



# Warming, not CO<sub>2</sub>-acidified seawater, alters otolith development of juvenile Antarctic emerald rockcod (*Trematomus bernacchii*)

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Received: 14 August 2020 / Revised: 3 March 2021 / Accepted: 26 July 2021 / Published online: 7 August 2021  
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## Abstract

The combustion of fossil fuels is currently causing rapid rates of ocean warming and acidification worldwide. Projected changes in these parameters have been repeatedly observed to stress the physiological limits and plasticity of many marine species from the molecular to organismal levels. High latitude oceans are among the fastest changing ecosystems; therefore, polar species are projected to be some of the most vulnerable to climate change. Antarctic species are particularly sensitive to environmental change, having evolved for millions of years under stable ocean conditions. Otoliths, calcified structures found in a fish's inner ear used to sense movement and direction, have been shown to be affected by both warming and CO<sub>2</sub>-acidified seawater in temperate and tropical fishes but there is no work to date on Antarctic fishes. In this study, juvenile emerald rockcod (*Trematomus bernacchii*) were exposed to projected seawater warming and CO<sub>2</sub>-acidification for the year 2100 over 28 days. Sagittal otoliths were analyzed for changes in area, perimeter, length, width and shape. We found ocean warming increased the growth rate of otoliths, while CO<sub>2</sub>-acidified seawater and the interaction of warming and acidification did not have an effect on otolith development. Elevated temperature also altered the shape of otoliths. If otolith development is altered under future warming scenarios, sensory functions such as hearing, orientation, and movement may potentially be impaired. Changes in these basic somatic abilities could have broad implications for the general capabilities and ecology of early life stages of Antarctic fishes.

**Keywords** Antarctic fish · Ocean acidification · Warming · Climate change · Otoliths

## Introduction

As anthropogenic greenhouse gases are emitted into the atmosphere, the temperature and partial pressure of carbon dioxide ( $PCO_2$ ) in seawater will continue to rapidly rise (IPCC 2013). The rates of observed and projected change of these two factors, however, varies across regional scales (IPCC 2013). The Southern Ocean is experiencing the highest rates of CO<sub>2</sub>-acidification due to the capacity of

cold water to absorb more dissolved gas and strong westerly winds driving upwelling of CO<sub>2</sub>-enriched deep water (Fabry et al. 2009; Xue et al. 2018). As a result, the Southern Ocean has absorbed roughly 75% of the ocean's total heat uptake between 1861 and 2005 (Frolicher et al. 2015), with the Western Antarctic Peninsula warming at unprecedented rates 3–4 times the global rates (Meredith and King 2005).

Antarctic fishes have evolved in a relatively stable, sub-zero thermal environment for millions of years (Eastman 1993). Because of their specialized stenothermal adaptations, the rapid speed at which environmental conditions in polar regions are currently changing and their slow population growth makes Antarctic fishes particularly vulnerable to climate change (Petricorena and Somero 2007; Barnes et al. 2009; Hauri et al. 2016). Polar species are unable to escape ocean warming by migrating to higher latitudes, have relatively long generation times (years to decades) and thus must rely on existing physiological plasticity and acclimation capacity to tolerate projected changes. The effects of CO<sub>2</sub>-acidification of seawater on fishes are far reaching,

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having been shown to affect physiology (Strobel et al. 2012; Enzor et al. 2013), behavior (Munday et al. 2010; Davis et al. 2017), olfactory signals (Munday et al. 2009), and otolith formation (Checkley et al. 2009).

Otoliths are calcified structures found in a fish's inner ear, which provide sensory functions of movement, balance, direction and sound detection as the otoliths move over sensory hair cells (Campana 1999; Popper and Lu 2000). The paired ear bones develop chemical and morphological signatures (Grønkvær 2016). Calcium carbonate is incrementally accreted to the primordium of each otolith as the fish grows (Fisher and Hunter 2018), thereby recording characteristics of a fish's life history and external environment. CO<sub>2</sub>-acidification of seawater results in less carbonate ions (CO<sub>3</sub><sup>2-</sup>) available to form calcium carbonate (CaCO<sub>3</sub>), and more hydrogen ions (H<sup>+</sup>) decreasing the saturation state of CaCO<sub>3</sub>, which should decrease skeletal accretion. However, paradoxically, otoliths have been shown to increase in size under CO<sub>2</sub>-acidified water (first shown by Checkley et al. 2009). During early life stages, the development of otoliths has been shown to be affected by both CO<sub>2</sub>-acidified seawater (Checkley et al. 2009; Pimentel et al. 2014; Martino et al. 2017) and elevated temperature (Otterlei et al. 2002; Folkvord et al. 2004; Pimentel et al. 2014; Martino et al. 2017); however, there has been little research to date on the combined effects of CO<sub>2</sub>-acidified seawater and temperature on otolith morphology (Melzner et al. 2009). Elevated PCO<sub>2</sub> and temperature have been shown to interact to affect other aspects of the physiology of Antarctic fishes (Strobel et al. 2012; Enzor et al. 2013; Flynn et al. 2015; Davis et al. 2017). If the morphology or development of these calcified structures is altered under projected ocean conditions, fish balance, movement and hearing may in turn be impaired.

