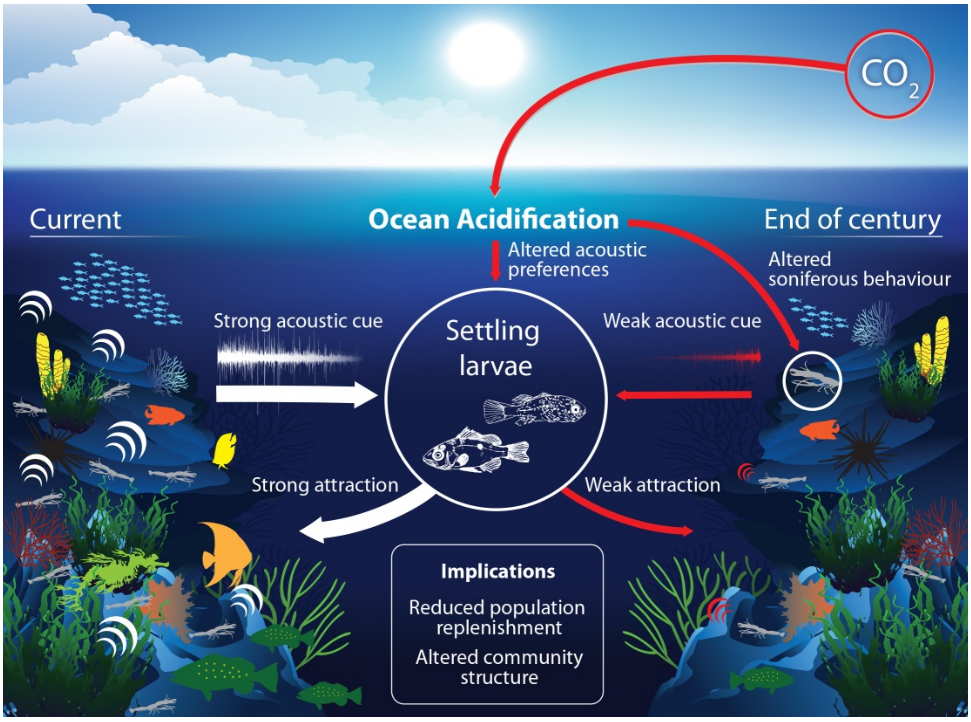
THE EFFECTS OF OCEAN ACIDIFICATION ON SOUND PRODUCTION AND RECEPTION IN MARINE ANIMALS



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Cover Image: Conceptual diagram showing the direct and indirect effects of ocean acidification on settlement-stage larvae in relation to marine sounds and hearing. Ocean acidification will directly impact settling fish larvae by altering their auditory preferences and indirectly by weakening the quantity and quality of the biological soundscape which is used as a long-distance cue for orientation by oceanic larvae of many species.

Artwork credit: Tullio Rossi

DECLARATION

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Tullio Rossi

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CHAPTER 2

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CHAPTER 3

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CHAPTER 4

This study was supported by Australian Research Council (ARC) Future Fellowship to Ivan Nagelkerken (grant no. FT120100183) and Sean D Connell (grant no. FT0991953), and a grant from the Environment Institute (The University of Adelaide). Figure 4 credit: Tullio Rossi and IAN University of Maryland Centre for Environmental Science.

CHAPTER 5

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ABSTRACT

The dispersal of larvae and their settlement is fundamental to replenishment of marine populations and their connectivity. During their ontogeny, larvae progressively develop behavioural and physiological capabilities that allow them to effectively identify, locate and reach suitable settlement habitats making the settlement process far from being a stochastic event. This process typically involves multiple senses, including audition, olfaction and vision. Sound propagates well in water with little attenuation and plays a critical part of this process because the larval stages of many species use it as a long distance cue for orientation towards suitable settlement habitat. Because marine coastal soundscapes are largely of biological origin, they not only carry information about the location and proximity of potential habitat, but also information about the quality of habitat. Ocean acidification has profound effects on marine life, but its effect on biological sound production and its reception by navigating oceanic larvae remains largely unknown. In this thesis I show that ocean acidification can profoundly decrease the total acoustic output of coastal habitats as measured at natural CO_2 vents. This change is largely due to the negative effect of ocean acidification on the soniferous behaviour and potentially the abundance of the noisiest invertebrate in the ocean, the snapping shrimp. Laboratory experiments showed that a quieter soundscape indirectly penalizes oceanic larvae by being a less attractive settlement cue. Remarkably, ocean acidification also caused a switch in role of preferred soundscape cues from attractor to repellent in the auditory preferences of fish larvae of two economically important species, barramundi and mulloway. Furthermore, ocean acidification boosted larval development but made fish larvae swim slower. This thesis reveals an increased risk to the complex process of larval settlement through a combination of direct and indirect effects driven by ocean acidification. Such alterations will likely have far-reaching consequences for the population

replenishment of marine organisms that utilize soundscapes orientation as part of their life history strategy.

CHAPTER I: GENERAL INTRODUCTION

THIS THESIS IN THE CONTEXT OF CLIMATE RESEARCH

More than two centuries of intensive use of fossil fuels and anthropization of the natural environment resulted in the increase of the atmospheric CO_2 concentration from ~280 µatm to ~ 400 µatm [1]. It is well known and accepted that this resulted in the warming of our planet. What is less known, is that about a third or the CO_2 that has been released by human activities dissolved in the ocean. This is on one hand beneficial, because without this service, the atmospheric concentration of CO_2, hence the greenhouse gas effect would be higher. However, on the other hand this resulted in a 30% increase in the acidity of the ocean. This process is known as ocean acidification or the "evil twin" of global warming. The current knowledge suggests that, if we do nothing about our carbon emissions and continue on the "business-as-usual" path, global warming and ocean acidification will severely affect the oceans in a variety of ways [2, 3]. However, data also suggest that if we successfully limit warming and ocean acidification to moderate levels we can avoid some of the worst impacts and buy time for species to adapt to the new conditions [2].

Despite the tremendous research effort that characterized the last few decades of climate research, some important questions remain unassessed [4]. It is however of fundamental importance to provide to the public and policy-makers the most comprehensive picture possible of how the marine environment is going to be affected if we do not manage our carbon emissions.

Ocean acidification lowers the saturation state of calcium carbonate. This makes the production of hard calcified parts in species like corals and shellfish harder. Therefore, it comes at no surprise that the most ocean acidification research focused on calcifying species. However, in the last decade new and unexpected effects of ocean acidification are being discovered in species that were considered to be capable to withstand high CO_2 concentrations.

Fish, for example, posses a highly flexible physiology that was expected to make this taxa particularly resistant to ocean acidification [5]. However, this view changed when new research revealed subtler effects mediated by neurological alterations. Ocean acidification has been found to alter the processing of sensory information in fish, which often translates in striking behavioural alterations [6]. A wide spectrum of behaviours and sensorial preferences have been found to be affected by CO_2 levels expected to be reached by the end-of-century or earlier [7, 8]. This effect of ocean acidification is now recognized to represent a serious risk for marine populations because of its potential to undermine important life history processes that are mediated by behaviour [8-10]. However, this field of research is still young and only few species, mostly with low economical importance, have been investigated. Furthermore, key processes, which regulate wild populations, such as larval dispersal and settlement, have been poorly investigated.

In this context, this thesis aims to push our knowledge forward on the effects of ocean acidification on marine species in a scarcely explored but highly important area. Furthermore, this thesis wants to focus on species with a high economical importance in order to be relevant to industry, the general public and policy makers. The approach I use is to study how ocean acidification might interfere with soundscape driven orientation in fish and how fish orientation might be indirectly affected by ocean acidification via an alteration of the soundscapes they rely on. As these ecological processes are poorly known even from a basic research perspective, this thesis tackles basic research questions and pushes forward our knowledge in the field of fish bioacoustics and soundscape ecology.

MARINE SOUNDSCAPES AND SOUNDSCAPE ORIENTATION

GENERAL INTRODUCTION TO SOUNDSCAPES

A soundscape is the set of biological, geophysical and anthropogenic sounds that originate from a landscape and which vary spatially and temporally, reflecting ecosystem processes [11]. Soundscape ecology, despite its value for applied research, remains a relatively young field undergoing rapid progress [12].

A soundscape can be seen as an organized multidimensional space that carries meaningful information about the location and quality of resources [13]. This can serve to inform both residents and dispersing species about the composition of the local community, the amount of available resources (such as food, territory and partners), and the presence of predators [13-15]. Soundscapes are not uniform and research showed how environmental heterogeneity translates into soundscape heterogeneity in both time and space [16, 17]. Research showed that not only different habitats produce distinct acoustic signatures [18, 19], but also that the quality of individual signals produced by soniferous animals is affected by stress levels [20], habitat condition [21] and climate [22, 23]. Hence, habitats impacted by anthropic perturbations produce distinct acoustic signatures associated with changes in biodiversity [24] and ecological condition such as coral cover [25] or habitat fragmentation [26].

MARINE SOUNDSCAPES

Marine coastal soundscapes are often dominated by biological sounds. The most common sources of marine biological sounds in coastal waters are snapping shrimp snaps, sea urchin raspings and fish vocalizations [16, 27]. Snapping shrimp (family Alpheidae), are certainly the most common and loud animals in tropical and temperate coastal areas [28-30]. These shrimp possess the unique ability to produce some of the loudest sounds in the ocean (up to 210 dB re 1 μ Pa) [28], which appear to be second only to sperm whale clicks (up to 236 dB re 1 μ Pa) [31]. Such extreme sound levels are achieved by the formation of cavitation bubbles upon rapid closure of the snapping claw [32]. Snapping shrimp use their "sonic gun" for a variety of purposes such as predation, agonistic behaviour and communication [33] and are responsible for the permanent characteristic background crackling sound that is so commonly found around reefs and mangroves [18, 34]. Snapping shrimp sounds have been studied since the Second World War because they can interfere with military communication and cause acoustical masking of submarines [30]. More recently, biological sounds and soundscapes in general, gained new research interest thanks to the recognition of their ecological importance as settlement cues for dispersing propagules [14]. The importance of snapping shrimp sounds for the orientation of larvae has earlier been shown in an *in situ* study where natural larval fish communities showed higher attraction towards soundscapes with high frequency (> 570 Hz) snapping shrimp-generated sound compared to non-snapping shrimp-generated low frequency soundscape (<570 Hz) [35].

Marine soundscape ecology is a promising but very young field where very little applied research has been conducted [12]. To date no studies investigated the effect of global stressors such as global warming and ocean acidification on the biotic component of marine soundscapes. Similarly, only one study investigated the effect of habitat degradation on marine soundscapes [25].

SOUNDSCAPE ORIENTATION

The pelagic dispersal of marine propagules connects populations via replenishment. Despite its ecological importance, this recruitment process has been poorly understood until recently and often referred to as a "black box" in the life history of marine species. Research showed that this process is far from being stochastic as larvae of many species are able to locate and selectively swim towards preferred settlement habitats using a set of sensory cues such as sounds, odours and light [36]. Sound is one of the most reliable directional cues in the ocean because it propagates to long distances (100 – 1000s meters) with little attenuation and in a predictable fashion [37]. Visual and olfactory cues instead, are easily confounded by light, turbidity and turbulence making them less reliable for long-distance orientation. In the marine realm, a variety of marine organisms use soundscape for orientation during the crucial and delicate process of dispersal and settlement. Studies have provided evidence that propagules of both fish [15, 38-40] and invertebrates such as corals, crustaceans and molluscs [41-43] can use sound to orient themselves towards suitable settlement habitats. This means that soundscapes play an important ecological functional role as mediators of connectivity and population replenishment. In the ocean, a variety of marine larval fish species had been shown to respond to specific soundscapes in a species-specific manner [15]. Two possible settlement strategies emerged. Some species orient towards a habitat by being selectively attracted to its soundscape while others select habitats by avoidance of non-preferred soundscapes [15, 44]. From the point of view of a dispersing larva, the spatial heterogeneity of soundscape can be used as a map containing qualitative (type of habitat) and directional information (what direction and how far). Despite this important ecological role that soundscapes plays in the ocean, it is surprising that the potential effects of ocean acidification on sound production have never been explored.

THE EFFECT OF OCEAN ACIDIFICATION ON ANIMAL BEHAVIOUR

CLIMATE CHANGE AND ANIMAL BEHAVIOUR

Animal behaviour is a mediator between the physical environment and the ecological processes that it influences [8]. Climate change, which leads to a major modification of the physical environment, is exerting pervasive alterations to both marine and terrestrial ecosystems [45-47]. In the face of a changing environment, behavioural plasticity represents the first line of defence that animals can adopt. In the marine environment most research focused on the study of the effects of increasing temperatures on marine organisms. However, anthropogenic carbon emissions are responsible for another major alteration of the physical environment known as ocean acidification. Ocean acidification is predicted to have profound effects on marine life [48, 49], and some of its most detrimental and at times striking effects are trough the direct alteration of animal behaviour and the processing of sensory information [7, 8].

Fish possess elevated physiological plasticity that enables them to prevent acidosis, even at very high levels (>5000 μatm) of $p\mathrm{CO}_2$ [5]. However, the same physiological mechanisms that fish rely on to prevent acidosis in high $p\mathrm{CO}_2$ conditions, result in altered processing of sensory information, which translates in often striking behavioural alterations [6]. A wide spectrum of behaviours and sensorial preferences have been found to be affected by CO_2 levels expected to be reached by the end-of-century or earlier [7, 8]. These include olfactory, auditory and visual preferences, which are all important for settling animals [50-52]. What seems to be the common denominator of these behavioural changes is an altered function of the $\mathrm{GABA_A}$ neuroreceptor [6, 53, 54], which is the major inhibitory receptor in the vertebrate brain. Despite the elevated vulnerability and ecological importance of larval stages, few studies to date investigated the effects of ocean acidification on behaviour in pre-metamorphic larvae [9, 55, 56] and most other studies on ocean acidification and fish behaviour have tested behavioural competencies in post-metamorphic juveniles. This skewed research emphasis has left the potential effects of ocean acidification on pre-metamorphic and metamorphic behavioural traits unexplored in most fish species. This is a major gap in our knowledge because it interests the critical transition from a pelagic to a demersal life style [36, 38] where the effects of ocean acidification on sound reception in larval fish have the potential to be most detrimental.

OCEAN ACIDIFICATION AND FISH SOUND DRIVEN ORIENTATION

The effect of ocean acidification on sound reception by free-swimming larvae remains largely untested. To date, only one study has directly investigated the effect of ocean acidification on auditory mediated behaviour, finding that elevated CO_2 (600-900 μatm) alters the auditory responses of juvenile clownfish to predator-rich daytime reef noise [44]. It is also known that elevated CO_2 conditions can increase the size of otoliths [57], and that this could potentially affect hearing sensitivity [58]. However, nothing is known about the effects of elevated CO_2 on the orientation of fishes to auditory cues during their pelagic larval stage, which represents a major gap in our knowledge because of the crucial importance of effective orientation during this delicate life stage.

THE EFFECT OF OCEAN ACIDIFICATION ON SONIFEROUS ANIMALS AND SOUNDSCAPES

Ocean acidification will likely change marine ecosystems within this century by affecting a wide range of marine organisms [59-61]. Coastal marine sounds are largely of biological origin, therefore, potentially susceptible to ocean acidification. As mentioned above, the main marine sound producing animals are snapping shrimp, fish and sea urchins [16, 19, 62]. We currently know that these taxa are directly affected by ocean acidification in their behaviour [8, 63], calcification [64], and physiology [65], but nothing is known about potential effects on their sound production capabilities. Furthermore, ocean acidification could exert indirect effects on sound producing animals by changing their habitat [60]. As elevated CO_2 also acts as a fertilizer it can favour opportunistic species like algal turfs to the expense of canopy forming algae and seagrass which tend to be overgrown resulting in a regime shift in the ecosystem [60]. Nevertheless, despite the important role that soundscapes play as cues for dispersing propagules, the effect of ocean acidification on sound production has never been investigated before.

THESIS AIM AND APPROACH

Many marine organisms possess a dispersive larval stage and anything that has the potential to decrease settlement success could have large implications for the replenishment of marine populations and their connectivity [66]. Soundscape orientation is a critical process that directly influences the success of larval settlement [14]. Given the importance of such process, it is puzzling that to date no studies investigated the effect of ocean acidification on soundscape orientation. This thesis aims is to fill this major gap in out knowledge in two ways (Fig. 1).

1. By investigating the direct effects that ocean acidification has on audition in fish.
2. By investigating the indirect effect that ocean acidification has on dispersing larvae by affecting marine soundscapes and sound producing animals.

The first objective was tackled by raising two species of fish under current and end-of-century business as usual seawater CO_2 levels [1] and testing their auditory preferences to settlement habitats sounds in the lab. The second objective was tackled by studying changes in the marine soundscapes at natural CO_2 vents, which were used as a natural laboratory and future analogue. The results showed that ocean acidification heavily depresses biological sounds mainly by reducing snapping shrimp sound. I then investigated possible explanatory mechanisms by doing laboratory experiments on snapping shrimp and by comparing the changes in soundscape caused by ocean acidification with those caused by habitat degradation alone.

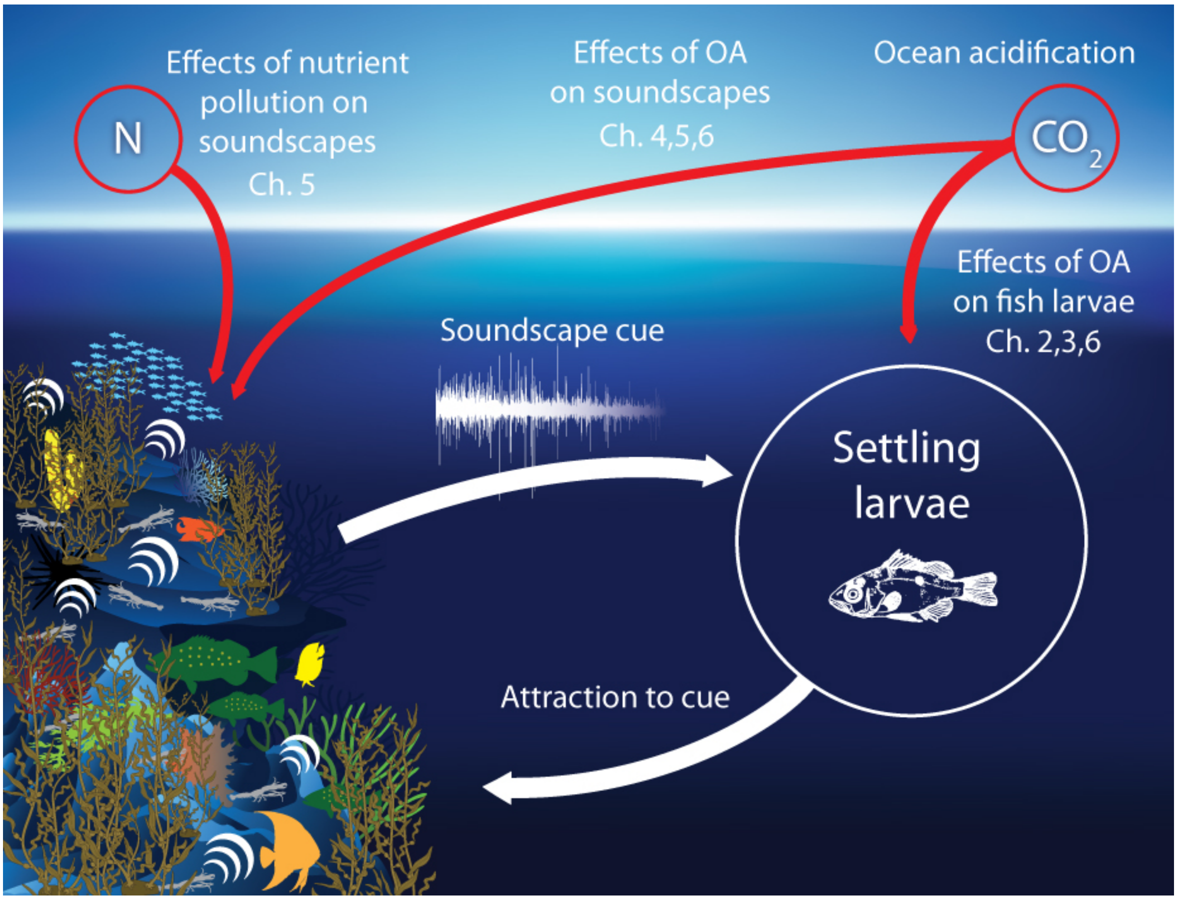

Fig.1 Conceptual diagram of the processes investigated in the various chapters of the thesis.

Artwork credit: Tullio Rossi.

THESIS OUTLINE

CHAPTER 2

This chapter aimed to investigate whether ocean acidification could cause changes in behavioural traits relevant to the process of settlement and post settlement life in barramundi larvae. The following hypotheses were tested:

1. Barramundi uses soundscape as a settlement cue during a its larval phase
2. The auditory preferences of barramundi towards its settlement habitat soundscape are altered by ocean acidification
3. Growth, swimming and metamorphosis in barramundi are affected by ocean acidification
4. Barramundi is also affected in the post-settlement phase by ocean acidification in important behavioural traits such as sheltering

CHAPTER 3

Soundscapes in coastal habitats, where many species of fish and invertebrates settle, are dominated by biological sounds. This chapter wanted to investigate whether ocean acidification could affect the process of biological sound production. This question was tackled by performing sound recordings at natural CO_2 vents, which were used as natural laboratories. The results showed that ocean acidification levels like those expected by the end of the century caused a significant decrease in total sound levels and number of snapping shrimp snaps, which dominated the sound spectrum in all recording. This finding led to the second part of the study, which by using of a series of laboratory experiments on snapping shrimp, provided a possible explanatory mechanism to the pattern observed in the field.

Chapter 4

In chapter 3 I established that ocean acidification can significantly reduce biological sound production by affecting the behaviour of snapping shrimp and possibly reduce their abundance via habitat degradation. Ocean acidification, despite being a stressor for most animals, also acts as a fertilizer for primary producers and has the capacity to make opportunistic algal turfs overgrow kelp forests and seagrass beds just like nutrient enrichment does. This typically results is a regime shift from complex and diverse ecosystem states to simplified states. In this chapter I wanted to compare soundscapes found in alternate ecosystem states where regime shifts were driven by CO_2 enrichment or nutrient enrichment. In this chapter, I not only provide the first evidence for regime-shifted soundscapes, but also reveal that the soundscapes of shifted ecosystems are similar regardless of spatial scale and type of environmental driver. Furthermore, as biological sounds can act as functional cues for orientation by dispersing propagules the observed shifts in soundscape loudness may affect this function. I conclude the chapter by proposing that soundscapes, by regulating the arrival of propagules, could be one of those feedback mechanisms that regulate the change or stasis in ecosystem state.

Chapter 5

This chapter builds on the findings of the previous chapters. I here wanted to test whether ocean acidification, by reversing fish auditory preferences at settlement stage and by affecting the quality of soundscapes, could have both direct and indirect effects on the process of larval settlement. To answer this question I used mulloway larvae raised under current and end of future CO_2 conditions and tested their response to normal soundscapes and to the soundscapes affected by CO_2 described in chapter 3 and 4. The results showed that mulloway larvae, like barramundi ones, were deterred by normal settlement habitat sounds when raised under future CO_2 conditions. Furthermore, an altered soundscape poorer in biological cues, indirectly penalized mulloway larvae at settlement stage because both control and CO_2 treated fish larvae showed lack of any response to such future soundscapes.

THESIS BY PUBLICATIONS FORMAT

Each data chapter (2 - 5) has been written in the form of an individual scientific paper and therefore uses the journal formatting. A list of co-authors and their contributions to the paper has been highlighted in the statement of authorship for each data chapter. Chapter 2, 3 and 5 are published journal articles. Chapter 4 is currently under review.

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CHAPTER II: OCEAN ACIDIFICATION BOOSTS LARVAL FISH DEVELOPMENT BUT REDUCES THE WINDOW OF OPPORTUNITY FOR SUCCESSFUL SETTLEMENT

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
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Ocean acidification boosts larval fish development but reduces the window of opportunity for successful settlement

Short title
Larval disorientation in a future ocean

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ABSTRACT

Locating appropriate settlement habitat is a crucial step in the life cycle of most benthic marine animals. In marine fish, this step involves the use of multiple senses, including audition, olfaction and vision. To date, most investigations of larval fish audition focus on the hearing thresholds to various frequencies of sounds without testing an ecological response to such sounds. Identifying responses to biologically relevant sounds at the development stage in which orientation is most relevant is fundamental. We tested for the existence of ontogenetic windows of reception to sounds that could act as orientation cues with a focus on vulnerability to alteration by human impacts. Here we show that larvae of a catadromous fish species (barramundi, *Lates calcarifer*) were attracted towards sounds from settlement habitat during a surprisingly short ontogenetic window of ~3 days. Yet, this auditory preference was reversed in larvae reared under end-of-century levels of elevated CO_2, such that larvae are repelled from cues of settlement habitat. These future conditions also reduced the swimming speeds and heightened the anxiety levels of barramundi. Unexpectedly, an acceleration of development and onset of metamorphosis caused by elevated CO_2 was not accompanied by the earlier onset of attraction towards habitat sounds. This mismatch between ontogenetic development and the timing of orientation behaviour may reduce the ability of larvae to locate habitat or lead to settlement in unsuitable habitats. The misinterpretation of key orientation cues can have implications for population replenishment, which are only exacerbated when ontogenetic development decouples from the specific behaviours required for location of settlement habitats.

Keywords soundscape, audition, behaviour, mangrove, fish larvae, orientation

INTRODUCTION

The transition from a pelagic to a benthic lifestyle is a crucial phase in the life cycle of many marine organisms. During early stages of development, dispersal of planktonic larvae may be mostly driven by currents; however, larvae progressively develop behavioural and physiological competencies that allow them to locate, orient towards and selectively settle in suitable benthic habitat [1-3]. Competencies acquired during development include increased body size, development of functional fins and strong musculature, as well as sensory capabilities necessary for navigation and habitat selection. This set of adaptations means that settlement is far from a stochastic event [1].

In the ocean, sound pressure waves carry qualitative (e.g. type of habitat) and quantitative (e.g. proximity) information over long distances (far-field) due to low attenuation of acoustic energy in water. Sound, in the form of particle acceleration, instead does not travel such long distances and is the dominant component in the vicinity of the sound source (near-field). In recent years acoustic cues have been shown to aid settlement of marine fishes and some invertebrates [2, 4-6]. Electrophysiological assessments of larval fishes show that auditory sensitivity increases during development [7, 8], suggesting acoustic cues may become more important through development. However, these studies demonstrate only that fish at a certain age can detect particular frequencies and do not explore how larval fish interpret and respond to biological sounds at different stages of development. Some species of fish appear to have a narrow window of competency for settlement and may be unable to delay settlement past this window [9, 10]. Despite the apparent role of sound in guiding settlement and the likely influence on patterns of connectivity among populations, the timing of attraction towards auditory cues remains unknown for most fish species. Identifying when fish become responsive to sensory cues from settlement habitat is critical for predicting settlement and connectivity patterns of marine species and how they could be affected by human impacts [11].

Ocean acidification is predicted to have profound effects on marine life [12, 13], but its potential effect on sound reception and swimming behaviour by free-swimming larvae remains largely untested. To date, only one study has directly investigated the effect of ocean acidification on auditory mediated behaviour, finding that elevated CO_2 (600-900 μatm) alters the auditory responses of juvenile clownfish to predator-rich daytime reef noise [14]. It is also known that elevated CO_2 conditions can increase the size of otoliths [15], and that this could potentially affect hearing sensitivity [16].

However, nothing is known about the effects of elevated CO_2 on the orientation of fishes to auditory cues during their pelagic larval stage. The effect of elevated CO_2 on fish swimming behaviour is also unresolved. Some studies suggest that swimming performances of larval fishes could be unaffected by ocean acidification [17, 18] whereas others find ocean acidification alters activity levels [19, 20] and one study suggests that ocean acidification can have an indirect effect on maximum swimming speed of settlement stage larvae through increasing body size [21]. However, most of these studies have used different swimming behaviour metrics, making comparisons difficult to interpret.

