lens was left exposed inside the nest, which limited intrusion, but opened it up to blockage by shifting objects- seaweed, shells, organisms, etc. However, lens obstruction occurred on only one other occasion (June 24th), and lasted only four seconds. Such events, though relatively rare, were unavoidable, and should be factored into schedules when conducting future experiments (e.g., allowing for study flexibility and additional experiment dates if necessary). On June 21st the dominant species in all three treatments was a sea cucumber *Cucumaria miniata*, and *P. notatus* was barely- if at all- visible in video clips. When other organisms dominate videos, accurate algorithm detection of *P. notatus* movement becomes unattainable as the majority of movement comes from the other organism. However, such events did not occur often: once June 21st was removed from analysis, *P. notatus* was the principal organism in videos >99% of the time.

In addition to events in which other organisms dominated the frame or lens obstruction occurred, there were two other instances where portions of videos had to be removed: video corruption (which occurred randomly across videos), and *P. notatus* absence. These events accounted for another 22 minutes and 35 seconds of video removed from analysis (see Appendix 5.3). Video corruption episodes were seldom, unpredictable, and generally a result of Microcam wiring interference. During these events, computer screens recording data were momentarily 'lit up' and the algorithm incorrectly interpreted these rapid lighting changes as *P. notatus* movement. As they were conspicuous sources of error, all 39 videos were examined manually to remove corruption events from analysis and improve accuracy scores. Future studies could potentially limit corruption events by better securing the Microcam's cable, as moving cables could increase wire malfunctions.

Porichthys notatus absence was defined as any time *P. notatus* was out of frame for >1 second. During this time, any movement attributable to *P. notatus* could not be picked up, manually or by algorithm, and was thus removed from analysis (Appendix 5.3). Such events were unavoidable, as *P. notatus* was not constrained in this field set-up and free to move/flee. However, despite this freedom in mobility, such events were rarely seen, accounting for only 3% of time across all videos included in the analysis, compared with 79% under the TOAD.
After all video deletion, a total of 573 minutes and 19 seconds of video were included in this study. All other problems or discrepancies were counted as sources of error and contributed to the accuracy scores (Appendix 5.2).

Sources of error

While the algorithm was effective at detecting movement, there were invariably events that were detectable only by a trained human eye. Two such events accounted for the majority of algorithm error: aeration-related movement and current-induced movement (Appendix 5.2). Other environmental variables that interfered with accurate algorithm detection were related to floating particles, such as seaweed, plankton, and sand. On some occasions, moving floating particles were falsely identified as *P. notatus* movement. In other instances, floating particles temporarily obscured *P. notatus* movement, for example when sand was kicked-up during *P. notatus* nest cleaning (Table 5.1).

Further sources of error depended on the speed and location of the movement: if a movement was too slow or too fast, it was not detected (though down-sampling increased accuracy for slower movements); likewise, if movements occurred too close or too far from the camera lens they were not detected (Table 5.1). These errors associated with movement location reveal perhaps the greatest failure of the algorithm: an inability to detect depth perception. This resulted in the inability to accurately measure the magnitude of movement events: movements that occurred further away from the lens but were of the same size as those closer were computed as smaller (and sometimes not detected at all). Thus, two other calculations, movement size and average movement, which were both contingent on accurate depth perception, were therefore not assumed to yield reliable estimates and were thus not included in the Results. For example, to determine how ‘big’ *P. notatus* movements were (i.e., movement size), movement was broken up into various increments of magnitude (e.g., 2-4 pixels, 4-6 pixels, etc.). Likewise, average movement was based on the average size of all movement in a sample, broken-down by pixel size. Total movement on the other hand did not take into account the size of movements and was therefore a more accurate and informative figure for this study.

As each *P. notatus* nest was somewhat different, and each required a slightly different camera angle arrangement, there was inevitably variation between videos regarding the appearance of *P. notatus* within frames. This effect was compounded by the ability of *P. notatus* to move freely during treatments. For example, in some nests *P. notatus* spent most of its time close to the lens while in others it strayed further from it. Luckily, for our study, problems with depth perception were limited to a short field of view- 0.3 meters- which represented the general dimensions of *P. notatus* nests. Further, videos taken on the same dates reflected the same *P. notatus* in the same nest, and variation of *P. notatus* location in frames between these videos was generally consistent.

Accuracy

For the most part, the algorithm was able to accurately detect movement by *P. notatus* in its nest in natural settings. Given the diversity of variables and potential sources of error at our uncontrolled field site, and considering the preliminary nature of our trial, our results proved the algorithm to be effective, with very high precision and recall rates both with averages above 80%, and scores above 90% in many videos. Further, when the main sources of error were accounted for -i.e., current and aeration-related errors (see Appendix 5.2)- results improved substantially, averaging above 94% across all scores. Considering the biases and drawbacks to manual analyses (discussed in Introduction), the possibilities for detection of aquatic animal movement using automated algorithms is clear. Further, our example demonstrates the potential for studies to implement computer processing in field-based research documenting behavioural responses of individual fish to real-life stressors.

Future studies

It is important to note that our study benefitted from a minimally-invasive field set-up and a fierce territoriality exhibited by nest-guarding *P. notatus*. Because our recording arrangement did not severely disturb the nest, and since *P. notatus* rarely left its nest, we were able to successfully capture *P. notatus* behaviours rarely observed in its natural habitat. However, other, less sedentary or territorial fish would be difficult to observe using similar equipment due to their movement over longer distances. Nevertheless, there is evidence from our research that this *in-situ* setup will work for other species beyond *P. notatus*. On the rare occasion when another organism dominated the video frame, the algorithm identified movement associated with those organisms. For example, on two occasions a gunnel (family Pholidae) dominated the video, and the algorithm detected and correctly identified movement associated with that gunnel; on another occasion, movement was correctly detected from a passing hermit crab (Superfamily Paguroidea). These examples show the algorithm's versatility in picking-up movement from a variety of marine organisms, and indicate its potential use in other biological studies.

