RESEARCH ARTICLE
Painted Goby Larvae under High-CO₂ Fail to Recognize Reef Sounds

Joana M. Castro¹, M. Clara P. Amorim¹, Ana P. Oliveira², Emanuel J. Gonçalves¹, Philip L. Munday³, Stephen D. Simpson⁴, Ana M. Faria¹*

¹ MARE–Marine and Environmental Sciences Centre, ISPA-Instituto Universitário, Lisbon, Portugal, ² IPMA-Instituto Português do Mar e da Atmosfera, Algés, Portugal, ³ ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland, Australia, ⁴ Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, United Kingdom

* afaria@ispa.pt

Abstract
Atmospheric CO₂ levels have been increasing at an unprecedented rate due to anthropogenic activity. Consequently, ocean pCO₂ is increasing and pH decreasing, affecting marine life, including fish. For many coastal marine fishes, selection of the adult habitat occurs at the end of the pelagic larval phase. Fish larvae use a range of sensory cues, including sound, for locating settlement habitat. This study tested the effect of elevated CO₂ on the ability of settlement-stage temperate fish to use auditory cues from adult coastal reef habitats. Wild late larval stages of painted goby (Pomatoschistus pictus) were exposed to control pCO₂ (532 μatm, pH 8.06) and high pCO₂ (1503 μatm, pH 7.66) conditions, likely to occur in nearshore regions subjected to upwelling events by the end of the century, and tested in an auditory choice chamber for their preference or avoidance to nighttime reef recordings. Fish reared in control pCO₂ conditions discriminated reef soundscapes and were attracted by reef recordings. This behaviour changed in fish reared in the high CO₂ conditions, with settlement-stage larvae strongly avoiding reef recordings. This study provides evidence that ocean acidification might affect the auditory responses of larval stages of temperate reef fish species, with potentially significant impacts on their survival.

Introduction
Ocean acidification, caused by the uptake of anthropogenic CO₂ from the atmosphere, is increasingly recognized as a serious threat to marine ecosystems [1,2]. Exposure to high CO₂ levels can affect physiological processes (e.g. [3,4]), calcification (e.g. [5,6]), development (e.g. [7,8]), and survival (e.g. [9,10]) of marine organisms, especially during their larval and juvenile stages. Furthermore, there is increasing evidence that larval behaviour can be disrupted by elevated CO₂ levels (e.g. [11,12,13]), which may ultimately affect species interactions and ecological processes [14].

Many benthic marine organisms spend an early developmental period in the pelagic environment before settling to benthic habitat at the end of this phase [15]. There are a number of sensory cues that are used for navigation and long distance orientation in the marine
environment (review by [16]). Auditory cues are valuable as sounds associated with habitat can travel over large spatial scales, and reflect the physical and biological characteristics and quality of the environment [17,18,19,20,21,22,23,24]. There is a growing list of studies that demonstrate that fish, crustacean and other invertebrate larvae orient and settle in response to habitat-related sounds [15,20,24,25,26,27,28,29,30,31,32,33,34,35,36,37].

Recent studies suggest that ocean acidification might interfere with the ability of fish larvae to detect or respond to ecologically important auditory cues that could be used for habitat selection at settlement. Exposure of fish larvae to high CO$_2$ induced changes in the directional response of individuals towards coastal soundscapes in a coral reef species [38], a catadromous species [39] and a temperate species [40]. Despite these similar results, there is also increasing evidence that the effects of elevated CO$_2$ on larval behaviour can vary greatly among species [41,42], highlighting the need for further research across a wide range of fish species with contrasting life histories and habitats. Furthermore, these few studies conducted to date [38,39,40] have tested laboratory-reared larvae, but studies on wild larvae are needed since behavioural capabilities may differ between captive reared and wild larvae [43].

In this study we tested the effect of simulated ocean acidification (elevated CO$_2$) on the auditory preferences of wild-caught, settlement-stage larvae of a common temperate reef fish species, the painted goby Pomatochistus pictus. The painted goby is a small benthic-coastal fish species that inhabits low-turbidity waters in rocky and sandy coastal areas of the Eastern Atlantic Ocean and the Mediterranean Sea [44]. In Portugal the reproductive season ranges from January to May. Males guard eggs in nests through 11–12 days embryonic development [44], after which larvae hatch at approximately 2.8 mm total length, develop in the pelagic environment [45], and then settle into coastal habitat at approximately 17–18 mm [46]. Settlement-stage larvae are usually found schooling close to the substrate (personal observations). Here, wild-caught larvae were exposed for a minimum of 10 days to local ambient conditions (532 μatm ± 58.16, pH 8.06 ± 0.04) and to an elevated pCO$_2$ condition (1503 μatm ± 71.42, pH 7.66 ± 0.02). The high pCO$_2$ level was chosen to be close to 1500 μatm, corresponding to a pH decrease of approximately 0.4 units, which is consistent with projections for the end of the century on the current CO$_2$ emissions trajectory [47]. Moreover, this species inhabits nearshore regions that already experience pCO$_2$ levels > 1000 μatm due to upwelling events [48,49], and pCO$_2$ values up to 1170 μatm have been recorded in the coastal waters where painted goby inhabits [50]. With the amplifying effects of anthropogenic ocean acidification, future pCO$_2$ could, therefore, easily exceed 1500 μatm. We used an auditory choice chamber to test for responses to night-time recordings of reef sound. If behaviour of settlement-stage larvae was affected by acoustic conditions, we predicted that control (537 μatm) fish would be attracted by these sounds, but that this attraction may be lost in fish reared in high CO$_2$ (1503 μatm) conditions.

