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

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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.

1. Introduction

Climate change has been forecast 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 by 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

Table 1. Summary of the water chemistry parameters. Average ($\pm$ s.e.) temperature (T), pH (National Bureau of Standards), total alkalinity (TA); SW, seawater. $p\mathrm{CO_2}$ values were calculated using $\mathrm{CO_2}$sys [12].

	treatment	T (°C)	pH_{NBS}	N (T, pH)	TA (μmol kgSW^{-1})	$p\mathrm{CO_2}$ (μatm)	N (TA, $p\mathrm{CO_2}$)	salinity	N (sal.)
$\mathrm{CO_2}$ vents	control	17.6 ($\pm$ 0.1)	8.06 ($\pm$ 0.02)	21	2295.7 ($\pm$ 10.7)	538.8 ($\pm$ 32.2)	4	35	1
(White Island)	elevated	17.9 ($\pm$ 0.1)	7.86 ($\pm$ 0.02)	33	2287.2 ($\pm$ 12.1)	929.6 ($\pm$ 54.1)	4	35	1
mulloway	control	22.2 ($\pm$ 0.2)	8.03 ($\pm$ 0.01)	25	2538.4 ($\pm$ 8.5)	606.5 ($\pm$ 17.6)	4	38.4 ($\pm$ 0.2)	25
experiment	elevated	22.2 ($\pm$ 0.1)	7.65 ($\pm$ 0.02)	25	2520.6 ($\pm$ 9.3)	1368.6 ($\pm$ 145.6)	4	38.3 ($\pm$ 0.2)	25

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 towards useful auditory cues, and indirect effects on larvae via altered quality of biological auditory cues (i.e. the underwater soundscape).

The effect of high $\mathrm{CO_2}$ 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 in deep holes [8]. These types of settlement habitat are known to currently experience elevated $p\mathrm{CO_2}$ levels [9] due to anthropogenic and natural processes such as eutrophication and coastal upwelling. As these processes will locally exacerbate the effect of ocean acidification, we expect these areas to reach $\mathrm{CO_2}$ 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 $p\mathrm{CO_2}$ 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.

2. Material and methods

(a) Effect of $\mathrm{CO_2}$ 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 $p\mathrm{CO_2}$ of approximately 1368 μatm as elevated-$\mathrm{CO_2}$ treatment (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 electronic supplementary material 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-$\mathrm{CO_2}$ 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 electronic supplementary material.

(b) Study site and soundscape

Natural $\mathrm{CO_2}$ vents in temperate waters at White Island (New Zealand) were used to assess the effect of end-of-century business-as-usual levels of $\mathrm{CO_2}$ [13] (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 $\mathrm{CO_2}$ vents (two in Italy and the one in New Zealand presented in this paper) and which verified, with a series of laboratory experiments, that sound-producing behaviour of snapping shrimps is reduced by ocean acidification (T Rossi, I Nagelkerken, SD Connell 2013–2014, 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 electronic supplementary material.

(c) 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 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 were tested using ANOVA.