5.4.3 Effect of boat noise on *P. notatus*

Study setbacks

The primary setback in this study proved to be with our ambient treatment. Ambient treatments involved the presence of a boat with its engine turned off and maintained in place by an anchor. As boat engine noise was not the purpose of this treatment, oars were used when moving the boat to and from the field site, and occasionally during treatments to orient the boat. It has been shown that prey can detect and distinguish between predator species based on acoustic predator calls (Hettena, Munoz, & Blumstein, 2014); here, field observations disclose a similar phenomenon by fish in response to paddle noise. Splashing emitted by boat oars directly before and during ambient treatments caused nest-guarding *P. notatus* to retreat deeper into their nests and lay motionless, a common threat-response behaviour exhibited by fish (Schwarz & Greer, 1984; Pearson, Skalski, & Malme, 1992; Sand *et al.*, 2000; Picciulin *et al.*, 2010). This same behaviour is employed by *P. notatus* in response to natural foraging predators such as harbour seals *Phoca vitulina* and river otters *Lontra canadensis* (S. Cullis-Suzuki, pers. obs.). Thus, the splashing emitted by boat oars in this study appeared to closely imitate the splashing from marine mammal predators, and resulted in analogous threat-response behaviour of *P. notatus*. This follows research showing fish exhibit increased heart rate and cardiac outputs under canoe and electric trolling motor boat conditions comparable to those when under attack from natural predators (Graham & Cooke, 2008). Due to this unforeseen paddle noise effect, behaviours of *P. notatus* during ambient treatments were likely biased towards less movement. Results show that indeed out of all three treatments, *P. notatus* moved least under ambient conditions. These results indicate that non-motorized boats can also impact fish, a finding echoed in Graham and Cooke (2008).

Another potential challenge of conducting live-boat treatments was the possibility of generating boat-induced current. This however did not prove to be particularly problematic for our study: nest guarding *P. notatus* are very well sheltered from waves as they remain underneath rocks (Arora, 1948). Further, being at the bottom of the ocean (as opposed to near the surface) they are less likely to be affected by surface waves. Nevertheless, all videos were reviewed manually for potential fish movement induced by the boat. When current was detected in the boat treatments, it was also generally apparent in the other treatments from that day, indicating it was likely due to a natural source and not from the boat. On June 12th however, there is indication that during the boat treatment, substrate may have been disturbed by the boat, as stirred sand was occasionally seen in the frame. On such rare occasions, 'moving sand' might be counted by the algorithm as movement, which would have contributed to error. However, during such events, *P. notatus* actually appeared to freeze in place and remain motionless, even when current was present. Therefore, counter to expectations, potential boat-induced current generally led to decreased movement of *P. notatus*, a trend which runs opposite to our results (showing more movement under boat conditions). Therefore, had such events been eliminated from our study, this trend would likely have increased.

Implications of results

Our results show that *P. notatus* moves inside its nest more in boat conditions than under ambient or control conditions. This supports results from studies on other species of fish. For example, laboratory tests on the red-mouthed goby, a territorial fish, showed increased movement under boat noise over quiet conditions (Sebastianutto *et al.*, 2011). Likewise, European silver eels *Anguilla anguilla* held in traps moved in response to loud conditions (Sand *et al.*, 2000), as did free-swimming coastal fish such as Atlantic herring *Clupea harengus* and blue whiting *Micromesistius poutassou* in an acoustically-measured study (Slotte *et al.*, 2004). Pearson, Skalski and Malme (1992) also suggest noise can induce movement in benthic fish like rockfish, *Sebastes* spp. (although because fish were enclosed in nets in that study, the full extent of movement is unknown).

This significant increase in movement of fish under boat noise conditions could lead to serious consequences. In a study on European eels, ventilation and oxygen rates increased when eels were exposed to ship noise (Simpson, Purser, & Radford, 2015). Increased muscle activity can also cause increased oxygen consumption (Buscaino *et al.*, 2010), a phenomenon also seen in crabs after ship noise exposure (Wale, Simpson, & Radford, 2013). Further, heart rate and cardiac output can increase, a trend already known to occur in fish in response to boat noise (Graham & Cooke, 2008). Such physiological changes raise associated energetic costs, which, in turn, can impact on fitness by limiting growth and decreasing survival and reproductive success (Thomson *et al.*, 2009; Slabbekoorn *et al.*, 2010; Kight & Swaddle, 2011; Slabbekoorn, 2012; Wale, Simpson, & Radford, 2013). Alpha male *P. notatus* would be particularly vulnerable: these morphs rarely eat over the several months spent nest-guarding (Arora, 1948), and are thus already energetically starved; therefore energy spent on additional behaviours during this time would almost certainly be costly.

Effects from our ambient treatment also confirm that responses of fish to threats are complex: not only are they species-specific (Voellmy *et al.*, 2014), they appear to be threat-specific as well. As shown here, *P. notatus* does not react to boat engine noise in the same way it reacts to known, natural predators. Helfman's hypothesis states that fish demonstrate threat-sensitivity during predator avoidance; i.e., the type of response elicited by fish will be determined by the severity of the threat (Helfman, 1989). However, while increasing gradients of the same disturbance might produce clear and proportional threat-responses (Vavrek & Brown, 2009), quantification of responses to new or unnatural threats- e.g. such as from anthropogenic sources like boats- becomes far more complicated. Therefore, as threat-responses cannot necessarily be predicted by the disturbance (especially if anthropogenic), research on behavioural reactions to specific man-made stressors is needed.

5.5 CONCLUSIONS

Most of the challenges associated with this study stemmed from its field-based nature. These environmentally-variable conditions however set it apart from previous studies attempting automated detection of fish movement in labs (Noldus, Spink, & Tegelenbosch, 2001), and provide original insight into animal behaviour-analysis in real-life conditions and ecosystems. In the near-future, computer programs such as the one presented here will undoubtedly help increase efficiency and improve accuracy of data analysis in biological research. Taken together with the results from Chapter 4, my research shows there are both positive and negative consequences of boat noise on *P. notatus*: boat noise increases *P. notatus* movement inside its nest (this chapter), and decreases presence of *P. notatus* predators (Chapter 4), suggesting contrasting consequences for *P. notatus*, and revealing the importance of noise studies on multi-trophic levels. Given the significant results regarding effects of boat noise on wild fish, and considering the rising impacts of anthropogenic stressors on biological systems around the world (Halpern *et al.*, 2008; Barber, Crooks, & Fristrup, 2010), such programs will become particularly important in helping to rapidly detect potential effects on wild species.