**Materials and Methods**

**Seawater manipulations**

Artificial seawater used in the experiments was adjusted to a salinity of 34 psu by blending a commercial salt mixture (Tropic Marin®) with filtered freshwater (reverse osmosis system). CO$_2$ conditions were maintained by dosing CO$_2$ in 200 l sumps to achieve set pH levels. A pH-controller (Tunze Aquarientechnik, Germany) maintained pH at pH$_{NBS}$ 8.0 in the control treatment and pH$_{NBS}$ 7.6 in the high pCO$_2$ treatment. One sump per pCO$_2$ treatment was used, each delivering seawater into two replicate 35 l rearing tanks, at ~600 ml min$^{-1}$. Each sump was equipped with biological, mechanical, chemical and ultraviolet filtration. Rearing tanks were sealed with a clear glass lid to limit CO$_2$ exchange with the atmosphere.
Temperature, salinity and pH in each aquarium were measured twice daily. pH was measured on the National Bureau of Standards (NBS) scale with a portable meter (SevenGo DuoPro, SG23) calibrated weekly with fresh buffers (Mettler Toledo). Oxygen levels were maintained above 90% saturation by the mixing action of the diffusion pumps in the sumps. Samples for determining total alkalinity (TA) were collected from experimental tanks on a weekly basis, placed in air-tight containers without air space, stabilized by mercuric chloride poisoning [51] and kept at +4˚C until further analysis. Analyses performed using automated Gran titrations, with certified reference material supplied by A. Dickson (Scripps Institutions of Oceanography, San Diego). pCO₂ was calculated from the in situ temperature, TA and pH, using the carbonic acid dissociation constants given by [52] and the CO₂ solubility coefficient of [53]. Errors associated with pCO₂ calculations were estimated to be ±10 μatm (accumulate errors on TA and pH). Estimated seawater parameters are shown in Table 1.

Due to logistical reasons, control water (i.e. not treated with additional CO₂) was used during all acoustic trials; preliminary tests indicated that there was no difference in behaviour when larvae were tested in control or treatment water. Moreover, a recent study by [54] has shown that predator avoidance behaviour is not altered by experimental test water.

Larvae

_Pomatochistus pictus_ settlement-stage larvae (lacking full squamation) were collected by SCUBA divers at the Arrabida Marine Park (38˚ 28' N; 8˚ 59’ W), Portugal on 14th July and 12th August 2015. Larvae were immediately transported to the laboratory and transferred to four 35 l tanks (~50 fish per tank) with a continuous supply of recirculating seawater, matching field temperature (~ 16˚C), and left for one day to recover from transfer and handling. Subsequently, larvae were randomly assigned to two replicate 35 l tanks (~50 fish per tank) per treatment (control or high pCO₂) and reared under these conditions for 10, 15 and 25 days, to test if larvae became acclimated to acidified conditions. The position of the two replicate tanks in control and high CO₂ treatment was reversed between the capture dates to account for possible confounding effects such as lighting conditions and position in the room. On test days, fish were randomly chosen from each tank/treatment, and used only once. Larvae were reared under locally relevant temperature and salinity conditions, with a summer light cycle of 14h light: 10h dark simulated using fluorescent lights. Larvae were daily fed with _Artemia_ nauplii ad libitum, with exception of the test day to avoid potential influence of variable recent feeding on performance.

Fish auditory response

Auditory preferences of larva were tested in acoustic choice chambers [38] using playbacks of recorded reef sound, playbacks of recordings with no biological significance (offshore sound), and control conditions (no playback). Thus, three dual-choice acoustic experiments were performed (fish lengths were measured for inclusion in statistical analyses, see below):

1. reef sound vs. no playback (n = 30, standard length 10.36–21.29 mm for control pCO₂;
   n = 28, standard length 11.13–22.45 mm for high pCO₂);

| pCO₂ condition | pH_{NBS} | T(˚C)      | S (psu)   | TA μmol kg⁻¹ | pCO₂ μatm |
|----------------|---------|------------|-----------|--------------|-----------|
| Control        | 8.06±0.04 | 16.03±0.28 | 34.43±0.84 | 2248.70±11.22 | 531.97±58.16 |
| High CO₂       | 7.66±0.02 | 16.05±0.23 | 34.58±0.80 | 2247.08±4.89 | 1503.65±71.42 |

Table 1. Mean (± SD) seawater parameters in the experimental system.

doi:10.1371/journal.pone.0170838.t001
2. reef sound vs. offshore sound (n = 30, standard length 10.45–19.55 mm for control pCO₂; 
n = 28, standard length 10.16–18.68 mm for high pCO₂);

3. offshore sound vs. no playback (n = 29, standard length 10.84–21.58 mm for control 
pCO₂; 
n = 27, standard length 10.07–20.93 mm for high pCO₂).

Details of acoustic stimuli. Sound recordings were conducted at the Arrábida Marine 
Park (38° 28’ N; 8° 59’ W), Portugal. Reef sounds were recorded at the same location where 
P. pictus larvae were collected. Three reef recordings of 3–4 minutes each were made at dusk (14/
07/2015, 20.30 hrs.), in the very nearshore, at a depth of ~10 meters (14/07/2015, 20.30 hrs.); another three offshore recordings of 3–4 minutes each were recorded at 3 miles from the 
coast, at ~5 meters depth (14/07/2015, 12.00 hrs.). Recordings in both habitats were made 
under calm conditions and therefore containing few abiotic sounds. Reef recordings were 
made at dusk, as the biological chorus in most reefs studied so far were found to peak at this 
time [18,19,55]. The offshore sound was chosen as it is associated with a habitat of low interest 
for reef-fish larvae, in contrast to reef sounds that represent suitable habitats for settlement 
and are used as biologically relevant acoustic cues [25,30].

Sounds were recorded with an omnidirectional hydrophone (HiTech HTI-96-MIN with 
inbuilt preamplifier, High Tech Inc., Gulfport MS; sensitivity-164.3 dB re 1 V/μPa, frequency 
range 0.02–30 kHz) connected to a digital Sony PCM-M10 recorder (96 kHz 24-bit Recording, 
Sony Corporation, Tokyo, Japan). All recordings were made using the same settings so as to 
allow comparison between their relative amplitudes. Reef sound was on average 12 dB re 1 μPa 
(RMS) louder than offshore sound (Fig 1).

