

Research



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Author for correspondence:

Antonio Di Franco

e-mail: difry@libero.it

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Ocean acidification affects somatic and otolith growth relationship in fish: evidence from an *in situ* study

Antonio Di Franco^{1,2}, Antonio Calò^{1,2}, Khalil Sdiri¹, Carlo Cattano^{2,3}, Marco Milazzo^{2,3} and Paolo Guidetti^{1,2}

¹Université Côte d'Azur, CNRS, UMR 7035 ECOSEAS, Parc Valrose 28, Avenue Valrose, 06108 Nice, France

²CoNISMa, Piazzale Flaminio 9, 00196 Roma, Italy

³Dipartimento di Scienze della Terra e del Mare (DiSTeM), Università di Palermo, Via Archirafi 20, 90123 Palermo, Italy

ADF, 0000-0003-3411-7015; AC, 0000-0001-6703-6751; CC, 0000-0002-9562-1330; MM, 0000-0002-2202-0542

Ocean acidification (OA) may have varied effects on fish eco-physiological responses. Most OA studies have been carried out in laboratory conditions without considering the *in situ* pCO₂/pH variability documented for many marine coastal ecosystems. Using a standard otolith ageing technique, we assessed how *in situ* ocean acidification (ambient, versus end-of-century CO₂ levels) can affect somatic and otolith growth, and their relationship in a coastal fish. Somatic and otolith growth rates of juveniles of the ocellated wrasse *Symphodus ocellatus* living off a Mediterranean CO₂ seep increased at the high-pCO₂ site. Also, we detected that slower-growing individuals living at ambient pCO₂ levels tend to have larger otoliths at the same somatic length (i.e. higher relative size of otoliths to fish body length) than faster-growing conspecifics living under high pCO₂ conditions, with this being attributable to the so-called 'growth effect'. Our findings suggest the possibility of contrasting OA effects on fish fitness, with higher somatic growth rate and possibly higher survival associated with smaller relative size of otoliths that could impair fish auditory and vestibular sensitivity.

1. Introduction

Ocean acidification (OA) is the ongoing decline of ocean pH owing to absorption of the increasing atmospheric CO₂ [1]. OA represents a major threat to marine ecosystems by impacting calcification, growth and survival of many organisms [2,3], and ultimately leading to changes in marine communities, including biodiversity loss [2,4].

Although fish actively regulate ionic concentrations in their body compartments [5], eco-physiological studies have reported varied OA effects in some species (see [4] for a recent review). For instance, otoliths are critical elements of the fish inner ear and vestibular system, and several studies have documented increased otolith size associated with elevated pCO₂ exposure. A smaller number of studies have shown increased somatic growth rate under elevated CO₂ [4]. Indeed, otolith sizes increase with body length and some evidence suggested that slower-growing fish tend to have larger otoliths than faster-growing conspecifics at the same somatic length (namely the 'growth effect') [6,7]. However, experimental evidence on such relation under OA conditions is presently scant (but see [8]).

In addition to this, most of the studies conducted so far were carried out in controlled laboratory conditions (but see [9–11]) and did not include the pCO₂

variability that fish may experience in coastal marine ecosystems (but see [12,13]), nor other potential OA-induced differences (e.g. food availability).

Here we attempt to address this potential drawback by assessing how elevated CO_2 conditions experienced *in situ* can affect somatic and otolith growth, and their relationship, for the ocellated wrasse *Symphodus ocellatus* living off a natural vent under different pH/CO_2 conditions.