Accretion of otolith rings occurs daily in the early life stages of many fish species (Fisher and Hunter 2018), allowing for detailed analyses of changes in otolith morphology in response to elevated PCO<sub>2</sub> and temperature conditions over relatively short periods of time. Though Antarctic fishes are relatively slow growing (La Mesa et al. 1996), daily increments of otolith rings have been documented in multiple Antarctic fish species such as *Notothenia coriiceps* and *Pseudochaenichthys georginus* (Kellermann et al. 2002), and *Trematomus newnesi* (Radtke et al. 1989). The present study provides the first evidence of how the otoliths of an Antarctic fish may change in response to projected ocean warming and acidification by examining the size and shape of otoliths from juvenile emerald rockcod, *Trematomus bernacchii*, when exposed to four weeks of elevated PCO<sub>2</sub> and temperature in isolation and combined. We predicted that the interaction of elevated PCO<sub>2</sub> and temperature would increase the size and alter the shape of otoliths, as these factors have been shown to influence otolith growth both as independent stressors (Otterlei et al. 2002; Folkvord et al.

2004; Checkley et al. 2009) and concurrent stressors in fishes (Pimentel et al. 2014; Martino et al. 2017).

## Materials and methods

### Study design

Otoliths were collected from juvenile *Trematomus bernacchii* as in Davis et al. (2017). Briefly, fish were collected from McMurdo Sound at Cape Evans Ice Wall, Ross Island, Antarctica (77°38.4070S, 166°31.0680E) by SCUBA divers and transported to the Crary Lab at McMurdo Station. Fish were assumed to be in their second year based on length distributions documented in previous studies (La Mesa et al. 1996) and sex was not determined. Fish were acclimated to two temperatures (Ambient: -1 °C, Increased: 2 °C) and three PCO<sub>2</sub> treatments (Ambient PCO<sub>2</sub>: 450 µatm, Moderate PCO<sub>2</sub>: 850 µatm, and High PCO<sub>2</sub>: 1,200 µatm PCO<sub>2</sub>), based on Representative Concentration Pathways RCP6.0 and RCP8.5 (IPCC 2013), for 28 days in a full factorial design, according to Davis et al. (2017). Ambient seawater of McMurdo Sound is approximately -1.9 °C; however, -1 °C was the coldest stable seawater temperature we could achieve in the lab. Each of the six treatment conditions were run in triplicate buckets and conditions remained relatively stable for the entire 28 days. Seawater chemistry was monitored every two days for total pH using m-cresol dye (Sigma-Aldrich, St. Louis, MO, USA) with a UV spectrophotometer (Shimadzu, Columbia, MD, USA) in each bucket and every four days for total alkalinity using open-cell titration (T50 titrator, Mettler Toledo Inc., Columbus, OH, USA; CRM and titrant from Dixon Laboratory, Scripps Institution of Oceanography, La Jolla, CA, USA) in each PCO<sub>2</sub> mixing reservoir bucket of each treatment (reported in Table 1 of Davis et al. 2017). Fish were fed frozen plankton (0.025 g/fish, Hikari BioPure Ocean Plankton, Hayward, CA, USA) twice daily.