Test protocol. The response of larvae to sounds was tested in an auditory choice cham-
ber (adapted from [38]). The chamber consisted of a transparent acrylic tubular chamber 
(50 cm long), within which the larva was released, supported inside a glass aquarium (60 x 
110 x 25 cm). The tubular chamber had mesh at the two ends, to prevent larvae from escaping. Acoustic stimuli were played back using two underwater speakers (Electrovoice UW-
30, Lubell Labs Inc., Columbus, OH, USA), each suspended above the substrate at opposite 
ends of the aquarium, and kept at a fixed distance of 7 cm from the tubular chamber. To 
reduce the acoustic resonance and reflections in the auditory choice chamber, aquarium 
walls were lined on the inside with air-bubble packing film. The aquarium was further insu-
lated from general building noise using layers of ROCKWOOL™ and ROOFMATE™ placed 
between the test arena and the research bench. Additionally, each leg of the bench was 
placed inside a container with sand and ROCKWOOL™ to avoid direct contact of the table 
with the floor.

Sound stimuli consisted of three reef and three offshore recordings, each cut to 3 min of 
duration. A low-pass filter of 3 kHz was applied to the recordings so that the frequency of 
sound stimuli was below the resonance frequency of the experimental tank [56] while match-
ing the auditory ability of the species [57].

In each trial the tested sound recording was assigned alternately to the left and right sides of 
the experimental tank. The sounds files were randomly chosen per pCO₂ condition. Sound stim-
uli were played back using an audio chain that consisted of two underwater speakers con-
ected to an amplifier (Phoenix Gold QX 4040, Portland, OR, U.S.A.) and fed through D/A 
device (Edirol UA25, Roland, Osaka, Japan) controlled by Adobe audition 2.0 (Adobe Systems 
Inc., Mountain View, CA, USA). The average intensity (RMS, full spectra) of sound playback, 
measured just in front of the speaker, was adjusted to that recorded in the field by regulating 
the output of the speakers. Electrical noise was reduced by grounding the experimental tank 
water and all audio equipment.
The average sound playback intensity decreased by approximately 11 dB re 1 μPa (RMS) from the end of the choice chamber near to the speaker to the center of the chamber, and an additional 2–3 dB from the centre to the far end of the tubular chamber (Fig 2). This sound gradient allowed us to test for auditory preferences, as we assumed larvae would spend more time close to the speaker if they experienced a soundscape that they found “attractive”. Ambient conditions in the tank during ‘no playback’ (the silent treatment in some of the experiments) was ~21 dB lower than reef sound playback.

At the beginning of each trial, one larva was placed in a central release chamber in the middle of the auditory choice chamber. We chose to test larvae individually as opposed to groups because behaviour is likely not independent between individuals, and group testing wouldn’t allow us to disentangle the response to a specific sensory cue (sound) from other possible sensorial mechanisms, such as odour or mechanoreception. After 1 min of acclimation, experimental sounds were played for 1 min, after which the larva was released into the tubular chamber, and its position recorded every 5 sec for 2 min. The tubular chamber was divided in half to define the position of the fish at each time interval relative to the playback speaker(s). To avoid changes in water temperatures and olfactory gradients that could influence larval behaviour, the chamber water was renewed between each trial. The sides of the tank with each sound playback was switched regularly to control for unwanted global chamber effects. After each trial, fish were euthanised with an overdose of anaesthetic (MS222 tricaine methane sulphonate; Pharmaq, Norway), fixed in 96% ethanol and measured.

Fig 1. Relative amplitude of reef and offshore sounds (mean of 3 recordings per habitat). Power spectra [fast Fourier transform (FFT), 8192 points, Hamming window].

doi:10.1371/journal.pone.0170838.g001
The percentage of time spent by each larvae in the half of the chamber near the test speaker was used as the dependent variable. Percentage data was logit-transformed [log natural(p/[1-p])] for analysis [58]. The ‘test’ speaker was considered to be the one broadcasting reef recordings in reef vs. no playback and reef vs. offshore experiments; and offshore recordings in offshore vs. no playback experiments. Generalized linear mixed models (GLMMs) were used to explore the relationship between the dependent variable and $pCO_2$ condition, time in treatment and standard length (SL). $pCO_2$ condition, time and SL were entered as fixed effects, and the tank and capture date entered as random effects to account for multiple fish sampled from the same tank and date. The model that best represented the data set, based on Akaike Information Criterion (AIC), was: \[ Var \sim pCO_2 \text{ condition} + \text{Time} + \text{SL} + (1|\text{Tank}) + (1|\text{Date}) \].

Preference or avoidance for specific auditory cues were tested with one-sample t-tests comparing the percentage of time spent near the test speaker with $H_0 = 0.5$, i.e. against a random response of 50%.

All statistical analyzes were done using the R software (version 3.2.2, RFoundation for Statistical Computing, Vienna, Austria) and the lme4 library was used to perform the GLMM analyses.

**Ethics statement**

This study was authorized by the Portuguese National Authority for Animal Health (Direccção-Geral de Alimentação e Veterinária), it was performed in strict accordance with the recommendations of the Animal Care and Use Committee of Ispa-Instituto Universitário, and
undertaken under the supervision of an accredited expert in laboratory animal science (following FELASA category C recommendations). Permission for capturing fish at the field site was granted by the National Institute for the Conservation of Nature and Forests (ICNF).

Fish were caught with hand nets, placed into stock tanks, provided with substrate and fresh surface sea water and immediately transported to the laboratory. At the end of the experiment fish were euthanized with an excessive dose of anaesthetics (MS222 tricaine methane sulphonate; Pharmaq, Norway).

**Results**

Time-in-treatment and size did not affect the response of larvae to sound in any of the three dual-choice sound experiments (Table 2). However, a significant effect of pCO$_2$ condition was detected, with the response of larvae reared in high pCO$_2$ levels being significantly different to the control reared larvae when experiencing reef recording vs. no playback (Table 2; Fig 3) and reef recording vs. offshore recordings (Table 2; Fig 4); by contrast, no significant pCO$_2$ condition effect was detected in the experiment of offshore recordings vs. no playback (Table 2; Fig 5).