Fish possess well-developed physiological mechanisms to prevent acidosis of the blood and tissues, even at very high levels (>5000 μatm) of CO_2 [22]. However, the same physiological responses that fish use to prevent acidosis in a high CO_2 environment appear to affect the processing of sensory information resulting in profound and often striking behavioural alterations [23]. A wide spectrum of behaviours have been found to be affected by CO_2 levels expected to be reached by the end-of-century [24], including the senses of olfaction, audition and vision [14, 25, 26]. The common driver for all these effects is altered function of the $GABA_A$ neuroreceptor [23, 27, 28], which is the main inhibitory receptor in the vertebrate brain. However, few studies to date investigated the effects of ocean acidification on behaviour in pre-metamorphic larvae [19, 20, 29] and only the olfactory ability of clownfish has been investigated throughout larval development [26]. Most other studies on ocean acidification and fish behaviour have tested behavioural competencies in post-metamorphic juveniles. This emphasis has left the potential effects of ocean acidification on behavioural traits in pre-metamorphic and metamorphic stages unexplored in most fish species. It is at this stage that the effects of ocean acidification on sound reception in larval fish may be most detrimental because it overlaps with the critical transition from a pelagic to benthic life style [1, 6].

To date, the majority of research on ocean acidification and fish behaviour has focused on coral reef species. Few behavioural studies have been conducted on species with euryhaline physiologies [30, 31]. Many estuarine environments normally experience high and fluctuating CO_2 levels due to processes such as tidal exchange, eutrophication and freshwater input [32, 33]. Therefore, the evolution of fish within these environments may have enabled adaptation to elevated levels of CO_2 [34]. However, adaptation to extreme ranges of natural variability in seawater chemistry may not automatically translate into resistance to future conditions. As local processes can exacerbate the effect of ocean acidification, these environments may attain future CO_2 levels that are much higher than currently experienced [35, 36]. Yet, little is known about whether estuarine species are better adapted to ocean acidification than marine species due to the dynamic and more extreme pH environment in which they live.

We here tested the effect of ocean acidification expected for estuarine environments on multiple behavioural traits of a catadromous fish (barramundi, *Lates calcarifer,* that migrates from fresh water to the ocean to spawn) known to have a highly flexible physiology and to be associated with marine and estuarine habitats during its larval development [37]. The larvae of barramundi settle in estuaries, embayments and near-shore coastal areas which can experience high pCO_2 due to natural and anthropogenic processes such as tidal exchange, freshwater input, eutrophication and runoff of acid sulphate soils. As these processes could locally exacerbate the effect of ocean acidification we expect that estuarine areas in the future will reach CO_2 levels that are substantially higher than projections for the open ocean [32, 33]. Future projections that take into account the combined effect of ocean acidification and heterotrophic degradation of organic matter in coastal and estuarine hypoxic regions estimate that pCO_2 values of 1700-3200 μatm could be reached by the end of the century [35]. Given this observation, we exposed barramundi to pCO_2 of ~1675 μatm to represent possible future elevated CO_2 conditions in their habitat.

The aims of the study were to: 1) identify ontogenetic patterns of attraction to sound cues from potential settlement habitats; 2) test the effects of ocean acidification on important behavioural traits characteristic of pre-settlement lifestyle, such as sound-driven orientation and swimming velocity, as well as post-settlement behavioural traits like sheltering; and 3) test the effect of CO_2 on development and timing of metamorphosis and how this might interact with the timing of attraction to settlement habitat sound and swimming velocity.

METHODS

MODEL SPECIES

Barramundi is a tropical fish whose range extends from the eastern Indian Ocean to the western Central Pacific and is highly valued both commercially and recreationally [38]. Barramundi is an obligatory catadromous species (migrates from fresh water to the ocean to spawn) whose eggs and larvae are typically found around river mouths and marine bays [37, 38]. In this species, successful gonadal and larval development requires saltwater (28-35 ppt) [38] and juveniles settle into mangroves and wetland habitats [37]. The egg stage duration is typically 12-17 hr and metamorphosis occurs at approximately 19 days post hatching (dph), depending on diet and environmental factors [39].

Barramundi like many other Perciformes possess a rostral extension of the swim bladder and a gas filled chamber in the otic region [40, 41]. This trait appears to have evolved multiple times independently in this group of fish and allows the transit of sound pressure oscillations captured by the swim bladder to the otolithic region [41]. Although the hearing sensitivity of barramundi has not been tested, experimental studies have shown that the extension of the swim bladder to the otic region is linked to enhanced acoustic sensory performance typical of hearing specialists [41]. Dissection under a stereomicroscope confirmed the presence of this gas filled chamber in the otic region of barramundi at settlement stage (n = 5, length 8 – 20 mm, T. Rossi personal observation, 2015).

Fertilized eggs were obtained from a commercial hatchery (Robarra, 7th generation broodstock) and reared at University of Adelaide. The larval rearing systems were duplicated for each treatment and comprised a 60 l rearing tank recirculating in a closed system with a 20 l sump that contained a biological filter, a protein skimmer WG-308 (Boyu, Guangdong, China) and a UV sterilizer UView (Blue Planet). Fish were fed *ad libitum* with rotifers for the first 12 days post hatching, then with *Artemia* nauplii and a dry feed (Otohime) of increasing granule size as development progressed. CO_2 treatments (Table 1) were initiated on the second day post hatching. Temperature was maintained at ~27 °C (Table 1). See Supplementary methods for details on CO_2 manipulation.

EFFECT OF CO_2 ON LARVAL FISH AUDITION AND SWIMMING VELOCITY

The attraction to acoustic cues of habitat was investigated daily for a duration of 15 days (from pre-metamorphosis at 13 dph to post-metamorphosis at 28 dph), by testing the response of naïve larval fish to recordings of estuarine soundscapes, which could act as a potential orientation cue leading to settlement habitat. The tests started at 13 dph because earlier stages showed low swimming competency during trials, which is typical of the early planktonic stages. For each individual fish we also visually assessed their developmental stage (Suppl. methods).

The response to soundscapes was tested in fish raised under control and high CO_2 conditions using an auditory choice chamber. A five minute recording of mixed tropical and temperate estuarine habitats was used as the biologically relevant acoustic cue (Fig. S1, S2, S3, S4). The recordings were dominated by typical broadband snapping shrimp sound [42], which rose sharply from zero to being the dominant acoustic feature in the soundscape around 500-800 Hz (Fig. S2). The recordings contained very few abiotic sounds because the sea state at the time of the recordings was always calm. As sounds like those produced by snapping shrimp are highly dependent on habitat characteristics [42] and are known to be associated with mangroves [43] they likely provide a potentially valuable directional cue for barramundi larvae attempting to locate suitable settlement habitat. We also tested the auditory preferences of barramundi against white noise (constant amplitude at every frequency), which acted as a biologically irrelevant control sound. Additional information on the sound recordings is provided in the Supplementary materials.

The auditory choice experiments were performed inside a plastic tank (100 x 50 x 20 cm) lined with polystyrene foam and containing a white acrylic auditory choice chamber (35 cm x 22 cm x 2 cm) divided in 8 parallel lanes with a triangular section (35 x 3 x 2 cm; Fig. S5). At each end of the chamber there was an underwater speaker (UW-30; maximal output 156 dB re 1 µPa at 1 m, frequency response 0.1–10 kHz, Lubell Labs Inc., Columbus OH). The sound pressure level was very similar between external and central lanes (Fig. S6). The auditory chamber had mesh at the two ends facing the speakers while the top was open to the surface. The fish larvae could not escape or see each other because the ridges between each lane were higher than the water level. Between each trial the chamber was flushed with fresh seawater in order to remove potential chemical cues left by the previous fish. During the experiments the chamber was placed at a fixed distance of 8 cm from the speakers. At the beginning of each trial one fish larva was randomly placed in a removable enclosure in the centre of each of the eight lanes and given a two min habituation period during which recorded estuary sounds were played. The sound during the habituation time was played by the same speaker used for the trial and was intended to avoid the potential confounding effect of a startle response triggered by the abrupt increase of sound level in the tank during the trial. At the beginning of each trial, 8 fish (4 control and 4 elevated CO_2, one per lane) were released simultaneously and their position was videotaped from above using a camcorder (HF R406 Legria, Canon, Japan).

The position and velocity of the fish in the choice chamber was tracked continuously using EthoVision XT10 (Noldus Information Technology, Wageningen, The Netherlands) for 7 min. The combined use of video recordings and automated tracking eliminated the risk of observer bias and external influences on behaviour caused by the presence of the observer. Within Ethovision each chamber was divided in two equal sections and the percentage of time that the fish spent in each section was obtained (Fig. S5). Assuming that a fish with no acoustic preference spends an equal amount of time in each section (50%), any percentage of time spent in one of the sections that significantly differs from 50% indicates a response to the cue. The sound pressure levels of the playback in the chamber (below 4 kHz) were set so that they matched levels recorded in the field near to the speaker, and decreased towards ambient levels along the chamber [44] (Figs. S1, S3). A response to sound does not necessarily demonstrate that fish can resolve the directional origin of the sound as they may simply sense the gradient of sound pressure along the chamber by sampling multiple positions, and consequently choosing to spend more time in the section of the chamber where they can hear a soundscape that they find “attractive”. In other words we assume that if the fish were deterred by our playback, and this playback is audible only in a section of the chamber, the fish would move to the other section. From a drifting propagule perspective, the selection of habitat based on soundscape spatial heterogeneity is possible by simply sampling sound pressure at multiple time points as the larva moves closer to the source (see Lillis et al. [45] for conceptual model). Particle acceleration was measured in the experimental chamber based on sound pressure measurements obtained simultaneously with two hydrophones (same as above) held at 5.5 cm distance and the Euler equation as in [46]. This established method is not prone to the limitations of using large accelerometers in tanks. The results show that particle acceleration was maximal in proximity of the speaker and decreases linearly along the chamber (Fig. S3). To test for possible side preference effects in the chambers we ran trials with control fish and no sound playing. The results showed the absence of a side preference (ANOVA, $F_{1,30} = 0.22$, $p = 0.643$, $n = 15$ at 23 dph).

We acknowledge the difficulty of replicating a far field acoustic cue in a small tank [47], however, in this study we do not attempt to determine absolute values of sensitivity but rather relative auditory preferences in larval fish that had been exposed to elevated CO_2 versus control conditions throughout their larval development.

A total of 20–24 (half from each treatment) randomly selected naïve larval barramundi that had been reared under control and elevated CO_2 conditions from the second day post hatching were utilized daily between 13 and 28 dph for trials. All testing took place between 14:00 and 18:00. Additional methods are present in the Supplementary methods.

SHELTERING BEHAVIOUR

Sheltering behaviour and thigmotaxis (edge-following) were tested as a proxy for boldness/anxiety [48]. The time to first emergence from the shelter and total time spent in the shelter were scored by human observer (see Supplementary methods for details). A total of 56 (half from each treatment) naïve post-settlement barramundi that had been reared under control and elevated CO_2 conditions from 2 dph were utilized at 35 dph for trials.

Statistical analysis

Percentage of time spent in the half of the chamber in proximity of the acoustic cue, fish standard length and swimming velocity data were pooled in 3-day blocks. Attraction or deterrence towards soundscape was then determined by testing each distribution of percentages of time spent in the half of the chamber close to the active speaker against the threshold for random response set at 50%. Percentage data were not normally distributed, as assessed by Shapiro-Wilk's test ($p < 0.05$); therefore a non-parametric One-Sample Wilcoxon Signed Rank Test was used. Fish standard length and swimming velocity were analysed using a 2-way ANOVA with CO_2 treatment and dph as factors. No significant tank effect was detected (3-way ANOVA with CO_2 treatment, dph and tank nested in treatment as factors).

A log-linear model was used to test the relationships between the number of individuals in each developmental stage (S), day post hatching (dph) and CO_2 treatment (CO_2). Starting from the saturated model (containing all main effects and their interactions), higher order terms were removed from the model until there was a significant increase in deviance from one model to the next.

The overall proportion of fish that emerged from the shelter during the trial was compared between treatments with a 2x2 Chi-square test. Differences in sheltering behaviour traits (time to first emergence from the shelter and total time outside the shelter) were tested with ANOVA. No significant tank effect was detected (ANOVA with CO_2 treatment and tank nested in treatment as factors). Logit transformation was applied to the percentage time in shelter data.

RESULTS

AUDITORY BEHAVIOUR, SWIMMING VELOCITY, AND DEVELOPMENT

There was a significant attraction in control fish towards playback of settlement habitat soundscapes for the 3-day period from 16–18 dph (Wilcoxon Signed Rank Test, $p = 0.022$; Fig. 1A). This pattern was largely driven by fish spending more time in the loudest part of the chamber (Fig. S7). The period of attraction towards settlement habitat in the control fish matched the period when most fish underwent metamorphosis (Fig. 1B). Swimming velocity of control fish was lowest during the early developmental stages (13–15 dph), before most fish had initiated metamorphosis, then significantly increased for a period of 9 days (16–24 dph) before decreasing again past day 25 when all fish had completed metamorphosis (Fig. 2A).

During the short temporal window when control fish showed attraction towards playback of settlement habitat sound, CO_2 treated fish were instead significantly deterred by habitat sounds (Wilcoxon Signed Rank Test, $p < 0.001$; Fig. 1A). This pattern was largely driven by fish spending more time in the quietest part of the chamber (Fig. S7). There was no significant attraction or avoidance exhibited towards the habitat acoustic cue, for either control or CO_2 treated fish, at any other developmental stage (Fig. 1A). Furthermore, no attraction or avoidance, at any stage, towards playback of white noise was observed (Fig. S8). Swimming velocity of CO_2 treated fish during the audition trials followed the same ontogenetic pattern exhibited by the control fish but at significantly lower values (ANOVA, CO_2 treatment: $F_{1,246} = 6.7$, $p = 0.011$; dph: $F_{4,246} = 14.7$, $p < 0.001$; Fig. 2A).

Fish in the high CO_2 treatment initiated metamorphosis significantly earlier than control fish. The log-linear model showed that developmental stage was dependent on days post hatching and CO_2 treatment, but not an interaction between the two. The best fitting model contained the interaction between developmental phase and dph and the two way interaction between developmental phase and CO_2 treatment (Likelihood chi-square = 3.338, d.f. = 8, p = 0.911). Removal of the three way interaction between developmental phase, dph and CO_2 treatment did not lead to a significant increase in deviance. However, removal of either of the two way interactions involving developmental phase caused a significant increase in deviance. Removal of the interaction between developmental phase and dph had a much greater effect on the model deviance (Chi-square = 398.52, d.f. = 8, $p < 0.001$) compared with removing the interaction between developmental phase and CO_2 treatment (chi-square = 25.76, d.f. = 2, $p < 0.001$), indicating that dph has a larger effect on developmental phase than did CO_2 treatment.

Fish length was significantly higher (ANOVA, CO_2 treatment: $F_{1,294} = 33.1$, $p < 0.001$, dph: $F_{4,294} = 119.8$, $p < 0.001$, interaction: $F_{4,294} = 98.1$, $p = 0.024$) in the elevated CO_2 treatment compared to the controls (Fig. 2B). A pair-wise test showed that the effect of CO_2 on growth was significant only from dph 19-21 onward (Fig 2B).

SHELTERING BEHAVIOUR

Elevated CO_2 had a significant effect on fish sheltering behaviour. The proportion of fish that emerged from the shelter during the trial was significantly lower in the elevated CO_2 treatment (13 out of 28 fish) compared to the controls (21 out of 28 fish) (Pearson's Chi-square = 4.791, d.f. = 1, p = 0.029; Fig. 3A). The mean percentage of time spent in shelter was significantly higher for the fish in the elevated CO_2 treatment (ANOVA, $F_{1,54} = 7.38$, p = 0.009; Fig. 3B). Furthermore, when only considering the fish that emerged from the shelter during the trial, the average time to first emergence was significantly longer in the elevated CO_2 treatment (ANOVA, $F_{1,32} = 17.8$, $p < 0.001$; Fig. 3C). Thigmotaxis did not significantly differ between treatments (ANOVA, $F_{1,31} = 2.71$, p = 0.11; Fig. S9).

DISCUSSION

The overarching goal of our study was to investigate whether a restricted ontogenetic window of opportunity exists during which larval fish respond to benthic habitat sound cues that could guide them from the ocean to their settlement habitat and whether ocean acidification might alter this critical process. Our approach addressed the effects of ocean acidification on sound-driven orientation, swimming behaviour and sheltering behaviour in the context of ontogenetic development. We expected barramundi to show some degree of tolerance to elevated CO_2 due to its residence in estuaries, however, our experiment revealed behavioural and life-history effects at CO_2 levels that can be considered moderate for estuarine environments [35]. Auditory preferences, development, growth, swimming speed and boldness were all affected by elevated CO_2 (~1675 μatm) conditions. As settlement to adult habitat is a crucial step in the life cycle of coastal marine species, these results have implications for the replenishment and connectivity of populations of this euryhaline species.

The ontogenetic timing of responsiveness towards settlement cues is poorly known in most fish [49]. However, some species appear to have a narrow window of competency for settlement [9, 10]. Our study found that under present-day CO_2 conditions larval barramundi exhibited attraction to playback of settlement habitat soundscape only during a short temporal window of approximately three days, which also matched the timing of metamorphosis from larval to juvenile morphology. To our knowledge, this is the first study that investigates the behavioural response to soundscapes across multiple developmental phases in larval fish and identifies a specific window of attraction. We also provide evidence that barramundi is selectively attracted to biologically relevant habitat sounds but not to artificial white noise. During metamorphosis, larvae of many species like barramundi undergo dramatic morpho-physiological changes at the same time that they transition from a pelagic to a demersal lifestyle. Larval fish also become highly competent swimmers as they approach metamorphosis, able to move directionally and travel many kilometres despite ocean currents [1]. As expected, swimming speed during auditory trials increased along with the onset of metamorphosis and decreased post-metamorphosis. The lowering of swimming speed after metamorphosis may reflect the transition from pelagic to demersal lifestyle typical of post-settlement ambush predators like barramundi [50].

Elevated CO_2 caused larval barramundi to grow faster and initiate metamorphosis earlier. However, a mismatch between the two occurred, because a significantly higher proportion of fish in the high CO_2 treatment initiated metamorphosis between 13–15 dph, whereas the increase in length appeared 6 days later. Despite the earlier onset of metamorphosis, the window of response to soundscape playback in CO_2 treated fish did not advance relative to the control fish. Instead, fish reared under elevated CO_2 significantly avoided the playback of settlement habitat sound during the same days when control fish were attracted to these sounds. This reversal of auditory preference is different to the only other study on ocean acidification and sound orientation, which showed that post-settlement stage juvenile clownfish that are normally deterred by daytime reef soundscape simply lose auditory preference when exposed to elevated CO_2 levels [14]. Response to habitat sound during the pelagic larval stage (for orientation), however, is very different to that for settled stages (e.g. for communication, predator avoidance and finding food) [2, 51, 52]. Here we show that ocean acidification can disrupt the window of opportunity for sound-driven orientation by oceanic larvae towards settlement habitats. Such a behavioural disruption could lead to decreased chances of finding suitable adult habitat, leaving larvae exposed to predation and starvation for longer periods of time potentially resulting in reduced population replenishment and connectivity.

Current knowledge suggests that ocean acidification affects larval growth in a species-dependent fashion. Some studies have found reduced growth [53], while others find increased growth [17, 54] and others no effect [18]. Similarly, swimming performances have been found to be unaffected by ocean acidification in some studies [17, 18] or decreased in others [20]. Here, contrary to expectations [1], increased length in high CO_2 reared fish was accompanied by a decrease in swimming velocity. This could be due to the fact that in our study (as in Pimentel et al.[20]), we quantified spontaneous swimming activity rather than forced maximum sustained swimming velocity (U_{crit}). Additionally, in this study the fish may have compensated the cost of hypercapnia by decreasing swimming velocity. Additionally, increased anxiety, as found in later developmental stages in this study (see below), might decrease the willingness of fish to explore the chamber resulting in a lower velocity. As swimming competency during late larval stages is an important trait that allows fish larvae to swim directionally, overcome currents and ultimately reach desired settlement habitats [1], a decrease could compromise successful settlement, with detrimental impacts on likelihood of survival.

Ocean acidification also had a negative effect on the behaviour of post-metamorphic fish. Juvenile barramundi live in mangroves and wetlands [37] and typically hide in submerged vegetation where they adopt an ambush predatory strategy [50]. Whereas some studies have found increased boldness in fish exposed to elevated CO_2 [55], other studies found decreased boldness [28, 30], indicating that ocean acidification might affect the same behavioural trait differently in different species. Our observations of sheltering behaviour showed that post-settlement barramundi exposed to elevated CO_2 are less bold (or more anxious) compared to control fish. From an ecological perspective, increased anxiety might result in decreased foraging success due to extended time spent hiding. As this anxiety test was performed with post-metamorphic fish that had experienced high CO_2 during most of their pre-metamorphic stage, it provides evidence for a lack of acclimation to elevated CO_2 as fish progress to their juvenile stage. Juvenile reef fish at natural CO_2 seeps exhibited some of the same suite of behavioural problems (e.g. reversal of olfactory and sheltering preferences, and reduced anti-predator behaviour) to those observed in laboratory experiments [56, 57], indicating that the fish did not acclimate despite continuous exposure to elevated CO_2 since settlement. Furthermore, another study [58] found that parental exposure to elevated CO_2 did not ameliorate behavioural impacts of elevated CO_2 on juveniles, as has been observed in some life history traits [31, 59], while a meta-analysis of studies based on short vs. long-term CO_2 exposure revealed little scope for acclimation for various species traits [12]. Consequently, rapid acclimation of behaviour to high CO_2 does not appear likely and fish may need to rely on slower mechanisms like selection and evolutionary adaptation to overcome future effects of ocean acidification.

Fish can use a suite of senses to locate suitable settlement habitat. This includes sound, olfaction and vision [2, 5]. Sound is one of the cues that propagates furthest, and it attenuates most predictably with distance from the source [2]. Whilst other senses like olfaction and vision could potentially compensate for the ineffective processing of auditory sensory information caused by CO_2, they too are likely to be impaired [26, 60, 61]. It is well known that larval fish settlement in many species is synchronized with lunar cycles [62]. However, ocean acidification has been shown to also affect timing of settlement by forcing fish to settle at unfavourable times [63]. The inability to successfully or quickly locate settlement habitat is likely to reduce survival and undermine successful establishment due to increased predation risk, delayed occupancy of suitable benthic habitats and settlement to unsuitable habitats. As orientation towards suitable settlement habitat is a key process in the life of most coastal marine organisms, our findings could have far-reaching implications for the replenishment of marine populations and ecosystems and for population connectivity.

Ethics statement

Research was carried out under approval of the University of Adelaide animal ethics committee (permit: S-2012-171) and according to the University's animal ethics guidelines.

Data accessibility

Data are available at datadryad.org (doi:10.5061/dryad.2cf6s)

Author's contributions

All authors contributed to the study design and writing of the article. T.R. collected and analysed the data. P.M. and L.M. contributed to data analysis.

Competing interests

We declare we have no competing interests.

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Table 1 | Summary of the water chemistry parameters measured in the laboratory experiment

Treatment	**T(°C)**	**pH NBS**	**N**	**TA (µmol/kg SW)**	**pCO$_2$ (µatm)***	**N**	**Salinity**	**N**
Control	27.07 (± 0.05)	8.19 (± 0.01)	34	2595.6 (± 25.4)	400.9 (± 43.6)	3	38.8 (± 0.2)	34
Elevated	27.11 (± 0.05)	7.70 (± 0.01)	34	2629.8 (± 17.6)	1675.1 (± 135.0)	3	38.8 (± 0.2)	34

*Average (± SE) temperature (T), pH and total alkalinity (TA) measured in the laboratory. * indicates values of pCO$_2$ calculated using CO2SYS.*

FIGURES

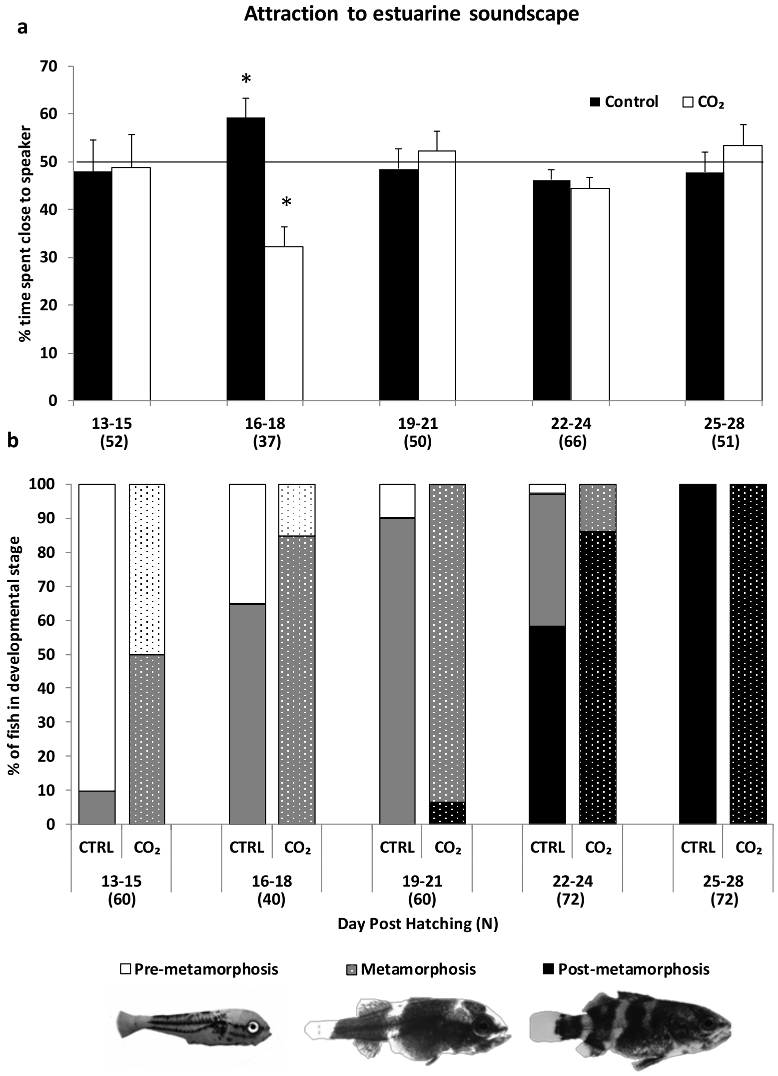

Figure 1. Effect of ocean acidification on fish auditory preferences throughout larval development. ***(a)*** *Mean (± SE) percentage of time spent in the half of the chamber closest to the speaker broadcasting an estuarine soundscape. Results are pooled in blocks of 3 days. Stars indicate statistically significant differences relative to a 50% threshold for random response represented by the horizontal bar.* ***(b)*** *Proportion of fish in each developmental stage as a function of number of days post hatching. Results are pooled in blocks of 3 days. The elevated* CO_2 *treatment is represented by dotted bars. Number of replicates for each 3-day block is reported in parentheses.*