### Otolith preparation and analysis

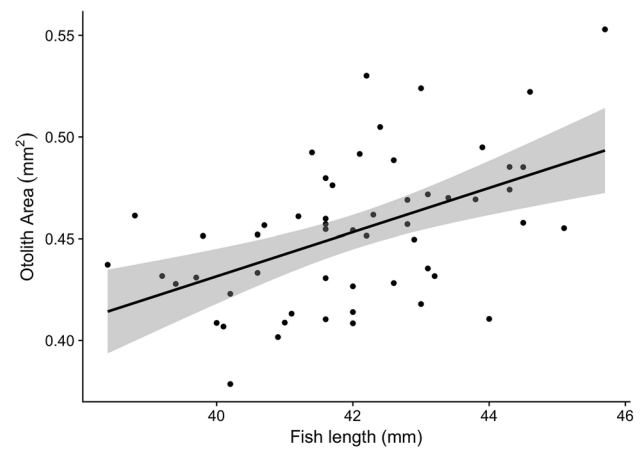
Following 28 days of exposure to PCO<sub>2</sub> and temperature treatments, fish were euthanized in 0.3% tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Redmond, WA, USA) and length and weight measurements were recorded prior to extraction of left and right sagittal otoliths ( $n = 7-11$  per treatment as indicated in Table 1). Individual otoliths were stored in 96-well polystyrene plates filled with 100% type B immersion oil until later analysis. Otolith preparation for image analysis was conducted at the University of California, Davis. Each otolith pair was individually imaged using a MU1000 digital microscope camera (AmScope, Los Angeles, CA, USA) mounted to a Leica Stereo Zoom seven stereomicroscope. A stage calibration slide was used to

**Table 1** Mean ( $\pm$ SD) otolith parameters of juvenile *Trematomus bernacchii* after 4 weeks of acclimation to ambient (-1 °C) and increased temperature (+2 °C) across three  $PCO_2$  treatments (Ambient [A], Moderate [M], and High [H])

Otolith Parameter	Temperature (°C)	$PCO_2$	<i>n</i>	Mean	SD
Area	-1	A	7	0.430	0.021
		M	11	0.428	0.030
		H	10	0.448	0.031
	2	A	8	0.463	0.030
		M	10	0.464	0.042
		H	10	0.486	0.026
Perimeter	-1	A	7	2.378	0.063
		M	11	2.369	0.088
		H	10	2.426	0.083
	2	A	8	2.477	0.082
		M	10	2.477	0.113
		H	10	2.541	0.073
Length	-1	A	7	0.841	0.033
		M	11	0.836	0.033
		H	10	0.854	0.029
	2	A	8	0.886	0.023
		M	10	0.879	0.043
		H	10	0.909	0.031
Width	-1	A	7	0.661	0.015
		M	11	0.666	0.021
		H	10	0.677	0.023
	2	A	8	0.683	0.024
		M	10	0.688	0.027
		H	10	0.695	0.021

provide a reference for deviations in magnification between imaging sessions. To account for differences in microscope magnification, all otolith images were resized to a selected reference calibration slide image in Photoshop CS6 (Adobe Systems, San Jose, CA, USA). Each otolith image was imported into R (v3.6.3, R Core Team, 2020) and analyzed using the package ‘ShapeR’ as described in Libungan and Pálsson (2015). Otolith outlines were traced in the ‘ShapeR’ package by creating a series of data points along the otolith’s perimeter (example shown in Online Resource 1). Contour smoothing and threshold functions in ‘ShapeR’ were set to 100 and 0.2, respectively. After all otoliths had been traced, ‘ShapeR’ calculated otolith area, perimeter, length, width and wavelet coefficients. ‘Shape R’ determines wavelet coefficients, which are used to assess shape, with polar coordinates that measure the distance from the otolith’s centroid to the otolith’s outline along a series of radials (Libungan and Pálsson 2015).

Data analyses were conducted in R (v3.6.3, R Core Team, 2020). All data were first tested for parametric assumptions using Shapiro Wilks for normality and Levene’s test for

**Fig. 1** Relationship between otolith area ( $mm^2$ ) and standard fish length (mm) of *Trematomus bernacchii* following a 28-day acclimation to warming (Ambient: -1 °C, Increased: 2 °C) and  $CO_2$ -acidified seawater (Ambient  $PCO_2$ : 450  $\mu atm$ , Moderate  $PCO_2$ : 850  $\mu atm$ , or High  $PCO_2$ : 1200  $\mu atm$   $PCO_2$ ). The shaded area around the black regression line represents the standard error