Under present-day ambient pCO$_2$ conditions (control), painted goby larvae showed significant attraction towards reef sound when tested against offshore sound (one-sample t-test, $H_0 = 0.5$, $t_{29} = 6.08$, $p < 0.001$) and no playback (one-sample t-test, $H_0 = 0.5$, $t_{29} = 3.65$, $p = 0.001$), spending, on average 80.69% and 66.67% of the time in the half of the chamber close to the test speaker, respectively (Figs 3 and 4). By contrast, no directional auditory response was detected when larvae were presented with offshore recordings vs. no playback (one-sample t-test, $H_0 = 0.5$, $t_{28} = 1.28$, $p = 0.21$), spending, on average 57.61% of the time close to the half of the chamber broadcasting offshore sounds (Fig 5).

Painted-goby larvae reared in high pCO$_2$ levels showed an opposite response to control reared larvae, significantly avoiding reef recordings when tested against offshore recordings (one-sample t-test, $H_0 = 0.5$, $t_{27} = -4.13$, $p < 0.001$; Fig 3) and spending less time (although not significant).
significant) close to reef recordings when tested against no playback (one-sample t-test, $H_0 = 0.5, t_{27} = -1.93, p = 0.06$; Fig 4). However, similar to control reared larvae, no preference or avoidance was detected when larvae were presented with offshore recordings vs. no playback (one-sample t-test, $H_0 = 0.5, t_{26} = 0.91, p = 0.37$; Fig 5).

Discussion

This study demonstrates a strong effect of elevated CO$_2$ conditions on the behaviour of wild-caught fish larvae towards suitable settlement habitat cues, by disrupting auditory responses to habitat-specific soundscapes. Underwater soundscapes contain information on habitat features and biological composition that can be used by marine larvae for orientation, habitat selection and settlement [21,22,23,29,30,32,36,59]. Our results show that exposure to high pCO$_2$ reverses the attraction towards dusk-time reef sounds in wild settlement-stage painted-goby larvae. Larvae exposed to high pCO$_2$ conditions strongly avoided reef sound, as opposed to larvae in control pCO$_2$ conditions which were strongly attracted by reef sound. Consistent with our results, settlement-stage larvae of barramundi (Lates calcarifer) and mulloway
(Argyrosomus japonicus) reared in acidified conditions were also repelled from auditory cues from settlement habitat [39,40], and juvenile clownfish (Amphiprion percula) showed no preference or actively avoided daytime reef sounds [38]. A note on the experimental set-up is needed here to address the possible issue of pseudoreplication, as the use of a single sump per treatment, feeding replicate tanks, might be considered a pseudoreplicated design (sensu [60]). We argue that pseudoreplication is unlikely to be associated with confounded effects as 1) we have accounted for such issues in our statistical analysis, by using an appropriate multilevel model (GLMM) [61]; 2) we have maintained very high standards of water quality, which made sure there were no other differences in the seawater between treatments, other than carbonate chemistry, that could be responsible for the differences observed; and 3) our results are highly significant, and consistent with other studies [38,39,40], which provides a degree of replication on its own.

In addition to auditory cues, fish use a range of other senses to locate suitable settlement habitat, including olfaction and vision [62]. However, recent studies indicate that these senses...
are also likely to be impaired by increased CO₂ [63,64,65,66,67,68]. Moreover, recent evidence suggests that sound production by snapping shrimps, which are among the noisiest invertebrates dominating coastal marine soundscapes, is substantially reduced by exposure to future ocean acidification conditions [69]. This trend towards silence, and the compromised ability to orient towards suitable habitat at settlement may have implications for survival and replenishment of marine populations [70].

Our results also provide evidence that painted goby larvae, from control conditions, are attracted to biologically relevant habitat sounds, but not to sounds deprived of biological significance, such as offshore soundscapes. This agrees with field observations which show that settlement stage larvae of reef fishes orientate away from the reef during the day [71,72,73], but are attracted to nocturnal coastal soundscapes [17,27,29,30,32], presumably due to predation risk being reduced at night [74,75]. Preliminary data on painted goby larvae seem to support this hypothesis, as settlement stage larvae tested in the laboratory avoided day time reef sounds (Faria AM, unpublished results). Reef sounds vary with time of the day and season, and in
most studied reefs, biological choruses peak during dusk hours and during the summer time [18,19,55,76,77], coinciding with the arrival of settlement stage larvae in higher densities [78,79]. In general, settlement-stage reef fishes are attracted by the high-frequency sounds of reefs (produced mainly by invertebrates) [80], contrary to juvenile and adult reef fishes that are attracted by low-frequency sounds produced by other fishes [32]. The reef sounds we broadcast in this study contain a mix of frequencies and amplitudes, and we don’t know the components of nocturnal reef sounds to which larvae of our studied species are attracted. Future work could address the sensitivity of this species to different frequencies of sound, using an electrophysiological technique such as the auditory brainstem response (ABR) [38,81], to determine the range of sounds to which this species likely responds. It is also important to note that playback experiments in tanks do not reflect real-world noise sources as the particle motion (the sound component to which larvae are more sensitive) occurs at higher levels in aquaria than in the open ocean [82]. Therefore it would be beneficial for future studies to test larval response to natural sounds in their natural habitat. Moreover, the ontogenetic timing of responsiveness towards sound cues should be investigated, as auditory sensitivity and motivation to respond to acoustic cues varies with ontogeny and among species [39,83]. What our data suggests is that painted goby larvae as small as 10 mm can detect and respond to sound cues, but when, during ontogeny, this capacity develops in unknown; a greater understanding of the species’ hearing abilities will provide valuable information for parameterising larval dispersal models.

