Science of the Total Environment 627 (2018) 95–103

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Transgenerational acclimation to seawater acidification in the Manila clam *Ruditapes philippinarum*: Preferential uptake of metabolic carbon

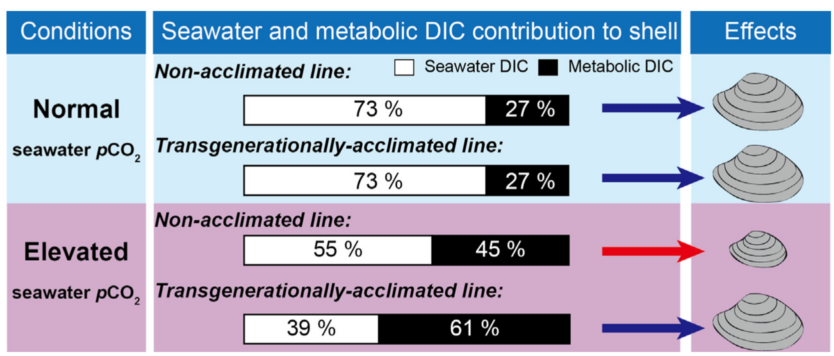
Liqiang Zhao [a,c,*], Feng Yang [b], Stefania Milano [d], Tiankun Han [b], Eric O. Walliser [a], Bernd R. Schöne [a]

[a] Institute of Geosciences, University of Mainz, Mainz 55128, Germany
[b] Engineering Research Center of Shellfish Culture and Breeding in Liaoning Province, Dalian Ocean University, Dalian 116023, China
[c] Atmosphere and Ocean Research Institute, The University of Tokyo, Chiba 277-8564, Japan
[d] Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig 04103, Germany

HIGHLIGHTS

- Rapid transgenerational acclimation can persist into adulthood.
- Stable carbon isotope analysis deciphers carbon sources of the shell.
- Transgenerational exposure elicits a large metabolic carbon contribution to shell carbonate.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 December 2017
Received in revised form 22 January 2018
Accepted 23 January 2018
Available online xxxx

Editor: D. Barcelo

Keywords:
Ocean acidification
Transgenerational effect
Shell growth
Metabolic carbon
Marine bivalve mollusks

ABSTRACT

Ocean acidification may interfere with the calcifying physiology of marine bivalves. Therefore, understanding their capacity for acclimation and adaption to low pH over multiple generations is crucial to make predictions about the fate of this economically and ecologically important fauna in an acidifying ocean. Transgenerational exposure to an acidification scenario projected by the end of the century (i.e., pH 7.7) has been shown to confer resilience to juvenile offspring of the Manila clam, *Ruditapes philippinarum*. However, whether, and to what extent, this resilience can persist into adulthood are unknown and the mechanisms driving transgenerational acclimation remain poorly understood. The present study takes observations of Manila clam juveniles further into the adult stage and observes similar transgenerational responses. Under acidified conditions, clams originating from parents reproductively exposed to the same level of low pH show a significantly faster shell growth rate, a higher condition index and a lower standard metabolic rate than those without prior history of transgenerational acclimation. Further analyses of stable carbon isotopic signatures in dissolved inorganic carbon of seawater, individual soft tissues and shells reveal that up to 61% of shell carbonate comes from metabolic carbon, suggesting that transgenerationally acclimated clams may preferentially extract internal metabolic carbon rather than transport external seawater inorganic carbon to build shells, the latter known to be energetically expensive. While a large metabolic carbon contribution (45%) is seen in non-acclimated clams, a significant reduction in the rate of shell growth indicates it might occur at the expense of other calcification-relevant processes. It therefore seems plausible that, following transgenerational acclimation, *R. philippinarum* can implement a less costly and more

* Corresponding author at: Institute of Geosciences, University of Mainz, Mainz 55128, Germany.
E-mail addresses: lzhao@aori.u-tokyo.ac.jp, liqiang@uni-mainz.de (L. Zhao).

https://doi.org/10.1016/j.scitotenv.2018.01.225
0048-9697/

efficient energy-utilizing strategy to mitigate the impact of seawater acidification. Collectively, our findings indicate that marine bivalves are more resilient to ocean acidification projected for the end of the century than previously thought.

1. Introduction

Rapid absorption of anthropogenic carbon dioxide (CO_2) by the ocean has significantly altered seawater carbonate chemistry, increasing the partial pressure of CO_2 (pCO_2) and bicarbonate ion (HCO_3^-) concentration and resulting in declines of pH and carbonate ions (CO_3^{2-}) (IPCC 2014). Ample evidence suggests that this ongoing process, termed ocean acidification (OA), can likely interfere with the calcification physiology of marine bivalves and many other marine calcifiers (Rodolfo-Metalpa et al. 2011; Gazeau et al. 2013; Parker et al. 2013), potentially impairing the ability to build skeletal structures and, in some extreme cases, causing dissolution of calcareous hard parts (Fitzer et al. 2014; Milano et al. 2016; Zhao et al. 2017a). Notably, detrimental OA effects are likely more pronounced at early life stages (especially pelagic larvae and newly settled juveniles) of marine bivalves (Scanes et al. 2014; Waldbusser et al. 2014; Thomsen et al. 2015; Frieder et al. 2017; Zhao et al. 2017b), thereby presumably influencing molluscan recruitment and population dynamics (Gobler and Talmage 2013).