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Chapter 6: Discussion

6.1 SUMMARY OF RESEARCH AIMS AND RESULTS

The primary objective of this research was to quantify the effects of boat noise on a wild and highly vocal species of fish, the plainfin midshipman, *Porichthys notatus*. To achieve this and to place results in an ecological context, primary research on *P. notatus*' behaviour and acoustic communication was conducted in the field in its natural habitat.

Chapter 2 described the technology that facilitated this research. The TOAD (Teamed Optic Acoustic Device) consisted of a cabled underwater video camera and hydrophone, which was created to obtain real-time audio/video recordings of *P. notatus* over long time frames. Obtaining audio/video data on natural behaviours of *P. notatus* would not have been possible without it, due to battery limits on conventional underwater recording systems, as well as viewing restrictions (inability to stream live data, for example). This chapter gave an exploratory look at data obtained through the TOAD, by analyzing species presence in daylight and nighttime conditions obtained in 30 hours of recording over 15 days. The TOAD proved to be an effective tool, documenting a total of 838 individual organisms from 30 different species. In addition, a new behaviour of *P. notatus* was characterized while using the TOAD: the grunge, a grunt and lunge enacted simultaneously by alpha male *P. notatus* towards heterospecific nest intruders, was observed to be an effective deterrent response towards predators. A lack of studies on fish *in situ* is due in part to the absence of affordable and accessible tools; the TOAD could offer a useful option.

Chapter 3 presents a two-pronged study investigating a) the acoustic footprint of *P. notatus* in its natural habitat, and b) the behavioural context behind its most common vocalization, the grunt. Long-term audio recordings from passive acoustics revealed high call numbers and call diversity. Diel patterns of vocal activity including call type and call duration contradicted previous assumptions about *P. notatus* behavior, showing hums occur at all times of the day as opposed to only at night. All *P. notatus*' four main vocalizations were detected in long-term recordings, and a fifth was detailed here for the first time. The behavioural component of this chapter revealed the context of grunt production was primarily to deter intruders from entering nests, and secondarily, in adventitious circumstances. It further quantified the high level of effectiveness of the grunge as a predator deterrent, previously defined in Chapter 2. By setting the behavioural results against the backdrop of the passive acoustic study, the importance of *P. notatus* calls was further put into context. Grunts, as revealed here to be produced in agonistic encounters with heterospecifics, were by far the most commonly detected call of *P. notatus*, audible at every hour of the day and night. Taken together, the findings suggest *P. notatus* uses sound both frequently and effectively to communicate beyond its own species.

Chapter 4 presents an experimental study on the effects of boat noise on *P. notatus* and its predators. Fish were filmed and recorded in their natural habitat in an intertidal nesting site under ambient, boat and control conditions. While no significant direct effect of boat noise on *P. notatus* was documented, an unforeseen effect of paddle noise in ambient conditions revealed *P. notatus* responds to boat oars in the same way as to marine mammal predators: by hiding and staying still. In addition, compared with control conditions, fewer fish predators were found to frequent *P. notatus* nests under boat noise scenarios. By investigating effects of boat noise on wild fish and their predators concurrently, this study reveals the complexity of noise pollution in ecological systems by including indirect effects from predator interactions; here for example, a decrease in the number of predators likely yields positive benefits for *P. notatus*. This study reinforces the necessity of field research when addressing noise impacts on marine life, a setting currently vastly underrepresented in the literature but paramount when predicting consequences of noise in natural systems, especially as they relate to policy recommendations.

Chapter 5 investigated how automated computer programs can facilitate the analysis of fish behaviour in the wild. Here, an automated approach was created to detect *P. notatus* movement in its nest under boat noise, ambient control conditions. The algorithm was trained with manual analyses and proved to be highly effective at detecting fish movements, despite uncontrolled field conditions. As data were obtained from a small Microcam fitted inside *P. notatus* nests, findings reflect a consistent interpretation of *P.*

notatus behaviours in their primary habitat during nesting season. They showed *P. notatus* moved more inside nests in boat noise conditions than under ambient or control conditions, suggesting boat noise can cause elevated stress in *P. notatus* and increase metabolic costs, thus negatively impacting on its health and fitness. This contrasts the indirect fitness advantage *P. notatus* gained in boat noise conditions through the reduced occurrence of its predators, as shown in Chapter 4, and reveals the importance of multi-trophic studies on noise. This chapter presents the first use of automated video analysis in an ecological study assessing impacts of anthropogenic noise on wild fish; such a tool has the potential to expand our understanding of noise effects on natural ecosystems at a quicker pace, and on a larger scale.

6.2 CURRENT OCEAN NOISE MANAGEMENT AND DIRECTIONS FOR FUTURE RESEARCH

My research adds to the growing evidence that anthropogenic noise affects marine life (DFO, 2004; Tyack *et al.*, 2006; Nowacek *et al.*, 2007; Chan *et al.*, 2010; André *et al.*, 2011; de Soto *et al.*, 2013; Goldbogen *et al.*, 2013; Wale, Simpson, & Radford, 2013a, 2013b). However, while recommendations have been made (e.g., Gray *et al.*, 2010; Boyd *et al.*, 2011; Erbe, Macgillivray, & Williams, 2012; Williams *et al.*, 2014) current regulations do not yet reflect the growing awareness of potential impacts: “To date, adverse effects of chronic sound sources (e.g., commercial shipping) at the level of individuals, populations, species’ habitats, or ecosystems have not been incorporated into management decisions” (Ellison *et al.*, 2012). Suggestions to limit noise impacts on marine life include ‘quiet’ protected areas (Barber, Crooks, & Fristrup, 2010), noise ‘buffers’ (Weilgart, 2007) or ‘barriers’ (Slabbekoorn & Ripmeester, 2008), and seasonal boating restrictions (Picciulin *et al.*, 2010); yet the vast majority of marine parks do not have any type of regulatory management in respect to noise (Hatch & Fristrup, 2009). By contrast, in terrestrial parks, protecting natural sounds and noise monitoring have been accepted into law in many places including the USA (Miller, 2008), Brazil (Szeremeta & Zannin, 2009) and Canada (Draper, 2000); in some cases visitors are even willing to pay more for quieter parks (Merchan, Diaz-Balteiro, & Soliño, 2014).

