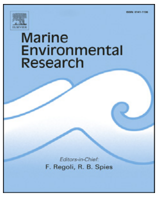
Marine Environmental Research 125 (2017) 110–117

Contents lists available at ScienceDirect

Marine Environmental Research

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



Low pH conditions impair module capacity to regenerate in a calcified colonial invertebrate, the bryozoan *Cryptosula pallasiana*

Chiara Lombardi [a, *, 1], Paul D. Taylor [b, 1], Silvia Cocito [a], Camilla Bertolini [c, 2], Piero Calosi [c, d]

[a] ENEA Marine Environment Research Centre, PO Box 224, 19100 La Spezia, Italy
[b] Department of Earth Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK
[c] Marine Biology and Ecology Research Centre, Plymouth University, Drake Circus, Plymouth, PL4 8AA, UK
[d] Département de Biologie, Chimie et Géographie, Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski, QC G5L 3A1, Canada

ARTICLE INFO

Article history:
Received 4 September 2016
Received in revised form
13 December 2016
Accepted 5 February 2017
Available online 7 February 2017

Keywords:
Phenotypic plasticity
Resource allocation
Climate change
Ocean acidification
Modular organism
Bryozoa

ABSTRACT

Many aquatic animals grow into colonies of repeated, genetically identical, modules (zooids). Zooid interconnections enable colonies to behave as integrated functional units, while plastic responses to environmental changes may affect individual zooids. Plasticity includes the variable partitioning of resources to sexual reproduction, colony growth and maintenance. Maintenance often involves regeneration, which is also a routine part of the life history in some organisms, such as bryozoans. Here we investigate changes in regenerative capacity in the encrusting bryozoan *Cryptosula pallasiana* when cultured at different seawater pCO_2 levels. The proportion of active zooids showing polypide regeneration was highest at current oceanic pH (8.1), but decreased progressively as pH declined below that value, reaching a six-fold reduction at pH 7.0. The zone of budding of new zooids at the colony periphery declined in size below pH 7.7. Under elevated pCO_2 conditions, already experienced sporadically in coastal areas, skeletal corrosion was accompanied by the proportional reallocation of resources from polypide regeneration in old zooids to the budding of new zooids at the edge of the colony. Thus, future ocean acidification can affect colonial organisms by changing how they allocate resources, with potentially profound impacts on life-history patterns and ecological interactions.

1. Introduction

The production of multiple copies of a basic body form characterizes clonal modular organisms, whose repeated units may separate or remain connected during their lifespans (Harper, 1977). Modular organisms are widespread, include both plants and colonial animals, and share many similar reproductive, defensive, competitive and life history traits. Marine invertebrates that grow as modular colonies, such as corals, hydroids, bryozoans and ascidians, jointly dominate the profuse sessile communities encrusting solid surfaces in the sea, and form a major component of global marine biodiversity.

The modules of colonial animals – zooids – are not always identical but may instead be polymorphic, meeting the various structural and functional needs of the colony, such as defence, feeding and sexual reproduction (Hughes, 1989). Communication and cooperation between individual zooids, which may involve neural connectivity, enables colonies to behave as integrated functional units and allows the translocation of substances/metabolites to facilitate feeding, growth, reproduction, response to threats, and recovery from localized damage (Mackie, 1986; Stuefer et al., 2004).

An important element of maintenance in modular organisms is regeneration. In fact, degeneration-regeneration cycles are characteristic of many modular organisms and enable: (1) replacement of ageing zooids; (2) excretion of waste products; and (3) shedding of fouling organisms (Hughes, 1989; Gordon, 1977). It is commonplace for the zooids of marine colonies to undergo degeneration-regeneration cycles, e.g. thecate hydroids, bryozoans and colonial ascidians (Crowell, 1953; Gordon, 1977; Berrill, 1935). In the

---

[*] Corresponding author. Marine Environment Research Centre ENEA, Via Forte Santa Teresa, 19032 Pozzuolo di Lerici (SP), Italy.
E-mail address: chiara.lombardi@enea.it (C. Lombardi).
[1] Joint first authors.
[2] Current addresses: School of Biological Sciences, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK.

http://dx.doi.org/10.1016/j.marenvres.2017.02.002
0141-1136/© 2017 Elsevier Ltd. All rights reserved.

exclusively colonial phylum Bryozoa, each feeding zooid possesses a polypide – the feeding structures and associated organs – that exhibits cycles of degeneration and regeneration in the majority of species. Degeneration of a bryozoan polypide results in the formation of a 'brown body', which is either expelled from the colony or retained in the coelomic cavity (Gordon, 1977; Hughes, 2005). Brown body formation can be triggered by adverse environmental factors, embryogenesis, or simply the accumulation of residual materials in the digestive and secretory cells of the stomach, causing the entire stomach to degenerate (Gordon, 1977). Tissues of the zooid that remain after polypide degeneration are used together with materials translocated from adjacent zooids to form a replacement polypide, a process known as polypide regeneration. Cycles of polypide degeneration-regeneration are rejuvenatory and extend the lifespans of individual zooids in bryozoan colonies (Hughes, 2005; Dyrynda, 1981).