2. Material and methods

(a) Study area, fish collection and otolith analysis

This study was carried out in Levante Bay at Vulcano Island (Aeolian Archipelago, Northeastern Sicily, Italy) where natural submerged CO_2 emissions create a pCO_2/pH gradient (see electronic supplementary material for details). During 27–30 July 2013, 50 and 59 juveniles *Symphodus ocellatus* were collected at 1–2 m depth using a hand net at ambient (pCO_2 : $428 \pm 47 \mu\text{atm}$; electronic supplementary material, table S1) and high (pCO_2 : $752 \pm 221 \mu\text{atm}$; electronic supplementary material, table S1) pCO_2 sites, respectively. Juveniles, which are strongly site-attached [14], ranged in age from 18 to 42 days post-settlement [11]. All individuals were gently euthanized by an overdose of clove oils dissolved into seawater to minimize suffering. Fish were left in the anaesthetic solution for at least five minutes following the cessation of opercular movement. Individuals were measured to estimate their total length (TL, to the nearest 0.1 mm) using a Vernier caliper.

For each juvenile, the two lapillar otoliths were removed and one was processed to determine the total number of daily rings as described in [15,16] (see electronic supplementary material for details). Single otoliths from a total of 99 individuals were retained for subsequent analyses (i.e. 55 from ambient- pCO_2 site and 44 from high- pCO_2 site).

For each individual we back-calculated spawning and settlement dates, and measured otolith radius (to estimate fish TL at settlement) and the area of the pre- and post-settlement otolith portion (to estimate otolith growth). Measurements of otoliths' area were obtained using the software Image Pro Plus. We estimated somatic growth rate and otolith growth rate of each individual both before and after the settlement (see electronic supplementary material for details).

(b) Data analyses

Variability in frequency distributions of spawning and settlement dates of fish sampled in the two sites was tested using a two-sample Kolmogorov–Smirnov test. To detect the potential effect of OA on somatic and otolith growth rates, we used two 2-way PerMANOVA and adopted a before-after-control-impact (BACI) design, where the settlement is considered the onset of exposure to local pCO_2 associated with the seep (impact) for the group of individuals settling in the high- pCO_2 site (see electronic supplementary material for details).

Two factors are considered: life stage (two levels: pre- and post-settlement) and site (two levels: ambient- and high- pCO_2). Therefore, OA effects can be detected as an interaction between stage and site. Pairwise tests (i.e. t -tests) were run on significant interaction.

We also investigated the effect of OA (i.e. during post-settlement phase) on the relationship between otolith and somatic growth by using an analysis of covariance (ANCOVA) on post-settlement otolith growth and considering post-settlement somatic growth as a covariate to account for the