homogeneity of variances. Since we included both left and right sagittal otoliths for each fish, we tested for differences in otolith metrics (asymmetry) using a linear mixed effect model with otolith side as an independent variable with individual fish identification as a random factor. Because otolith length showed asymmetry while otolith area, perimeter, and width did not (Online Resource 2), all analyses were conducted on the average value of the left and right sagittal otoliths (area, perimeter, length, width) for each individual (Škeljo and Ferri 2012). To determine if elevated temperature and  $PCO_2$  altered otoliths, a two-way analysis of variance (ANOVA) was conducted on fish length standardized otoliths including dependent variables of otolith area, perimeter, length, and width, with fixed factors of temperature,  $PCO_2$ , and nested replicate buckets. Otoliths were standardized by plotting each metric against the fish’s standard length and taking the residuals along a linear regression (Fig. 1). Lastly, to assess sagittal otolith shape differences among the six treatments a canonical analysis of principal coordinates (CAP) of otolith wavelet coefficients standardized by fish length was conducted in ‘ShapeR’. Using the R package, ‘vegan’ (Oksanen et al. 2018), wavelets were tested by an ANOVA with 1000 permutations (Libungan and Pálsson 2015). A cluster plot of wavelet coefficients from each otolith with two discriminating axes was then produced to visualize shape differences among treatments.

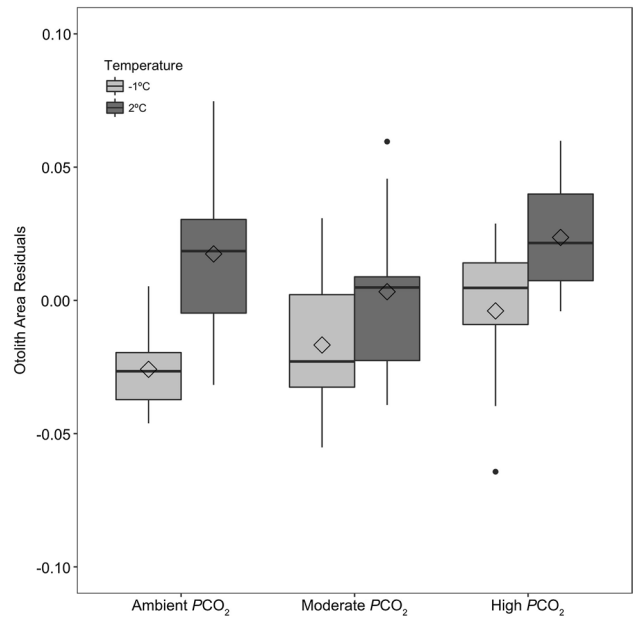
## Results and discussion

Increased temperature significantly increased otolith area ( $P < 0.001$ ) but elevated  $PCO_2$  had no significant effect on otolith area ( $P = 0.114$ ). There was no significant interaction

between elevated temperature and  $PCO_2$  on otolith area ( $P=0.600$ ) (Table 2). Similarly, increased temperature significantly increased otolith perimeter, length and width, while elevated  $PCO_2$  had no effect on these metrics, nor did the interaction between temperature and  $PCO_2$  (Table 1 for means  $\pm$  SD, Table 2 for ANOVA results). There was a positive relationship of fish standard length (range 38.4 to 45.7 mm) on otolith size ( $P < 0.001$ ,  $R^2 = 0.235$ ) (Figs. 1, 2).

Canonical values from otolith wavelets for each of the six treatments were calculated using a canonical analysis of principle coordinates. The first discriminating axis (CAP1) accounted for 59% of the variation among treatments and the second discriminating axis (CAP2) accounted for 22% of the variation among treatments (Fig. 3). Along the first discriminating axis (CAP1), there is very little divergence between the  $PCO_2$  treatments at  $-1\text{ }^\circ\text{C}$ , with the second discriminating axis (CAP2) also showing little divergence. The relatively tight cluster of canonical values indicates little difference in otolith shape between  $-1\text{ }^\circ\text{C}$  temperature treatments, despite differences in  $PCO_2$ .  $PCO_2$  treatments at  $2\text{ }^\circ\text{C}$  displayed more variation in otolith shape relative to  $-1\text{ }^\circ\text{C}$  treatments, but still formed a broad cluster. These treatments displayed less variance along the first discriminating axis. Just as seen in area and perimeter analysis, there was a large discrepancy in otolith structure between temperatures, and very little change across  $PCO_2$  levels. The canonical values of  $-1\text{ }^\circ\text{C}$  and  $2\text{ }^\circ\text{C}$  treatments clustered together while  $PCO_2$  levels did not cluster together, demonstrating that temperature had a larger effect on otolith shape.