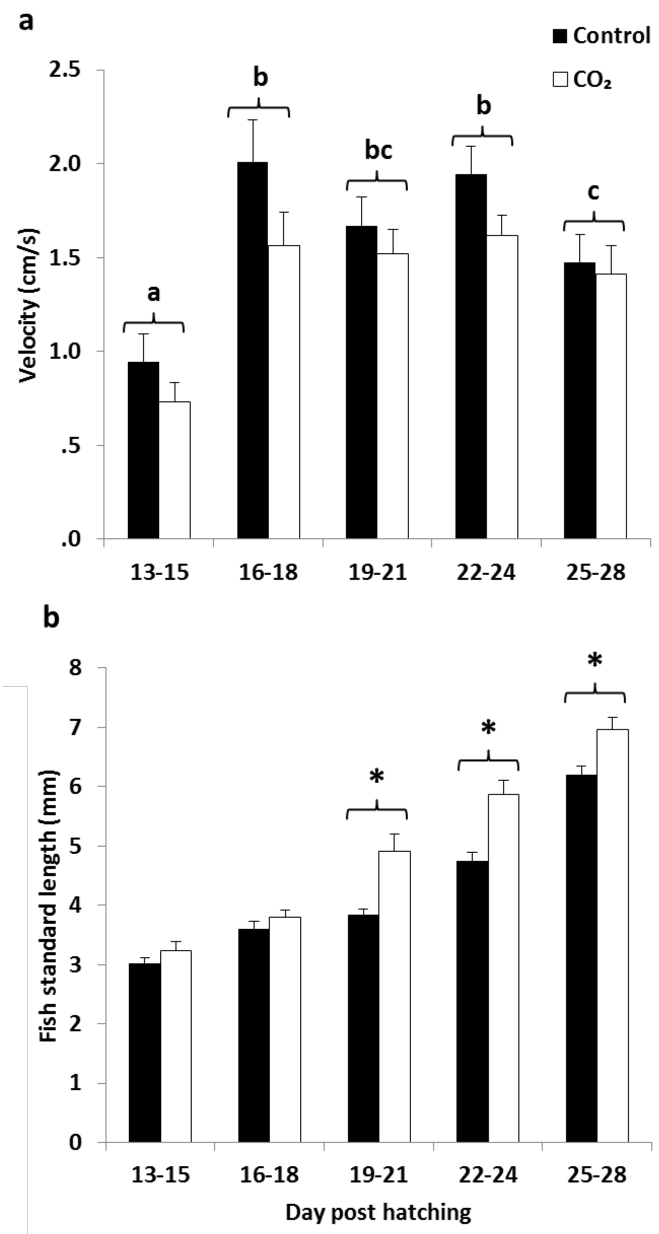

Figure 2. Effect of ocean acidification on swimming velocity and fish size throughout larval development. ***(a)*** *Mean (± SE) swimming velocity during audition trials; and* ***(b)*** *Mean (± SE) fish standard length during development. Asterisks indicate significant ($p < 0.05$) differences between distributions. Letters indicate significant differences between groups of dph for combined CO_2 treatments (pair-wise tests).*

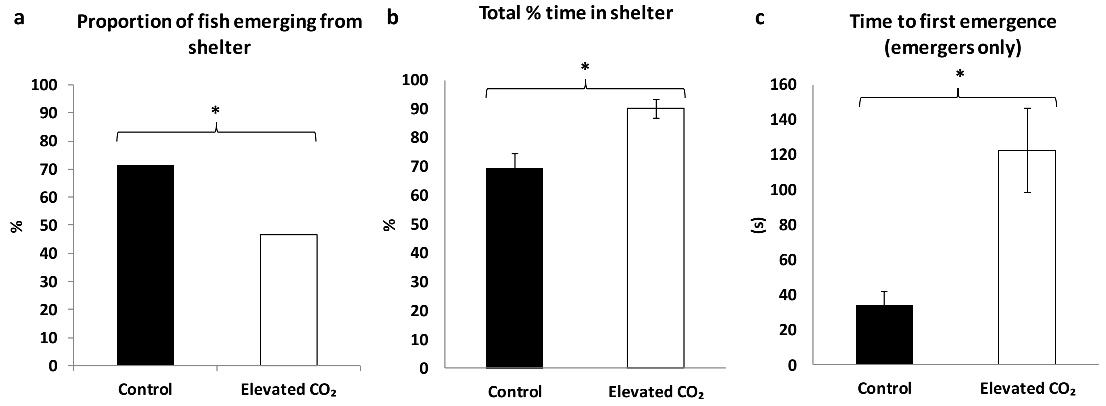

Figure 3. Fish sheltering behaviour during the post-settlement stage. (a) *Percentage of fish that emerged at least once from shelter during the trials.* ***(b)*** *Mean percentage (± SE) time (seconds) spent in shelter during trials.* ***(c)*** *Mean (± SE) time to first emergence from shelter (emergers only). Asterisks indicate significant ($p < 0.05$) differences.*

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SUPPLEMENTARY METHODS

CO_2 MANIPULATION

Treatment conditions were achieved by bubbling CO_2-enriched air into the rearing tanks and in the sumps with a Pegas 4000 MF gas mixer (Columbus). pH_{NBS} was measured daily with a SG2-ELK SevenGo pH probe (Mettler Toledo) calibrated with a three point calibration. The CO_2 partial pressure in the seawater was calculated using measured values of temperature, salinity, pH_{NBS} and total alkalinity (TA) in the larval holding tanks, using the software CO2SYS [1] with constants K1 and K2 from Mehrbach (1973) and refit by Dickson & Millero (1987) [2,3]. Alkalinity was measured by Dynamic Endpoint Titration using an 888 Titrando (Metrohm) titrator. During the course of the study alkalinity values were within 1% accuracy of certified standards (reference materials from Dr A. Dickson, Scripps Institution of Oceanography). Seawater samples for alkalinity were analysed immediately upon collection. Salinity was measured with a SR6 refractometer (Vital Sine).

Egg and first day post hatching are very fragile stages. At this stage barramundi larvae can withstand only a minimal amount of bubbling and water circulation in the rearing tank. In order to minimize stress to the larvae we waited until the second day post hatching for full CO_2 treatment which required moderate bubbling in the tank. Bubbling CO_2 enriched air in the rearing tank was necessary to achieve the target pCO_2 because the low recirculation rate between sump and rearing tank in the first few days of rearing was not enough to maintain the target pCO_2.

EFFECT OF CO_2 ON LARVAL FISH AUDITION AND SWIMMING VELOCITY

Playback tracks consisted in a mix of randomly selected 30 sec fragments of recordings obtained in mangrove channels from both tropical (water depth 1 m, calm sea, Kunduchi, Tanzania, daytime 15:30–19:00, 16–19 February 2007) and temperate (depth 3 m, hydrophone 1 m above the bottom, calm sea, Barker Inlet, South Australia, dusk 19:30¬–20:00, 10 March 2013 and 31 October 2013) locations. As our mangrove recordings from temperate and tropical locations were very similar (e.g. snapping shrimp sound was always the dominant feature), we used both for the purpose of providing a more generic cue not specific to one location, despite barramundi being a tropical species.

Recordings were made with a hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.3 dB re 1V/µPa; frequency range 0.02–30 kHz; calibrated by manufacturers; High Tech Inc., Gulfport MS) and digital recorder (2007 recordings: Edirol R-1, 44kHz sampling rate, Roland Systems Group, Bellingham WA; 2013 recordings: PCM-M10, 48 kHz sampling rate, Sony Inc., Tokyo, Japan).

Fish are known to retain the behavioural effects of CO_2 for over 24h when placed back in control conditions [4], therefore, we tested all fish in water equilibrated with ambient pCO_2 at the same temperature and salinity in which they had been reared. Every day, the side of the active speaker was switched to the opposite side of the tank to additionally control for potential tank side effects. Ethovision was also used to calculate mean swimming velocity and number of crossings between the boundaries of the two sections for each fish during trials (Fig. S10). Playback equipment failure prevented data collection on 2 days within the sampling period (17 and 27 dph). Each fish was used only once. Fish that were unresponsive (played dead) or showed a startle response at the start of the trial were excluded from the analysis (15% of all individuals).

Development

The standard length of every fish used for the auditory trials was measured from video screenshots with a length reference using Photoshop CS6 (Adobe). Additionally each fish was classified into three developmental stages based on morphological features: pre-metamorphosis, metamorphosis (onset of body pigmentation and body thickening), and post-metamorphosis (occurrence of three lateral white bands; Fig. 1) [5,6].

SHELTERING BEHAVIOUR

The test arena was made of white acrylic with dimensions of 12 x 5 x 4 cm. One third of the chamber was closed by a removable gate and lined with black tape on all sides including the top so that it acted as a dark shelter while the rest of the chamber was white and illuminated. Similar protocols that measure light/dark preferences are widely used in fish as a behavioural test for anxiety [7]. At the beginning of each trial, a fish was placed inside the dark area of the chamber and allowed to habituate to the new conditions for two minutes. At the start of each trial the fish was released by manually opening the gate and the behaviour of the fish was videotaped from the top with a camcorder (HF R406 Legria, Canon, Japan) for five minutes. The time to first emergence from the shelter and total time spent in the shelter were scored by human observer using the software J Watcher [8]. Thigmotaxis (edge-following) was scored from videos every 5 seconds during the sheltering experiment when the fish were exploring the unsheltered part of the chamber. Between each trial the chamber was flushed with fresh seawater and rotated 180° to control for potential side effects. All testing took place between 14:00 and 18:00.

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SUPPLEMENTARY FIGURES

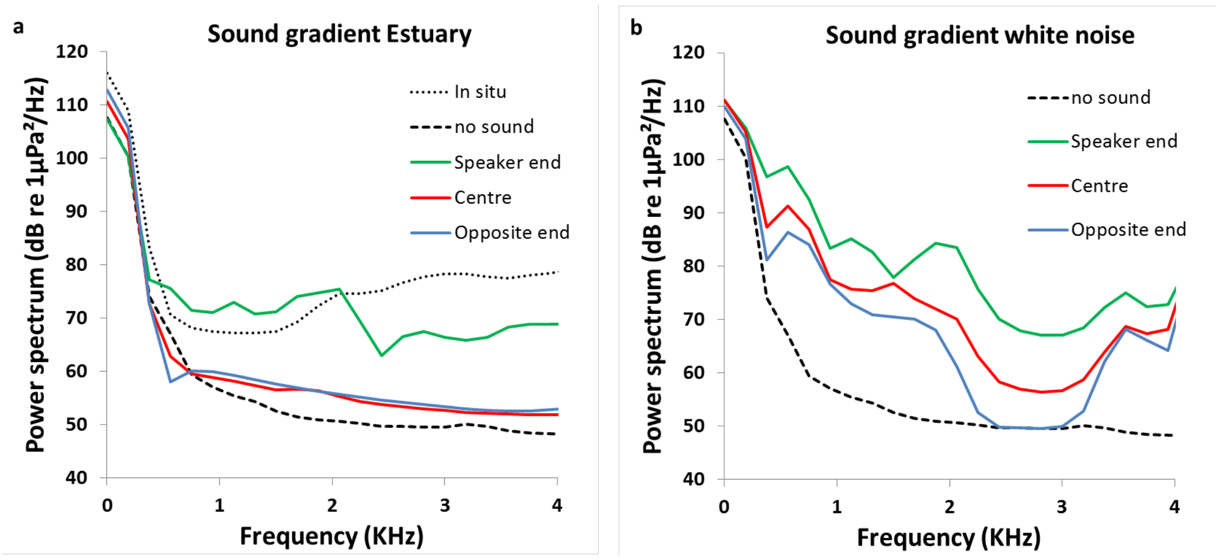

Figure S1. Sound gradients during playbacks in the experimental choice chamber. *Sound pressure gradient from estuarine soundscapes (a) and white noise (b). "Speaker end" represents the part of the choice chamber closest to the speaker, "Centre" represents the middle section of the chamber and "Opposite end" refers to the section of the chamber opposite the speaker. "No sound" refers to the background sound level present in the chamber without playback and "In situ" refers to original field recordings. All measurements were obtained below the surface and along the centre of the chamber. Energy below 500 Hz was constant throughout the experiment and originated from noise in the building.*

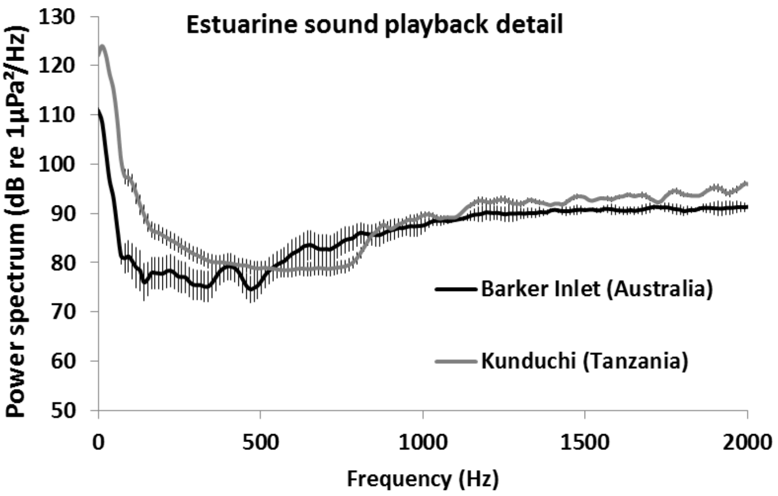

Figure S2, Acoustic spectrum of recordings from different locations mixed in playback. *Snapping shrimp sound had comparable levels in the two locations with the difference that in the Barker Inlet some sound was in the range 500–800 Hz, while in Kunduchi the shrimp sounds were >800 Hz. In both locations there were high levels of abiotic noise < 100 Hz. Temporal variability is represented by SE bars (N = 3 recordings for Barker Inlet and N = 4 for Kunduchi). Mean sound pressure level in the hearing range of fish (< 5 kHz) was 113.3 dB re 1 µPa.*

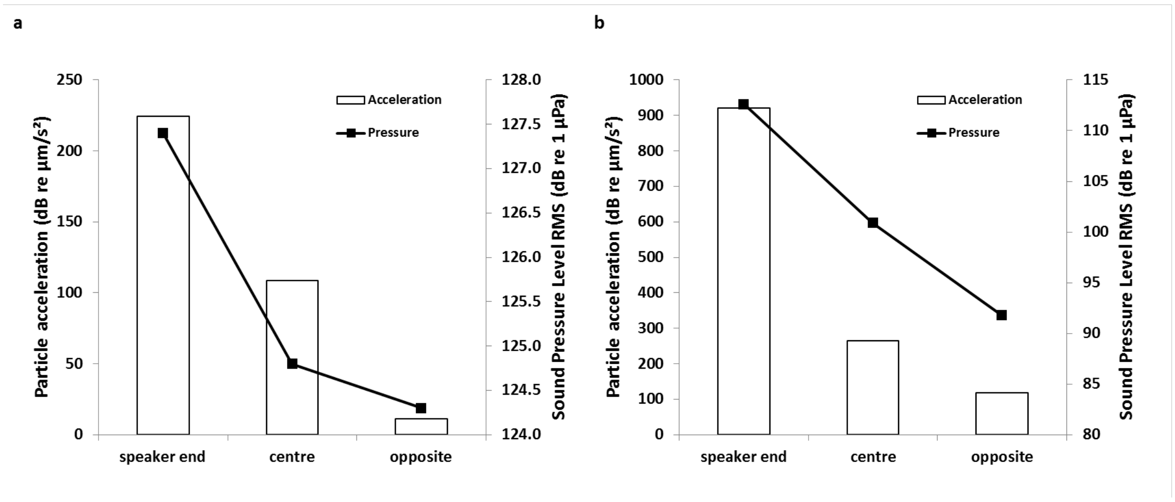

Figure S3, Particle acceleration and sound pressure gradient during playbacks of estuarine soundscape in the experimental choice chamber. *(a) Analysis across the full spectrum, and (b) Band pass filtered (0.3-5 kHz) measurements. The band pass filter was used to show the sound pressure and particle acceleration patterns in the likely hearing range of fish filtering* *out higher frequencies (> 5 kHz) and low frequency vibrations in the building (< 0.3 kHz). "Speaker end" represents the part of the choice chamber closest to the speaker, "Centre" represents the middle section of the chamber and "Opposite end" refers to the part of the chamber opposite to the speaker. The sound pressure level in the part of the chamber closest to the speaker was set to match the in situ sound pressure level in the hearing range of fish (113.3 dB re 1 µPa, < 5 kHz).*

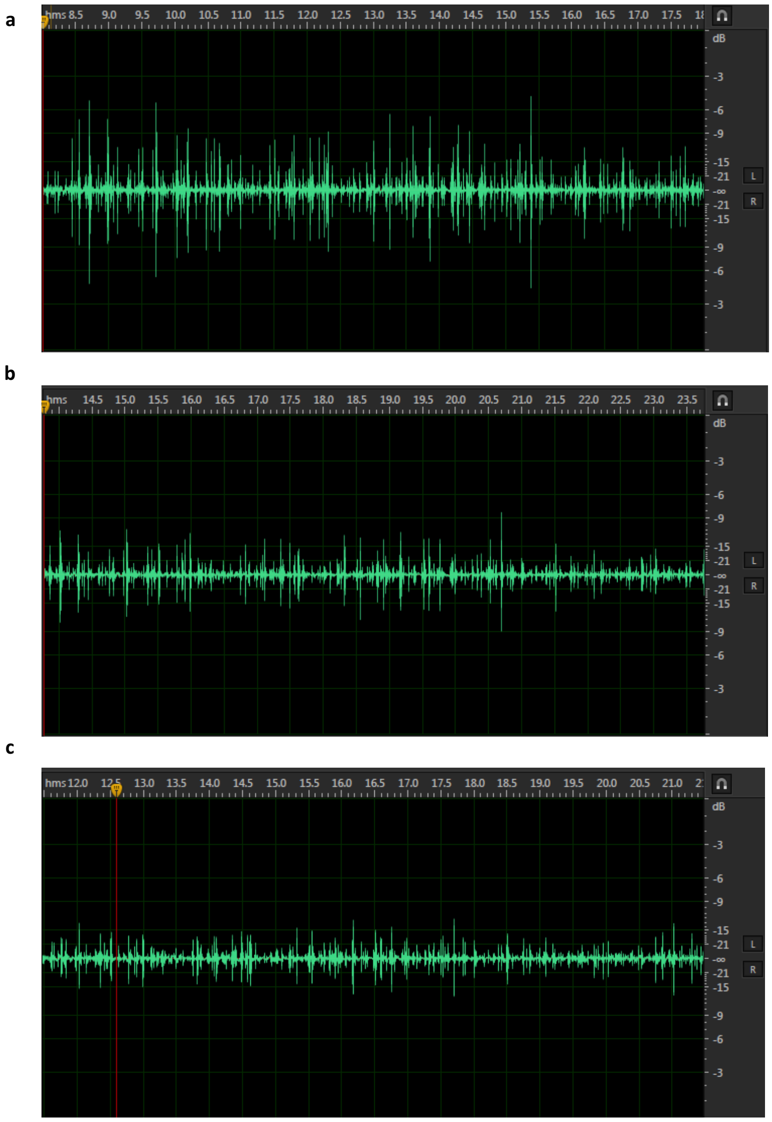

Figure S4, Waveform of estuarine sound playback (10 sec) at different positions of the experimental choice chamber. *(a) speaker end, (b) centre, (c) opposite end.*

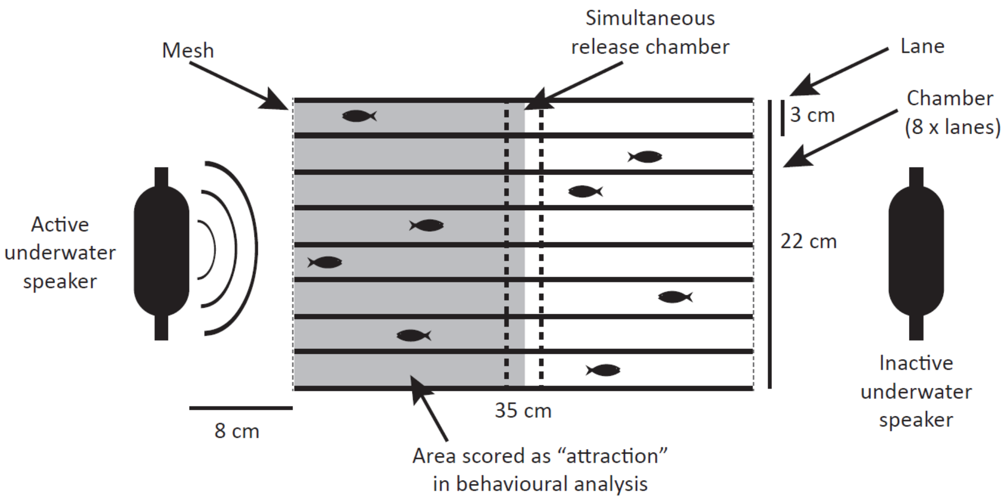

Figure S5. Schematic representation of the auditory chamber as seen from the top. *The sections close to the active speaker that represent attraction to sound by fishes is shown in grey.*

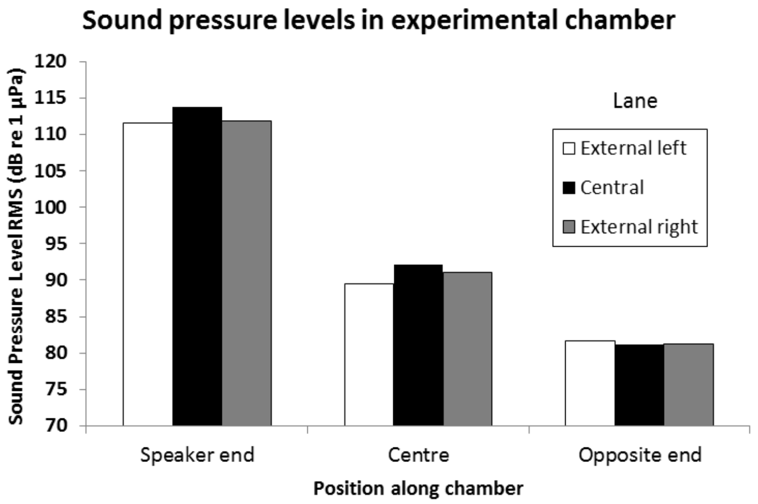

Figure S6, Sound pressure levels in different lanes of the experimental choice chamber. *Single measurements of sound pressure levels of white noise (same amplitude at every frequency) played back in the experimental chamber show that the sound pressure level decreases along the length of the chamber but does not differ between different lanes* *(external left, central, external right). All measurements were processed with a band pass filter 0.5–4 kHz.*

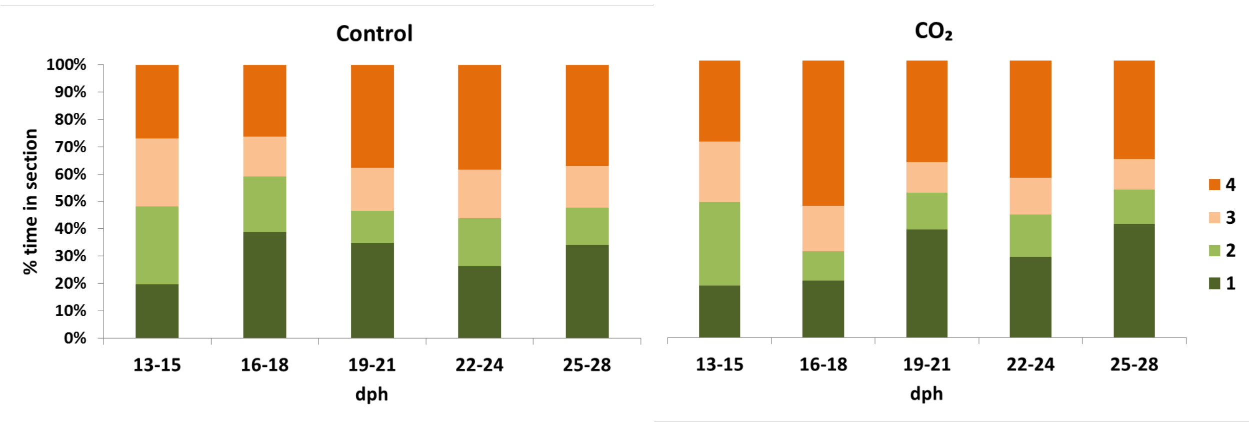

Figure S7, Distribution of fish in the chamber during estuarine sound playback. *Section 1 in dark green is represented by the ¼ of the chamber closest to the active speaker, section 2 in light green by the second ¼ of the chamber in the half closest to the active speaker and so on till section 4 which is the ¼ of the chamber farthest from the active speaker.*

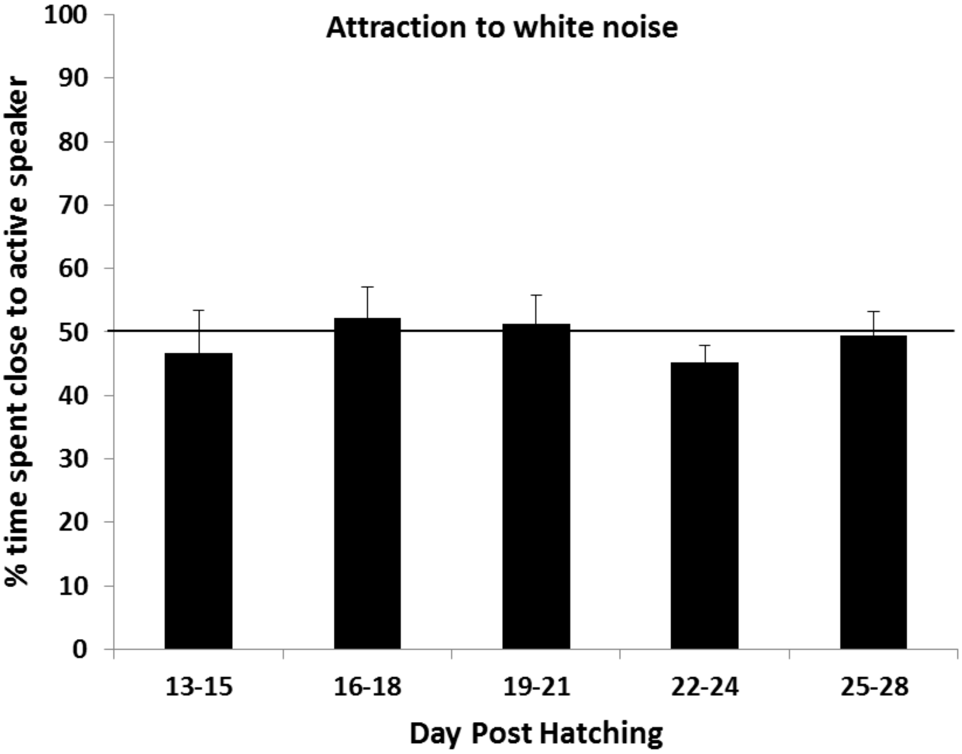

Figure S8, Response to white noise. *Mean (± SE) percentage of time that control fish spent in the half of the chamber close to the speaker broadcasting white noise. Results are pooled in blocks of 3 days. The horizontal line represents the threshold of random response (50%).*

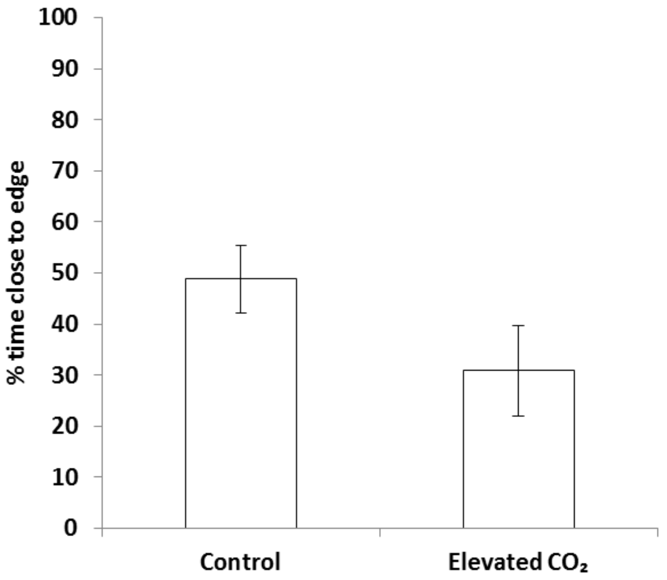

Figure S9, Thigmotaxis. *Edge following behaviour during sheltering experiment reported as mean percentage of time spent close to one of the tank edges ± SE. Total number of replicates n = 33 (controls: n = 20, CO_2: n = 13).*

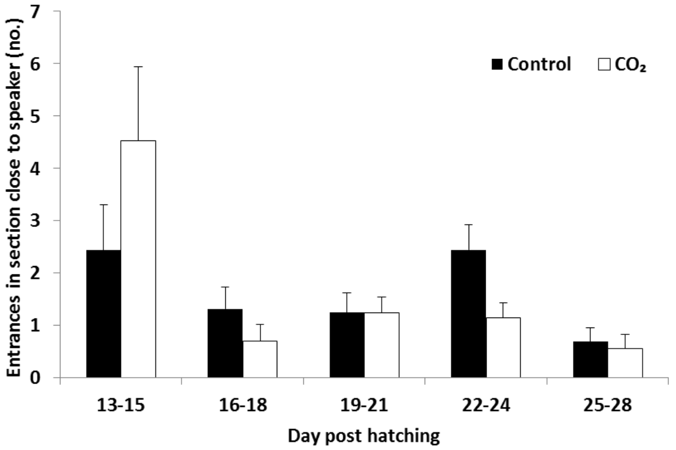

Figure S10, Explorative behaviour. *Number of times the fish larvae crossed the boundary between the two sections of the chamber during estuarine sound playback (average ± SE). Values are based on entrances into the section closest to the active speaker and do not count the times the fish was in that section at the beginning of the trial.*

SUPPLEMENTARY DATA

Table S1: *Two-way analysis of variance of length data*

Source	DF	MS	F	P
dph	4	1017.4	119.81	0.0001
CO_2	1	280.98	33.089	0.0001
dph x CO_2	4	24.538	2.8897	0.0244
Residual	294	8.4916		
Total	303			

dph = Day post hatching pooled in 3 day blocks, CO_2 *= treatment*

Table S2: *Two-way analysis of variance of velocity data*

Source	DF	MS	F	P
dph	4	7.8077	14.728	0.0001
CO_2	1	3.5656	6.726	0.0106
dph x CO_2	4	0.26557	0.50097	0.744
Residual	246	0.55355		
Total	255			

dph = Day post hatching pooled in 3 day blocks, CO_2 = treatment

CHAPTER III: SILENT OCEANS: OCEAN ACIDIFICATION IMPOVERISHES NATURAL SOUNDSCAPES BY ALTERING SOUND PRODUCTION OF THE WORLD’S NOISIEST MARINE INVERTEBRATE

Statement of Authorship

Statement of AuthorshipTitle of Paper	Silent oceans: ocean acidification impoverishes natural soundscapes by altering sound production of the world's noisiest marine invertebrate
Publication Status	Published
Publication Details	Proceedings of the Royal Society B

Principal Author

Name of Principal Author (Candidate)	Tullio Rossi		
Contribution to the Paper	Study design, data collection, data analysis, writing		
Overall percentage (%)	85		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	12/11/15

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

iv. the candidate's stated contribution to the publication is accurate (as detailed above);
v. permission is granted for the candidate in include the publication in the thesis; and
vi. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ivan Nagelkerken		
Contribution to the Paper	study design and writing		
Signature		Date	12/11/15

Name of Co-Author	Sean D. Connell		
Contribution to the Paper	study design and writing		
Signature		Date	11/11/15

Please cut and paste additional co-author panels here as required.