Considering anthropogenic activities have touched every part of the global ocean (Halpern *et al.*, 2008), and have contributed to an overall decline in ocean resiliency (Jackson, 2008), acoustic pollution is a relatively new marine stressor that warrants investigation with a view to possible regulation. In the meantime, application of the Precautionary Principle- which could be economically prudent in the long run (McCarthy *et al.*, 2012)- should be implemented into marine activities and management protocol while investigations and quantifications of effects of noise at an ecosystem level are conducted (McCarthy, 2007); this will help to identify the geographic extent and effect of noise (Barber *et al.*, 2011). Concurrently, research at the species-specific level must continue (Popper & Hastings, 2009). Although a growing number of studies have looked at noise effects on fish in short-term laboratory studies, long-term impacts of anthropogenic noise are lacking and require demonstration (e.g., Picciulin *et al.*, 2010). Future studies should place particular consideration on wild or natural settings (Slabbekoorn *et al.*, 2010): through conducting this research it became evident that basic knowledge of marine species, interactions, and ecosystems, remains limited. Field-based studies are needed to provide context to findings, and to understand complex interactions between species groups and habitats, as well as to obtain insights on natural characteristics of acoustic communication. Research must diversify to include a much wider variety of taxa (especially given the persistent focus of past research on charismatic animals; Griffiths & Dos Santos, 2012) and to unite disciplines (e.g., ecology and computer sciences) which will help to expand the scale and speed on which we can work on such problems.

6.3 CONCLUSIONS

The acoustic marine world has been largely overlooked in conservation science, and a lack of studies and data reflect this (Halfwerk *et al.*, 2011). My research sheds light on the quantity and complexity of calls observed from *P. notatus* in its natural habitat, and describes how it uses sound to communicate with heterospecifics. It further demonstrates that boat noise interferes with its natural behaviours and the behaviour of its predators, through negative direct and positive indirect effects, and reveals the complexity of ecosystem interactions and the importance of considering multi-trophic effects of noise.

Interestingly, unlike many global ocean threats, noise has a comparatively simple solution: the majority of low frequency ocean noise could be alleviated by changes to boat propellers (Malakoff, 2010), as the propeller creates the primary source of boat noise (Nowacek, Johnson, & Tyack, 2004; Rolland *et al.*, 2012). Indeed cavitation from the propeller blade produces considerable noise across all frequencies (Hildebrand, 2009). While initial costs are likely to prevent immediate change in the shipping industry, long-term savings could be a strong economic incentive, as quieter engines are more fuel efficient (Malakoff, 2010), and can even reduce biofouling (Wilkens, Stanley, & Jeffs, 2012).

Such proactive measures would be wise in the face of mounting marine ecological impacts. New evidence suggests that as climate change increases ocean acidity, it could affect fish both directly, e.g., by interfering with development of carbonate hearing organs (Simpson *et al.*, 2011), and indirectly, e.g., by altering the properties of sound absorption (Hester *et al.*, 2008; Ilyina, Zeebe, & Brewer, 2009). Further, changing water temperatures could interfere with fish orientation and communication (Papes & Ladich, 2011). By reducing the additional stressor of noise, oceans would become more resilient to other, perhaps more 'complex' or less easily managed sources of anthropogenic pressures (see Halpern *et al.*, 2008). Given our global dependence on fish (FAO, 2012), the impacts of noise on fish in their natural habitats thus becomes an ultimate consideration in an increasingly noisy ocean.

6.4 REFERENCES

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Appendix 3.1

Table 3A1. Data table of all manual annotations taken over five dates in June, categorized by vocalization type and time of day.

	Grunt					Growl				
Time of day	01-Jun	07-Jun	15-Jun	22-Jun	30-Jun	01-Jun	07-Jun	15-Jun	22-Jun	30-Jun
12:00 AM	0	0	0	4	5	0	0	3	3	1
1:00 AM	0	0	0	6	4	0	0	0	1	3
2:00 AM	0	0	0	11	8	0	0	0	3	4
3:00 AM	0	0	0	16	9	1	0	1	4	6
4:00 AM	3	21	49	42	47	3	8	6	10	15
5:00 AM	58	77	56	153	153	4	11	16	38	57
6:00 AM	88	115	80	148	111	14	14	31	80	51
7:00 AM	62	125	49	161	86	4	28	25	52	44
8:00 AM	43	61	9	104	59	4	22	22	44	31
9:00 AM	23	39	13	22	20	4	2	9	25	12
10:00 AM	49	30	42	166	40	9	2	34	57	27
11:00 AM	27	45	20	63	27	3	14	10	10	11
12:00 PM	33	42	26	74	44	3	12	11	15	11
1:00 PM	12	34	41	152	20	10	3	6	12	2
2:00 PM	33	37	45	157	70	7	5	7	23	9
3:00 PM	18	9	30	118	29	10	2	8	9	16
4:00 PM	33	37	31	67	50	11	2	16	6	12
5:00 PM	16	29	18	77	26	4	12	2	14	5
6:00 PM	6	51	12	33	87	1	14	2	9	23
7:00 PM	2	22	21	7	77	2	6	3	1	32
8:00 PM	3	27	41	18	118	0	8	8	6	50
9:00 PM	0	38	5	48	57	0	8	1	19	16
10:00 PM	0	3	1	7	14	0	0	2	3	15
11:00 PM	1	2	0	5	24	0	0	3	3	5
				TOTAL	*4,789*				*TOTAL*	*1,397*