Plasticity in module regeneration has the potential to improve the ability of a species to cope with the low pH conditions sporadically experienced by coastal organisms (Arnaud-Haond et al., 2012; Hofmann et al., 2011), as well as ocean acidification (OA) predicted to occur at a greater frequency and more chronically over the coming centuries (IPCC, 2014). Resulting from anthropogenically elevated levels of atmospheric carbon dioxide (CO_2), OA has been manifested by a drop of 0.1 units in average surface seawater pH, as well as a reduction in carbonate ion concentration during the past 150 years. According to expected fossil-fuel consumption, a further pH decline of 0.3–0.5 units (400 μatm–1000 μatm pCO_2) is predicted by 2100 (IPCC, 2014), and a cumulative drop of up to 0.7 units or more (540 μatm to *c.* 1990 μatm) by 2300 (Kawaguchi et al., 2013).

The aim of the present study is to investigate alterations in regenerative capacity in a calcifying colonial invertebrate under future ocean conditions. The cheilostome bryozoan *Cryptosula pallasiana*, cultured in a mesocosm for periods of up to four weeks at pH levels of 8.1 (current ocean), 7.7, 7.4 and 7.0, was used as a model organism to infer changes in the relative investment to maintaining existing zooids by polypide regeneration *vs.* the budding of new zooids. We here investigate: (1) frequency of polypide regeneration (regenerated polypides/total number of polypides); (2) changes in the number of 'active generations' of zooids (i.e. the number of rows of mature zooids in the active generation band involved in polypide cycles); and (3) changes in ontogenetic zonation (i.e. the relative extent of the budding band, active generation band and moribund/corroded zooid band) under different pH scenarios. Our results identify a previously unrecognised biological response – diminution of regenerative capacity – that may occur as a consequence of OA, with likely impacts on marine functional diversity.

2. Material and methods

2.1. Study species

The cheilostome bryozoan *Cryptosula pallasiana* (Moll, 1803) is an encrusting species forming sheet-like colonies comprising numerous zooids, each about 0.8 mm long, which feed by extending the tentaculate organ (lophophore) of the polypide, produce gametes and brood embryos. New zooids are added by budding at the colony periphery, leaving increasingly older zooids at greater distances from the growing edge. The calcareous skeleton of the zooid body walls comprises calcite and aragonite, with calcite having an intermediate/low content of $MgCO_3$ predominating (Poluzzi and Sartori, 1974; Smith et al., 2006). Polypides survive for 2–10 weeks in aquarium conditions, then completely regress in 6–17 d (Gordon, 1973). A new polypide may begin to form during the process of regression (Gordon, 1973). Widely distributed in the North Atlantic, Mediterranean and Black Sea, *C. pallasiana* inhabits littoral and shallow sublittoral environments (<50 m deep) and is a globally successful invasive fouling species in docks and harbours.

2.2. Biological material and experimental design

Colonies of *C. pallasiana* were collected from marinas in Brixham, Plymouth and Falmouth, southwest UK, during late summer 2012. In the laboratory, larvae released from the wild colonies were settled onto acetate sheets, grown to 20–50 zooids while fed daily with the microalgae *Isochrysis galbana* and *Rhinomonas reticulata*, and then excised and glued, two colonies per slide, to 76 × 38 mm microscope slides using cyanoacrylate adhesive (48 slides in total). The slides were then placed back in the original culture vats. Twelve days later, two initial batches of 16 slides each were transferred to the experimental apparatus described below and kept at constant temperature (15 °C) and at one of four pH levels: pH 8.1 as control (ambient) conditions, and 7.7, 7.4 and 7.0, to mimic the predictions of various models of future oceanic pH (Feely et al., 2004; IPCC, 2014). One of the initial batches of 16 slides was kept for one week (= '1-week Batch'), and the second batch was kept for four weeks (= '4-week Batch'). The third batch of 16 slides was introduced into the experimental apparatus on Day 8, replacing the 1-week Batch, and maintained for the next two weeks (= '2-week Batch'). The exposure times of one, two and four weeks used for the experiment were based on our experience of growth rates in the bryozoan culture system (Pistevos et al., 2011). Orthogonal designs were employed to test the null hypotheses that polypide regenerative capability (regenerated polypides/total number of polypides), the number of active zooid generations (i.e. the number of rows of mature zooids in the active generation band involved in polypide cycles) and ontogenetic zonation (i.e. the relative extent of the budding band, active generation band, and moribund/corroded zooid band) in *C. pallasiana* did not vary when exposed to lowered pH and after different lengths of time.

2.3. Determining polypide regeneration, active zooid generations and ontogenetic zonation

Polypide degeneration is the complete regression of the polypide within a zooid, leaving a residual brown body (which may subsequently be defecated by the new polypide), whereas regeneration is the reverse transition to restore a complete polypide (Fig. 1).

Just prior to introducing the colonies of *C. pallasiana* to the experiment (start: time 0 for the different batches), they were placed on sheets of graph paper and photographed using image capture and processing software (Infinity Analyze, Lumenera, Ottawa, Canada) connected to a digital camera (Infinity 1, Lumenera, Ottawa, Canada) attached to a microscope (MZ12, Leica, Heerbrugg, CH) at 0.8 x magnification. Further images of each colony were taken after 7 d for colonies of the 1-week Batch, after 14 d for colonies of the 2-week Batch, and after 21 and 30 d for colonies of the 4-week Batch. Following photographic recording at 21d, colonies from the 4-week Batch were put back into the experimental apparatus for the final week without being exposed to air or significant temperature or pH/pCO_2 fluctuations.

Polypide regeneration was here investigated only in the 4-week batch by comparing the states of the zooids at the start of the experiment and after three and four weeks. In order to quantify the frequency of polypide regenerations, zooids were separately numbered on digital images of the colonies; regeneration was scored for any zooid, previously lacking a polypide, in which a new polypide appeared either between week 1 and week 3 or between