Silent oceans: ocean acidification impoverishes natural soundscapes by altering sound production of the world's noisiest marine invertebrate

Running title: The silence of acidified oceans

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Keywords: climate change, ocean acidification, sound, soundscape, snapping shrimp, noise,

ABSTRACT

Soundscapes are multidimensional spaces that carry meaningful information for many species about the location and quality of nearby and distant resources. Because soundscapes are the sum of the acoustic signals produced by individual organisms and their interactions, they can be used as a proxy for the condition of whole ecosystems and their occupants. Ocean acidification resulting from anthropogenic CO_2 emissions is known to have profound effects on marine life. However, despite the increasingly recognised ecological importance of soundscapes, there is no empirical test of whether ocean acidification can affect biological sound production. Using field recordings obtained from three geographically separated natural CO_2 vents, we show that forecasted end-of-century ocean acidification conditions can profoundly reduce biological sound level and frequency of snapping shrimp snaps. Snapping shrimp were among the noisiest marine organisms and the suppression of their sound production at vents was responsible for the vast majority of the soundscape alteration observed. To assess mechanisms that could account for these observations, we tested whether long-term exposure (2-3 months) to elevated CO_2 induced a similar reduction in the snapping behaviour (loudness and frequency) of snapping shrimp. The results indicated that the soniferous behaviour of these animals was substantially reduced in both frequency and sound level of snaps produced. As coastal marine soundscapes are dominated by biological sounds produced by snapping shrimp, the observed suppression of this component of soundscapes could have important and possibly pervasive ecological consequences for organisms that use soundscapes as a source of information. This trend towards silence could be of particular importance for those species whose larval stages use sound for orientation towards settlement habitats.

INTRODUCTION

A soundscape is a mosaic of biological, geophysical and anthropogenic sounds that encompasses various landscape features and ecosystem processes [1]. Soundscape ecology, despite its value for applied research, remains a relatively young field of research undergoing rapid progress [2].

The soundscape generated by a specific habitat represents a multidimensional space that carries decision-making information needed for locating and evaluating resources [3]. This information informs both residents and dispersing species about the composition of the local species community, the amount of available resources (such as food, territory and partners), and the presence of potential predators [3-5]. Research showed that the quality of individual signals produced by soniferous animals is affected by stress levels [6], habitat condition [7] and climate [8, 9]. Hence, habitats impacted by anthropogenic perturbations produce distinct acoustic signatures associated with changes in biodiversity [10] and ecological condition [11, 12].

The pelagic dispersal of marine propagules connects populations via replenishment. Despite its ecological importance, this recruitment process has been poorly understood until recently and often referred to as a “black box” in the life history of marine species. Research showed that this process is far from being stochastic as larvae of many species are able to locate and selectively swim towards preferred settlement habitats using a set of sensory cues such as sounds, odours and light [13]. Sound is one of the most reliable directional cues in the ocean because it propagates to up to thousands of kilometres with little attenuation and in a predictable fashion [14]. Visual and olfactory cues instead, are easily confounded by light, turbidity and turbulence making them less reliable for long-distance orientation. In the marine realm, a variety of marine organisms use soundscape for orientation during the crucial and delicate process of dispersal and settlement. Studies have provided robust evidence that propagules of both vertebrates [5, 15-17] and invertebrates [18-20] can use sound to orient themselves towards suitable settlement habitats, underpinning the important functional role of soundscapes as mediators of connectivity and population replenishment. Despite this important ecological role that soundscapes plays in the ocean, it is puzzling that the potential effects of ocean acidification on sound production have never been explored.

Ocean acidification will likely change marine ecosystems within this century [21-23], but the processes through which this will occur are not always obvious. Coastal marine sounds are largely of biological origin, hence, potentially vulnerable to ocean acidification. The most common sources of marine biological sounds are snapping shrimp snaps, sea urchin raspings and fish vocalizations [24, 25]. Snapping shrimp (family Alpheidae), are the most ubiquitous and noisy soniferous animals in coastal ecosystems [26-28]. These shrimp possess the unique ability to produce some of the loudest sounds in the ocean (up to 210 dB re 1 μ Pa) [26], which appear to be second only to sperm whale clicks (up to 236 dB re 1 μ Pa) [29]. Such extreme sound levels are achieved by the formation of cavitation bubbles upon rapid closure of the snapping claw [30]. The snapping behaviour is used by these animals for a variety of purposes such as predation and agonistic behaviour [31], and they are responsible for the permanent characteristic broadband background crackling sound that propagates long distances offshore [32]. The importance of snapping shrimp sounds for the orientation of larvae has earlier been shown in an *in situ* study where natural larval fish communities showed higher attraction towards soundscapes with high frequency (> 570 Hz) snapping shrimp-generated sound compared to non-snapping shrimp-generated low frequency soundscape (<570 Hz) [33].

We here tested whether the functional role of biological soundscapes might be jeopardised by global change. We studied how ocean acidification can affect coastal marine soundscapes by making *in situ* audio recordings at three disparate natural CO_2 vents in temperate waters of both the northern and southern hemispheres. Results indicated a substantial reduction in snapping shrimp sound quantity and quality in areas where the pCO_2 concentration of seawater matched end-of-century projections of human CO_2 emissions. Using long-term (2-3 months) CO_2 exposure laboratory experiments on sound production by snapping shrimp we then provide a potential mechanism that could explain the loss of sound quantity and quality in marine ecosystems due to projected ocean acidification.

MATERIALS & METHODS

STUDY SITES

Three natural CO_2 vents in temperate waters - Vulcano and Ischia in Italy, and White Island in New Zealand (Fig. S1) - were used to assess the effect of forecasted end-of-century CO_2 levels, based on the RCP 8.5 business-as-usual scenario of human greenhouse gas emissions [34] (pCO_2: Vulcano ~ 720 μatm , Ischia ~ 930 , White Island ~ 849 μatm , see Table S1 for water chemistry details) on the acoustic output generated by sound producing organisms. Natural CO_2 vents, unaffected by elevated temperatures, are present in the northern part of Levante Bay at Vulcano Island, Sicily, Italy (38°25’ N, 14°57’ E) (Fig. S1). A pH gradient is present with values that range from extreme (> 2,000 µatm pCO_2) to roughly ambient levels along a 700-m stretch of rocky coast along the northern side of Levante Bay [35]. We selected an extreme CO_2, an elevated CO_2 (representing mid-century projections) and a control area (Table S1) along this gradient and made sound recordings at 7 stations (Fig. S1). Even though we included areas representing extreme pCO_2 values, all our conclusions are based on forecasted realistic end-of-the century pCO_2 levels. Similarly, at the island of Ischia, shallow cold CO_2 vents are present around Castello Aragonese (40°43'51.87"N, 13°57'47.13"E) where we made recordings at three well characterized stations based on previous studies [36, 37] (Fig S1). White Island is a volcanic island located in the Bay of

Plenty, North Island of New Zealand (37°27'S,177°17' E) (Fig. S1). CO_2 vents unaffected by temperature are located along the north-east rocky coast of the island (Table S1) [38, 39]. We selected an elevated CO_2 and a control area and made sound recordings at 4 stations (Fig. S1). Carbonate chemistry measurement and analysis are described in the Supplementary methods.

SOUNDSCAPE RECORDINGS AND ANALYSIS

Snapping shrimp snaps, sea urchins rasping and fish vocalizations are the most common sources of marine biological sound and are known to peak at dusk during the so called "dusk chorus" and remain active until morning [24, 25]. For this reason we focused the sampling effort at the immediate hours after dusk. The recordings were made with a Hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.1 dB re $1V/\mu Pa^{-1}$; frequency range 0.02–30 kHz; calibrated by manufacturers; High Tech Inc., Gulfport, USA) and a calibrated audio recorder (PCM-M10, 48 kHz sampling rate, Sony Inc., Tokyo, Japan). At Vulcano and Ischia five minutes recordings were obtained at each station along the CO_2 gradient at 1 m from the bottom of a mixed rocky and seagrass habitat ranging in depth between 1 and 4 m. Recordings were obtained in late summer (between 14 and 18 September 2013 in Vulcano and between 27 and 28 September 2014 in Ischia) under calm sea conditions in the first hour after sunset. During the sampling period the moon phase was between waxing gibbous and full moon for Vulcano and waxing crescent for Ischia. At White Island, the same recording apparatus as described above was enclosed in a waterproofed barrel fixed to an anchored buoy for overnight recordings. The hydrophone was deployed at 5 meters depth over a rocky seafloor whose sea surface ranged between 8 to 12 m depending on station and tide. Recordings were made over rocky reef bottom characterized by kelp, turf-forming algae, and urchin barrens. The abundance of sea urchins at White Island did not vary between control and elevated CO_2 areas (Sean Connell personal observation, 2013). Night recordings were performed over four consecutive nights under full moon (18-21 November 2013). During the recordings the swell height ranged between 0.7 and 1.5 m. All audio files were high pass filtered at 300 Hz in order to remove wave noise. Additional methods for the soundscape analysis are described in the Supplementary methods.

SNAPPING SHRIMP EXPERIMENTS

Preliminary experiments showed that snapping shrimp produce snaps spontaneously when left undisturbed without contact with conspecifics or other external stimuli. This suggests that the snapping behaviour is not only used as a defence/offense mechanism as suggested by the literature. Furthermore, snapping shrimp were able to widely modulate the intensity of the snaps when left undisturbed but produced consistently loud snaps upon simulated aggression. Based on these observations we conducted two types of behavioural experiments on snapping behaviour. First, we recorded number and intensity of snaps produced spontaneously overnight in absence of external stimuli, and second we measured the intensity of snaps produced upon simulated aggression as a proxy for maximum snapping performance achievable. The Supplementary methods contain detailed descriptions of these experiments.

Snapping shrimp of the species *Alpheus novaezelandiae* (Miers, 1876) were collected using SCUBA in a stony pebbled bottom habitat at Rapid Bay, South Australia on 7 and 13 February 2014. Following one week of acclimation to the laboratory conditions, the animals were assigned to control and end-of-century CO_2 conditions targeting a pCO_2 level of ~ 1000 μatm [34] (Table S1). Shrimps were housed in 40 l tanks (2 tanks per treatment) where each individual was enclosed in a 10 cm x 5 cm container and fed pelleted feed for crustaceans every other day. Overnight shrimp snapping frequency was recorded in the period of 23 April - 22 May 2014, after long-term (two months) exposure to elevated CO_2. Intensity of defensive snaps of shrimp was measured after 3 months of exposure to elevated CO_2. All shrimp moulted within the first month of exposure. Shrimp activity levels, moulting time, claw and carapace length were also measured and are described in details in the Supplementary materials.

RESULTS

SOUNDSCAPE ALTERATION

In situ sound recordings in the CO_2-affected areas revealed a marked decline in sound intensity in the frequencies corresponding to snapping shrimp sound (above ~ 500 Hz with peak at ~ 4 KHz) [40] at all sites, as observed by acoustic spectra (Fig. 1A, B, S2). Likewise, the full bandwidth sound pressure level and the number of snaps by snapping shrimp significantly decreased at elevated as well as extreme CO_2 levels compared to the controls (ANOVA, SPL, $F_{(2,5)} = 27.8$, $p = 0.002$; snaps, $F_{(2,5)} = 12.4$, $p = 0.012$) (Fig. 2A, B). This pattern holds when analysed at the site level (Fig. S3). Regression analysis showed that the number of snaps explained 84% of the variation in full bandwidth sound pressure level < 10 kHz, with a positive significant relationship between the two variables (ANOVA, $F_{(1,16)} = 85.5$, $p < 0.001$) (Fig. 2C). At Vulcano, fish vocalizations with peak frequency ~ 800 Hz were detected at the elevated CO_2 area (Fig. 1B), but the mobility of soniferous fish makes this result hard to link to a possible effect of high CO_2. Sounds around 1.2 kHz, likely originating from sea urchin rasping [41]**,** were detected at White Island but showed no difference between treatments (Fig 1A, B). This was consistent with a uniform presence of sea urchins at White Island control and elevated CO_2 areas (Sean Connell personal observation, 2013). Furthermore, at the extreme CO_2 areas of Vulcano and Ischia, noise below 2 kHz originated from CO_2 bubbles seeping trough the seafloor as confirmed by aural and visual observation of the spectrograms.

SNAPPING SHRIMP EXPERIMENTS

Diminished sound level and frequency of snaps at CO_2 vents may be driven by at least four potential mechanisms. First, ocean acidification could directly reduce the soniferous behaviour of shrimp in terms of frequency and/or intensity of snaps produced. To test this hypothesis we exposed snapping shrimp to elevated CO_2 (~ 1074 μatm, Table S1) and monitored their sound production in the laboratory. The results indicate that the soniferous behaviour of these animals was substantially reduced in both frequency and peak sound level (peak amplitude) of snaps produced (snap frequency: ANOVA, $F_{(1,18)} = 6.3$, $p = 0.015$; undisturbed snap sound level: ANOVA, $F_{(1,18)} = 16.36$, $p = 0.001$) (Fig. 3). Second, a decrease in snapping shrimp sound production at CO_2 vents could also be the result of a physico-mechanical impairment of the snapper claw due to altered calcification caused by elevated CO_2. We tested the sound level of defensive snaps in the laboratory, as a proxy of maximum snap intensity achievable, but detected no significant differences after three months of elevated CO_2 exposure (ANOVA, $F_{(1,12)} = 0.74$, $p = 0.41$) (Fig. 3C). Third, a decrease in snapping shrimp sound at CO_2 vents could result from sparser abundance of these animals at the vents. Quantification of snapping shrimp abundance in the field is difficult due to their highly cryptic nature, yet other studies have shown reduced shrimp abundances at both temperate and tropical natural CO_2 vents [36, 42]. Finally, habitat change due to high CO_2 [39] might alter the shrimps snapping behaviour and/or abundance. Carapace length, claw length, moulting time, and activity levels were not affected by treatment conditions (Fig. S4).

DISCUSSION

Biological sounds are recognized as important orientation cues for various marine organisms due to their long propagation distance and richness of biological information that they carry[16]. Snapping shrimp crackle is certainly the most common biological feature of marine coastal soundscapes and often dominates background abiotic noise. In this study, using a combination of field and laboratory experiments, we found elevated CO_2 to reduce snapping shrimp sound production. Our results suggest that this is caused by CO_2-induced disruption of snapping shrimp soniferous behaviour, and not through a physico-mechanical impairment of their claw. This result is consistent with recent findings that show how ocean acidification can impair key behaviours in invertebrates, such as predator escape responses [43-45]. However, additional non-exclusive explanations exist for this pattern. Elevated CO_2 levels could indirectly penalize shrimps by changing their habitat quality through reduction of the cover of canopy forming algae and by favouring opportunistic turf forming algae [22, 39]. This could potentially be responsible for a reduction in shrimp population abundance and further changes in their behaviour.

At the natural vents, CO_2 levels representative of end-of-century ocean acidification scenarios caused a substantial decrease not only in the number of snapping shrimp snaps but also in the total acoustic output of the reef. Extrapolating this result from the localized CO_2 vents to future oceans, where there will be few to no refuges to global acidification, we can expect potentially detrimental effects for population replenishment by oceanic larvae that rely on sound for orientation. Furthermore, as not all fish rely equally on sound this might promote changes in community structure of recent settlers.

Models suggest that ocean acidification levels expected by the end of the century will significantly decrease sound absorption in the ocean [46], potentially compensating for the loss in biogenic sound caused by ocean acidification found in this study. However, by considering the main energy loss mechanisms in shallow waters and the distances likely to be relevant for fish orientation (<10 km), the effect of pH on sound absorption is negligible because geometric spreading accounts for most of the sound loss from the source [47, 48]. If biological sounds in oceans become poorer, but richer in anthropogenic noise [49], then biological sound cues are likely to become less important for orientation. Larvae will have to either rely on other relevant environmental cues or extend their oceanic larval stage until they come within reach of the reduced sound detection zone of potential settlement habitats. A prolonged oceanic life phase, due to delayed settlement, is likely to increase predation risk, delay occupancy of food-rich benthic habitats, and undermine successful establishment. As orientation towards suitable settlement habitat is a key process in the life of most marine organisms, the findings of our study have important implications for the replenishment of marine populations and ecosystems, and connectivity between source and sink areas. Future research could investigate the effect of CO_2 not only on sound producing animals but also on those species that are known to use sound as a settlement cue [19, 50]. This aspect of research is of particular priority because CO_2 can reverse auditory preferences in fish [51, 52]. The potential for adaptation to ocean acidification remains largely unknown, but recent findings show lack of trans-generational acclimation in fish [53]. Furthermore, compensatory effects through dependence on other senses are possible, but a wide range of studies have demonstrated negative effects of ocean acidification on vision, olfaction, and other senses in fishes and invertebrates [54]. In conclusion, the results of our study show that ocean acidification significantly reduces the amount of biological sound in coastal soundscapes by affecting snapping shrimp behaviour. Given the important role of soundscapes for the orientation of propagules our result suggests that ocean acidification could have negative indirect effects on the process of larval orientation and settlement in marine organisms.

Ethics statement. Research was carried according to the University of Adelaide animal ethics guidelines.

Data Accessibility

Data will be deposited on datadryad.org upon acceptance

Author contributions: All authors contributed to the design of the study, collection of the data, and writing of the article. T.R. analysed the data.

Competing interests

We declare we have no competing interests.

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FIGURES

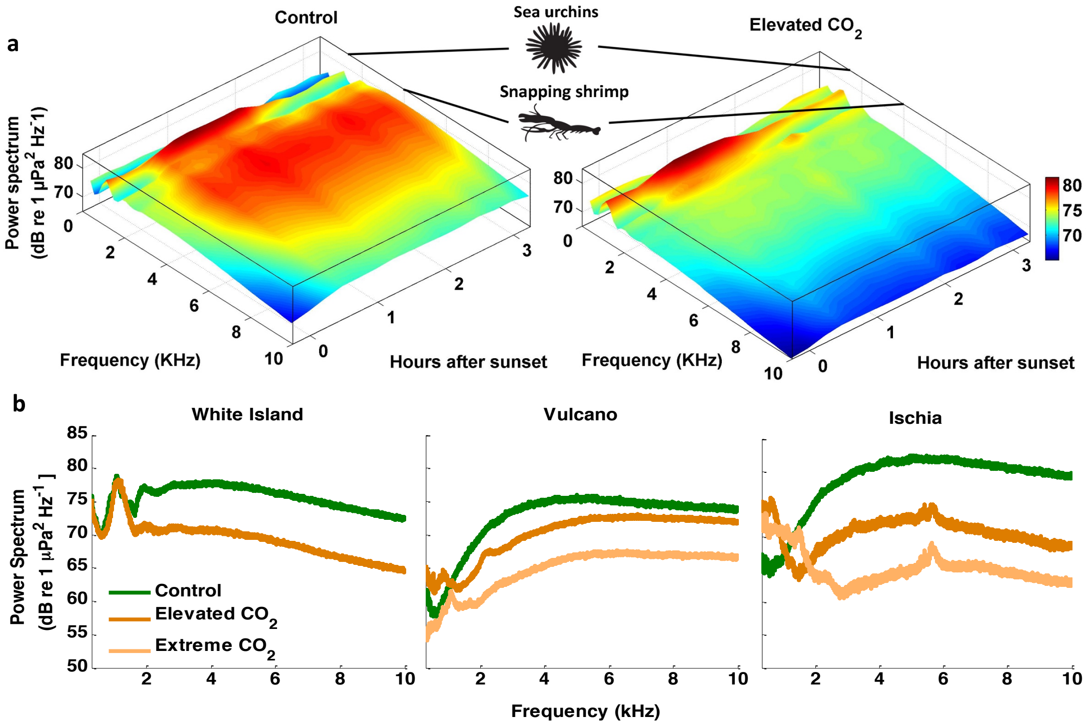

Figure 1. Acoustic spectra for a current vs. a future high-CO$_2$ marine soundscape. *(A), represents dusk and evening chorus of snapping shrimp (peak frequency ~ 4 kHz) and sea urchins (peak frequency ~ 1.2 kHz) at White Island. Acoustic power was averaged among multiple days of sampling in multiple sites (N = 2 per treatment) and is reported along a colour scale. (B) Median acoustic power spectra for recordings obtained at dusk averaged among multiple days of sampling at White Island (New Zealand) (N = 2 per treatment), Vulcano (Italy) (N = 3 per treatment) and Ischia (Italy) (N = 2 per treatment). Spectrograms are shown in Fig. S1.*

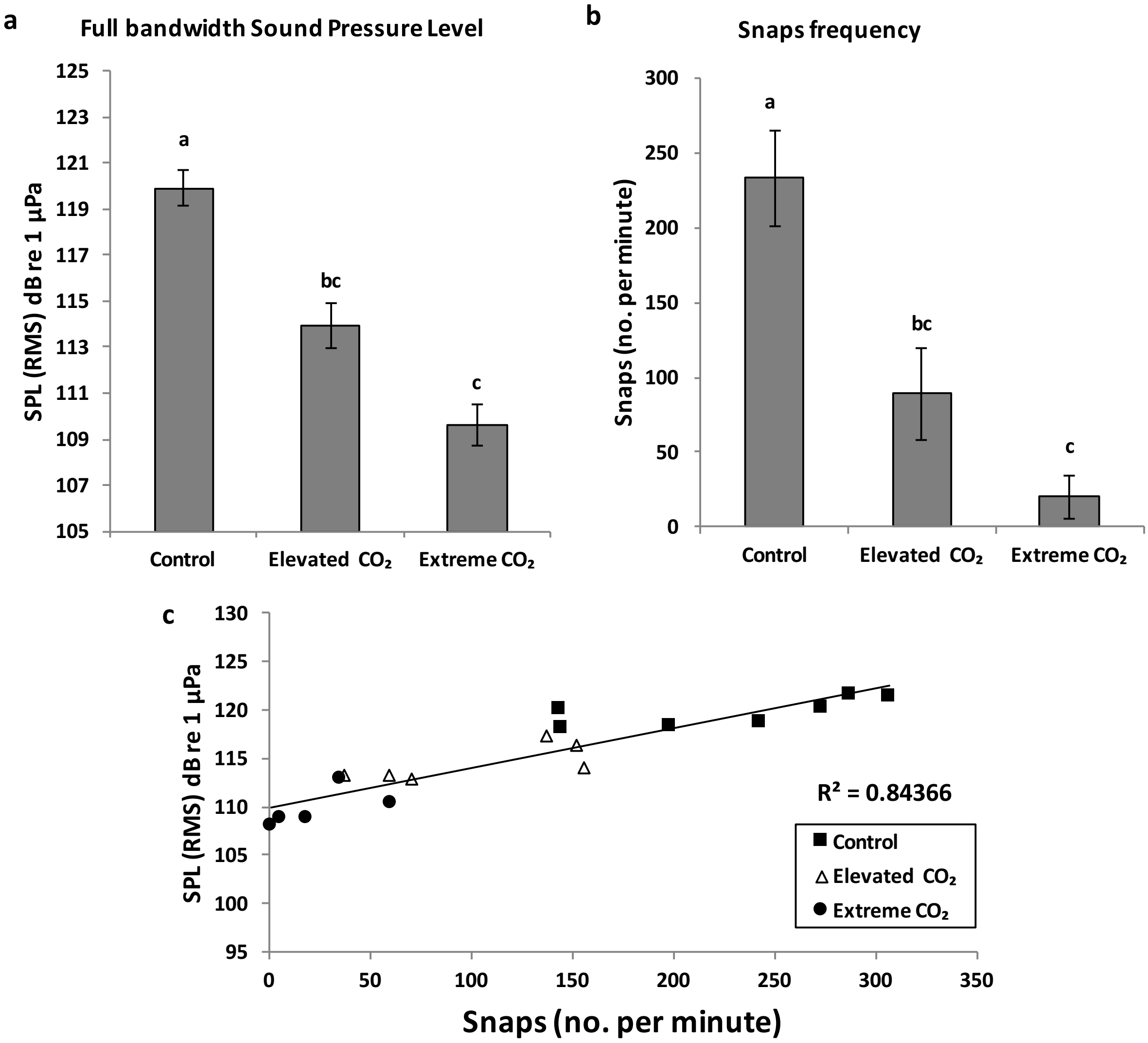

Figure 2. Effect of ocean acidification on biological soundscapes and snapping shrimp noise. *(A) Full bandwidth sound pressure level and (B) mean number of snaps per minute ± SE in field recordings at Vulcano and Ischia (Italy) and White Island (New Zealand) at dusk (sunset to 1 h past sunset) over 5 minutes recordings. Different letters indicate statistically significant ($p < 0.05$) differences. (C) Scatterplot showing the relationship between frequency of snaps and full bandwidth root mean squared sound pressure level. Data points represent single recordings replicated across both time and space.*

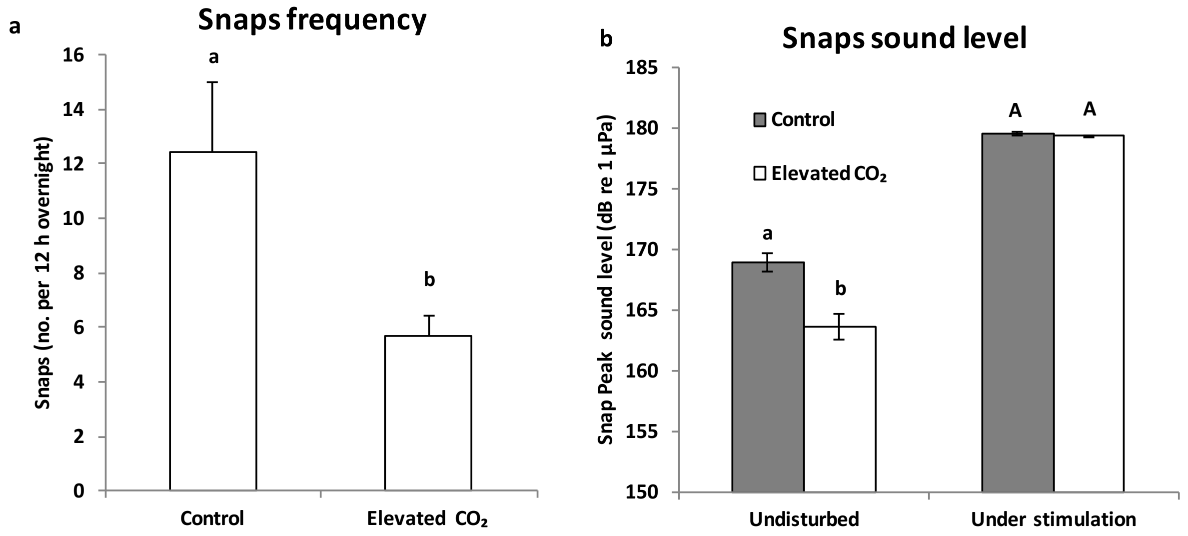

Figure 3. (A) Mean (± SE) number of snaps produced overnight in laboratory experiments by individual snapping shrimp (N = 10 per treatment). *(B) Mean (± SE) peak sound pressure level (SPL) of defensive snaps produced upon stimulation (N = 7 per treatment) and of snaps produced overnight in undisturbed conditions (N = 10 per treatment). Different letters indicate statistically significant ($p < 0.05$) differences.*

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SUPPLEMENTARY METHODS

CO_2 VENTS: TOPOGRAPHIC CONSIDERATIONS

Topographic conditions such as depth range, bottom type and inclination were maintained constant along the pH gradient at all sites. All pH gradients were located along uniform rocky coasts so we do not expect potential effects of topography to confound the sound recordings. The only exception was the control station of Ischia, which was surrounded by rocky outcrops, which might have different reverberation properties compared to the other stations. This particular station was, however, the only true control site available nearby the vents (see Kerrison et al., [1] for a detailed description). Therefore, we felt that it was a necessary compromise. Nevertheless, our study was done at 3 disparate vent sites, with each showing the same patterns so it is unlikely that a potential confounding factor present in one station out of 8 would compromise the value of this study.