The mechanisms responsible for behavioural impairment in fish larvae exposed to high CO$_2$ are still uncertain, although it appears to be, at least partially, caused by a disturbance in the GABA-A receptor; the primary inhibitory neurotransmitter receptor in the vertebrate brain [84,85,86]. In the case of auditory sensitivity, changes in otoliths may also account for altered auditory preferences. Otoliths are sensory aragonite structures involved in balance, orientation and sound detection in fishes. However, the few available studies on the effects of acidification on size and shape of otoliths have produced conflicting results—while some species exhibit little effect of near-future CO$_2$ levels on otolith development [38,87,88], others show increased otolith growth with increasing CO$_2$ [40,89,90], potentially affecting auditory sensitivity [90]. Otoliths of larvae tested in the present study were not analyzed, but previous results of painted goby larvae exposed to CO$_2$ levels exceeding the levels used here indicate that neither size nor shape are affected (Faria AM, unpublished results). If otoliths are not affected, the observed altered auditory preference in painted goby under high CO$_2$ may be related to altered neurotransmitter function. Future studies should address this hypothesis by treating larvae with an antagonist of GABA-A receptor, such as gabazine [85,86].

The lack of a time-in-treatment effect provides evidence for a lack of acclimation to elevated CO$_2$, at least over the time frame of 10–25 days. Consistent with these findings, larvae of a catadromous fish reared from hatching to post-metamorphosis in high CO$_2$ conditions did not acclimate to these conditions, despite continuous exposure [40], and juvenile reef fish at natural CO$_2$ vents showed similar behavioural disturbances (e.g. bolder behaviour, reversal of olfactory preferences) as those observed in laboratory experiments, indicating that fish did not acclimate despite presumed continuous exposure to elevated CO$_2$ since settlement [91]. Furthermore, a review based on short- and long-term experiments and studies at natural CO$_2$ vents revealed little evidence of acclimation to acidification for several species [14]. Despite a current lack of evidence for the ability of fish to acclimate to elevated CO$_2$ conditions, there remains a need for long-term multigenerational experiments to determine whether species have the capacity to adapt to the predicted ocean acidification over the next century. To date, the few studies on transgenerational acclimation to climate change on fishes yields conflicting results, as some suggest improved growth and survival [92,93], while others suggest that
cognitive functions have limited plasticity [94,95]. Determining which traits show transgenerational acclimation, and which mechanisms may be used by species to overcome rapid climate change (including the synergistic effects of ocean acidification and warming), should be a priority for future research.

Supporting Information
S1 Dataset. The file summarizes all the relevant data that have been used in the statistical analyses.
(XLSX)

Acknowledgments
The authors would like to thank A. Lopes, D. Rodrigues, H. Folhas, P. Coelho for support in the laboratory and field.

Author Contributions
Conceptualization: AMF SDS PLM EJG.
Formal analysis: AMF MCPA.
Funding acquisition: AMF.
Investigation: JMC MCPA AMF.
Methodology: AMF MCPA SDS PLM EJG.
Project administration: AMF.
Resources: APO.
Visualization: JMC MCPA AMF.
Writing – original draft: JMC MCPA SDS PLM EJG AMF.
Writing – review & editing: JMC MCPA SDS PLM EJG AMF.

References
1. Hoegh-Guldberg O, Bruno JF. The impact of climate change on the world's marine ecosystems. Science. 2010; 328: 1523–1528. doi: 10.1126/science.1189930 PMID: 20558709
2. Doney SC, Ruckelshaus M, Duffy JE, Barry JP, Chan F, English CA, et al. Climate change impacts on marine ecosystems. Ann Rev Mar Sci. 2012; 4: 11–37. doi: 10.1146/annurev-marine-041911-111611 PMID: 22457967
3. Pörtner HO, Langenbuch M, Reipschläger A. Biological impact of elevated ocean CO$_2$ concentrations: lessons from animal physiology and earth history. J Oceanogr. 2004; 60: 705–718.
4. Pörtner HO, Farrell AP. Physiology and climate change. Science. 2008; 322: 690–692. doi: 10.1126/science.1163156 PMID: 18974339
5. Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, et al. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying org1anisms. Nature. 2005; 437: 681–686. doi: 10.1038/nature04095 PMID: 16193043
6. Hofmann GE, Barry JP, Edmonds PJ, Gates RD, Hutchins DA, Klinger T, et al. The effect of ocean acidification on calcifying organisms in marine ecosystems: An organism-to-ecosystem perspective. Ann Rev Ecol Evol System. 2010; 41: 127–147.
7. Kurita H. Effects of CO$_2$-driven ocean acidification on the early developmental stages of invertebrates. Mar Ecol Prog Ser. 2008; 373: 275–284.
8. Frommel AY, Maneja R, Lowe D, Malzahn AM, Geffen AJ, Folkvord A, et al. Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. Nat Clim Chang. 2012; 2: 42–46.

9. Dupont S, Havenhand J, Thorndyke W, Peck L, Thorndyke M. Near-future level of CO$_2$-driven ocean acidification radically affects larval survival and development in the brittlestar Ophiothrix fragilis. Mar Ecol Prog Ser. 2008; 373: 285–294.

10. Baumann H, Talma GE, Gobler CJ. Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. Nat Clim Chang. 2012; 2: 38–41.

11. Briffa M, de la Haye K, Munday P. High CO$_2$ and marine animal behaviour: potential mechanisms and ecological consequences. Mar Poll Bull. 2012; 64: 1519–1528.

12. Heuer R, Grosell M. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. Am J Physiol Regul Integr Comp Physiol. 2014; 307(9): R1061–R1084. doi: 10.1152/ajpregu.00064.2014 PMID: 25163920

13. Clements JC, Hunt HL. Marine animal behaviour in a high CO$_2$ ocean. Mar Ecol Prog Ser. 2015; 536: 259–279.

14. Nagelkerken I, Connell SD. Global alteration of ocean ecosystem functioning due to increasing human CO$_2$ emissions. Proc Natl Acad Sci USA. 2015; 112: 13272–13277. doi: 10.1073/pnas.1510856112 PMID: 26460052

15. Leis JM, Carson-Ewart BM, Cato DH. Sound detection in situ by the larvae of a coral-reef damselfish (Pomacentridae). Mar Ecol Prog Ser. 2002; 232: 259–268.

