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

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Abstract
Ocean acidification (OA) may have varied effects on fish eco-physiological responses. Most OA studies were 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 pCO2 concentration (ambient vs. end-of-century CO2 levels) can affect somatic and otolith growth, and their relationship in a coastal fish. Using juveniles from a population of the ocellated wrasse Symphodus ocellatus living off a Mediterranean CO2 seep, somatic and otolith growth rates 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, with this being attributable to the so-called “growth effect”. Our findings suggest contrasting OA effects on fish fitness, with higher somatic growth rate and possibly higher survival associated to smaller relative size of otoliths that could impair fish auditory and vestibular sensitivity.

Keywords: CO2 seeps, coastal fish, Ocean acidification, otoliths, pCO2 variability, somatic growth,

1. Introduction
Ocean acidification (OA) is the ongoing decline of ocean pH due 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].

Despite 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. A smaller number of studies has shown increased somatic growth rate under elevated CO₂ [4]. Indeed, otolith sizes increase with body length with some evidence suggesting 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 fish may experience in coastal marine ecosystems [but see 12, 13]. Here we attempt to address this potential drawback by assessing how elevated CO₂ conditions experienced in situ can affect somatic and otolith growth, and their relationship for the ocellated wrasse Symphodus ocellatus living under different pH/CO₂ conditions.

2. Materials and Methods

2.1. 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 CO₂ emissions create a pCO₂/pH gradient (see Electronic Supplementary for details). During July 27th-30th 2013, 50 and 59 juveniles Symphodus ocellatus were collected at 1–2 m depth using a hand net at two sites with Ambient (pCO₂: 428 ±47 μatm; Table S1) and High (pCO₂: 752 ±221 μatm; Table S1) pCO₂, respectively. Juveniles, which are strongly site-attached [14], ranged in age from 18 to 42 d post-settlement [11]. All individuals were gently euthanized soon after sampling and 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 for details). Single otoliths from a total of 99 individuals were retained for subsequent analyses (i.e., 55 from ambient-pCO₂ site and 44 from high-pCO₂ 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 for details).

2.2. 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 Permanovas and adopted a before-after-control-impact (BACI) design, where the settlement is considered the onset of exposure to local pCO₂ associated with the seep (impact) for the group of individuals settling in the high-pCO₂ site (see Electronic Supplementary for details).

Two factors are considered: life stage (2 levels: pre- and post-settlement) and site (2 levels: ambient and high-pCO₂). 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 influence of fish TL on otolith area. Covariate-adjusted means were derived using the emmeans package in R.

3. Results

In situ pCO₂ levels continuously measured at the high-pCO₂ site revealed that juveniles were exposed on average to 752 (±221) μatm pCO₂ (min: 429 μatm; max 1407 μatm), and to 428 (±47) μatm pCO₂ (min: 333 μatm; max 530 μatm) at the ambient-pCO₂ site (see Supplementary Table 1). Life stage and site significantly interacted in their effect on somatic growth (pseudo-F: 7.7747, p= 0.0066, see Supplementary table 1 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-pCO₂ 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 Supplementary table 2 for further details), and a greater average growth occurring at high-pCO₂ 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 high-pCO₂ (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 show that post-settlement otolith growth (normalized for post-settlement somatic growth) is 4.43% higher in ambient than in high-pCO₂ site (Figure 2).

4. Discussion

Our study highlights that in situ exposures to highpCO₂ levels is related to higher somatic and otolith growth rates of Symphodus ocellatus juvenile fish. This finding supports previous evidence mostly arising from laboratory studies carried out under stable pCO₂ conditions [4]. We explain the increased somatic growth under OA conditions by two non-mutually exclusive mechanisms: 1) the increased energetic costs to restore homeostatic balance stimulating appetite and increased foraging activity [17]; and 2) 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 that would finally increase the aragonite saturation state of the endolymph increasing otolith growth rates [18,19].
We show that individuals exposed to high-$p$CO$_2$ levels, in addition to displaying higher somatic growth rate, exhibit smaller otoliths at equal body length than individuals exposed to ambient-$p$CO$_2$. 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 [21].

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 fish auditory response of fish impairing hearing, navigation and balance [20].

However, given that our results are based on two sites (high and ambient-$p$CO$_2$) which, despite being only 500 m apart, could also differ for other environmental and biological features (e.g. food availability, competition, predation), alternative explanations could not be ruled out conclusively. Additional research would be thus required to conclusively elucidate the underlying process(es).

In conclusion, our study raises 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 [21], while smaller relative size of otoliths could impair auditory response of fishes, with potentially detrimental impacts on their survival [20].
Figure 1. Violin plots of a) somatic growth rate and b) otolith growth rate of individuals collected in ambient and high-pCO₂ 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 SE for ambient and high-pCO₂ respectively. Letters indicate statistically significant differences according to a t-test (p < 0.05).
Figure 2. Figure 2. Relationship between post-settlement otolith growth and post-settlement somatic growth of individual juveniles collected at a high-pCO$_2$ and ambient-pCO$_2$ site. Bands around regressions are the 95% confidence intervals. Insert: post-settlement otolith areas (ANCOVA-adjusted mean ± 1 SE) of juveniles from the high- and ambient-CO$_2$ sites.

References


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Author contributions. MM and CC conceived the study, experimental design and collected samples. ADF, AC, KS carried out otolith analyses. ADF and AC 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.

Data accessibility. Data are available as Electronic Supplementary material.

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Competing interests. We declare we have no competing interests.

Ethical statement. All experiments were carried out in accordance with institutional and national (law 116/1992) guidelines concerning the use of animals in research.