CARBONATE CHEMISTRY MEASUREMENT AND ANALYSIS

NATURAL CO_2 VENTS

For all experiments, the CO_2 concentration in the seawater was calculated using the measured values of temperature, salinity, pH_{NBS} and total alkalinity (TA) using the software CO2SYS [2] with constants K1 and K2 from Mehrbach (1973) [3] and refit by Dickson & Millero (1987) [4]. Alkalinity was measured by Dynamic Endpoint Titration using an 888 Titrando (Metrohm) titrator in all experiments. During the course of the study values for standards were successfully maintained within 1% accuracy from certified reference materials from Dr. A. Dickson (Scripps Institution of Oceanography).

At the vent sites all seawater samples were collected between 14/9/13 and 18/9/14 at Vulcano, between 27/9/14 and 28/9/14 in Ischia and between 18/11/13 and 21/11/13 at White

Island. The pH_{NBS} was measured daily at Vulcano and Ischia with a portable SG2-ELK SevenGo probe (Mettler Toledo) from the surface and at White Island with a Sonde 6600 multi-meter logger (YSI) calibrated daily positioned directly above the substratum. The difference between surface and benthic pH was addressed for the vents at Ischia by Kerrison et al., [1]. The authors concluded that the surface and benthic pH were closely coupled. TA samples were collected in Vulcano and White Island on three different days, fixed with mercury chloride and preserved in Duran glass bottles (Schott) pending analysis at the University of Adelaide according to the Standard Operating Procedures [5]. For Ischia, previously published alkalinity values were used instead [1]. For all laboratory experiments seawater samples for alkalinity were analysed immediately upon collection. Salinity was measured with a SR6 refractometer (Vital Sine, Dartmouth, Canada). At Vulcano, previous research showed that hydrogen sulphide released in the main bubbling area does not extend to the northern side of the bay and that sulphate levels are typical of oceanic waters making this stretch of coast suitable for realistic ocean acidification studies [6]. At Ischia previous studies did not detect sulphur [7]. At White Island, hydrogen sulphide was absent in the study area and sulphate levels were slightly higher than the oceanic average (mean ± SE: 1137 ppm ± 9.6 versus 904 ppm) but uniform throughout the study area [8].

SNAPPING SHRIMP EXPERIMENT

Treatment conditions were achieved by bubbling CO_2 enriched air into the shrimp tanks with a Pegas 4000 MF gas mixer (Columbus, USA). pH_{NBS} was measured every other day with a SG2-ELK SevenGo pH probe (Mettler Toledo, Greifensee, Switzerland) calibrated with a three point calibration. Alkalinity was measured as described above.

MEASUREMENT OF SHRIMP SNAP-FREQUENCY AND INTENSITY

A total of 20 shrimp, 10 per treatment (control vs. elevated CO_2), were used to estimate the frequency and intensity of snaps produced over a 12 h period. Each shrimp was randomly selected from its treatment tank and enclosed in an acoustically transparent 2 L plastic container with holes placed inside a 60 L plastic tank at a fixed distance of 10 cm to the hydrophone and at 10 cm depth. Based on the formulas reported in Akamatsu et al., [9] this tank had a resonant frequency of ~ 1.6 kHz which falls outside the dominant frequency of snapping shrimp sounds (~4 kHz). An air stone was placed inside the tank in order to maintain oxygenation, treatment pH, and water circulation. The noise produced by the aerator was detectable by the hydrophone below 10 kHz but it did not prevent the identification of the snaps due to their broadband frequency. Shrimp were randomly selected from their tanks and each trial started with a two hours acclimation period with the recording starting at sunset. The recorders were stopped the following morning after 12 hours and the animals returned to their original tanks. Two calibrated hydrophones and a calibrated audio recorder (same as above) were used to record the snaps at a sampling rate of 48 kHz/ 24 bit. Gender did not have an effect on number of snaps produced (Independent samples Mann-Whitney *U* Test, $p = 0.856$). The waveforms and spectrograms of the recordings were visually and aurally inspected for artefacts then the number of snaps was counted and their peak amplitude measured with the software Audition CS6 (Adobe, San Jose, USA). The peak amplitude of the signal was used instead of its RMS amplitude in order to limit the effect of sound reflections.

MEASUREMENT OF MAXIMUM SHRIMP SNAP-INTENSITY

To determine whether ocean acidification affects the physico-mechanical capability of snapping shrimp to produce loud defensive snaps, we measured the peak amplitude of the snaps produced in response to a simulated aggression. Previous research showed that snapping shrimp produce consistently loud snaps [10] when stimulated but, as found in our study, are able to widely modulate their spontaneous snapping behaviour when left undisturbed. This suggests that defensive snaps are a measure of the maximum sound intensity that the animal can reach when snapping. In each of the trials (n = 14, half in each treatment: control vs. elevated CO_2), one shrimp was placed inside an acoustically transparent plastic container with mesh sides (10 x 5 x 5 cm), suspended below the water surface of a larger tank (150 x 50 x 30 cm). Based on the formulas reported in Akamatsu et el., [9] this tank had a resonant frequency of ~ 3 kHz which falls outside the dominant frequency of snapping shrimp sounds (~4 kHz). The calibrated hydrophone was placed 1 m away from the shrimp at the same water depth of 5 cm. Each shrimp was stimulated with a soft brush until at least two snaps were produced by the shrimp. The number of replicated snaps used for each individual was set at two because most individuals were reluctant at snapping more than twice upon stimulation. Shrimp that did not produce any defensive snap were excluded from the experiment. The shrimp that did not produce at least 2 snaps and those who had molted two days previous to or after the sound measurement were excluded from the analysis. The waveforms and spectrograms of the recordings were visually and aurally inspected and the snaps peak amplitude was measured with the software Audition CS6 (Adobe, San Jose, USA). Claw size and gender did not have an effect on snap peak amplitude (respectively, linear correlation: $R^2 = 0.10$ and Independent samples Mann Whitney *U* Test: $p = 0.945$). We acknowledge that the spectral characteristics, such as peak frequency, of the snaps recorded may be distorted by resonance in the experimental tank, therefore we limit our analysis to the count of snaps produced and the relative comparison of their peak amplitude between treatments.

SNAPPING SHRIMP ACTIVITY LEVELS

Snapping shrimp activity levels (velocity and total distance moved) were recorded in an open field white plastic arena (30 cm × 20 cm × 15 cm) and videotaped from the top using a camcorder (HF R406 Legria, Canon, Japan). One shrimp at the time were positioned in the chamber which was filled with treatment water. The position and velocity of the shrimp in the choice chamber was tracked continuously using EthoVision XT10 (Noldus Information Technology, Wageningen, The Netherlands) for 4 minutes. The combined use of video recordings and automated tracking eliminated the risk of observer bias and external influences on behaviour caused by the presence of the observer.

SNAPPING SHRIMP SIZE AND MOULTING

The animals were checked daily for moulting but the moult was not removed from the tank as these shrimp usually consume it within a couple of days. The carapace and claw length were measured at the end of the experiment from photos after 3 months of exposure to treatment conditions.

SOUNDSCAPE ANALYSIS

All the audio recordings were obtained at a sampling rate of 96 kHz and 32 bit. Power spectra and sound levels (RMS) were calculated with PAMguide in MATLAB® [11] and calibrated in SASLab Pro (Avisoft bioacoustics) using a reference signal at full (1 kHz) scale and the hydrophone sensitivity. Power spectra were calculated using the Hann window (length 1 second and 50% overlap). For White Island, power spectral measurements were produced for every recording by subsampling the first 5 minutes of every quarter hour between 20:00 and 00:00. The measurements were then averaged per site for comparison. The frequencies <300 Hz were excluded from the analysis because they were dominated by wave noise. Due to scratching of the hydrophone cord against the anchor line only the recordings between 20:00 and 00:00 are comparable among all sampling days. For Vulcano and Ischia, the same power spectral analysis was applied to the 5-minute recordings obtained at each station and the results averaged per CO_2 level (Vulcano only) and across sampling days. The number of snapping shrimp snaps was measured over 5 minutes (2.5-30 kHz band pass filtered) recordings at dusk in both sites using the pulse train analysis function in the software SAS lab pro (Avisoft bioacoustics). An artificially generated full scale snap was inserted at the beginning each recording in order to allow valid comparisons between different recordings. The number of snaps was counted for each recording obtained at dusk replicated over days of sampling ($N = 3$ for Vulcano, $N = 2$ for Ischia, $N = 2$ for White Island per treatment). One recording obtained at the elevated CO_2 site in Ischia had to be discarded due to the presence of boat noise.

The moon phase differed between the three vent sites (as described in the method section) but not within sites as the recordings were performed over a short period of time relative to changes in moonphase at each of the sites: 4 days at White Island, 3 days at Vulcano, and 2 days at Ischia. We are aware that the moon phase regulates cycles of biological sounds in marine soundscapes and we believe that by sampling the same site (control and high CO_2) over just a few consecutive days we avoided this confounding factor within site. We do not exclude that moon phase could confound the comparisons between sites but given that all three sites showed an identical pattern in SPL and snap frequency, it is clear that high CO_2 has an overarching effect compared to moon phase differences.

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SUPPLEMENTARY FIGURES

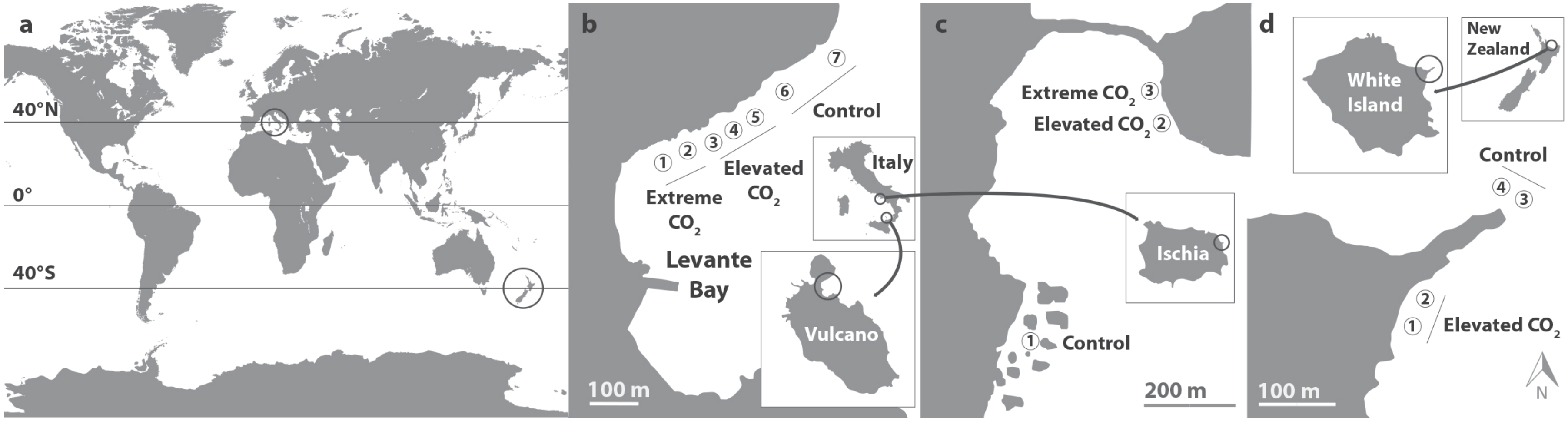

Figure S1, a) Location of the CO_2 vent sites shown on a global map. *Site maps of b) Levante Bay, Vulcano (Italy), c) Ischia (Italy), and d) White Island (New Zealand). Numbers represent stations where sound recording were performed.*

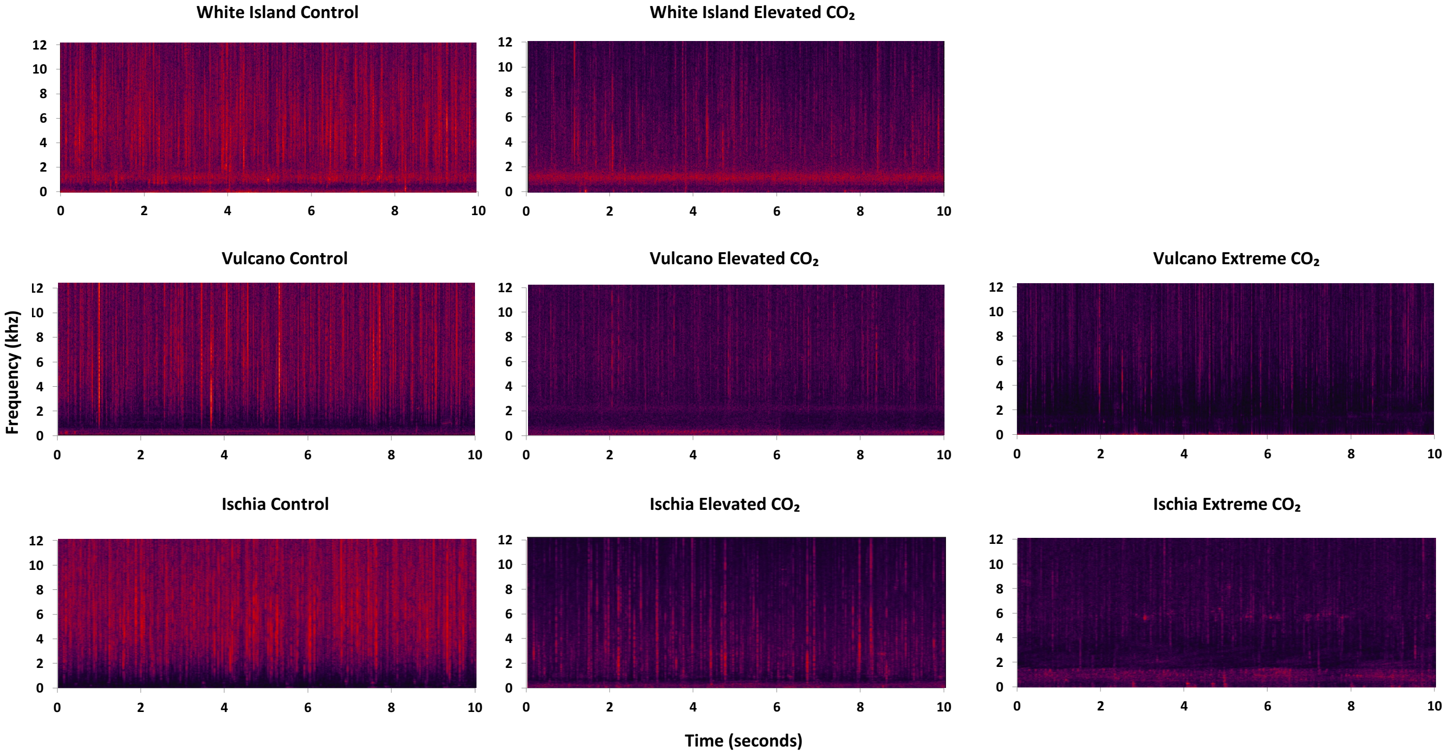

Figure S2, Spectrograms of randomly selected 10 sec subsamples from each site at dusk. *Time is represented on the X-axis, frequency on the Y-axis and amplitude in colour (black/purple = low amplitude, red = high amplitude). Short broadband vertical bars (<1/10 of a second) are snapping shrimp snaps while the horizontal bar centred around 1.2 kHz is probably sea urchin sound (White Island only).* CO_2 *bubbles seeping through the seafloor produced an easily recognisable sound below 2 kHz at Ischia (elevated and extreme* CO_2 *stations).*

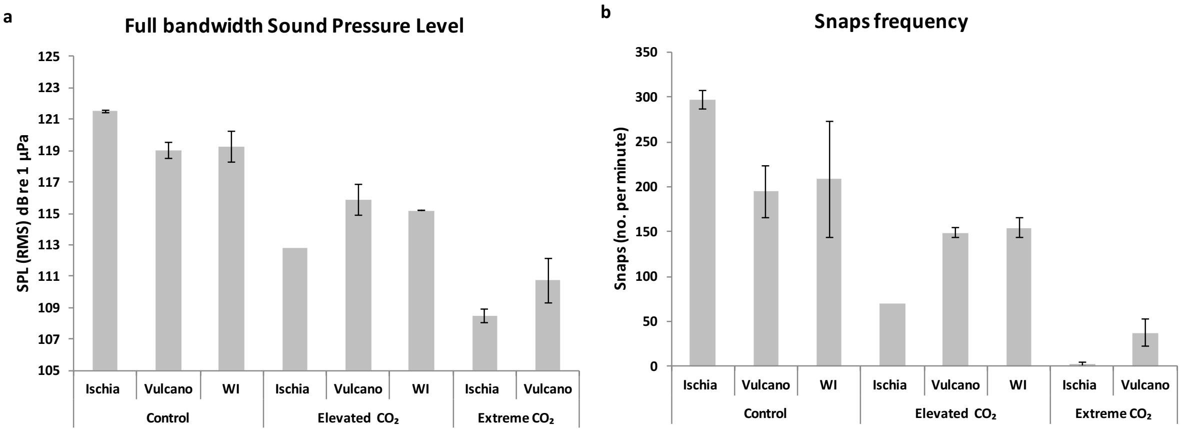

Figure S3. Effect of ocean acidification on biological soundscapes and snapping shrimp noise. *a) Full bandwidth sound pressure level and b) mean number of snaps per minute ± SE. This figure reports the same data of Fig. 2 analyzed by site.*

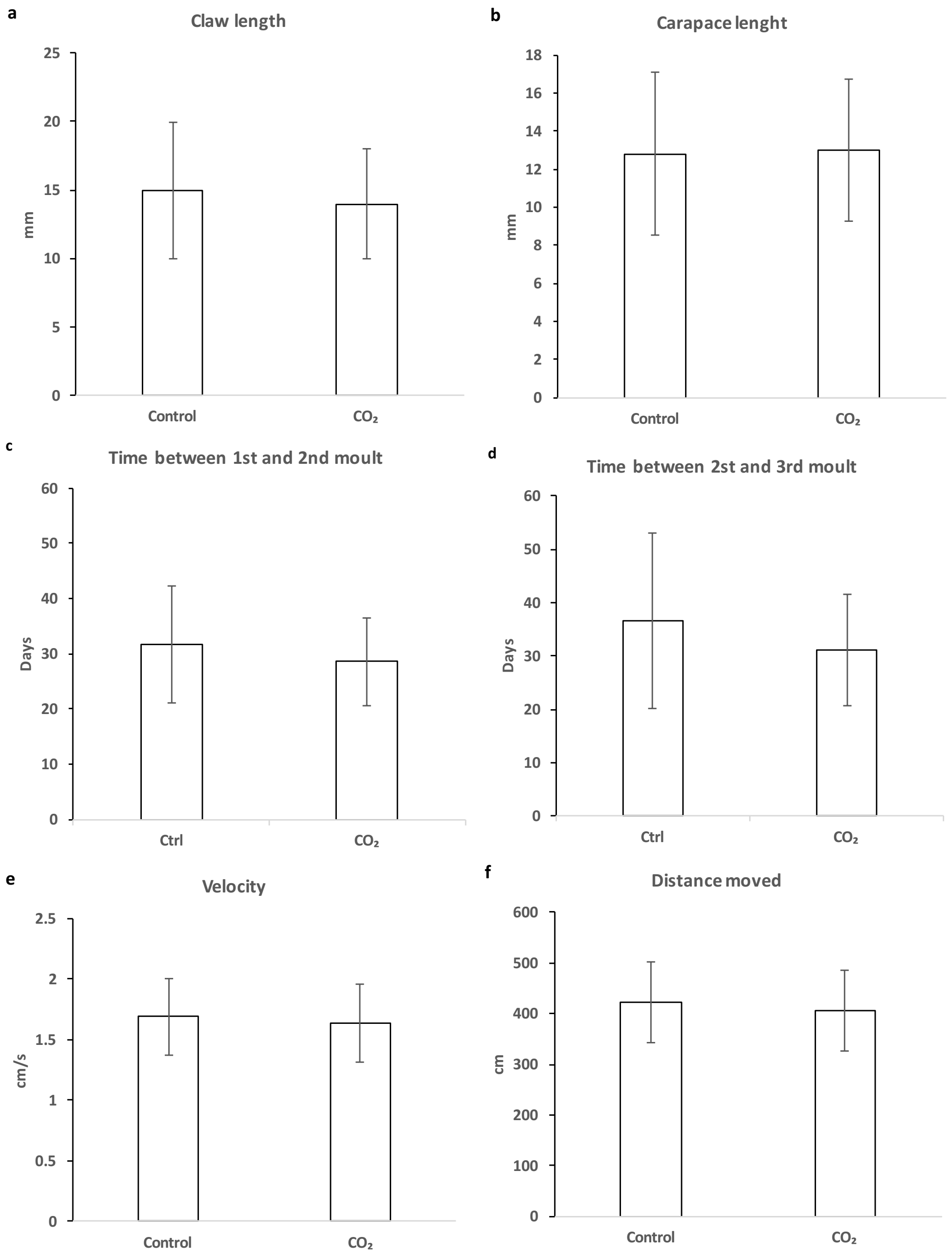

Figure S4, *biometric parameters and activity levels of snapping shrimps after 3 months of exposure to treatment conditions. a) snapping claw length, b) carapace length, c-d) molting time, e-f) activity levels measured in an open field arena.*

Table S1. Summary of the water chemistry parameters measured in the field and in the laboratory experiments.

Location	**Area**	**T(°C)**	**pH NBS**	**N**	**TA (µmol/kgSW)**	**$p\mathrm{CO}_2$ (µatm)**	**N**	**Salinity**	**N**
Vulcano	Control	25.5 (± 0.1)	8.06 (± 0.02)	8	2524.8 (± 2.3)	586.6 (± 39.1)	8	41	1
	Elevated	25.5 (± 0.2)	7.99 (± 0.03)	8	2546.1 (± 12.1)	720.0 (± 59.0)	8	41	1
	Extreme	25.8 (± 0.3)	7.76 (± 0.12)	8	2541.4 (± 9.5)	2116.1 (± 1143.1)	8	41	1
White Island	Control	17.6 (± 0.1)	8.06 (± 0.02)	21	2287.2 (± 12.1)	538.8 (± 32.2)	4	35	1
	Elevated	17.9 (± 0.1)	7.86 (± 0.02)	33	2295.7 (± 10.7)	929.6 (± 54.1)	4	35	1
Ischia	Control	24.2 (±0.4)	8.21 (± 0.01)	4	2519 (± 4)*	381 (± 7.7)	4	40	1
	Elevated	24.3 (±0.4)	7.94 (± 0.08)	4	2518 (± 6)*	849.5 (± 146.6)	4	40	1
	Extreme	24.6 (±0.4)	7.26 (± 0.15)	4	2525 (± 4)*	5369.1 (± 2220.1)	4	40	1
Species	**Treatment**								
Snapping shrimp	Control	21.2 (± 0.1)	8.20 (± 0.01)	42	2598.0 (± 16.5)	393.5 (± 7.8)	16	40.0 (± 0.1)	12
	Elevated	21.3 (± 0.1)	7.81 (± 0.01)	42	2538.0 (± 13.3)	1074.1 (± 27.6)	16	40.0 (± 0.1)	12

Average (± SE) temperature (T), pH and total alkalinity measured in the field from 14-18 September 2013 at Vulcano (Italy), from 18-21 November 2013 at White Island (New Zealand), and between February and May 2014 for snapping shrimp in the laboratory. pCO_2 *values were calculated using CO2sys. *TA values taken from Kerrison et al. 201*

CHAPTER IV: THE SOUNDS OF SILENCE: REGIME SHIFTS IMPOVERISH MARINE SOUNDSCAPES

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Tullio Rossi		
Contribution to the Paper	Study design, data collection, data analysis, writing		
Overall percentage (%)	85		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	12/11/15

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

vii. the candidate's stated contribution to the publication is accurate (as detailed above);

viii. permission is granted for the candidate in include the publication in the thesis; and

ix. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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The sounds of silence: regime shifts impoverish marine soundscapes

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ABSTRACT

Context. Regime shifts are well known for driving penetrating ecological change, yet we do not recognise the consequences of these shifts much beyond species diversity and productivity. Sound represents a multidimensional space that carries decision-making information needed for dispersing species to locate resources and evaluate their quantity and quality.

Objectives. Here we assessed the effect of regime shifts on marine soundscapes, which we propose has the potential function of strengthening the positive or negative feedbacks that mediate ecosystem shifts.

Methods. We tested whether biologically relevant cues are altered by regime shifts in kelp forests and seagrass systems and how specific such shifted soundscapes are to the type of driver; i.e. local pollution (eutrophication) vs. global change (ocean acidification).

Results. Here, we not only provide the first evidence for regime-shifted soundscapes, but also reveal that the modified cues of shifted ecosystems are similar regardless of spatial scale and type of environmental driver. Importantly, biological sounds can act as functional cues for orientation by dispersing larvae, and observed shifts in soundscape loudness may alter this function.