16. Leis JM, Siebeck U, Dixon DL. How Nemo finds home: the neuroecology of dispersal and of population connectivity in larval marine fishes. Integr Comp Biol. 2011; 51(5): 826–843. doi: 10.1093/icb/icr004 PMID: 21562025

17. Montgomery JC, Jeffs A, Simpson SD, Meekan M, Tindale N. Sound as an orientation cue for the pelagic larvae of reef fishes and decapod crustaceans. Adv Mar Biol. 2006; 51: 143–196. doi: 10.1016/S0065-2881(06)51003-X PMID: 16905427

18. Radford CA, Jeffs AG, Tindale CT, Montgomery JC. Resonating sea urchin skeletons create coastal cho-eruses. Mar Ecol Prog Ser. 2008; 362: 35–47.

19. Radford CA, Jeffs AG, Tindale CT, Montgomery JC. Temporal patterns in ambient noise of biological origin from a shallow water temperate reef. Oecologia. 2008; 156: 921–929. doi: 10.1007/s00442-008-1041-y PMID: 18461369

20. Radford CA, Stanley JA, Tindale CT, Montgomery JC, Jeffs AG. Localised coastal habitats have distinct underwater sound signatures. Mar Ecol Prog Ser. 2010; 401: 21–29.

21. Kennedy EV, Guzman HM, Holderied MW, Mair J, Simpson SD. Spatial patterns in reef-generated noise relate to habitats and communities: Evidence from a Panamanian case study. J Exp Mar Bio Ecol. 2010; 305: 85–92.

22. Piercy JJB, Codling EA, Hill A, Smith DJ, Simpson SD. Habitat quality affects sound production and likely distance of detection on coral reefs. Mar Ecol Prog Ser. 2014; 516: 35–47.

23. Nedelec SL, Simpson SD, Holderied MW, Radford AN, Lecellier G, Radford C, et al. Soundscape and living communities in coral reefs: temporal and spatial variation. Mar Ecol Prog Ser. 2015; 524: 125–135.

24. Bertucci F, Parmentier E, Lecellier G, Hawkins AD, Lecchini D. Acoustic indices provide information on the status of coral reefs: an example from Moorea Island in the South Pacific. Scientific Reports. 2016; 6: 33326. doi: 10.1038/srep33326 PMID: 27629650

25. Tolimieri N, Haine O, Montgomery J, Jeffs A. Ambient sound as a navigational cue for larval reef fish. Bioacoustics. 2002; 12(2–3): 214–217.

26. Tolimieri N, Jeffs A, Montgomery JC. Ambient sound as a cue for navigation by the pelagic larvae of reef fishes. Mar Ecol Prog Ser. 2000; 207: 219–224.

27. Leis JM, Carson-Ewart BM, Hay AC, Cato DH. Coral-reef sounds enable nocturnal navigation by some reef-fish larvae in some places and at some times. J Fish Biol. 2003; 63: 724–737.

28. Leis JM, Lockett MM. Localization of reef sounds by settlement-stage larvae of coral-reef fishes (Pomacentridae). Bull Mar Sci. 2005; 76: 715–724.

29. Simpson SD, Meekan MG, McCauley RD, Jeffs A. Attraction of settlement-stage coral reef fishes to reef noise. Mar Ecol Prog Ser. 2004; 276: 263–268.

30. Simpson SD, Meekan M, Montgomery J, McCauley RD, Jeffs A. Homeward sound. Science. 2005; 308: 221–221. doi: 10.1126/science.1107406 PMID: 15821083

31. Simpson SD, Yan HY, Wittenrich ML, Meekan MG. Response of embryonic coral reef fishes (Pomacentridae: Amphiprion spp.) to noise. Mar Ecol Prog Ser. 2005; 287: 201–208.
32. Simpson SD, Jeffs A, Montgomery JC, McCauley RD, Meekan MG. Nocturnal relocation of adult and juvenile coral reef fishes in response to reef noise. Coral Reefs. 2008; 27: 97–104.

33. Wright KJ, Higgs DM, Belanger AJ, Leis JM. Auditory and olfactory abilities of pre-settlement larvae and post-settlement juveniles of a coral reef damselfish (Pisces: Pomacentridae). Mar Biol. 2005; 147(6): 1425–1434.

34. Stanley J, Radford C, Jeffs A. Induction of settlement in crab megalopae by ambient underwater sound. Behav Ecol. 2010; 21: 113–120.

35. Stanley JA, Radford CA, Jeffs AG. Behavioral response thresholds in New Zealand crab megalopae to ambient underwater sound. PLoS ONE. 2011; 6(12): e28572. doi: 10.1371/journal.pone.0028572 PMID: 22163314

36. Lillis A, Eggleston DB, Bohnenstiehl DR. Oyster larvae settle in response to habitat-associated underwater sounds. PLoS ONE. 2013; 8(10): e79337. doi: 10.1371/journal.pone.0079337 PMID: 24205381

37. Parmentier E, Berten L, Rigo P, Aubrun F, Nedelec S, Simpson SD, et al. The influence of various reef sounds on coral reef fish behaviour. J Fish Biol. 2015; 86: 1507–1518. doi: 10.1111/jfb.12651 PMID: 25786340

38. Simpson SD, Munday PL, Wittenrich ML, Manassa R, Dixon DL, Gagliano M, et al. Ocean acidification erodes crucial auditory behaviour in a marine fish. Biol Lett. 2011; 7(6): 917–920. doi: 10.1098/rsbl.2011.0293 PMID: 21632617

39. Rossi T, Nagelkerken I, Simpson SD, Pistevos JCA, Watson S, Merillet L, et al. Ocean acidification boosts larval fish development but reduces the window of opportunity for successful settlement. Proc R Soc B: Biol Sci. 2015; 282: 20151954.