After only four weeks of exposure to projected increases in temperature and  $PCO_2$ , otolith development patterns in

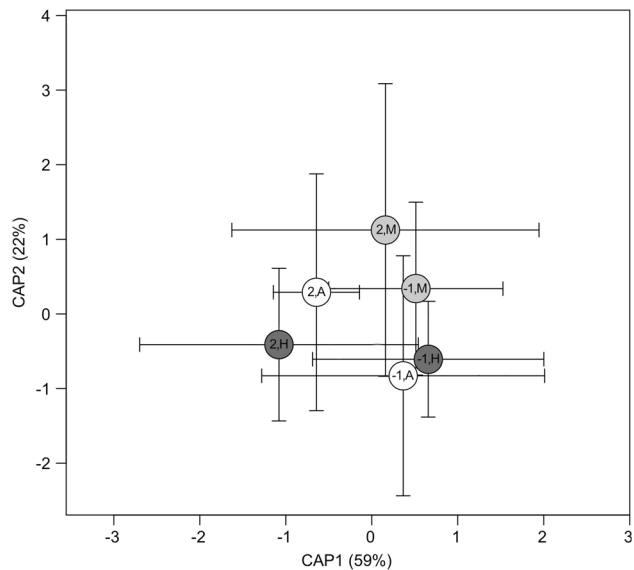


**Fig. 2** Otolith area ( $\text{mm}^2$ ) of *Trematomus bernacchii* following 28 days of acclimation to  $\text{CO}_2$ -acidified seawater and warming. Data are presented as boxplots ( $n=7\text{--}11$  per box as indicated in Table 1) with  $-1\text{ }^\circ\text{C}$  (light grey) and  $2\text{ }^\circ\text{C}$  (dark grey). Boxplots of the data represent the median as the center line and the interquartile range (IQR) as the box. Whiskers extend 1.5 times IQR, and outliers are greater than 1.5 times the IQR as black points. Diamonds indicate the mean value

**Table 2** Analysis of variance (ANOVA) results table

Predictor	Dependent variable	F	DF1	DF2	P
Temperature	Otolith area	17.538	1	46	<0.001*
	Otolith perimeter	21.319	1	46	<0.001*
	Otolith length	29.712	1	46	<0.001*
	Otolith width	9.411	1	46	0.004*
$PCO_2$	Otolith area	2.282	2	46	0.114
	Otolith perimeter	2.391	2	46	0.103
	Otolith length	2.346	2	46	0.107
	Otolith width	1.307	2	46	0.280
Temperature: $PCO_2$	Otolith area	0.517	2	46	0.600
	Otolith perimeter	0.495	2	46	0.613
	Otolith length	0.695	2	46	0.504
	Otolith width	0.487	2	46	0.618

The results are given for each dependent variable (otolith parameter) by the F-statistic ( $F$ ), degrees of freedom ( $DF1$  [numerator] and  $DF2$  [denominator]), and the  $p$  value ( $P$ ). Asterisks indicate a significant effect of the predictor ( $P < 0.05$ )



**Fig. 3** Canonical analysis of principal coordinates (CAP) using wavelet coefficients from left *Trematomus bernacchii* otoliths following 28 days of acclimation to  $\text{CO}_2$ -acidified seawater and warming. Dark grey, light grey and white circles represent the mean canonical value for each treatment after 4 weeks of acclimation to ambient ( $-1\text{ }^\circ\text{C}$ ) and increased temperature ( $2\text{ }^\circ\text{C}$ ) across three  $PCO_2$  treatments (Ambient [A], Moderate [M], and High [H]) with standard error bars (mean  $\pm$  ISE). 59% of the variation is explained in CAP1 while 22% of the variation is explained in CAP2

juvenile *T. bernacchii* began to diverge. In contrast to what was seen in previous studies (Checkley et al. 2009; Pimentel et al. 2014; Martino et al. 2017), otolith area and perimeter remained relatively similar across  $PCO_2$  treatment groups, whereas elevated temperature had a larger effect on otolith morphology (Fig. 2). If these otolith changes under warming waters continue through ontogenetic development, it may in turn impact sensory functions such as hearing (Bignami et al. 2013). Ocean warming is known to increase biochemical and metabolic rates in fishes (Martino et al. 2017), which has been suggested to influence the accretion rate of otoliths independently of whole organism growth (Wright 1991). An increase in metabolic rates associated with elevated temperature during the acclimation period (see Fig. 2 in Davis et al. 2017) may be responsible for the larger otoliths found in this study.