Conclusions. These results open the question as to whether shifted soundscapes provide a functional role in mediating the positive or negative feedbacks that govern the arrival of species associated with driving change or stasis in ecosystem state.

Keywords: Regime shift, kelp, seagrass, soundscape, snapping shrimps, orientation, population replenishment, ocean acidification, climate change

INTRODUCTION

Surprisingly large shifts towards a simplification of species composition and ecosystem function, often known as regime shifts, occur within human-dominated landscapes of the terrestrial (Walker 1993), freshwater (Carpenter et al. 1999) and marine realms (Bellwood et al. 2004). The consequences of simplification of species diversity are often well known, generally resulting in less productivity than comparable assemblages with more species (Cardinale et al. 2006; Stachowicz et al. 2007). Yet, there remains less appreciation of change among other ecosystem properties that may yield and maintain such large shifts. Indeed, the question of how regime shifts affect other key ecosystem functions remains relatively understudied. One such function is the biological sounds that ecosystems produce (soundscapes, *sensu* Krause 1987) as potential orientational cues for dispersing taxa that replenish populations (Lillis et al. 2015; Simpson et al. 2005; Stanley et al. 2015; Vermeij et al. 2010) and as biological cues to evaluate habitat quantity and quality (Piercy *et al.* 2014).

In human-dominated landscapes, the positive and negative feedbacks that hasten or maintain an ecosystem in a particular state are partly a function of the ability for recovery of inhabitants that comprised the previous state (Scheffer and Carpenter 2003). We propose that soundscapes could act as positive or negative feedback by mediating the arrival of species associated with alternate ecosystem states. A soundscape is a mix of biological, geophysical and anthropogenic sounds that reflect various landscape features and ecosystem processes (Pijanowski et al. 2011). Soundscape ecology remains a relatively young field of research undergoing rapid progress (Servick 2014), with particular value for applied ecology, including the study of regime shifts. The quality of individual signals produced by soniferous animals is affected by stress levels (Buchanan et al. 2003), habitat condition (Laiolo et al. 2008) and climate (Botero et al. 2009; Narins and Meenderink 2014). Hence, ecosystems produce distinct acoustic signatures associated with their biodiversity (Sueur et al. 2008) and their ecological condition such as coral cover (Piercy et al. 2014; Tucker et al. 2014).

Sound represents a multidimensional space that carries decision-making information needed for locating resources (Farina et al. 2011). This information informs both resident and dispersing species about the composition of the local species community, the amount of available resources (such as, food, territory and partners), and the presence of potential predators (Farina et al. 2011; Parmentier et al. 2015; Simpson et al. 2011; Slabbekoorn and Bouton 2008) . Sound is one of the most reliable directional cues in the ocean because it propagates to long distances with little attenuation and in a predictable fashion. In the marine realm, a variety of marine larval fish species respond to specific soundscapes in a species specific manner to mediate two possible settlement strategies. Some species orient towards a habitat by being selectively attracted to its soundscape while others select habitats by avoidance of non-preferred soundscapes (Parmentier et al. 2015; Simpson et al. 2011). From the perspective of a dispersing propagule, the spatial heterogeneity of soundscape can therefore be used as a qualitative and directional guide towards the most appropriate adulthood habitat.

We hypothesized that ecosystems which shift in state, regardless of the identity and scale of driver, produce distinct soundscapes that reflect their particular state. If regime-shifted soundscapes exist, such a finding would support the proposition that sound may well represent a key function that changes with regime shifts. In this study we focus on regime shifts in kelp forests and seagrass beds as model systems for assessing the consequences of locally and globally driven change. Locally driven shifts were assessed in systems in which nutrient pollution drives shifts from a highly diverse and productive state to a less diverse and less productive state (Connell et al. 2008; Gorman et al. 2009; Neverauskas 1987). Globally driven shifts, such as resulting from ocean acidification, were assessed in systems in which natural CO_2 vents mimic future change due to increasing human greenhouse gas emissions (Nagelkerken et al. 2015).

MATERIALS & METHODS

STUDY SITES.

Eutrophication is the main driver of ecosystem regime shifts in marine systems on the metropolitan coast of Adelaide (South Australia) (Connell et al. 2008; Gorman et al. 2009). Rocky reefs in this region are typically dominated by canopy forming kelp forests consisting of *Eklonia radiata* (Connell and Irving 2008). However, excessive nutrient input into this naturally oligotrophic system has driven a shift from kelp to algal turf domination over the last 40 years (Connell et al. 2008). Kelp forests are highly productive carbon storage systems (Copertino et al. 2005) and create a complex three dimensional habitat with their blades and convoluted holdfasts. Snapping shrimps (family Alpheidae), which are the most ubiquitous and noisy soniferous animal in coastal systems (Johnson et al. 1947; Knowlton and Moulton 1963), commonly inhabit the holdfasts of these canopy-forming algae (Thiel and Vásquez 2000). A previous study that investigated the crypto fauna assemblages living in *E. radiata* holdfasts in southern Australia found snapping shrimps present in 26% of the holdfasts collected (62/240) (Connell unpublished data, see Fowler-Walker *et al.* 2005 for experimental design).

We identified an area between Horseshoe reef and Port Noarlunga (South Australia) where kelp forests and turf dominated areas are present in close proximity and with comparable abiotic factors (Connell et al. 2008) (depth between 1.5 and 9 m depending on site and tide, sedimentary rocks). In this area we selected three kelp forests (continuous canopy patch for a minimum diameter of ~20 m punctuated by small gaps of 1-2 m) and three algal turf dominated (uninterrupted turf forming algae for a minimum diameter of ~20 m punctuated by small kelp remnants) sampling stations where we made audio recordings (Table S1). Recordings at each control and degraded site were replicated three times during different months (Table S2).

Seagrass meadows (*Posidonia* spp. and *Amphibolis* spp.) extensively cover sandy substrate in the Spencer Gulf (South Australia). However, excessive eutrophication caused by sewage discharge resulted in large areas of seagrass meadows to be overgrown by epiphytes and ultimately die causing the system to shift to a bare sandy substratum (Neverauskas 1987; Walker and McComb 1992). Based on the most recent (year 2013) seagrass cover maps and aerial images (Hart 2013) we identified an area in front of Semaphore beach where extensive patches of seagrass have receded due to eutrophication (sandy patch minimum diameter: 500 m). Surrounding areas with the same water depth (12-14 m), distance to shore and sediment type (Jason Tanner personal communication) were covered by seagrass meadows that we used as controls. We selected three seagrass and three sand sampling stations for our audio recordings (Table S1). Recordings at each control and degraded site were replicated two times (Table S2).

NATURAL CO_2 VENTS.

Three natural CO_2 vents in temperate waters – Vulcano Island and Ischia Island (Italy) and White Island (New Zealand) (Table S1) – were selected to assess the effect of ocean acidification on marine soundscapes, resulting from end-of-century levels of anthropogenic CO_2 as forecasted under a business-as-usual emission scenario (Meinshausen et al. 2011) (Table S2). Natural CO_2 vents, unaffected by elevated temperatures (Table S3), are present in the northern part of Levante Bay at Vulcano Island (Italy). A pH gradient was present with values that range from extreme (> 2000 µatm pCO_2) to roughly ambient levels along a 700-m stretch of rocky coast along the northern side of Levante Bay (Boatta et al. 2013; Nagelkerken et al. 2015). We selected an elevated CO_2 and a control area (Table S3) along this gradient for our sound recordings, which consisted of three temporal replicates for each area (Table S2). Similarly, at the island of Ischia (Italy), shallow CO_2 vents are present around Castello Aragonese where sound recordings were done at two well characterized sites based on previous studies (Hall-Spencer et al. 2008; Kerrison et al. 2011) (Table S1). White Island is a volcanic island located in the Bay of Plenty, North Island of New Zealand. CO_2 vents are located along the north-east rocky coast of the island (Brinkman and Smith 2015; Nagelkerken et al. 2015). We selected an elevated CO_2 and a control site for the sound recordings, which consisted of two temporal replicates for each site (Table S2). Details of the sampling design are shown in Table S2 and the characterization methods of the carbonate chemistry (as shown in Table S3) are described in the Supplementary methods.

SOUNDSCAPE RECORDINGS AND ANALYSIS.

The most common source of marine biological sounds are from snapping shrimps snaps, sea urchins rasping and fish vocalizations all of which are known to peak at dusk during the so called “dusk chorus” and remain active until dawn (Staaterman et al. 2014) . For this reason we kept the sampling time constant across all recordings (i.e. from 1 h before to 1 h after sunset). To enable comparison among sites with different water depths we positioned the hydrophone (held by hand from a craft) at one meter elevation above the substratum in all recordings with the exception of White Island where the same recording apparatus described above was enclosed in a waterproofed barrel fixed to an anchored buoy for overnight recordings. Here the hydrophone was deployed at 5 meters depth over a rocky seafloor whose sea surface ranged between 8 to 12 m depending on site and tide. The recordings were made with a Hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.1 dB re $1V/\mu Pa^{-1}$; frequency range 0.02–30 kHz; calibrated by manufacturers; High Tech Inc., Gulfport, USA) and a calibrated audio recorder (PCM-M10, 48 kHz sampling rate, Sony Inc., Tokyo). All recordings were high pass filtered at 300 Hz in order to remove the noise of breaking waves (present in White Island). Duration of audio recordings were 5 minutes and acquired at a sampling rate of 96 kHz and 32 bit. Power spectra and sound levels (RMS) were calculated with the package PAMguide (Merchant et al. 2015) in MATLAB® (Natick, MA, USA) and calibrated in SASLab Pro (Avisoft bioacoustics) using a reference signal at full (1 kHz) scale and the hydrophone sensitivity. Power spectra were calculated using the Hann window (length 1 second and 50% overlap). The frequency of snapping shrimp snaps was measured on band-pass filtered recordings (2.5-30 kHz) using the pulse train analysis function in the software SAS lab pro (Avisoft bioacoustics), where an artificially generated full scale snap was inserted at the beginning of each recording in order to allow valid comparisons between recordings with different noise levels. Sound pressure level (SPL) and frequency of snaps were averaged across multiple days of sampling for each site. Additional details on the experimental design are shown in Table S2. The waveforms and spectrograms of the recordings were visually and aurally inspected in Audition CS6 (Adobe, San Jose, USA) for other sources of biological sound. Boat noise and artefacts were deleted if present.

FISH AND INVERTEBRATE HEARING RANGE

Sound in water is characterized by two components: sound pressure and particle displacement. All fish are able to detect particle displacement with their otoliths and some are sensitive to both particle displacement and sound pressure due to a series of anatomical specializations such as gas chambers and connections between the swim bladder and the inner ear (Popper and Fay 2011). It is worth noting that some fish species can detect sound pressure even if not equipped with such anatomical specializations, suggesting that our morpho-functional understanding of fish hearing is still incomplete (Popper and Fay 2011). The same is true for invertebrates where often, despite showing behavioural and physiological evidence of hearing ability, we cannot associate this sensitivity to any obvious anatomical specialization (Montgomery et al. 2006). Studies suggest that the sound pressure sensitivity in fish ranges widely from a few Hertz to 1 kHz, 5 kHz or even higher in some species (Montgomery et al. 2006; Popper and Fay 2011). In order to capture this wide range of sensitivities we calculated the sound pressure levels in our recordings in three overlapping frequency bands (< 1 kHz, <3 kHz, 5< kHz)

STATISTICAL ANALYSIS

The sound pressure level in the hearing range of fish (<1 kHz, < 3 kHz and <5 kHz), full bandwidth (< 10 kHz) and the frequency of snaps were averaged across days of sampling and then tested for the factor habitat condition with one-way ANOVAs using spatial replication (n = 3 sampling stations per treatment for each of the rocky reef and seagrass system) (Table S2). For ocean acidification, the three natural CO_2 vents served as spatial replicates. In order to allow multiple comparisons (3) the Bonferroni correction was applied and α adjusted from 0.05 to 0.017. The interaction between the factor stressor (nutrient/CO_2) and habitat condition (control/degraded) was tested with a two-way ANOVA on data standardized by habitat. Each habitat group (kelp, seagrass and mixed) was standardized to its mean before the computation. The link between full bandwidth sound pressure level and frequency of snapping shrimp snaps was analysed with a simple regression.

RESULTS

Regime shifts triggered by nutrient pollution and ocean acidification correspond to reduced levels of sound pressure in kelp-dominated and mixed macroalgae-seagrass habitats (nutrient pollution on kelp forests, ANOVA, $F_{(1,4)} = 39.3$, $p = 0.003$, ocean acidification on macroalgae-seagrass, ANOVA, $F_{(1,4)} = 24.7$, $p = 0.008$) (Fig. 1A). Likewise, the frequency of snapping shrimp snaps decreased following the same pattern suggesting that snapping shrimp sound is a key component of biological sound in both nutrient driven change to kelp forests (ANOVA, $F_{(1,4)} = 150.2$, $p < 0.001$) and ocean acidification driven change in macroalgae-seagrass habitats (ANOVA, $F_{(1,4)} = 32$, $p = 0.005$) (Fig. 1B). Despite the low levels of biogenic noise that characterized seagrass beds, nutrient pollution caused a reduction in the frequency of snapping shrimp snaps and a marginal reduction in level of full bandwidth sound pressure (snaps, ANOVA, $F_{(1,4)} = 103.1$, $p < 0.001$, full bandwidth sound pressure level, ANOVA, $F_{(1,4)} = 8.34$, $p = 0.045$, $\alpha = 0.017$ with Bonferroni correction). The reduction in number of snaps and full bandwidth sound pressure level was independent of the type of stressor when habitat specific differences were standardized (ANOVA, snap frequency: condition × stressor interaction, $F_{(1,14)} = 0.55$, $p = 0.46$, sound pressure level: $F_{(1,14)} = 0.55$, $p = 0.46$). Regression analysis incorporating all recordings across habitat types, seasons, and locations showed that the variation in frequency of snapping shrimp snaps significantly explained 84% of the variation ($F_{(1,41)} = 271.8$, $p < 0.001$) in full bandwidth sound pressure level (Fig. S1).

The sound pressure level was also analysed for three frequency bands relevant to fish hearing: 1 kHz, <3 kHz and <5 kHz. Sound levels were reduced in the frequency range < 5 kHz due to regime shifts triggered by nutrient pollution and ocean acidification on rocky and mixed rocky-seagrass bottoms (kelp forest, ANOVA, $F_{(1,4)} = 35$, $p < 0.004$, ocean acidification, ANOVA, $F_{(1,4)} = 76.7$, $p < 0.001$) but not on seagrass beds (Fig. 2). In the two lower frequency ranges, however, the sound levels did not decrease significantly due to habitat degradation.

DISCUSSION

We propose a new concept; that the alternate states associated with regime shifts, regardless of the driver, produce profoundly distinct soundscapes. We found that although each ecosystem was associated with distinct soundscapes, the shift from diverse macroalgae like kelp to low lying turfs (Connell et al. 2008) or from diverse seagrass to sand (Bryars et al. 2011), was associated with a reduction in biological sound. Importantly, although these ecosystem shifts are caused by resource enhancement, the driving mechanisms are different; i.e. local nitrogen enrichment (seagrass, Bryars *et al.* 2011; kelp, Gorman *et al.* 2009) versus global CO_2 enrichment (kelp, Connell *et al.* 2013; kelp and seagrass, Nagelkerken *et al.* 2015). It was striking that regardless of the driver of regime shift (eutrophication or ocean acidification) the magnitude of changes in sound was remarkably similar. It is possible that despite the diversity in drivers of regime shifts, they not only result in similar ecological outcomes of reduced species diversity and productivity, but also reduced soundscapes and the orientational cues they offer. Common to these shifts is a reduction in resources (Cardinale et al. 2006; Stachowicz et al. 2007), such as food and habitat to inhabiting species, which dispersing animals may be able to detect. As underwater coastal sounds are largely biological, the degradation of the habitat is reflected in an impoverished soundscape. This also suggests that soundscapes are a promising, rapid and cost effective monitoring tool for ecosystem condition (Piercy et al. 2014; Tucker et al. 2014).

Soundscapes were dominated by snapping shrimp crackle. Despite being more intense on rocky reefs than soft sediment, snapping shrimp crackle was not only ubiquitous, but it also dominated the soundscapes of all types of habitat and their shifts to lower diversity and complexity. This predominance is evidenced by the correlation (84%) between the frequency of snapping shrimp snaps and the total loudness of all seascape sounds. Kelp forests are not only more productive than turf dominated reefs (Copertino et al. 2005), but they also create habitat for cryptic animals like snapping shrimps with their convoluted holdfasts (Fowler-Walker et al. 2005). Similarly, soft sediment covered by seagrass has a much higher productivity compared to bare sand and creates habitat and shelter for a number of organisms (Hemminga and Duarte 2000). Although the quantification of the density of shrimps is difficult due to the highly cryptic nature of these animals, their snapping frequency and intensity (i.e within their main frequency band) are commonly used as an index of their abundance (Kennedy et al. 2010; Lillis et al. 2014; Nedelec et al. 2015; Piercy et al. 2014; Staaterman et al. 2014). In both kelp and seagrass ecosystems, a decrease in snapping reflected the regime-shifts that result in a simplification of habitat complexity.

The ecological implications of impoverished soundscapes may depend on the extent to which a species relies on sound for navigation. Many marine vertebrates (Huijbers et al. 2012; Montgomery et al. 2006; Parmentier et al. 2015; Simpson et al. 2005) and invertebrates (Lillis et al. 2015; Stanley et al. 2015; Vermeij et al. 2010) can use sound as directional cue towards settlement habitats or away from undesired habitats. Based on the reduction of soundscapes measured, we suggest that those organisms that rely more heavily on higher frequency sound (i.e. snapping shrimp sounds > 500 Hz) are likely to be more penalized by shifted soundscapes. However, this effect remains hard to quantify because of our incomplete knowledge about sound pressure hearing sensitivities in fish and invertebrate larvae and the complexity of sound propagation in shallow waters.

Models suggest that ocean acidification levels expected by the end of the century will significantly decrease sound absorption in the ocean (Ilyina et al. 2010), potentially compensating for the loss in biogenic sound caused by ocean acidification. However, by considering all important energy loss mechanisms the effect of pH on sound absorption is negligible in shallow waters and at the distances likely to be relevant for larval orientation (<10 km) because geometric spreading accounts for most of the sound loss from the source (Au and Hastings 2008; Reeder and Chiu 2010)~~.~~

We propose that if regime shifts produce distinct soundscapes that reflect their particular state, sound may represent a functional change of regime shifts. Shifted soundscapes may act as one of the mechanisms that control resistance or resilience of ecosystems, notably where sound mediates the supply of keystone species. For example, shifted soundscapes could hamper the recovery of ecosystems by directly attracting a lower number of engineer species' propagules such as coral and oyster larvae (Lillis et al. 2015; Vermeij et al. 2010). Analogously, chemical cues from coral or seaweeds reinforce the persistence of coral or seaweed dominated alternative states respectively by acting as attractor or repellent for dispersing coral and fish larvae (Dixson et al. 2014).

Another example is that of simplification of soundscapes could lead to reduced supply of herbivores that resist ecosystem shifts (e.g. Ghedini *et al,.* 2015), and that would act as negative feedbacks to stabilise the shifted state characterised by low herbivory (i.e. resistance to positive change: Connell & Ghedini 2015). Shifted soundscapes could also reduce the settlement of keystone predators, such as lobsters (Ling et al. 2009; Stanley et al. 2015), which are crucial to limit catastrophic overgrazing by sea urchins and maintain the system in its kelp-dominated state. Either way, we propose that if shifted soundscapes exist as a functional ecosystem trait, then the possibility that they function as positive or negative feedbacks to dispersing taxa that govern regime shifts also exists.

Dispersing propagules may be able to compensate for lowered soundscape value by using alternative cues such as olfaction and vision (Dixson et al. 2010; Ferrari et al. 2012). However, these cues have a limited reach relatively to sound which is one of the cues that propagate furthest and most predictably with distance from the source (Montgomery et al.

2006; Simpson et al. 2013). Furthermore, the value of these cues is not immune to habitat degradation and is likely to similarly decrease (Dixson et al. 2014). This will be further exacerbated in the future, where regardless of the type of cue used, ocean acidification will disrupt propagule orientation by causing ineffective processing of all these sensory cues (Dixson et al. 2010; Ferrari et al. 2012; Munday et al. 2009; Rossi et al. 2015).

In conclusion, the simplification of habitats within human-dominated landscapes has similarly profound effects on their soundscapes. The specific consequences remain elusive, but this concept is likely to attract considerable attention given its potential to mediate a diversity of key ecological processes and become a new cost effective monitoring tool. Importantly, our study reveals that regime shifts within human-dominated landscapes impoverish natural soundscapes, tending towards ‘sounds of silence’.

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Author contributions: All authors contributed to the design of the study, collection of the data, and writing of the article. T.R. analysed the data.

Conflict of Interest: The authors declare that they have no conflict of interest.

FIGURES

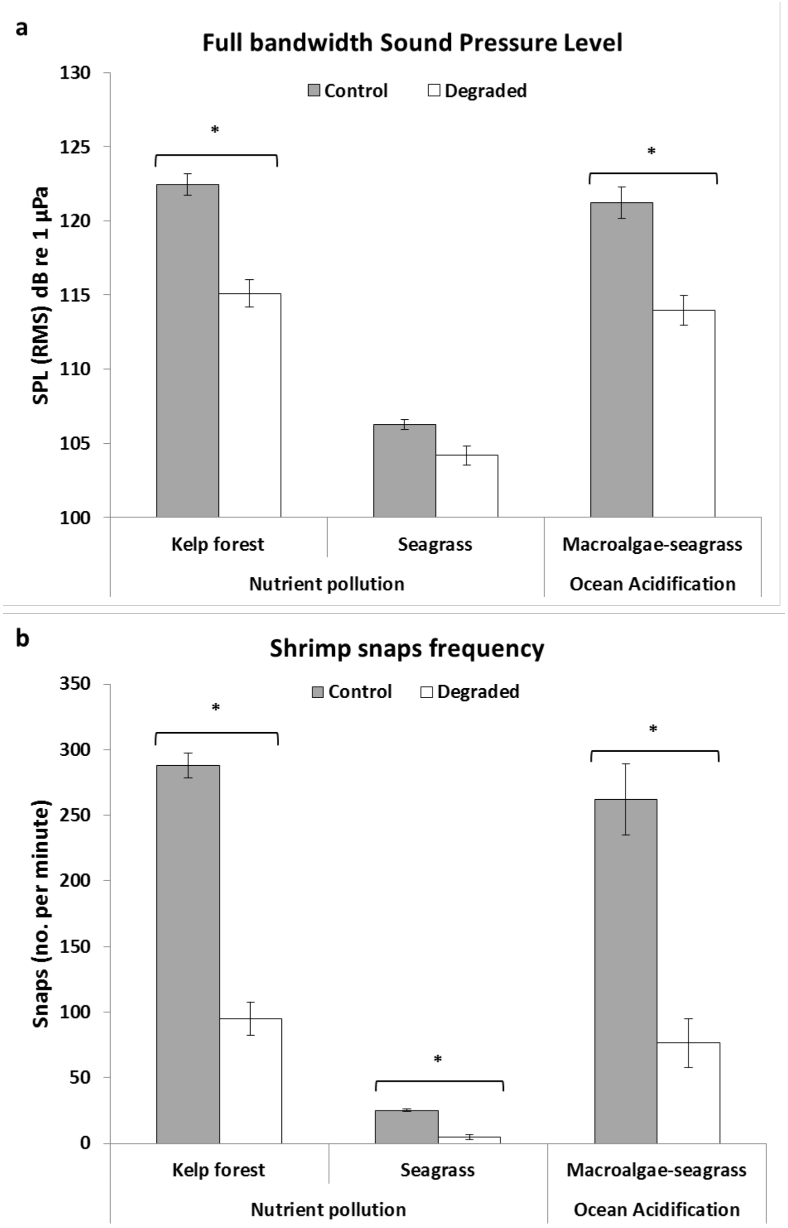

Figure 1. *Responses of soundscapes to regime shifts (A) full bandwidth root mean square sound pressure level (> 300 Hz, < 10 kHz) ± SE. (B) Mean frequency of snapping shrimp snaps counted per minute ± SE. Error bars represent variability due to spatial replication (N = 3 per treatment). Stars indicate significant differences between bar under the brackets.*

Figure 2

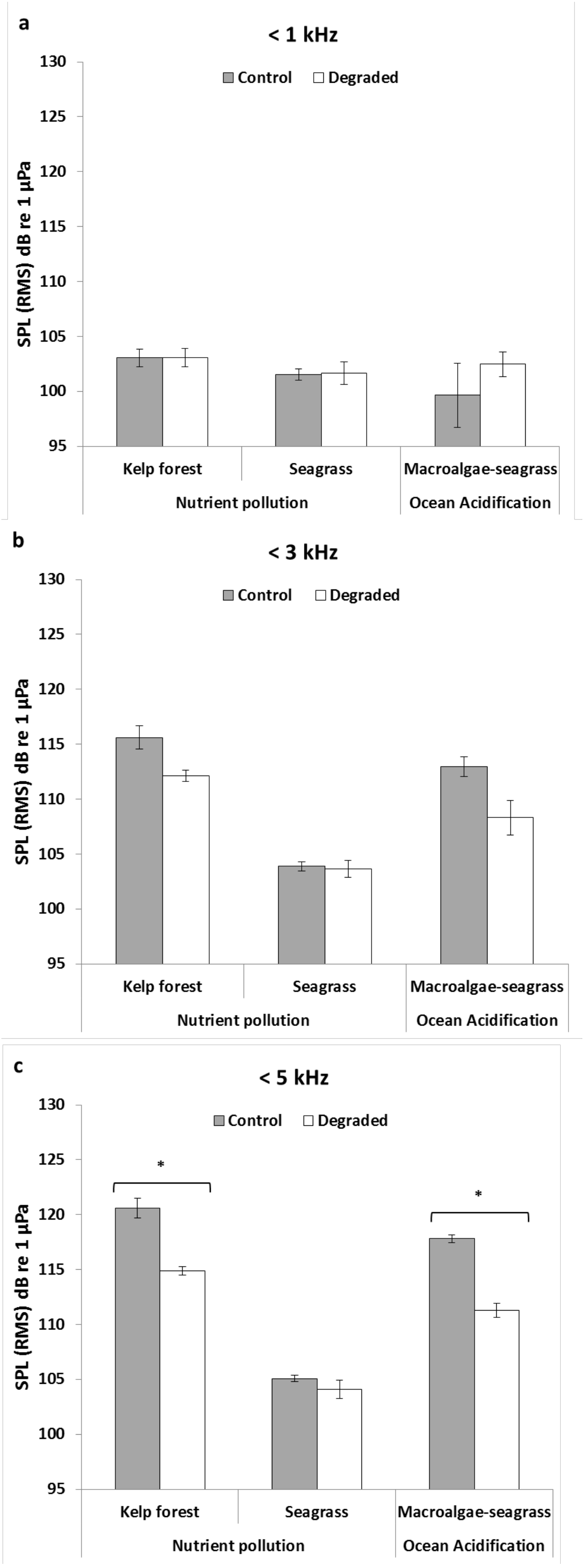
a
< 1 kHz
c
< 3 kHz
c
< 5 kHz
▨ Control
☐ Degraded
SPL (RMS) dB re 1 µPa
130
125
120
115
110
105
100
95
*
*
Kelp forest
Seagrass
Macroalgae-seagrass
Nutrient pollution
Ocean Acidification

Figure 2. *Effect of regime shifts and ocean acidification on sound levels in three frequency bands relevant to fish hearing: a) < 1 kHz, b) < 3 kHz, and c) <5 kHz. Error bars represent variability due to spatial replication (N = 3 per treatment). Asterisks indicate significant differences between bars under the brackets.*

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SUPPLEMENTARY MATERIALS & METHODS

CARBONATE CHEMISTRY MEASUREMENT AND ANALYSIS

The CO_2 concentration in the seawater was calculated using the measured values of temperature, salinity, pH_{NBS} and total alkalinity (TA) using the software CO2SYS (Pierrot 2006) with constants K1 and K2 from Mehrbach (1973) and refit by Dickson & Millero (1987) . Alkalinity was measured by Dynamic Endpoint Titration using an 888 Titrando (Metrohm) titrator in all experiments. During the course of the study values for standards were successfully maintained within 1% accuracy from certified reference materials from Dr. A. Dickson (Scripps Institution of Oceanography).