Cont’d **Table 3A1**

	Hum					Grunt Train				
Time of day	01-Jun	07-Jun	15-Jun	22-Jun	30-Jun	01-Jun	07-Jun	15-Jun	22-Jun	30-Jun
12:00 AM	1	1	1	2	1	0	0	0	0	4
1:00 AM	1	1	1	3	1	0	0	0	0	0
2:00 AM	1	1	1	2	1	0	0	0	0	4
3:00 AM	0	1	1	1	1	0	0	0	0	0
4:00 AM	1	3	1	2	4	0	0	0	0	0
5:00 AM	4	1	0	5	1	5	0	1	0	0
6:00 AM	1	6	5	0	0	2	0	0	0	0
7:00 AM	2	3	0	0	0	0	0	0	0	0
8:00 AM	5	4	0	0	1	5	3	0	0	0
9:00 AM	0	10	0	0	0	0	0	0	0	0
10:00 AM	2	2	0	0	0	0	0	0	0	3
11:00 AM	6	6	0	0	0	0	0	0	0	0
12:00 PM	5	1	2	2	0	0	0	0	0	0
1:00 PM	2	1	2	0	3	0	0	0	0	0
2:00 PM	1	0	3	0	0	0	0	0	0	0
3:00 PM	2	0	3	3	2	0	0	0	0	0
4:00 PM	2	4	2	4	3	3	0	0	0	0
5:00 PM	4	5	2	0	3	0	0	0	0	0
6:00 PM	1	3	9	3	0	0	0	0	0	0
7:00 PM	2	2	4	7	2	0	0	5	0	0
8:00 PM	2	5	5	1	1	0	0	1	0	0
9:00 PM	2	2	1	6	4	0	0	0	0	0
10:00 PM	1	1	1	3	3	0	0	0	0	0
11:00 PM	1	1	1	3	1	0	0	0	0	0
				TOTAL	*236*				*TOTAL*	*36*

Cont’d **Table 3A1**

	All				
Time of day	01-Jun	07-Jun	15-Jun	22-Jun	30-Jun
12:00 AM	1	1	4	9	11
1:00 AM	1	1	1	10	8
2:00 AM	1	1	1	16	17
3:00 AM	1	1	2	21	16
4:00 AM	7	32	56	54	66
5:00 AM	71	89	73	196	211
6:00 AM	105	135	116	228	162
7:00 AM	68	156	74	213	130
8:00 AM	57	90	31	148	91
9:00 AM	27	51	22	47	32
10:00 AM	60	34	76	223	70
11:00 AM	36	65	30	73	38
12:00 PM	41	55	39	91	55
1:00 PM	24	38	49	164	25
2:00 PM	41	42	55	180	79
3:00 PM	30	11	41	130	47
4:00 PM	49	43	49	77	65
5:00 PM	24	46	22	91	34
6:00 PM	8	68	23	45	110
7:00 PM	6	30	33	15	111
8:00 PM	5	40	55	25	169
9:00 PM	2	48	7	73	77
10:00 PM	1	4	4	13	32
11:00 PM	2	3	4	11	30
				TOTAL	*6,458*

Appendix 3.2

Table 3A2. Nest penetration events by predator species.

Date	Predator	Grunt?	Attempt	Response by *P. notatus*
June 7	Black-clawed crab	No	Crab enters nest entrance sideways. *P. notatus* remains perfectly motionless because harbour seal in nest vicinity.	Once harbour seal leaves, *P. notatus* faces crab and assumes defensive posture; crab departs.
June 21	Mottled star	Unclear	Mottled star enters nest and sticks to ceiling.	Mottled star succeeds in entering nest; stays attached to nest ceiling.
June 21	Red rock crab	No	Crab enters nest sideways.	Crab departs a while later. Unable to see *P. notatus*' response.
June 24	Shore crab	No	Crab disappears inside nest.	No sign of *P. notatus*.
June 25	Perch spp.	No	Perch swims directly into shallow part of nest entrance.	No sign of *P. notatus*. As perch departs, *P. notatus* sticks head out nest entrance and assumes defensive posture.
June 27	Gunnel	No	Gunnel slithers into shallow part of nest entrance.	*P. notatus* remains in defensive posture; gunnel departs.
June 27	Ochre star	No	Ochre star gradually moves into nest opening; one arm pokes into nest entrance.	*P. notatus* nips at ochre star's arm; ochre star immediately responds by moving its arm away from nest entrance and departing area.

Appendix 3.3

Table 3A3. Description of each grunge vocalization and associated predator. A(R) = Adventitious grunt during rock removal; A(E) = Adventitious grunt during egg care.