In light of the impact of OA on marine bivalves, there is growing concern about the fate of this economically and ecologically important fauna in an acidifying ocean (Mabardy et al. 2015). Such concern has also promoted an increasing number of studies to test whether and to what extent bivalves are able to acclimate and adapt to near-future OA scenarios over few generations (Ross et al. 2016). Without exception, all three hitherto studied bivalve species used in transgenerational experiments, including the Sydney rock oyster *Saccostrea glomerata* (Parker et al. 2012, 2015), the blue mussel *Mytilus edulis* (Fitzer et al. 2014; Thomsen et al. 2017) and the Manila clam *Ruditapes philippinarum* (Zhao et al. 2017b), exhibit a positive transgenerational acclimation to OA projected by the end of the century. Evidently, such acclimation is crucial for marine bivalves to mitigate the impact of OA as it occurs over a very short period of time. However, the mechanisms underlying beneficial transgenerational effects are not well understood. Parker et al. (2015) found that the capacity of adult *S. glomerata* to regulate extracellular pH at elevated pCO_2 of 856 μatm is significantly improved following transgenerational acclimation, in line with the assumption by Zhao et al. (2017b) according to which *R. philippinarum* with a prior history of exposure to elevated pCO_2 of 980 μatm likely implements less costly and more ATP-efficient ion regulatory mechanisms to maintain acid-base homeostasis at the calcifying front. Moreover, transgenerational exposure of *M. edulis* to elevated pCO_2 of 1000 μatm might constrain shell mineralogy from bi-mineralic (aragonitic and calcitic) to mono-mineralic (calcitic) assemblages (Fitzer et al. 2014), which may be partly attributed to the fact that calcite is less susceptible to dissolution than aragonite (Mucci 1983). Evidently, a mechanistic understanding of how transgenerational exposure functions as a source of rapid acclimation requires more knowledge of the processes involved in shell formation.

At the end of the pelagic larval stage, bivalves start to build their shells in the extrapallial space (EPS), where the precipitation of calcium carbonate ($CaCO_3$) is governed by the $CaCO_3$ saturation state, and the crystallization of $CaCO_3$ is orchestrated by the organic matrix secreted from the outer mantle epithelium (Wheeler 1992). Given that the EPS is a microenvironment isolated from the ambient environment, external substrates used for shell mineralization such as calcium ions (Ca^{2+}) and dissolved inorganic carbon (DIC, the sum of CO_2, HCO_3^- and CO_3^{2-}) must be actively transported to the EPS, and the protons (H^+ ions) generated during the $CaCO_3$ deposition have to be excreted (reviewed in McConnaughey and Gillikin 2008). As seawater becomes acidic (i.e., H^+ concentration increases), the electrochemical gradient between the EPS and seawater will decrease which limits the ability of the bivalve to maintain pH homeostasis at the calcifying front. The rate of shell formation will thus decrease (Ries 2011). As reviewed by Hendriks et al. (2015), this is the generalized concept behind the majority of OA studies. Likewise, such concept may pave the way for elucidating the underlying mechanisms of rapid transgenerational acclimation in *S. glomerata* (Parker et al. 2015) and *R. philippinarum* (Zhao et al. 2017b), i.e., their capability to maintain acid-base homeostasis is substantially improved following transgenerational exposure to OA.

However, a fundamental basis largely lacking in our current knowledge is the speciation and dynamic variation of the DIC in the EPS - a key but poorly understood determinant of the carbonate chemistry and hence the acid-base status at the calcifying front and the rate of shell deposition. On the basis of the fact that OA has a fundamental impact on seawater carbonate chemistry, several reviews have recently emphasized, but hotly debated, the role of CO_2-driven DIC variability in constraining the response of shell calcification to OA (Bach 2015; Cyronak et al. 2015, 2016; Fassbender et al. 2016; Waldbusser et al. 2016). It is worth noting that all these studies appear to build upon the assumption that bivalves mainly use carbon from the DIC pool of the ambient water to precipitate $CaCO_3$, but none of them took into account the incorporation of metabolic carbon (C_M) – which is primarily composed of respired carbon (McConnaughey et al. 1997) – into shell carbonate. In the case of marine bivalves, the latter likely contributes approximately 5–37% of precipitated carbon (McConnaughey and Gillikin 2008), lending support to the assumption that bivalves may exert a tight control over carbonate chemistry at the calcifying front (Cyronak et al. 2015). In particular, given the highly variable nature of metabolic responses of the bivalve under acidified conditions (which can be positive, neutral or negative; summarized in Gazeau et al. 2013), it is reasonable to assume that OA may induce a significant shift of the calcifying carbon pool sourced from seawater DIC and C_M (Waldbusser et al. 2013; Brunner et al. 2016). Therefore, an explicit determination of the respective proportion of seawater DIC and C_M used for $CaCO_3$ precipitation will help to better understand how the calcifying physiology of marine bivalves responds and acclimates to OA.

The Manila clam *R. philippinarum* is natively distributed along the Pacific coast of Asia, where it is one of the most important bivalve species for local fisheries (FAO, 2012). Due to its high adaptability to various coastal environments, this species has been intentionally introduced and now widely cultivated in Europe and North America (see Cordero et al. 2017 for a short summary). However, the introduction and expansion of the nonindigenous Manila clam may pose a potential threat for native biodiversity. In several European coastal waters, for example, the ecological niche of the indigenous grooved carpet shell clam *Ruditapes decussatus* has been almost completely occupied by *R. philippinarum* (Bidegain and Juanes 2013), thereby resulting in substantial loss of native biodiversity and ecological services.

A recent study by Xu et al. (2016) demonstrated the impact of OA on the reproduction of *R. philippinarum*, one of the most important determinants of the population maintenance of this species. In particular, Manila clams inhabit highly variable estuarine and coastal waters, where the progress of OA occurs far more rapidly than in the open ocean due to intensive human activities and local oceanographic processes (Duarte et al. 2013). Therefore, to safeguard future fisheries of this important shellfish species, it is becoming imperative to make predictions about the capacity and evolutionary rate of *R. philippinarum* to acclimate and adapt to OA. Exposure of adult Manila clams to reduced