At the vent sites all seawater samples were collected between 14/9/13 and 18/9/14 at Vulcano and between 18/11/13 and 21/11/13 at White Island. The pH_{NBS} was measured daily at Vulcano and Ischia with a portable SG2-ELK SevenGo probe (Mettler Toledo) and at White Island with a Sonde 6600 multi-meter logger (YSI) calibrated daily. TA samples were collected at Vulcano and White Island on three different days, fixed with mercury chloride and preserved in Duran glass bottles (Schott) pending analysis at the University of Adelaide according to the Standard Operating Procedures (Dickson *et al.,* 2007). For Ischia previously published alkalinity values were used instead (Kerrison et al. 2011).

For all laboratory experiments seawater samples for alkalinity were analysed immediately upon collection. Salinity was measured with a SR6 refractometer (Vital Sine, Dartmouth, Canada).

SULPHUR AT NATURAL VENT SITES

At Vulcano, previous research showed that hydrogen sulphide released in the main bubbling area does not extend to the northern side of the bay and that sulphate levels are typical of oceanic waters making this stretch of coast suitable for realistic ocean acidification studies (Boatta et al. 2013). At Ischia previous studies did not detect sulphur (Hall-Spencer et al. 2008). At White Island, hydrogen sulphide was absent in the study area and sulphate levels were slightly higher than the oceanic average (mean ± SE: 1137 ppm ± 9.6 versus 904 ppm) but uniform throughout the study area (Brinkman and Smith 2015).

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Table S1. GPS position of recording sites

Driver	Habitats	Location	Condition	Latitude	Longitude
Nutrient pollution	Kelp vs turf	Port Noarlunga and Horseshoe Reef (South Australia)	Control	35° 8'15.00"S	138°27'47.00"E
				35° 8'55.01"S	138°27'48.82"E
				35° 9'2.00"S	138°27'51.00"E
			Degraded	35° 8'16.23"S	138°27'50.62"E
				35° 8'38.58"S	138°27'56.85"E
				35° 8'54.61"S	138°27'50.95"E
	Seagrass vs sand	Adelaide metropolitan coast (South Australia)	Control	34°53'5.69"S	138°25'15.23"E
				34°53'32.51"S	138°25'28.30"E
				34°53'55.30"S	138°25'23.73"E
			Degraded	34°49'33.53"S	138°25'44.31"E
				34°50'25.47"S	138°25'0.19"E
				34°51'12.10"S	138°25'41.52"E
Ocean Acidification	Kelp vs turf	White Island (New Zealand)	Control	37°30'55.84"S	177°11'43.27"E
			Elevated CO_2	37°31'0.60"S	177°11'36.54"E
	Macroalgae vs seagrass	Ischia (Italy)	Control	40°43'36.05"N	13°57'37.13"E
			Elevated CO_2	40°43'48.93"N	13°57'49.28"E
	Macroalgae vs sand	Vulcano (Italy)	Control	38°25'17.97"N	14°57'59.93"E
			Elevated CO_2	38°25'12.99"N	14°57'49.98"E

Table S2. Recordings metadata and replication

Regime shift driver	Bottom type	Habitat	Condition	Location	Spatial replication (N)	Temporal replication (N)	Total replication (N)	Season	Moon phase range	Sea State (Beaufort)	Sampling dates	Depth
Nutrient pollution	Rocky	Kelp	Control	Port Noarlunga and Horseshoe Reef (South Australia)	3	3	9	Summer/Autumn	Full, New, Waning Crescent	1-2	6/1/15 - 14/2/15 - 17/5/15	1.5-9 m
		Turf	Degraded		3	3	9	Summer/Autumn		1-2		
	Soft	Seagrass	Control	Adelaide metropolitan coast	3	2	6	Autumn	New, First Quarter	1-2	18/5/15 - 22/5/15	12-14 m
		Sand	Degraded		3	2	6	Autumn		1-2		
Ocean Acidification	Rocky	Kelp	Control	White Island	1	2	2	Summer	Full	up to 5	18-20/11/13	8-12 m
		Turf	Elevated CO_2		1	2	2	Summer				
	Rocky and soft	Macroalgae / seagrass	Control	Ischia	1	2	2	Summer	First quarter	1-2	27-28/9/14	1-4 m
		Macroalgae / seagrass	Elevated CO_2		1	1	1	Summer				
	Rocky and soft	Macroalgae /seagrass	Control	Vulcano	1	3	3	Summer	Full	1-2	14-18/9/13	1-4 m
		Macroalgae / sand	Elevated CO_2		1	3	3	Summer				

Table S3. Summary of the water chemistry parameters measured at natural CO_2 vents.

Location	**Area**	**T(°C)**	**pH NBS**	**N**	**TA (*m*mol/kgSW)**	***p*CO_2 (*µ*atm)**	**N**	**Salinity**	**N**
Vulcano	Control	25.5 (± 0.1)	8.06 (± 0.02)	8	2524.8 (± 2.3)	586.6 (± 39.1)	8	41	1
	Elevated	25.5 (± 0.2)	7.99 (± 0.03)	8	2546.1 (± 12.1)	720.0 (± 59.0)	8	41	1
White Island	Control	17.6 (± 0.1)	8.06 (± 0.02)	21	2295.7 (± 10.7)	538.8 (± 32.2)	4	35	1
	Elevated	17.9 (± 0.1)	7.86 (± 0.02)	33	2287.2 (± 12.1)	929.6 (± 54.1)	4	35	1
Ischia	Control	24.2 (±0.4)	8.21 (± 0.01)	4	2519 (± 4)*	381 (± 7.7)	4	40	1
	Elevated	24.3 (±0.4)	7.94 (± 0.08)	4	2518 (± 6)*	849.5 (± 146.6)	4	40	1

*Average (± SE) temperature (T), pH and total alkalinity measured in the field from 14-18 September 2013 at Vulcano (Italy) and from 18-21 November 2013 at White Island (New Zealand). pCO_2 values were calculated using CO2sys. *TA values taken from Kerrison et al. (2011)*

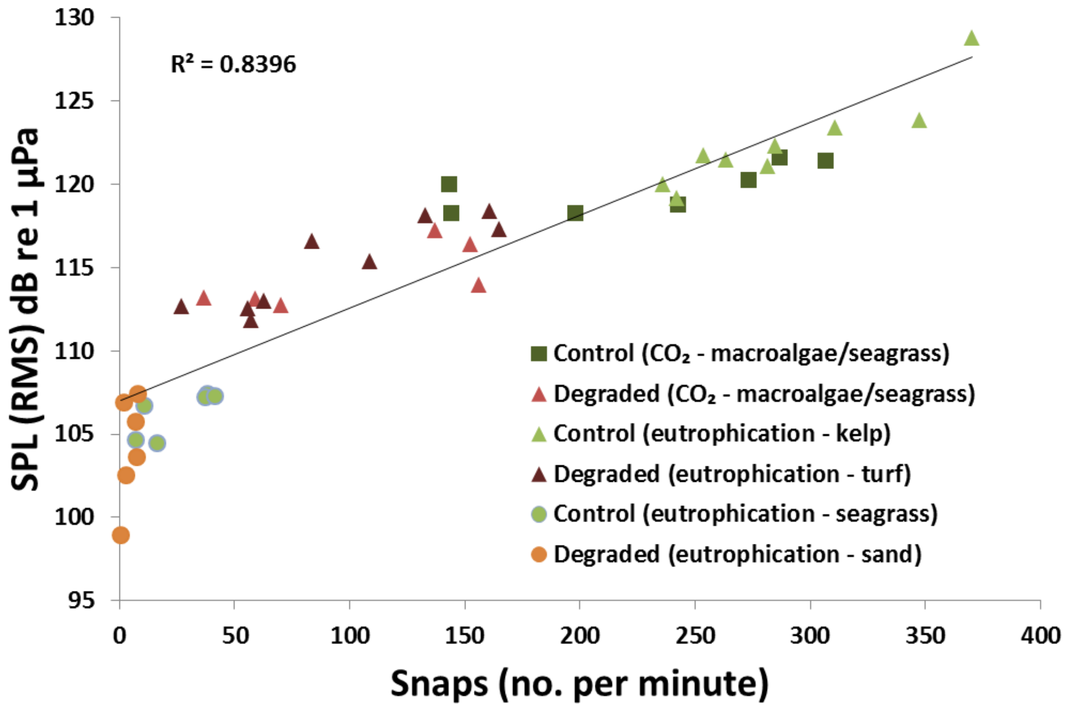

Figure S1. *Scatterplot showing the relationship between frequency of snaps and full bandwidth root mean squared sound pressure level. Data points represent single recordings replicated across both time and space*

CHAPTER V: LOST AT SEA: OCEAN ACIDIFICATION UNDERMINES LARVAL FISH ORIENTATION VIA ALTERED HEARING AND MARINE SOUNDSCAPE MODIFICATION

Statement of Authorship

Title of Paper	Lost at sea: ocean acidification undermines larval fish orientation via altered hearing and marine soundscape modification
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Principal Author

Name of Principal Author (Candidate)	Tullio Rossi		
Contribution to the Paper	Study design, data collection, data analysis, writing		
Overall percentage (%)	85		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

x. the candidate's stated contribution to the publication is accurate (as detailed above);

xi. permission is granted for the candidate in include the publication in the thesis; and

xii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Lost at sea: Ocean acidification undermines larval fish orientation via altered hearing and marine soundscape modification

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ABSTRACT

The dispersal of larvae and their settlement to suitable habitat is fundamental to the replenishment of marine populations and the communities in which they live. Sound plays an important role in this process because for larvae of various species it acts as an orientational cue towards suitable settlement habitat. Because marine sounds are largely of biological origin, they not only carry information about the location of potential habitat, but also information about the quality of habitat. While ocean acidification is known to affect a wide range of marine organisms and processes, its effect on marine soundscapes and its reception by navigating oceanic larvae remains unknown. Here we show that ocean acidification causes a switch in role of present-day soundscapes from attractor to repellent in the auditory preferences in a temperate larval fish. Using natural CO_2 vents as analogues of future ocean conditions, we further reveal that ocean acidification can impact marine soundscapes by profoundly diminishing their biological sound production. An altered soundscape poorer in biological cues indirectly penalizes oceanic larvae at settlement stage because both control and CO_2 treated fish larvae showed lack of any response to such future soundscapes. These indirect and direct effects of ocean acidification put at risk the complex processes of larval dispersal and settlement.

INTRODUCTION

Climate change has been forecasted to substantially change marine ecosystems within this century [1], but the processes through which this will occur are not always obvious. Larval dispersal and settlement are crucial and delicate processes that regulate marine community structuring and connectivity via population replenishment [2]. How this inherently complex process is affected ocean acidification remains largely unknown.

Oceanic propagules of various marine organisms rely on sound for orientation towards suitable adulthood habitat [3] and this sound is largely derived from biological sources, such as snapping shrimps and fish, which are vulnerable to ocean acidification themselves.

One of the most remarkable effects of ocean acidification on marine animals is its interference with a ubiquitous neurotransmitter, and this has been linked to a range of CO_2-driven behavioural alterations [4], which include auditory mediated behaviour [5]. The findings of these laboratory experiments have been recently confirmed in the field on natural CO_2 vents where fish communities, continuously exposed to elevated CO_2, failed to acclimate and showed striking behavioural abnormalities, such as attraction towards predator odour and increased boldness [6, 7]. Nevertheless, CO_2 vents are not a perfect analogue for the future ocean because they are influenced by larval supply from nearby unaffected populations. The question of how ocean acidification affects population replenishment therefore remains unassessed.

We studied how ocean acidification may affect settlement-stage oceanic larvae that use sound as a cue to find their adult habitat. We tested two potential pathways that could alter successful settlement: direct effects of ocean acidification on larvae via altered behavioural preferences toward useful auditory cues and indirect effects on larvae via altered quality of biological auditory cues (i.e. the underwater soundscape).

The effect of CO_2 on fish auditory preferences was tested in the laboratory by exposing settlement-stage larvae of a common temperate fish (mulloway, *Argyrosomus japonicus*) to coastal soundscapes as a potential orientation cue to settlement habitats. Mulloway is a highly valued fish in the Indo-West Pacific which typically spawns at sea and whose larvae settle in near shore coastal waters and estuaries on hard substratum and deep holes [8]. These type of settlement habitats are known to currently experience elevated $p\text{CO}_2$ levels [9] due to anthropogenic and natural processes such as eutrophication, runoff of acid sulphate soils and coastal upwelling. As these processes will locally exacerbate the effect of ocean acidification we expect these areas to reach CO_2 levels higher than what is expected for the global ocean average [10]. Future projections that take into account the combined effect of ocean acidification and eutrophication in coastal and estuarine hypoxic regions estimate that $p\text{CO}_2$ values of 1700-3200 μatm can be easily reached [11]. Mulloway is a soniferous fish with large otoliths [11]. To our knowledge the hearing range of this species is unknown and this is the first study that investigates auditory responses in this species at the larval stage.

MATERIALS & METHODS

EFFECT OF CO_2 ON LARVAL FISH AUDITORY RESPONSE.

Based on future projections for coastal and estuarine hypoxic regions [11] we conservatively exposed our fish larvae to a target $p\text{CO}_2$ of ~1368 μatm as elevated CO_2 treatments (Table 1). The response to settlement habitat sound was tested in an auditory choice chamber using an established method [5] and videorecorded for analysis (see Supplementary methods for an in-depth description). A total of 128 mulloway larvae at settlement stage (25-28 days old) that had been reared under control and elevated CO_2 conditions (Table 1) from the egg stage were used (half in each treatment). Subsequent to the behavioural tests, fish were euthanized using a clove oil overdose and frozen until dissection.

Additional details for the behavioural experiments and otolith preparation are provided in the Supplementary methods.

STUDY SITE AND SOUNDSCAPE

Natural CO_2 vents in temperate waters at White Island (New Zealand) were used to assess the effect of end-of-century business-as-usual levels of CO_2 [12] (Table 1) on settlement habitat soundscapes. The soundscape data presented in this paper represent a part of a broader study that characterized the effect of ocean acidification on marine soundscapes using three natural CO_2 vents (two in Italy and the one in New Zealand presented in this paper) and which verified, with a series of lab experiments, that sound producing behaviour of snapping shrimps is reduced by ocean acidification (Rossi T., Nagelkerken I., Connell S.D., unpublished data). Sound recordings from White Island were chosen here as cues for mulloway because they provided a more realistic settlement cue than soundscapes from the northern hemisphere. Additional methods for the soundscape analysis are described in the Supplementary methods.

STATISTICAL ANALYSIS

Attraction or deterrence of larval fish towards soundscapes was determined by testing the percentage of time spent in the half section of the choice chamber closest to the active speaker against the threshold for a random response of 50% in each half. Data were not normally distributed, as assessed by a Shapiro-Wilk's test ($p < 0.05$), and therefore a non-parametric One-Sample Wilcoxon Signed Rank Test was used, to test each of the four distributions separately. Differences in fish otolith size was tested using ANOVA.

RESULTS

EFFECT OF CO_2 ON LARVAL FISH AUDITION

Under present-day ambient seawater $p\mathrm{CO}_2$ conditions (~ 606 μatm), mulloway larvae showed significant attraction towards present-day acoustic habitat cues (Wilcoxon signed rank tests, Z = 2.565, p = 0.010), whereas CO_2-treated fish showed significant avoidance towards these cues (Wilcoxon signed rank tests, Z = -2.280, p = 0.023) (Fig. 1a).

In situ sound recordings on the elevated CO_2vent areas revealed a marked decline in sound intensity in the frequencies corresponding to snapping shrimp sound as observed by acoustic spectra (Fig. 2) (above ~ 500 Hz with a peak at ~ 4 KHz). A peak in sound pressure level centred around 1.2 kHz, likely originating from sea urchin rasping sound, did not show differences between treatment sites.

Furthermore, control as well as CO_2-treated mulloway larvae lost their responsiveness towards high-CO_2 affected soundscapes (respectively Z = 0.041, p = 0.968 and Z = -0.848, p = 0.396) (Fig. 1a).

LARVAL FISH OTOLITHS

Mulloway larvae possessed significantly enlarged otoliths (20.9 % increase in surface area standardized by body length; ANOVA, $F_{(1,42)} = 4.3$, p = 0.04) under future CO_2 conditions (Fig. 1b).

DISCUSSION

The results of our study suggest that ocean acidification could have negative direct and indirect effects on the process of larval orientation and settlement. Biological sounds are recognized as important orientation cues for various marine organisms due to their long propagation distance and richness of biological information that they carry [3]. Snapping shrimp crackle is certainly the most common biological feature of marine coastal soundscapes and often dominates over background abiotic noise and other biological sound sources. In this study, using field recordings, we find elevated CO_2 to reduce snapping shrimp sound intensity which occupies a substantial part of the sound frequency spectrum (> 500 Hz) including part of the hearing range of fish [3]. This pattern was also observed at two CO_2 vents in the northern hemisphere (Rossi et al. unpubl. data).

We also find that elevated CO_2 directly impacts larvae by reversing the innate attraction of settlement-stage fish larvae towards coastal soundscapes. Furthermore, fish larvae showed lack of attraction to future soundscapes whether or not they were treated with elevated CO_2. This suggests that fish are able to discern between soundscapes of different qualities and that present-day larvae lose selective attraction towards a high CO_2-degraded future soundscape even when unaffected by elevated CO_2 themselves. Our study therefore indicates that the decrease in sound quality and quantity of marine habitats in a high-CO_2 world can result in diminished value of an important biological cue used by marine species for orientation and navigation.

Ocean acidification increases the size of fish ear bones (otoliths) [13], which are used by fish for hearing, orientation and balance [3]. Using a modelling approach it has been hypothesized that enlarged otoliths under future CO_2 conditions might increase the hearing range of larval fish [14], but this effect has not been empirically validated. In our study, the mulloway that were raised under future CO_2 conditions throughout their larval development, showed an enlargement of their otoliths. Nevertheless, these mulloway larvae failed to respond to ecologically relevant habitat sounds from both present-day and future soundscapes. This failure in response suggests that even when ocean acidification leads to enlarged otoliths and potentially increases hearing sensitivity, fish cannot compensate for altered auditory preferences resulting from elevated CO_2.

If in a future ocean biological acoustic cues become of lower value for orientation, larvae will have to compensate by using other environmental cues such as chemical. But even other senses such as olfaction are impaired in many fishes by high CO_2 [4], as well as larval traits such as swimming speeds and development [15]. A prolonged oceanic life phase, due to delayed settlement, is likely to increase predation risk, delay occupancy of food-rich benthic habitats, and undermine population replenishment.

Ethics statement. Research was carried out under approval of the University of Adelaide animal ethics committee (permit: S-2013-005) and according to the University's animal ethics guidelines.

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Conflict of interests

The authors declare no conflict of interests.

Data Accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4t8c7 [16]

Table 1. Summary of the water chemistry parameters.

	Treatment	**T(°C)**	**pH $_{NBS}$**	**N**	**TA (*µ*mol/kgSW)**	***p*CO$_2$ (*µ*atm)**	**N**	**Salinity**	**N**
CO$_2$ vents	Control	17.6 (± 0.1)	8.06 (± 0.02)	21	2295.7 (± 10.7)	538.8 (± 32.2)	4	35	1
(White Island)	Elevated	17.9 (± 0.1)	7.86 (± 0.02)	33	2287.2 (± 12.1)	929.6 (± 54.1)	4	35	1
Mulloway	Control	22.2 (± 0.2)	8.03 (± 0.01)	25	2538.4 (± 8.5)	606.5 (± 17.6)	4	38.4 (± 0.2)	25
Experiment	Elevated	22.2 (± 0.1)	7.65 (± 0.02)	25	2520.6 (± 9.3)	1368.6 (± 145.6)	4	38.3 (± 0.2)	25

Average (± SE) temperature (T), pH (National Bureau of Standards) and total alkalinity. pCO$_2$ values were calculated using CO2sys.

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FIGURES

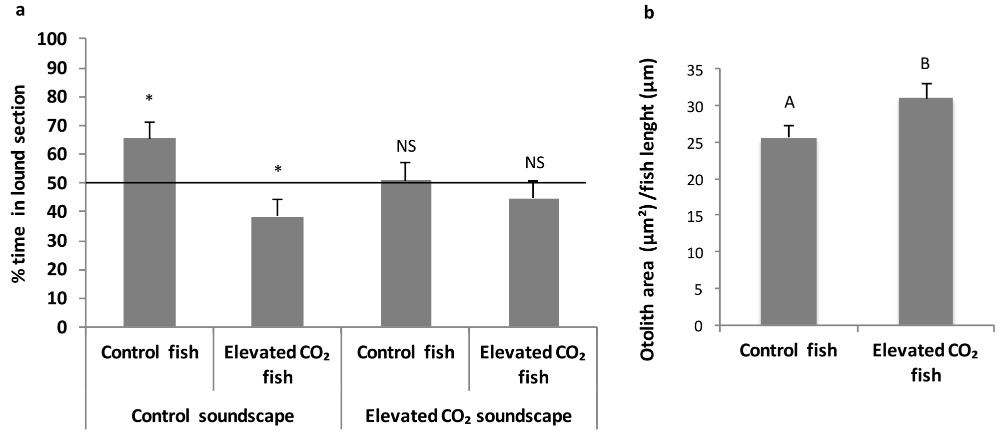

Figure 1. Effect of ocean acidification on settlement-stage larval fish auditory preferences and otolith (earbone) size. (a), Mean (± SE) percentage of time spent by mulloway larvae (N = 64 per treatment) in the half of the choice chamber closest to the broadcasting speaker playing elevated CO_2 or control temperate reef sounds. Stars indicate distributions significantly different ($p < 0.05$) to a random distribution of 50%. (b), Mean otolith surface area of mulloway (N = 22 per treatment) standardized to larval body length. Different letters indicate statistically significant ($p < 0.05$) differences.

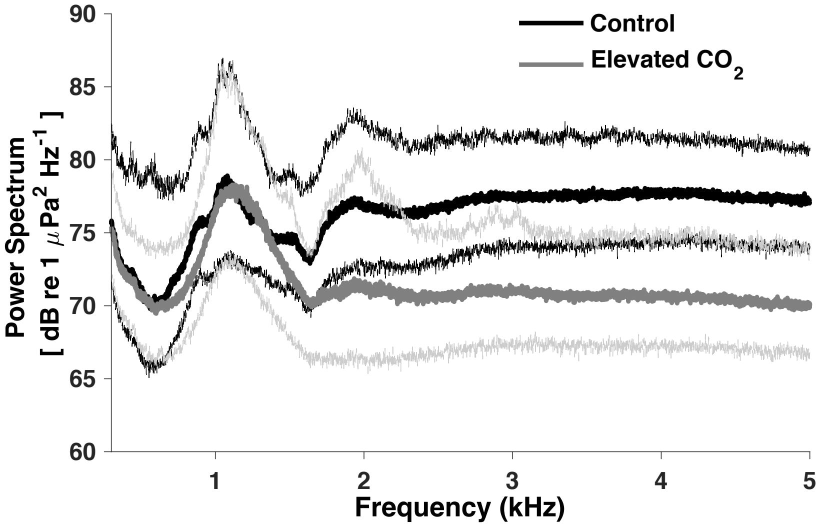

Figure 2. Acoustic spectra of a current vs. a future high-CO_2 *marine soundscape, based on median (thick line) ± 95 % CI (thin line) acoustic power spectra representing dusk chorus of snapping shrimps (peak frequency ~ 4 kHz) and sea urchins (peak frequency ~ 1.2 kHz) at White Island. Acoustic power was averaged among multiple days of sampling at multiple sites (N = 2 per treatment).*

SUPPLEMENTARY METHODS

FISH REARING

Fertilized eggs were obtained from the South Australian Research and Development Institute (1st generation broodstock) and reared at the University of Adelaide. The larval rearing systems were duplicated for each treatment and comprised a 60 l rearing tank recirculating in a closed system with a 20 l sump that contained a biological filter, a protein skimmer WG-308 (Boyu, Guangdong, China) and a UV sterilizer UView (Blue Planet, China). Fish were fed *ad libitum* with rotifers for the first 12 days post hatching, then with *Artemia* nauplii and a dry feed (Otohime, Japan) of increasing granule size as development progressed. Fish were reared under current and future CO_2 levels (Table 1). High CO_2 treatment conditions were achieved by bubbling CO_2-enriched air into the rearing tanks and in the sumps with a Pegas 4000 MF gas mixer (Columbus). pH_{NBS} was measured daily with a SG2-ELK SevenGo pH probe (Mettler Toledo) calibrated with a three point calibration. The CO_2 partial pressure in the seawater was calculated using measured values of temperature, salinity, pH_{NBS} and total alkalinity (TA) in the larval holding tanks, using the software CO2SYS [1] with constants K1 and K2 from Mehrbach (1973) and refit by Dickson & Millero (1987) [2, 3]. Alkalinity was measured by Dynamic Endpoint Titration using an 888 Titrando (Metrohm) titrator. During the course of the study alkalinity values were within 1% accuracy of certified standards (reference materials from Dr A. Dickson, Scripps Institution of Oceanography). Seawater samples for alkalinity were analysed immediately upon collection. Salinity was measured with a SR6 refractometer (Vital Sine).

FISH BEHAVIOUR QUANTIFICATION

Recordings from White Island control and elevated CO_2 sites at natural CO_2 vents (see below under 'CO_2 vent') were used as auditory cues. Although mulloway does not occur in New Zealand, where the audio recordings were obtained, it occurs in areas where shallow rocky reefs with similar kelp forests occur (e.g. South and South-East Australia). The playback of White Island sounds consisted of a 10-minute recording of the dusk chorus composed by mixing 30 seconds fragments collected over the four days of field recordings. Playbacks were dominated by typical broadband snapping shrimp sound that dominated the entire frequency spectrum above 500 Hz. Control and elevated CO_2 field recordings also included sound in the sea urchin frequency band (Figure 2).

The auditory choice experiments were performed inside a plastic tank (100 cm x 50 cm x 30 cm) lined with polystyrene foam where two underwater speakers UW 30 (Lubell) were placed oriented towards the centre. In-between the two underwater speakers a white acrylic auditory choice chamber (35 cm x 22 cm x 2 cm) divided in 8 parallel triangular lanes (35 cm x 3 cm x 2 cm) was used as a behavioural arena (Figure S1). The sound pressure level was almost equal between external and central lanes (Figure S2). The auditory chamber had mesh and the two ends facing the speakers while the top was open to the surface. The fish larvae could not escape or see each other because the ridges between each lane were higher than the water level. During the experiments the chamber was placed at a fixed distance of 8 cm from the active speaker. At the beginning of each trial one fish larva was placed in a removable enclosure in the centre of each lane until the end of a two minutes acclimation period with the sound. At the beginning of each trial, 8 fish (one per lane) were released simultaneously and their position was videorecorded using a HF R406 Legria camcorder (Canon, Tokyo, Japan) for 7 minutes. The position of the fish in the choice chamber was recorded every 5 seconds from the video recordings for 1 minute. The justification for a 1-min trial is that mulloway showed loss of interest after the first minute of trial. We were able to test this by partitioning the results in 1st, 2nd, 3rd, minute and so on. The response was found to be strongest in the first minute of the trial. It is reasonable for a fish to lose interest in a directional cue if response to that cue does not provide any benefits (i.e. being able to find relevant habitat in an experimental flume). The behavioural response was analysed by sectioning the arena in two halves and calculating the percentage of time that the fish spent in each section. As the sound pressure levels of the playback in the chamber (below 4 kHz) decreased rapidly from *in situ* levels to background levels (Figures S3-S4) we conservatively scored the percentage of time spent in the half of the chamber close to the speaker as attraction to the cue and time spent in the opposite half as avoidance, with 50% as threshold for random response. Unresponsive individuals (<1 %) were excluded from the analysis.