Date	Species	Call length (sec)	Call description
25-Jun	Red rock crab	0.085	Quick single grunt
09-Jun	Red rock crab	0.1	Quick single grunt
21-Jun	Red rock crab	0.1	Single grunt
23-Jun	Red rock crab	0.15	Single grunt
23-Jun	Red rock crab	0.22	Single grunt
23-Jun	Red rock crab	0.28	Growl
12-Jun	Red rock crab	0.3	Double peaked grunt
23-Jun	Red rock crab	0.3	Double peaked grunt
23-Jun	Red rock crab	0.35	Single grunt
12-Jun	Red rock crab	0.4	Double peaked grunt
26-Jun	Red rock crab	0.4	Double peaked grunt
23-Jun	Red rock crab	0.45	Double peaked grunt
12-Jun	Red rock crab	0.5	Double peaked grunt
23-Jun	Red rock crab	0.5	Triple peaked grunt
12-Jun	Red rock crab	0.55	Double peaked grunt
23-Jun	Red rock crab	0.55	“Ee-aw” grunt
07-Jun	Red rock crab	0.6	“Ee-aw” grunt
11-Jun	Red rock crab	0.6	“Ee-aw” grunt
23-Jun	Red rock crab	0.6	Two single grunts
11-Jun	Red rock crab	0.75	“Ee-aw” grunt
23-Jun	Red rock crab	0.75	“Ee-aw” grunt
22-Jun	Red rock crab	0.8	Double peaked grunt
23-Jun	Red rock crab	0.8	Five peaked grunt
07-Jun	Red rock crab	0.85	Four peaked grunt (“ee-ee-ee-ee-aw")
12-Jun	Red rock crab	0.9	Double peaked grunt
11-Jun	Red rock crab	0.95	Double peaked grunt (“ee-ee-aw”)
07-Jun	Red rock crab	1	Double peaked grunt (“ee-ee-aw”)
12-Jun	Red rock crab	1.1	Double peaked grunt followed by single grunt
22-Jun	Red rock crab	1.3	“Ee-aw” grunt followed by double grunt
23-Jun	Red rock crab	1.5	Long single growl-grunt
11-Jun	Red rock crab	3.3	Growl
27-Jun	Sculpin	0.17	Quick single grunt
27-Jun	Sculpin	0.1	Quick single grunt
27-Jun	Sculpin	0.2	Quick single grunt
27-Jun	Sculpin	0.2	Quick single grunt
27-Jun	Sculpin	0.2	Quick single grunt
27-Jun	Sculpin	0.2	Quick single grunt
09-Jun	Gunnel	0.1	Quick single grunt

26-Jun	Gunnel	0.05	Quick single grunt
26-Jun	Gunnel	0.05	Quick single grunt
12-Jun	Helmet crab	0.2	Single grunt
07-Jun	A(R)	0.3	Quick single grunt followed by residual grunt
09-Jun	A(E)	0.1	Quick single grunt
09-Jun	A(E)	0.1	Quick single grunt
09-Jun	A(E)	0.09	Quick single grunt
09-Jun	A(E)	0.09	Quick single grunt
09-Jun	A(E)	0.1	Quick single grunt
12-Jun	A(E)	0.1	Quick single grunt
12-Jun	A(E)	0.1	Quick single grunt
Total predator average		***1.5 (SE = ± 0.09, n = 41)***	
Red rock crab average		*0.68 (SE = ± 0.11, n = 31)*	
Sculpin average		*0.18 (SE = ± 0.02, n = 6)*	
Gunnel average		*0.07 (SE = ± 0.02, n = 3)*	
Adventitious average		*0.12 (SE = + 0.03, n = 8)*	

Appendix 5.1: Technical description of algorithm

All computer programs were written in the Python language with several open-source libraries to provide pre-written algorithms, including SURF from openCV, small utility functions from numpy (for calculations, e.g., computing averages, etc.) and k-means from scipy. k-means is a non-supervised approach, which means that it does not need any 'training' data. It is a classification method that aims to cluster the data so that there is minimal variance within each cluster (MacQueen, 1967). The k-means method used here is set to find five clusters of feature points based on the amount of movement and the location of each feature point, where the cluster with the largest movement should be representing fish movement.

As recordings were made *in situ*, the dataset contained varying background environments and conditions, including differences in lighting, currents, and shadows. To complement these conditions, a robust and model-free motion detection method- i.e., not based on a 3D model specific to the organism under investigation- was implemented. Speeded Up Robust Features (SURF) is a well-known and commonly used method in computer sciences to detect relevant features in images found on a frame-by-frame basis (Bay, Tuytelaars, & Van Gool, 2006). Figure 5A1 shows 'markers' picked up in two frames: one without motion detection and the other with motion detection. In our study, fish movement of the plainfin midshipman *Porichthys notatus* was determined between video frames based on assigned 'features' (Jean, Albu, & Dumoulin, 2011). The SURF

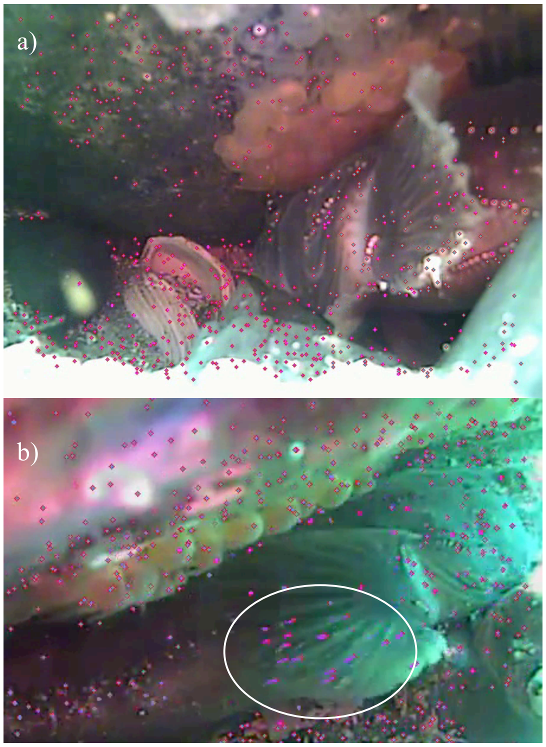

Figure 5A1. Example of two unfiltered images: a) Many markers but no movement detected. b) Many markers, and motion detected (in circle on right pectoral fin): points with 'tails' show movement between current and previous frame.

method is scale and rotationally invariant to find interest points on the image. These interest points are significantly different in image properties, including colour and colour intensity, which tend to be corners, edges and other distinctive locations. Such 'descriptors' generated by SURF are at the core based on differences in color intensities near the interest point (with some transformations steps in between to speed up processing and to increase accuracy). Because the descriptors are based on a local area, we were then able to match features found on one frame with features on the next, even if *P. notatus* had moved around in the scene, rotated, or changed in depth with respect to the camera.