40. Rossi T, Nagelkerken I, Pistevos JC, Connell SD. Lost at sea: ocean acidification undermines larval fish orientation via altered hearing and marine soundscape modification. Biol Lett. 2016; 12: 20150937. doi: 10.1098/rsbl.2015.0937 PMID: 26763221

41. Ferrari MCO, Dixon DL, Munday PL, McCormick MI, Meekan MG, Sih A, et al. Intrageneric variation in antipredator responses of coral reef fishes affected by ocean acidification: implications for climate change projections on marine communities. Glob Chang Biol. 2011; 17: 2980–2986.

42. Jutfelt F, Hedga˚ rde M. Juvenile Atlantic cod behaviour appears robust to near-future CO$_2$ levels. Front Zool. 2015; 12: 11. doi: 10.1186/s12983-015-0104-2 PMID: 27408612

43. Smith ME, Fuiman LA. Behavioural performance of wild-caught and laboratory-reared red drum Sciaenops ocellatus (Linnaeus) larvae. J Exp Mar Biol Ecol. 2004; 302: 17–33.

44. Miller PJ. Gobiidae. In: Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortonese E, editors. Fishes of the North-eastern Atlantic and the Mediterranean. Paris: UNESCO; 1986. vol. III, 1019–1085.

45. Lebour ML. The eggs of Gobius minutus, pictus and microps. J Mar Biol Assoc U.K. 1920; 12: 253–260. http://dx.doi.org/10.1017/S0025315400000060

46. Petersen CGJ. Our gobies (Gobiidae) from the egg to the adult stages. Rep Dan Biol Stn. 1919; 26: 47–66.

47. Caldeira K, Wickett ME. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. J Geophys Res. 2005; 110, C09S04.

48. Feely RA, Sabine CL, Hernandez-Ayon JM, Ishii D, Hales B. Evidence for upwelling of corrosive "acidified" water onto the continental shelf. Science. 2008; 320(5882): 1490–1492. doi: 10.1126/science.1155676 PMID: 18497259

49. Reum JCP, Alin SR, Harvey CJ, Bednarsek N, Evans W, Feely RA, et al. Interpretation and design of ocean acidification experiments in upwelling systems in the context of carbonate chemistry co-variation with temperature and oxygen. ICES J Mar Sci. 2015; 528–536.

50. Cabeçadas L, Oliveira AP. Impact of a Coccolithus braarudii bloom on the carbonate system of Portuguese coastal waters. J Nannoplankton Res. 2005; 27(2), 141–147.

51. Dickson AG, Sabine CL, Christian JR. Guide to best practices for ocean CO$_2$ measurements. PICES Special Publication. 2007; 3: 191.

52. Millero FJ, Graham TB, Huang F, Bustos-Serrano H, Pierrot D. Dissociation constants of carbonic acid in seawater as a function of salinity and temperature. Mar Chem. 2006; 100: 80–94.

53. Weiss RF. Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Mar Chem. 1974; 2: 203–15.

54. Munday PL, Welch M, Allan B, Watson S-A, McMahon S, McCormick MI. Effects of elevated CO$_2$ on predator avoidance behaviour by reef fishes is not altered by experimental test water. PeerJ 2016; 4: e2501. doi: 10.7717/peerj.2501 PMID: 27761317
55. McCauley RD, Cato DH. Patterns of fish calling in a nearshore environment in the Great Barrier Reef. Philos Trans R Soc Lond B Biol Sci. 2000; 355: 1289–1293. doi: 10.1098/rstb.2000.0686 PMID: 11079417

56. Akamatsu T, Okumura T, Novarini N, Yan Y. Empirical refinements applicable to the recording of fish sounds in small tanks. J Acoust Soc Am. 2002; 112: 3073–3082. PMID: 12509030

57. Bolgan M, Pedroso SS, Vasconcelos RO, Jordão JM, Amorim MCP, Fonseca PJ. Hearing sensitivity of the painted goby, Pomatoschistus pictus. Adv Exp Med Biol. 2012; 730: 109–111. doi: 10.1007/978-1-4419-7311-5_24 PMID: 22278461

58. Warton DI, Hui FK. The arcsine is asinine: the analysis of proportions in ecology. Ecol. 2011; 92: 3–10.

59. Radford CA, Stanley JA, Simpson SD, Jeffs AG. Juvenile coral reef fishes use sound to locate habitats. Coral Reefs, 2011; 30: 295–305.

60. Huribert SH. Pseudoreplication and the design of ecological field experiments. Ecol Monographs 1984; 51: 187–211.

61. Millar RB, Anderson MJ. Remedies for pseudoreplication. Fish Res. 2004; 70: 397–407.

62. Huijbers CM, Nagelkerken I, Lo’sbroek PA, Schulten IE, Siegenthaler A, Holleder MW, et al. A test of the senses: fish select novel habitats by responding to multiple cues. Ecology, 2012; 93: 46–55. PMID: 22486086

63. Munday PL, Dixon DL, Donelson JM, Jones GP, Pratchett MS, Devitsina GV, et al. Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. Proc Natl Acad Sci USA, 2009; 106: 1848–1852. doi: 10.1073/pnas.0809996106 PMID: 19188598

64. Munday PL, Dixon DL, McCormick MI, Meekan MG, Ferrari MCO, Chivers DP. Replenishment of fish populations is threatened by ocean acidification. Proc Natl Acad Sci USA. 2010; 107: 12930–12934. doi: 10.1073/pnas.1004519107 PMID: 20615968

65. Dixon DL, Munday PL, Jones GP. Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. Ecol Lett. 2010; 13(1): 68–75. doi: 10.1111/j.1461-0248.2009.01400.x PMID: 19917053

66. Ferrari MCO, Manassa RP, Dixon DL, Munday PL, McCormick MI, Meekan MG, et al. Effects of ocean acidification on learning in coral reef fishes. PLoS One. 2012a; 7: e11478.

67. Ferrari MCO, McCormick MI, Munday PL, Meekan MG, Dixon DL, Lovnessedt O, et al. Effects of ocean acidification on visual risk assessment in coral reef fishes. Funct Ecol, 2012b; 26(3): 553–558.