We do not assume that fish can extract the directional origin of the sound but that they can sense the gradient of sound pressure along the chamber by sampling sound pressure from multiple positions and consequently spend more time in the section of the chamber where they can hear a soundscape that they find “attractive”. In other words we assume that if the fish were deterred by our playback, and this playback is audible only in one section of the chamber, the fish would move to the other section. From a drifting larval fish perspective, the selection of habitat based on soundscape spatial heterogeneity is possible by simply sampling sound pressure at multiple time points as the fish moves closer to the source (see [4] for a conceptual model).

We acknowledge the difficulty of replicating a far field acoustic cue and measurement of the particle displacement component of sound in a small tank [5], however, in this study we do not attempt to determine absolute values of sensitivity but rather general auditory preferences in larval fish that had been exposed to CO_2 throughout their larval development. Particle acceleration in the experimental chamber was calculated based on sound pressure measurements obtained simultaneously with two hydrophones held at 5.5 cm distance and the Euler equation [6]. The results show that particle acceleration was maximal in proximity of the speaker and decreased gradually along the length of the chamber (Figure S3).

Fish were tested in water with the same temperature and salinity in which they had been reared. Half way through each series of trials the side of the active speaker was switched to the opposite side of the tank in order to control for potential tank effects.

OTOLITH MEASUREMENT

44 mulloway settlement-stage larvae (total across both CO_2 treatments) were used for otolith size analysis. The larvae were bleached through a 24 hr immersion in 5% solution of laundry pre-soak [7], measured for total length, and then dissected. Otoliths were photographed with an EOS 1100D camera (Canon, Tokyo, Japan) connected to a dissecting microscope, and the otolith surface area was measured from the photographs obtained using the analysis tool of Photoshop CS6 (Adobe, San Jose, USA). Because the size of fish differed slightly between treatments the otolith surface area was standardized to fish length by dividing the otolith surface area by total fish length. No data transformation was needed because our data approximated a linear relationship.

CO_2 VENTS

White Island is a volcanic island located in the Bay of Plenty, North Island of New Zealand (37°27'S, 177°17' E). CO_2 vents unaffected by temperature are located along the north-east rocky coast of the island [8]. All seawater samples were collected between 18/11/13 and 21/11/13 at White Island. The pH_{NBS} was measured daily with a Sonde 6600 multi-meter logger (YSI) calibrated daily. TA samples were fixed with mercury chloride and preserved in Duran glass bottles (Schott) pending analysis at the University of Adelaide according to standard operating procedures [9]. For all laboratory experiments seawater samples for alkalinity were analysed immediately upon collection. Salinity was measured with a SR6 refractometer (Vital Sine, Dartmouth, Canada). At White Island, hydrogen sulphide was absent in the study area and sulphate levels were slightly higher than the oceanic average (mean ± SE: 1137 ppm ± 9.6 versus 904 ppm) but uniform throughout the study area [10].

We selected an elevated CO_2 and a control area and made sound recordings at 4 stations. The hydrophone was deployed at 5 meters depth over a rocky seafloor whose sea surface ranged between 8 to 12 m depending on site and tide. Recordings were performed over four consecutive days under full moon (18-21 November 2013). The most common sources of marine biological sounds are known to peak at dusk during the so called "dusk chorus" and remain active until morning [11]. For this reason we focused the sampling effort at dusk and in the immediate hours after dusk. All recordings were made over rocky reef bottom characterized by kelp, turf-forming algae, and urchin barrens. During the recordings the swell height ranged between 0.7 and 1.5 m. The recordings were made with a Hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.1 dB re $1V/\mu Pa^{-1}$; frequency range 0.02–30 kHz; calibrated by manufacturers; High Tech Inc., Gulfport, USA) and a calibrated audio recorder (PCM-M10, 48 kHz sampling rate, Sony Inc., Tokyo, Japan). The recording apparatus was enclosed in a waterproofed barrel fixed to an anchored buoy. All the audio recordings were obtained at a sampling rate of 96 kHz and 32 bit. Power spectra and sound levels (RMS) were calculated with PAMguide in MATLAB® [12] and calibrated in SASLab Pro (Avisoft bioacoustics) using a reference signal at full (1 kHz) scale and the hydrophone sensitivity. Power spectra were calculated using the Hann window (length 1 second and 50% overlap).

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SUPPLEMENTARY FIGURES

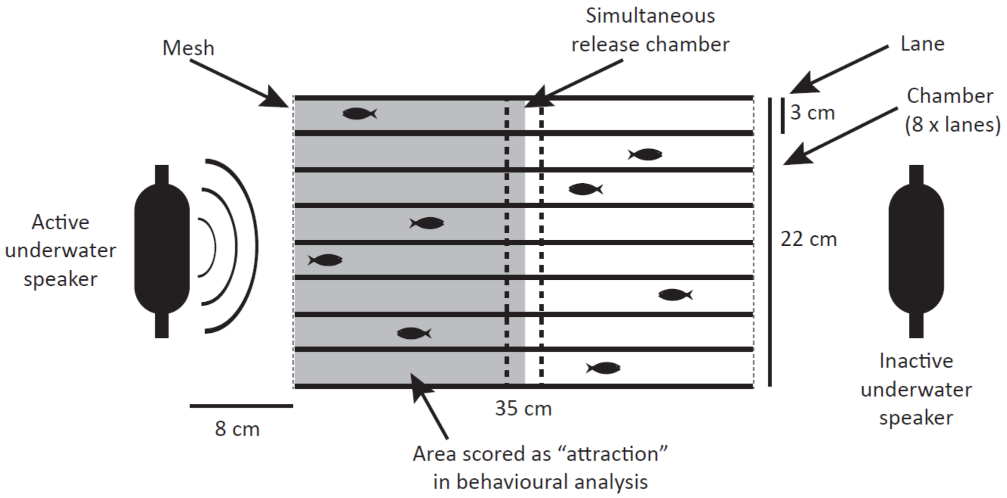

Figure S1, Schematic representation of the auditory chamber as seen from the top. *The sections close to the active speaker that represents attraction to sound by fishes is represented in grey.*

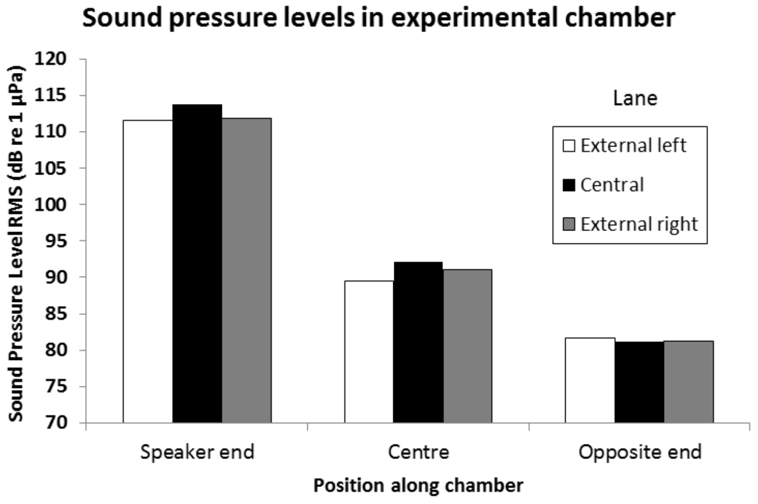

Figure S2, Sound pressure level at different positions of the experimental choice chamber. *Single measurements of sound pressure levels of white noise (same amplitude at every frequency) played back in the experimental chamber showing that the sound pressure level decreases exponentially along the length of the chamber but does not differ between different* *lanes (external left, central, external right). All measurements were processed with a band pass filter 0.5-4 KHz.*

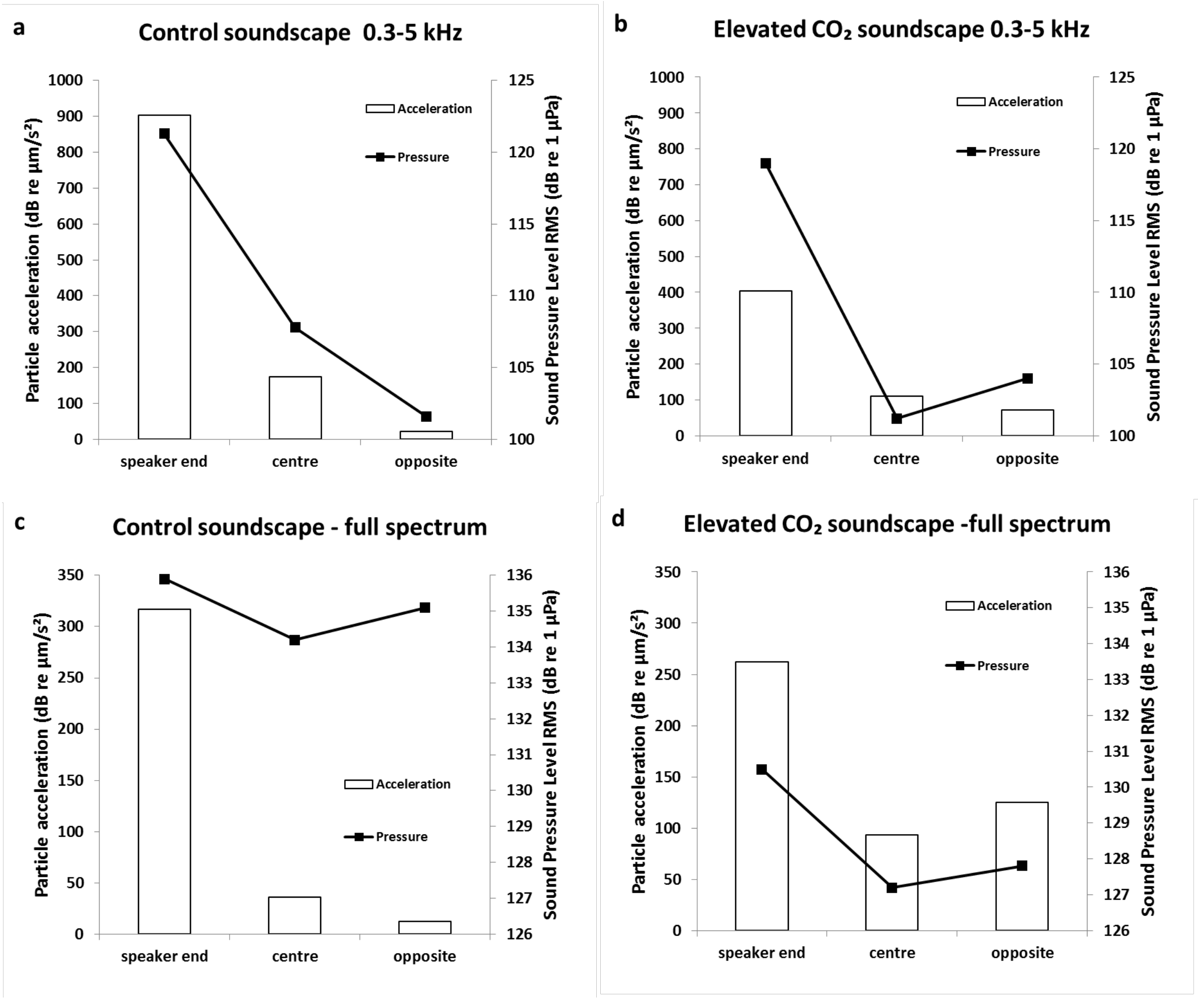

Figure S3, Particle acceleration and sound pressure gradient during playbacks in the experimental choice chamber. *(a-b) Band pass filtered (0.3-5 kHz) measurements, (c-d) Full spectrum measurements. (a-c) control soundscape playback. (b-d) elevated* CO_2 *soundscape. The band pass filter was used to show the sound pressure and particle acceleration patterns in the likely hearing range of fish filtering out higher frequencies (> 5 kHz) and low frequency vibrations in the building (< 0.3 kHz). "Speaker end" represents the part of the choice chamber closest to the speaker, "Centre" represents the middle section of the chamber and "Opposite end" refers to the part of the chamber opposite to the speaker. The sound* *pressure level in the part of the chamber closest to the speaker was set to match the in situ sound pressure level in the hearing range of fish.*

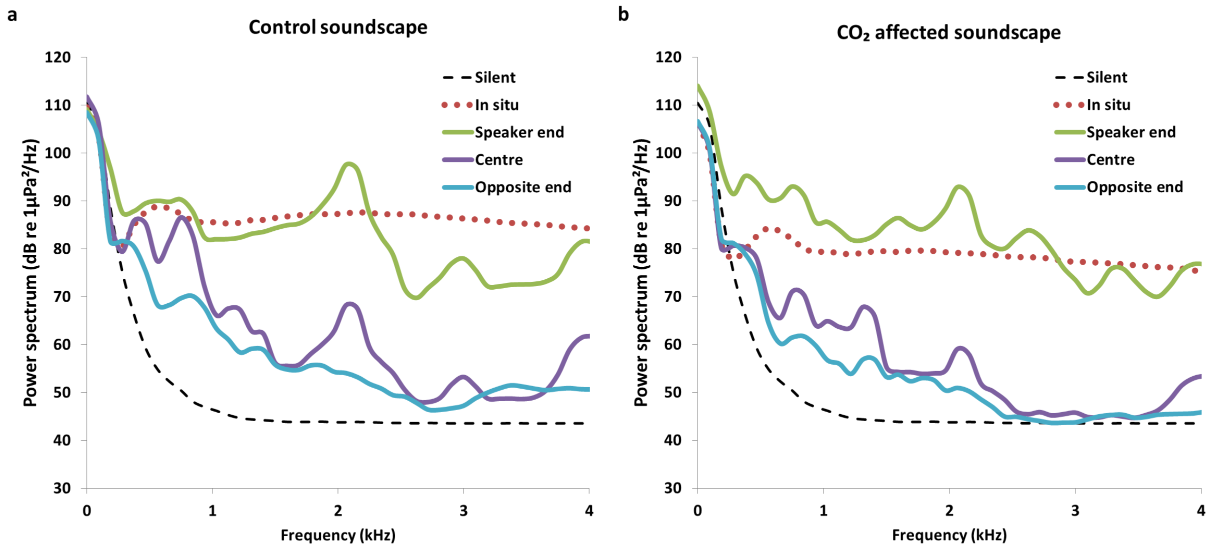

Figure S4, Sound gradients during playbacks in the experimental choice chamber. *(a) Sound gradient from recordings at the control site of White Island. (b) Sound gradient from recordings at elevated* CO_2 *site at White Island. "Speaker end" represents the part of the choice chamber closest to the speaker, "Centre" represents the middle section of the chamber and "Opposite end" refers to the section of the chamber opposite the speaker. "Silent" refers to the background sound level present in the chamber without playback and "In situ" refers to original field recordings. All measurements were obtained below the surface and along the centre of the chamber. Energy below 500 Hz was constant throughout the experiment and originated from noise in the building.*

CHAPTER VI: GENERAL DISCUSSION

GENERAL DISCUSSION

The overarching purpose of this thesis was to understand how ocean acidification might affect important ecological processes that undermine fish population replenishment by the end of the century if humanity continues on the current path of carbon emissions. We knew that ocean acidification can affect fish and in particular their behaviour in a variety of ways [1]. However, we still lacked decisive evidence suggesting that this could translate in tangible consequences for the sustainability of fish population and therefore economic costs for the human kind. This thesis gave a significant contribution to our knowledge in this direction by unravelling novel direct and indirect ways in which ocean acidification might affect the process of larval sound-driven orientation (Fig. 1). As larval orientation to settlement habitats undermines the replenishment and connectivity of marine populations this thesis provided important new information, not only for the scientific community, but also for the fishing industry, general public and policy makers. Furthermore, most of this research was conducted in relatively new and poorly explored scientific fields such as fish bioacoustics and soundscape ecology. Consequently, this thesis also significantly advanced our knowledge of some basic ideas in these disciplines.

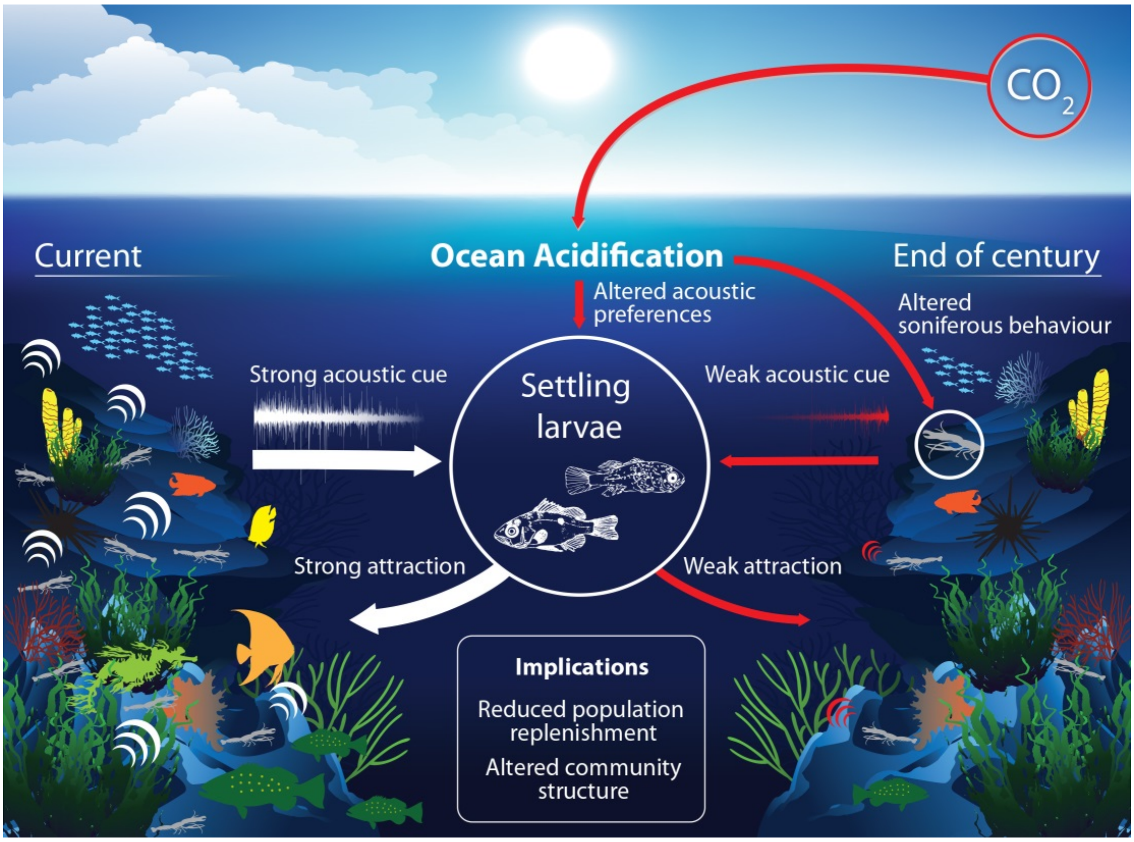

Fig. 1 Conceptual diagram showing the direct and indirect effects of ocean acidification on settlement-stage larvae in relation to marine sounds and hearing. Ocean acidification will directly impact settling fish larvae by altering their auditory preferences and indirectly by weakening the quantity and quality of the biological soundscape which they rely on as a long-distance cue for orientation.

Artwork credit: Tullio Rossi

THE EFFECT OF OCEAN ACIDIFICATION ON LARVAL FISH BEHAVIOUR

Ocean acidification is known to alter the behaviour of fish in radical and often surprising ways. Scientists have accumulated evidence that elevated levels of CO_2 like those expected by the end of the century or earlier can alter the normal function on the GABA neuro-receptor, which has a central importance in the brain by regulating a whole range of behaviours [1, 2]. Despite this has been known to scientists for years, only one study to date investigated the effects of ocean acidification on fish auditory preferences [3] and the soundscape orientation process in larval fish remains poorly known in general [4]. For example, some studies suggest that larval fish posses a short ontogenetic window of opportunity to settlement [5, 6], but this has never been empirically demonstrated.

This thesis presents three major novel findings in regard to fish bioacoustics and ocean acidification, which advance our knowledge in both the basic and applied science domains. First, I here showed that the larvae of two highly economically valued species respond to natural soundscapes during their settlement stage. This is important because most research on soundscape orientation in fish has been conducted on small coral reef fish with little economic importance. Second, I showed that barramundi larvae possess a short window of attraction to settlement habitat sounds. Some studies suggested that fish larvae possess a short window of responsiveness to settlement cues but no studies directly proved this empirically [5, 6]. Hence, this represents a major advancement in our understanding of the settlement mechanisms in fish. Third, ocean acidification causes the auditory preferences of barramundi and mulloway to settlement habitat soundscapes to switch from attraction to avoidance. This finding is analogous to what has been observed with olfactory settlement cues [7] but novel in terms of sound driven orientation where the only existing study on the topic found clownfish juveniles to lose response to auditory cues when exposed to elevated CO_2 [3]. The strong similarity between the behavioural responses to sensory cues of different kind as a result of elevated CO_2 exposure suggests that a common underlining mechanism is present.

All these findings were relatively unexpected in these species. Barramundi larvae settle in estuaries, which are normally characterized by elevated and variable CO_2 concentrations [9], therefore, some degree of tolerance to ocean acidification was expected. Conversely, barramundi showed to be sensitive to levels of ocean acidification that can be considered moderate for estuarine environments [10] in multiple behavioural traits, both before and after metamorphosis. Similarly, mulloway, whose larvae settle in near-shore coastal waters and estuaries on hard substratum and deep holes [11], was sensitive to ocean acidification levels that can be considered moderate for such environments. These findings together with another recent study [12], which found pink salmon larvae being sensitive to elevated CO_2 in terms of both growth and behaviour, suggest that coastal, catadromous and anadromous fish are more sensitive to elevated CO_2 than we initially thought and that brackish and fresh waters may not represent a refuge from elevated CO_2 [13].

THE EFFECT OF OCEAN ACIDIFICATION ON MARINE SOUNDSCAPES

In order to reach the most comprehensive picture on the effects of ocean acidification on fish sound-driven orientation I studied the effect of ocean acidification on the very same cue they rely on: soundscapes. This thesis provides two major elements of novelty in the field of soundscape ecology. First, I show that elevated CO_2 levels cause a significant decrease in the amount of biological noise recorded in coastal environments. Second, I show that habitat degradation, resulting from nutrient pollution, also causes a significant and similar decrease in biological sound. In both cases this pattern was largely explained by a reduction in snapping shrimp noise. This was probably due to a decreased abundance of these animals mediated by habitat change and a decreased soniferous behaviour in elevated CO_2 conditions. The relative importance between the two is however still unclear and would need further investigation.

These findings represent a major breakthrough in the young field of marine soundscape ecology where no previous studies to date investigated the effect of climate change on biological sound production. This thesis not only revealed that ocean acidification depresses biological sound production, but also provided a mechanistic explanation of the pattern. This opens up many exciting questions that future research could pursue as discussed in the following section of this chapter.

FUTURE RESEARCH

Auditory preferences can be studied in marine animals in the field and in the lab. Each approach has its advantages and limitations. The auditory preferences of barramundi and mulloway were studied in the lab due to the obvious difficulties of bringing fish that had been reared under elevated CO_2 conditions in the lab to the field for testing. However, this approach has some limitations in terms of realism as discussed in Chapter 2 and 5. Future research should validate the auditory preferences of barramundi and mulloway in field experiments (as in [14]) where the sound field can be realistically replicated and does not suffer from the limitations of tank experiments.

In this thesis the value of normal and degraded soundscapes for fish larvae was tested in the lab (Chapter 5). However, as stated above, tank based bioacoustics experiments have limitation of realism. Future research should validate the importance of soundscape quality for dispersing larvae of both fish and invertebrates in the field. This could be done in various ways. Light traps could be deployed with underwater speakers broadcasting soundscapes of different qualities and the difference in catch could be used to quantify the difference in value between the soundscapes. Analogously, this could be done with patches of habitat (as in [15]) or SMURFs [16]. The use of light traps with underwater speakers was attempted in South Australia in winter and summer as part of my PhD but provided no useful data due to the extremely low number of fish larvae caught.

Future research should also be directed to the study of the effect of ocean acidification on tropical marine soundscapes, which were not investigated in this thesis. Multiple tropical CO_2 vents are known today and regularly used for ecological experiments [17, 18]. However, the variation of biological noise along the CO_2 gradients that these sites offer has never been investigated. This research would be particularly promising because it is already known that the number of crustaceans decreases in elevated CO_2 areas at the natural vents in Papua New Guinea [17].

In recent years growing evidence suggests that soundscapes could represent a valuable cost-effective monitoring tool for habitat quality [19, 20]. It is hard to imagine soundscape monitoring to completely replace traditional techniques but this new tool offers new opportunities that should be explored. The findings of this thesis provide a strong argument in favour of the idea that soundscapes, and in particular snapping shrimp noise, are sensitive to habitat quality changes and could be employed on a vast scale and for long periods of time as monitoring tools in a cost effective manner.

Another exciting prospective that future ecological research should investigate is the idea proposed in this thesis that soundscapes, by being an important settlement cue for many marine organisms, might mediate the persistence of a certain ecosystem state. In chapter 4 we provide evidence that ecosystems that underwent regime shifts produce significantly different soundscapes and introduce the concept of shifted soundscapes. Future research could test the idea that shifted soundscapes act as one of those mechanisms that control resistance or resilience of ecosystems. This is particularly promising in the cases where sound mediates the supply of keystone species. We already know that degraded coral reefs dominated by algae are poor attractors of coral larvae because of the odors that algae produce [21]. This facilitates the persistence of the algal dominated state and hampers the recovery of the coral cover. It may well be that soundscapes exert the same function. For example soundscapes produced by degraded ecosystems would attract fewer keystone species, hence favoring the persistence of the degraded conditions. Alternatively, a healthy ecosystem would produce a strong and attractive soundscape, which would facilitate the input of new recruits hence facilitating the persistence of the ecosystem in its healthy state.

Finally, in the field of climate change, the study of acclimation and adaptation represent a high priority area for future research. Given the fast pace of human induced climate change, it initially seemed unlikely that evolutionary responses would be of any use for species. However, recent developments in the field of epigenetics showed that rapid acclimation to changing climatic conditions are possible [22]. For example, thanks to trans-generational acclimation mechanisms, the effects of increased temperature and CO_2 levels on metabolism and growth seem to have scope for acclimation, at least in some species [23]. However, when it comes the behavioural effects of ocean acidification on fish there seems to be little scope for acclimation [24]. Although, as only one study to date investigated the possibility of trans-generational acclimation to behavioural changes in fish, this represents an area to be prioritized.

OUTREACH

This work does not have any direct commercial application but serves a very important function, which is informing the industry, the general public and the policy makers of the risks of unabated carbon emissions. I believe that a scientist's job does not end when the results get published in peer-reviewed journals, but that reaching out to the general public is just as important. I therefore invested considerable time in the communication of my findings in the form of outreach videos, which have now been seen by thousands of people worldwide and won multiple awards. Furthermore the publication of all chapters was accompanied by a media release which has been picked up by numerous media outlets both nationally and internationally.

CONCLUSIONS

This thesis provides evidence that, if our CO_2 emission rate remains unabated, the process of larval settlement could be seriously affected in multiple ways by the end of the century. The main finding of this thesis is that ocean acidification will negatively affect the process of larval settlement by affecting the ability of larvae to find settlement habitats using sound via direct and indirect pathways. The inability to rapidly locate and reach suitable settlement habitat could result in dire consequences for fish larvae such as elevated risk of predation, starvation and settlement in unsuitable habitat. The likely result will be a decrease in population replenishment and connectivity, which will likely have ecological as well as economic and societal impacts. Given the high risks that these results reveal it is imperative that significant action towards the reduction of CO_2 emissions is taken at a global level in order to ensure the preservation of our natural resources for the generations to come.

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