As this study was conducted on juvenile fish over the course of only four weeks, it is unknown how otoliths would continue to develop through later life stages if exposure conditions persisted. Though this study did not find an influence of  $CO_2$ -acidified seawater, an effect may have been seen over a longer exposure as there were physiological differences in the metabolic capacity to acclimate in these fish, as evidenced by the inability of juvenile rockcod exposed to both elevated  $PCO_2$  and temperature to compensate through adjustments in routine metabolic rate (Davis et al., 2017). In developing fishes, the cost of maintaining homeostasis of elevated  $PCO_2$  is high, thereby increasing vulnerability to rapid abiotic changes (Heuer and Grosell 2014); however, once sensitivity to elevated  $PCO_2$  decreases in later life stages as fish develop acid–base regulation, impacts of elevated  $PCO_2$  on otolith growth rates may be diminished. If this is the case, a slight enhancement of otolith growth rates in larval fish life stages may be compensated as development progresses. Alternatively, if temperature is determined to increase the growth rate of otoliths throughout the fish's lifespan, regardless of acid–base regulation, a host of unknown fitness and behavior implications may result. Enhanced temperature has been documented to increase the rate of deformities in juvenile fishes in conjunction with faster growth (Pimentel et al. 2014), which may extend to otolith accretion patterns. In McMurdo Sound, current summer ocean temperatures rarely exceed 0.5 °C in areas where juvenile fishes are found and therefore the projected ocean warming to 2 °C, which *T. bernacchii* experienced in this study, represents a temperature higher than what the species has needed to accommodate for millions of years (Eastman 1993).

While the size of otoliths changed in response to warming, asymmetry of otolith area was unaffected, except for otolith length. Labidi et al. (2020) showed otolith asymmetry in relation to width but not length or area in *Boops boops*, which they suggest may indicate certain metrics are more

susceptible to environmental stress. It has been hypothesized that otolith asymmetry begins to develop under more severe stressor states (Vandenbussche et al. 2018) and therefore the general lack of a significant effect on asymmetry could be evidence that the magnitude of warming was moderate. It has also been suggested that otolith asymmetry is unrelated to organismal stress or fitness and acts as a poor proxy for assessing performance (Díaz-Gil et al. 2015).

Previous research of otolith growth under ocean acidification has shown a wide range of results. For example, one study showed elevated  $PCO_2$  significantly effects the growth of otoliths of white seabass (*Atractoscion nobilis*) larvae (Checkley et al. 2009). In a second study, otoliths from two different coral reef fishes (*Acanthochromis polyacanthus* and *Amphipiron percula*) did not show differences in size, shape, or symmetry under projected  $PCO_2$  (Jarrold and Munday 2018). To our knowledge, the only other two studies assessing the combined effect of elevated temperature and  $PCO_2$  on otolith growth demonstrated that both stressors influenced otolith development in Senegalese sole larvae (*Solea senegalensis*) (Pimentel et al. 2014) and barramundi juveniles (*Lates calcarifer*) (Martino et al. 2017). In the current study with juvenile *T. bernacchii*, which is the first to show otolith growth changes in Antarctic fish, temperature was the main driver of enhanced otolith growth. The varying results of the studies documenting effects of warming and elevated  $PCO_2$  may suggest that otolith development patterns will respond differently to future ocean conditions dependent on species, life stage, and geographical range. How changes in otolith development in early life stages of fishes will impact balance and hearing in the long-term is an unanswered question to date. Effects to hearing and balance could influence species interactions and general mobility, leading to impacts on organismal fitness and would be an important component of predicting vulnerability of fishes to climate change.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00300-021-02923-3>.

**Acknowledgements** This research was funded by the National Science Foundation (NSF ANT-1142122, ANT-1744999 to AET) and the University of California Agricultural Experimental Station (Grant Number CA-D-ASC-2252-H to AET and CA-D-ASC-2098-H to NAF). We are grateful to all the Antarctic Science Support who made this study possible, including the U.S. Antarctic Program, Lockheed Martin, the Cray Lab staff, ASC SCUBA divers Rob Robbins and Steve Rupp, and Trinh Nguyen for his R coding assistance. We would also like to thank our reviewers, Joyce Ong and an anonymous contributor, whose comments strengthened the publication.

**Author contributions** BED, AET and NAF conceived and designed research. BED, AET and NAF conducted experiments. AWN analyzed the otoliths, with help from JAH. AWN and BED conducted the statistical analyses. AWN wrote the manuscript, with editorial feedback from BED, NAF, JAH and AET. All authors read and approved the manuscript.

## Declarations

**Conflict of interest** The authors have no conflict of interest to report.

**Ethical approval** The research project was conducted in accordance with US federal animal welfare laws by approval by the University of California Davis Institutional Animal Care and Use Committee (Protocol #18248).

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