We performed the matching steps for all features, where not all features from the previous frame were necessarily paired with a feature from the current frame. A current feature point was said to 'match' with a point in the previous frame if the two sets of descriptors corresponded significantly better than the second closest feature point. For example, a very green corner would only get matched to the green corner in the previous frame if it matched better than the second best match, e.g., a light green corner. We then calculated the motion of each individual feature point based on the displacement between the previous and current feature location. Since we assumed that each local area of *P. notatus* generally moved consistently- i.e., the moving body part was a component of the same fish- we excluded as outliers points that moved more than two standard deviations away from the average of its neighbours (defined here as any feature within a 50 pixel radius of a feature). Further, only matching-features 40 or fewer pixels away[1] were counted, as initial trials revealed $\leq$40 pixels to be the best balance between quicker fish movements and false matchings. When the point had no nearby neighbours, matching features were lowered dramatically to three pixels away, as such points were more likely to be outliers.

Based on the distance moved and the current location of each feature point, we performed an unsupervised machine-learning algorithm (k-means) to automatically group the features. This was done to seek out clusters with the largest movement, which presumably consisted of features from *P. notatus*, as we assumed that *P. notatus* was the main moving object in all videos (an assumption that was verified manually). To find the cluster with the largest movement, we first excluded any cluster with fewer than five feature points: as determined

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[1] Using euclidean measurements.

through empirical trials, ≥five feature points best reflected typical movement made by *P. notatus*; detections with ≤five feature points were likely too small to be made by *P. notatus*, as the fish generally took up a substantial amount of video frame, and at any rate, would be considered too small to be a reliable measure of *P. notatus* movement.

After this exclusion, the cluster with the largest median movement was then chosen. Although the median measure was used to determine which cluster was picked to represent the movement of *P. notatus*, it was the mean used in the final calculation of the movement for that frame. This was because a cluster with only a few large movements could skew the mean and cause the wrong cluster to be picked while the median was resistant to outliers in this way. The same method was repeated for all frames.

A 'down-sampling' technique was employed in this analysis whereby two other analyses were run in addition to the initial analysis: one sampling every other frame, the other every four frames. By taking in fewer frames, the video was essentially 'sped up', which proved useful in capturing slower, more subtle fish movements. These three techniques- i.e., normal and down-sampled two and four times- were performed on all videos, and the results were then added together to obtain final results. For example, if no movement was detected in the initial trial (i.e., video at normal speed) but was detected in a down-sampled analysis, the movement from the down-sampled analysis would be counted in the results. This method sometimes led to false positives but overall increased accuracy.

Given the dynamic medium of water, and the uncontrolled conditions of this study, there was always some ambient background motion in videos (e.g., seaweed, plankton, etc.). Thus, when *P. notatus* was not moving, we eliminated the motion calculated from all frames that recorded two or fewer pixels of average mean movement.

One particular challenge encountered in this study was the algorithm's failure to detect SURF features in dark or blurry conditions. This drawback has the potential for improvement in the future by considering other feature-finding methods and feature-matching (for example, using more than the previous few frames for motion detection) which would increase detection of SURF.

As this algorithm was developed model-free- i.e., it was not based on a 3D model of *P. notatus*- it could potentially be used on biological videos focused on other species (as long as assumptions listed above were met). This greatly opens up the possibilities for research projects in future biological studies.

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Appendix 5.2

Table 5A1. Precision, recall and F1 scores for all nine manual versus automated annotations under different error scenarios. Shaded cells denote results from all errors included in analysis. Note scores increase substantially when both sources of dominant errors are excluded (current and aeration-related errors).

Sample video	Precision	Recall	F1		Sample video	Precision	Recall	F1
Including all errors					**Excluding current + aeration-related errors**			
Ambient 1	0.696	0.904	0.786		Ambient 1	0.930	0.926	0.928
Ambient 2	0.988	0.749	0.852		Ambient 2	0.988	0.918	0.951
Ambient 3	0.689	0.954	0.800		Ambient 3	0.986	0.967	0.977
Boat 1	0.576	0.975	0.724		Boat 1	0.983	0.985	0.984
Boat 2	0.917	0.446	0.600		Boat 2	0.917	0.929	0.923
Boat 3	0.966	0.980	0.973		Boat 3	0.966	0.980	0.973
Control 1	0.871	1.000	0.931		Control 1	0.943	1.000	0.971
Control 2	0.991	0.533	0.693		Control 2	0.991	0.790	0.879
Control 3	0.980	0.980	0.980		Control 3	0.980	0.980	0.980
Average	0.853	0.836	0.816		*Average*	0.965	0.942	0.952
Excluding aeration-related errors					**Excluding current-related errors**			
Ambient 1	0.696	0.904	0.786		Ambient 1	0.930	0.926	0.928
Ambient 2	0.988	0.918	0.951		Ambient 2	0.988	0.749	0.852
Ambient 3	0.689	0.954	0.800		Ambient 3	0.986	0.967	0.977
Boat 1	0.576	0.975	0.724		Boat 1	0.983	0.985	0.984
Boat 2	0.917	0.929	0.923		Boat 2	0.917	0.446	0.600
Boat 3	0.966	0.980	0.973		Boat 3	0.966	0.980	0.973
Control 1	0.871	1.000	0.931		Control 1	0.943	1.000	0.971
Control 2	0.991	0.790	0.879		Control 2	0.991	0.533	0.693
Control 3	0.980	0.980	0.980		Control 3	0.980	0.980	0.980
Average	0.853	0.937	0.883		*Average*	0.965	0.841	0.884

Appendix 5.3

Table 5A2. All sources and times of video deletion. Shaded cells denote videos excluded entirely from analysis.