68. Chung W-S, Marshall NJ, Watson S-A, Munday P, Nilsson G. Ocean acidification slows retinal function in a damselfish through interference with GABAA receptors. J Exp Biol, 2014; 217(3): 323–326.

69. Rossi T, Connell SD, Nagelkerken I. Silent oceans: ocean acidification impoverishes natural soundscapes by altering sound production of the world’s noisiest marine invertebrate. Proc R Soc B: Biol Sci. 2016; 283: 20153046.

70. Nagelkerken I, Munday PL. Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. Glob Chang Biol. 2016; 22: 974–989. doi: 10.1111/gcb.13167 PMID: 26700211

71. Leis JM, Sweatman HPA, Reader SE. What the pelagic stages of coral reef fishes are doing out in blue water: daytime field observations of larval behavioural capabilities. Mar Freshw Res 1996; 47: 401–411.

72. Leis JM, Carson-Ewart BM. Orientation of pelagic larvae of coral-reef fishes in the ocean. Mar. Ecol. Prog. Ser. 2003; 252: 239–253.

73. Heenan A, Simpson SD, Braithwaite VA. Testing the generality of acoustic cue use at settlement in larval coral reef fish. In: Proceedings of the 11th International Coral Reef Symposium, International Society for Reef Studies. Ft Lauderdale, Florida, U.S.A; 2009. 554–558.

74. Williams DMCB, Daily, monthly and yearly variability in recruitment of a guild of coral reef fishes. Mar Ecol Prog Ser. 1983; 10: 231–237.

75. Robertson DR, Green DG, Victor BC. Temporal coupling of production and recruitment of larvae of a Caribbean reef fish. Ecology, 1988; 69: 370–81.

76. Cato DH. Marine biological choruses observed in tropical waters near Australia. J Acoust Soc Am. 1978; 64: 736–743.

77. Cato DH. The biological contribution to the ambient noise in waters near Australia. Acoust Aust. 1992; 20: 76–80.

78. Dufour V, Galzin R. Colonization patterns of reef fish larvae to the lagoon at Moorea Island, French Polynesia. Mar Ecol Prog Ser. 1993; 102: 143–152.
79. Irisson JO, Lecchini D. In situ observation of settlement behaviour in larvae of coral reef fishes at night. J Fish Biol. 2008; 72: 2707–2013.
80. Simpson SD, Meekan MG, Jeffs A, Montgomery JC, McCauley RD. Settlement-stage coral reef fishes prefer the higher frequency invertebrate-generated audible component of reef noise. Anim Behav. 2008; 75: 1861–1868.
81. Kenyon TN, Ladich F, Yan HY. A comparative study of hearing ability in fishes: the auditory brainstem response approach. J Comp Physiol A. 1998; 182: 307–318. PMID: 9528109
82. Parvulescu A. The acoustics of small tanks. In: Tavolga WN, editors. Marine bioacoustics II. Pergamon, Oxford; 1967. pp 7–13.
83. Wright KJ, Higgs DM, Leis JM. Ontogenetic and interspecific variation in hearing ability in marine fish larvae. Mar Ecol Prog Ser. 2011; 424: 1–13.
84. Nilsson GE, Dixon DL, Domenici P, McCormick MI, Sorensen C, Watson S-A, et al. Near-future CO₂ levels alter fish behaviour by interference with neurotransmitter function. Nat Clim Chang. 2012; 2: 201–204.
85. Hamilton TJ, Holcombe A, Tresguerres M. CO₂-induced ocean acidification increases anxiety in Rockfish via alteration of GABA-A receptor functioning. Proc R Soc B: Biol Sci. 2014; 281(1775).
86. Lai F, Jutfelt F, Nilsson GE. Altered neurotransmitter function in CO₂-exposed stickleback (Gasterosteus aculeatus); a temperate model species for ocean acidification research. Conservation Physiology; 2015. vol. 3 (1).
87. Munday PL, Gagliano M, Donelson JM, Dixon DL, Thorrold SR. Ocean acidification does not affect the early life history development of a tropical marine fish. Mar Ecol Prog Ser. 2011; 423: 211–221.
88. Munday PL, Hernaman V, Dixon DL, Thorrold SR. Effect of ocean acidification on otolith development in larvae of a tropical marine fish. Biogeosciences. 2011; 8: 2329–2356.
89. Checkley DM, Dickson AG, Takahashi M, Radich JA, Eisenkolb N, Asch R. Elevated CO₂ enhances otolith growth in young fish. Science. 2009; 324: 1683. doi: 10.1126/science.1169806 PMID: 19556502
90. Bignami S, Enochs I, Manzello D, Sponaugle S, Cowen RK. Ocean acidification alters the otoliths of a pan-tropical fish species with implications for sensory function. Proc Natl Acad Sci USA. 2013; 110: 7366–7370. doi: 10.1073/pnas.1301365110 PMID: 23589887
91. Munday PL, Cheal AJ, Dixon DL, Rummer JL, Fabricius KE. Behavioural impairment in reef fishes caused by ocean acidification at CO₂ seeps. Nat Clim Chang. 2014; 4: 487–492.
92. Miller GM, Watson SA, Donelson JM, McCormick MI, Munday PL. Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. Nat Clim Chang. 2012; 2: 858–861
93. Murray CS, Malvezzi A, Gobber CJ, Baumann H. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. Mar. Ecol. Prog. Ser. 2014; 504: 1–11.
94. Allan BJM, Domenici P, McCormick MI, Watson SA, Munday PL. Elevated CO₂ affects predator-prey interactions through altered performance. PLoS One. 8. 2013; e58520. doi: 10.1371/journal.pone.0058520 PMID: 23484032
95. Welch MJ, Watson SA, Welsh JQ, McCormick MI, Munday PL. Effects of elevated CO₂ on fish behaviour undiminished by transgenerational acclimation. Nat. Clim. Chang. 4. 2014; 1086–1089.