3. Results

(a) Effect of $\mathrm{CO_2}$ on larval fish audition

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

In situ sound recordings in the elevated-$\mathrm{CO_2}$ vent areas revealed a marked decline in sound intensity in the frequencies corresponding to snapping shrimp sound as observed by acoustic spectra (figure 2) (above approx. 500 Hz with a peak at approx. 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 $\mathrm{CO_2}$-treated mulloway larvae lost their responsiveness towards high-$\mathrm{CO_2}$-affected soundscapes
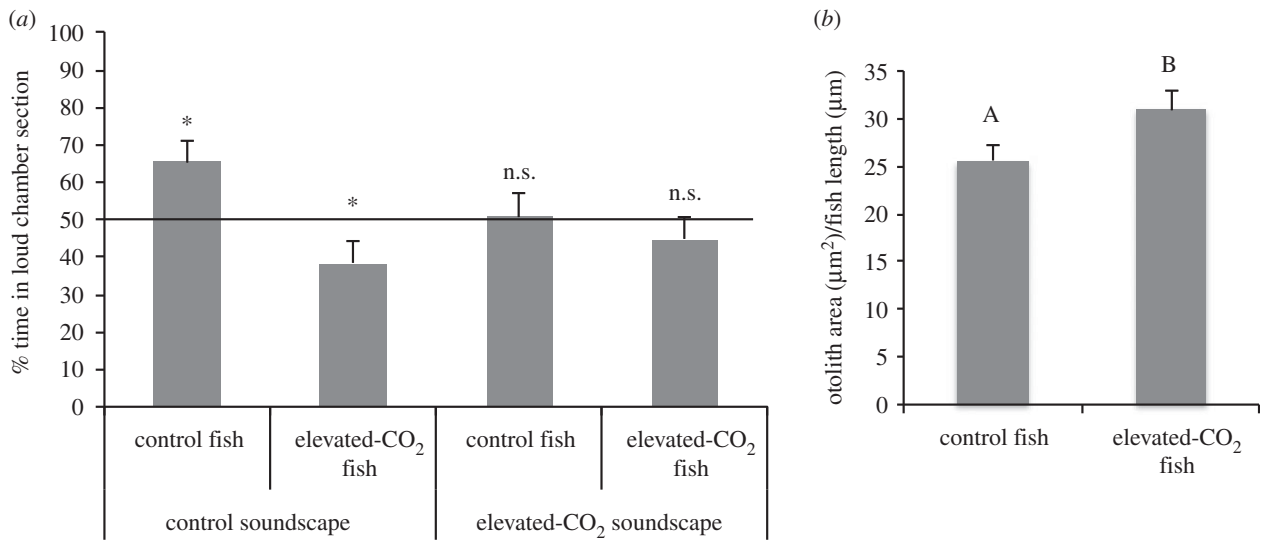
Figure 1. Effect of ocean acidification on settlement-stage larval fish auditory preferences and otolith (earbone) size. (a) Mean ($\pm$ s.e.) percentage of time spent by mulloway larvae ($N = 64$ per treatment) in the half of the choice chamber closest to the broadcasting speaker playing elevated-CO_2 or control temperate reef sounds. Stars indicate distributions significantly different ($p < 0.05$) from 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. n.s., not significant.
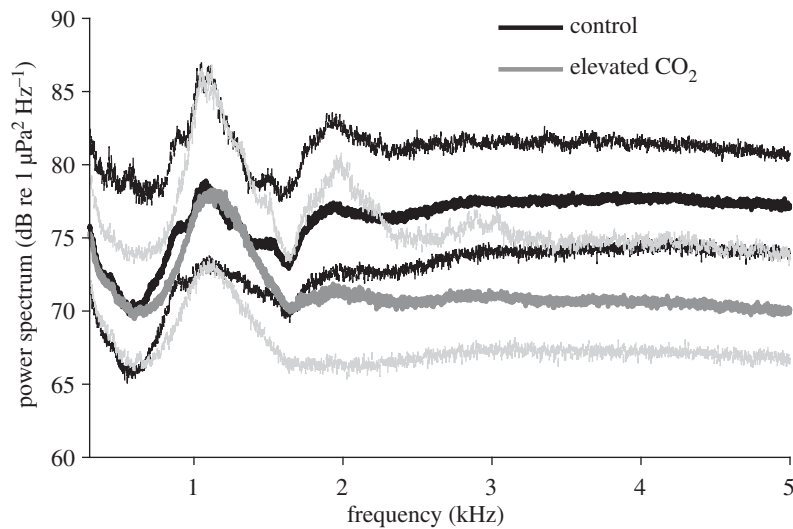

Figure 2. Acoustic spectra of a current versus a future high-CO_2 marine soundscape, based on median (thick lines) $\pm$ 95% CI (thin lines) acoustic power spectra representing dusk chorus of snapping shrimps (peak frequency approx. 4 kHz) and sea urchins (peak frequency approx. 1.2 kHz) at White Island.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

(respectively, $Z = 0.041$, $p = 0.968$ and $Z = -0.848$, $p = 0.396$) (figure 1a).

(b) 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 (figure 1b).

4. 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 owing to their long propagation distance and the richness of biological

information they carry [3]. Snapping shrimp crackle is certainly the most common biological feature of marine coastal sounds-capes and often dominates over background abiotic noise and other biological sound sources. In this study, using field record-ings, we found elevated CO_2 to reduce snapping shrimp sound intensity, which occupies a substantial part of the sound fre-quency spectrum (starting at $\sim$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 (T Rossi, I Nagelkerken, SD Connell 2013–2014, unpublished 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 directly by elevated CO_2. 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 earbones (otoliths) [14], 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 [15], 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 biological acoustic cues become of lower value for orientation in a future ocean, 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 [16]. 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.

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