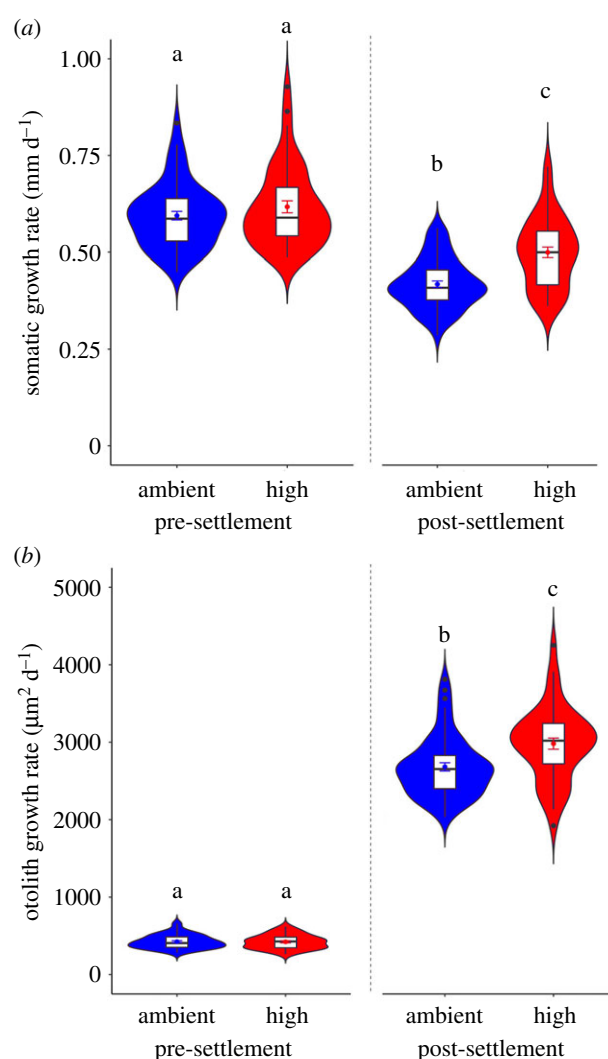


Figure 1. Violin plots of (a) somatic growth rate and (b) otolith growth rate of individuals collected in ambient and high- pCO_2 sites, for pre- and post-settlement phases. The outline of each plot represents the probability density of the variable, and embeds a box plot. Blue and red dots and bars within the boxplots represent means ± 1 s.e. for ambient and high- pCO_2 sites, respectively. Letters indicate statistically significant differences according to a t -test ($p < 0.05$).

influence of fish TL on otolith area. Covariate-adjusted means were derived using the emmeans package in R.

3. Results

In situ pCO_2 levels continuously measured at the two sites revealed that juveniles from the high- pCO_2 site experienced wider daily CO_2 fluctuation (min and max CO_2 concentrations: $429 \mu\text{atm}$ and $1407 \mu\text{atm}$, respectively) than juveniles from the ambient- pCO_2 site (min and max CO_2 concentrations: $333 \mu\text{atm}$ and $530 \mu\text{atm}$, respectively) (see electronic supplementary material, table S1). Life stage and site significantly interacted in their effect on somatic growth (pseudo- F : 7.7747, $p = 0.00666$, see electronic supplementary material, table S2 for further details), with pairwise-tests revealing no difference between the two sites for the pre-settlement phase. Conversely, a significant difference in somatic growth between the two sites was observed for the post-settlement phase, with the group average growth rate 19.47% greater in the high- pCO_2 site (figure 1a). A similar

pattern was observed for otolith growth rate, with a significant interaction between site and life stage (pseudo- F : 9.4736, p = 0.0030, see electronic supplementary material, table S3 for further details), and a greater average growth occurring at high- $p\text{CO}_2$ site during post-settlement, with a 11% difference between the two sites (figure 1b).

Otolith growth was linearly related to somatic growth for the post-settlement phase (ANCOVA, pseudo- F : 583.26, p = 0.0001). Despite a lower slope at the high- $p\text{CO}_2$ site (figure 2), no significant interaction between somatic growth and site was detected (pseudo- F : 2.262, p = 0.14). A significant variability in otolith growth between the 2 sites was detected (pseudo- F : 6.598, p = 0.012) and ANCOVA adjusted means showed that post-settlement otolith growth (normalized for post-settlement somatic growth) is 4.43% higher in ambient than in high- $p\text{CO}_2$ site (figure 2).

4. Discussion

Our study highlights that *in situ* exposure to high $p\text{CO}_2$ levels is related to higher somatic and otolith growth rates of *Symphodus ocellatus* juveniles. This finding supports previous evidence mostly arising from laboratory studies carried out under stable $p\text{CO}_2$ conditions [4]. We explain the increased somatic growth under OA conditions by two non-mutually exclusive mechanisms: (i) the increased energetic costs to restore homeostatic balance stimulating appetite and increased foraging activity [17]; and (ii) the increased production of growth hormone as a response to environmental stress [11].

Increase in otolith size with OA has been generally attributed to a series of acid–base regulation processes between fish plasma and the endolymph surrounding the otoliths, with this finally increasing the aragonite saturation state of the endolymph and otolith growth rates [18,19].