Date	Treatment	Event	Time (h:m:s)
07-Jun	Ambient	*P. notatus* out of sight	0:00:02
07-Jun	Ambient	*P. notatus* out of sight	0:00:10
07-Jun	Boat	Other organism (crab)	0:00:05
11-Jun	Ambient	Video corruption	0:00:09
11-Jun	Ambient	Video corruption	0:00:04
11-Jun	Ambient	Video corruption	0:00:01
11-Jun	Ambient	Video corruption	0:00:01
11-Jun	Ambient	Video corruption	0:00:03
11-Jun	Ambient	Video corruption	0:00:03
11-Jun	Ambient	Video corruption	0:00:01
11-Jun	Ambient	Video corruption	0:00:01
11-Jun	Boat	Other organism (hermit crab)	0:01:19
11-Jun	Boat	Video corruption	0:00:01
11-Jun	Boat	Video corruption	0:00:01
11-Jun	Boat	Video corruption	0:00:01
11-Jun	Boat	Video corruption	0:00:01
11-Jun	Boat	Video corruption	0:00:01
11-Jun	Control	Video corruption	0:00:01
19-Jun	Ambient	Lens obstruction (shell)	0:15:00
19-Jun	Boat	Lens obstruction (shell)	0:15:00
19-Jun	Control	Lens obstruction (shell)	0:15:00
20-Jun	Ambient	Video corruption	0:00:02
20-Jun	Ambient	Video corruption	0:00:03
20-Jun	Ambient	Video corruption	0:00:02
20-Jun	Ambient	Video corruption	0:00:02
20-Jun	Boat	Video corruption	0:00:03
20-Jun	Boat	Video corruption	0:00:01
20-Jun	Control	*P. notatus* out of sight	0:00:02
20-Jun	Control	*P. notatus* out of sight	0:00:14
20-Jun	Control	*P. notatus* out of sight	0:00:25
21-Jun	Ambient	Other organism (sea cucumber)	0:15:00
21-Jun	Boat	Other organism (sea cucumber)/*P. notatus* out of sight	0:15:00

21-Jun	Control	Other organism (sea cucumber)/*P. notatus* out of sight	0:15:00
22-Jun	Boat	*P. notatus* out of sight	0:00:34
22-Jun	Boat	*P. notatus* out of sight	0:00:16
22-Jun	Boat	*P. notatus* out of sight	0:00:01
22-Jun	Boat	*P. notatus* out of sight	0:00:05
22-Jun	Boat	*P. notatus* out of sight	0:00:15
23-Jun	Boat	Video corruption	0:00:01
24-Jun	Boat	Lens obstruction (seaweed)	0:00:04
24-Jun	Boat	Video corruption	0:00:26
24-Jun	Boat	Video corruption	0:00:12
26-Jun	Ambient	Other organism (gunnel)	0:00:11
26-Jun	Ambient	*P. notatus* out of sight	0:00:19
26-Jun	Ambient	*P. notatus* out of sight	0:00:11
26-Jun	Ambient	*P. notatus* out of sight	0:00:28
26-Jun	Ambient	*P. notatus* out of sight	0:00:04
26-Jun	Ambient	*P. notatus* out of sight	0:00:03
26-Jun	Ambient	*P. notatus* out of sight	0:00:33
26-Jun	Ambient	*P. notatus* out of sight	0:00:01
26-Jun	Ambient	*P. notatus* out of sight	0:00:24
26-Jun	Ambient	*P. notatus* out of sight	0:00:07
26-Jun	Ambient	*P. notatus* out of sight	0:00:02
26-Jun	Ambient	*P. notatus* out of sight	0:00:01
26-Jun	Ambient	*P. notatus* out of sight	0:00:05
26-Jun	Ambient	*P. notatus* out of sight	0:00:14
26-Jun	Ambient	*P. notatus* out of sight	0:00:02
26-Jun	Boat	*P. notatus* out of sight	0:00:16
26-Jun	Boat	*P. notatus* out of sight	0:00:03
26-Jun	Boat	*P. notatus* out of sight	0:00:01
26-Jun	Boat	*P. notatus* out of sight	0:00:06
26-Jun	Boat	*P. notatus* out of sight	0:00:01
26-Jun	Boat	*P. notatus* out of sight	0:00:05
26-Jun	Boat	*P. notatus* out of sight	0:00:01
26-Jun	Boat	*P. notatus* out of sight	0:00:10
26-Jun	Boat	Video corruption	0:00:01
26-Jun	Boat	Video corruption	0:00:08
26-Jun	Boat	Video corruption	0:00:01
26-Jun	Boat	Video corruption	0:00:01
26-Jun	Boat	Video corruption	0:00:01

26-Jun	Control	Other organism (gunnel)		0:00:23
26-Jun	Control	*P. notatus* out of sight		0:00:04
26-Jun	Control	*P. notatus* out of sight		0:00:02
26-Jun	Control	*P. notatus* out of sight		0:00:41
26-Jun	Control	*P. notatus* out of sight		0:00:20
26-Jun	Control	Video corruption		0:00:03
26-Jun	Control	Video corruption		0:00:04
26-Jun	Control	Video corruption		0:00:03
26-Jun	Control	Video corruption		0:00:05
26-Jun	Control	Video corruption		0:00:03
27-Jun	Ambient	*P. notatus* out of sight		0:00:47
27-Jun	Ambient	*P. notatus* out of sight		0:00:03
27-Jun	Ambient	*P. notatus* out of sight		0:00:37
27-Jun	Ambient	*P. notatus* out of sight		0:01:16
27-Jun	Boat	*P. notatus* out of sight		0:02:14
27-Jun	Boat	*P. notatus* out of sight		0:00:11
27-Jun	Boat	*P. notatus* out of sight		0:00:17
27-Jun	Boat	Video corruption		0:00:01
27-Jun	Boat	Video corruption		0:00:01
27-Jun	Control	*P. notatus* out of sight		0:00:56
27-Jun	Control	*P. notatus* out of sight		0:00:06
27-Jun	Control	*P. notatus* out of sight		0:00:03
27-Jun	Control	*P. notatus* out of sight		0:02:06
27-Jun	Control	*P. notatus* out of sight		0:01:19
27-Jun	Control	*P. notatus* out of sight		0:00:11
27-Jun	Control	*P. notatus* out of sight		0:01:53
27-Jun	Control	Video corruption		0:00:03
27-Jun	Control	Video corruption		0:00:08
27-Jun	Control	Video corruption		0:00:02
			Total	*1:52:35*
			Ambient (total)	*0:36:12*
			Boat (total)	*0:37:06*
			Control (total)	*0:39:17*