We show that individuals exposed to high- $p\text{CO}_2$ levels, in addition to displaying higher somatic growth rate, exhibit smaller otoliths at equal body length than individuals exposed to ambient- $p\text{CO}_2$ concentrations. In other words, faster somatic growth leads to smaller relative otolith size. This evidence is in agreement with the so-called ‘growth effect’, indicating that slower-growing individuals tend to have larger otoliths than faster-growing conspecifics at the same somatic length [6,7]. To the best of our knowledge, this is the first evidence of this process related to OA impact. No conclusive and detailed information exists about the mechanisms inducing the growth effect. However, it has been postulated that an uncoupling (i.e. loss of proportionality) between somatic and otolith growth occurs because somatic growth tends to be more variable and more susceptible to external factors than otolith growth [20].

It has been hypothesized that the relative size of otoliths to fish body length could affect fish auditory and vestibular sensitivity, and the higher the relative size the higher the sensitivity [19]. Based on previous hypotheses, we speculate that OA could affect the auditory response of fish, then impairing hearing, navigation and balance [21].

However, given that our results are based on two sites (high- and ambient- $p\text{CO}_2$) that, despite being only 500 m apart, could also differ in other environmental and biological features (e.g. food availability, competition, predation), alternative explanations could not be ruled out conclusively.

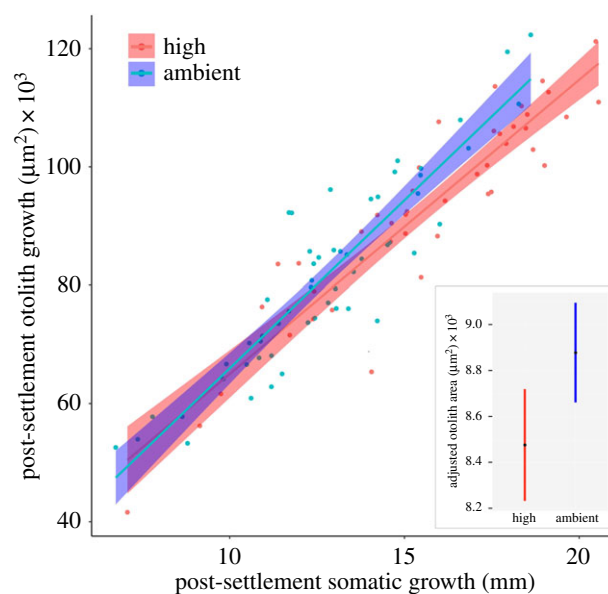


Figure 2. Relationship between post-settlement otolith growth and post-settlement somatic growth of juveniles collected at a high- $p\text{CO}_2$ and ambient- $p\text{CO}_2$ sites. Bands around regressions are the 95% confidence intervals. Insert: post-settlement otolith areas (ANCOVA-adjusted mean \pm 1 s.e.) of juveniles from the high- and ambient- $p\text{CO}_2$ sites.

Additional research would be thus required to elucidate the underlying process(es).

In conclusion, our study suggests the possibility of two potentially contrasting OA effects on fish ecological fitness: higher somatic growth rate can increase survival, as bigger individuals are generally more efficient in avoiding predators [20], while the smaller relative size of otoliths could impair fishes’ auditory response, with potentially detrimental impacts on their survival [21].

Ethics. Animal collection was carried out in accordance with institutional and national (law 116/1992) guidelines concerning the use of animals in research. For the case of animals collected in the wild and not reared, the law prescribed that individuals have to be collected using humane methods by operators who understand animals behaviours and habitats, and sacrificed using humane methods. Animals were sacrificed following specific guidelines contained in EU Recommendation 2007/526/EC on humane killing methods to minimize suffering. At the date of the sampling no additional ethical approval was required by law, and no ethical committee was in place at the institution where the study was carried out.

Data accessibility. Data are available as electronic supplementary material.

Authors’ contributions. A.D.F., A.C., P.G., M.M. and C.C. conceived the study; M.M. and C.C. planned the experimental design and collected samples. A.D.F., A.C., K.S. carried out otolith analyses. A.D.F. and A.C. carried out the statistical analyses and led the drafting of the manuscript. All authors contributed to the manuscript, gave final approval for publication and agree to be held accountable for all aspects of the work.

Competing interests. We declare we have no competing interests.

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