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First description of polyp bail-out in cold-water octocorals under aquaria maintenance

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Abstract

Cnidarians, characterized by high levels of plasticity, exhibit remarkable mechanisms to withstand or escape unfavourable conditions including reverse development which describes processes of transformation of adult stages into early developmental stages with higher mobility. Polyp bail-out is a stress-escape response common among scleractinian species, consisting of massive detachment of live polyps and subsequent death of the mother colony. Here we describe two cases of polyp bail-out in the cold-water octocoral species *Acanthogorgia armata* and *Acanella arbuscula*. During maintenance in aquaria, specimens of both species presented coenosarc withdrawal and loss of sclerites, followed by detachment of intact polyps. This is a strong indication of reverse development which can be a very important strategy under stress conditions and has not been reported before in cold-water octocorals.

Key words

Reverse development, deep-sea, stress response, life history traits, cnidaria, alcyonacea

Introduction

Cnidarians, due to their structural simplicity and modular nature, are characterized by diverse and complex life histories (Jackson and Coates, 1986). Owing to their plasticity, cnidarians can employ a variety of mechanisms to survive unfavourable conditions. Such mechanisms include transformation into resting developmental stages with inert metabolic functions (Piraino et al., 2004) and reverse development of adults into earlier

developmental stages (Richmond 1985; Jackson and Coates, 1986) which might involve higher probabilities of dispersal and escape.

Reverse development has been reported in a number of hydrozoan (e.g. Bavestrello et al., 2000; Piraino et al., 2004), scyphozoan (e.g. Lesh-Laurie and Corriel, 1973) and anthozoan species (e.g. Pearse, 2000), which are able to create mobile planula-like forms by autonomizing adult parts, such as tentacles or even entire polyps, which subsequently create new colonies. In scleractinian corals the concept of reverse development appears, among others, in the process of polyp bail-out (first described by Goreau and Goreau, 1959; termed by Sammarco, 1982) described as an extreme response to stress conditions and involving massive detachment of living polyps very often followed by the subsequent death of the mother colony. Polyp bail-out has been described in scleractinian species both in tropical, e.g. *Pocillopora damicornis* (Shapiro et al., 2016; Fordyce et al., 2017) *Tubastraea coccinea* (Capel et al., 2014) and temperate waters, e.g. *Cladocora caespitosa* (Kruzic, 2007), *Astroides calycularis* (Serrano et al., 2017), highlighting their ability to alternate between soft bodied and calcifying forms. The ability of some scleractinians to survive without a skeleton has been suggested as a possible developmental pathway that allowed the taxa to persist throughout periods of extremely unfavourable conditions during their evolutionary history (the “naked coral hypothesis”, Medina et al., 2006; Kvitt et al., 2015), highlighting the potential importance of such processes as survival mechanisms in hostile conditions.

While bail-out processes have been reported for a number of scleractinian species, polyp bail-out has not been reported before for cold-water octocoral species. Here, we describe for the first time two distinct cases of polyp bail-out from aquaria observations

performed in two cold-water alcyonacean species, the holaxonian *Acanthogorgia armata* (Verrill, 1878) and the bamboo coral *Acanella arbuscula* (Johnson, 1862)

Materials & Methods

Three colonies of *Acanthogorgia armata* were collected from the “Coral Garden Hill” in the Pico-Faial channel in Azores (38° 29.374’N 028° 37.262’W) at 350 m deep with the manned submersible Lula (Rebikoff-Niggeler Foundation) in September 2009. Corals were transported in coolers and transferred to aquaria at the DeepSeaLab facilities, IMAR/DOP, within hours after collection. The aquaria facilities consisted of a continuous flow-through open system supplied from a reservoir containing sand-filtered water pumped from 5 m depth (salinity 36%) in an unpolluted bay (Cruz et al., 2010) in Horta, Azores. This seawater was further treated by UV-light (Vecton 600, TMC™) before distributed to aquaria. Aquaria were set up in a thermostatic room in darkness and seawater was chilled to match the average annual temperature experienced by corals in their natural habitat ($14 \pm 0.9^{\circ}\text{C}$) by cooling systems connected to temperature controllers. Specimens were maintained in a 35 L aquarium with seawater renewal of approximately 12 L per hour, mixed with submersible Iwaki pumps (4.5 W, 280 L h⁻¹) and were fed daily with frozen zooplankton and a liquid mixture of marine particles between 10 and 450 μm (Marine Active Supplement, Bentos Nutrition Maim, Vic, Spain). During the five month maintenance period, coral colour and polyp expansion were closely monitored while measurements of temperature and oxygen were performed daily.

Four colonies of *Acanella arbuscula* were collected from the Formigas Bank off the Azores (37° 12.140’N 024° 26.717’W and 37° 12.059’N 024° 27.317’W) at depths between 1094 and 1127 m, using the Remotely Operated Vehicle (ROV) Liropus 2000

(IEO), during the MEDWAVES cruise on board the Research Vessel (RV) Sarmiento de Gamboa in October 2016. Upon emersion, which lasted approximately 40 minutes, colonies were transferred into buckets with refrigerated seawater ($\sim 4^{\circ}\text{C}$) and subsequently into a 20 L tank where they were fixed on plastic bases with epoxy. The tank was kept inside a dark, thermoregulated room on-board with a stable temperature of $12.0 \pm 0.5^{\circ}\text{C}$ while renewal of the water was made daily with water collected from 1000 m with a CTD rosette and kept refrigerated at 4°C . Corals were fed every other day with the Marine Active Supplement described above. Maintenance of all specimens lasted for 4-5 days after collection. During this period, no measurements of aquaria parameters were possible, however, observations on the colour, polyp expansion and tissue state of coral specimens were made daily.

Results & Discussion

Colonies of *A. armata* did not show signs of stress during the first two months of maintenance (September, October) (Fig. 1A, B). By November 2009, the colour of the colonies changed from vibrant yellow to dark brown and polyps retracted (Figure 1C, 1D). Thereafter, sporadic coenosarc withdrawal (Figure 1C) and sclerite release, accompanied by polyp detachment was observed. These events coincided with an unexpected rise in water temperature from 14°C to 16.5°C , caused by short malfunction of the refrigerating system, accompanied by a decrease in oxygen saturation from an average of 85% to 70%. Coenosarc withdrawal continued progressively and disassociated polyps, solitary or in small aggregates, were found at the bottom of the aquarium. By January 2010, only small traces of coenosarc were left on the colony.

Detached polyps were negatively buoyant and contained sclerites, with exposed aboral sides, i.e. open and free of sclerites (Fig. 1F).

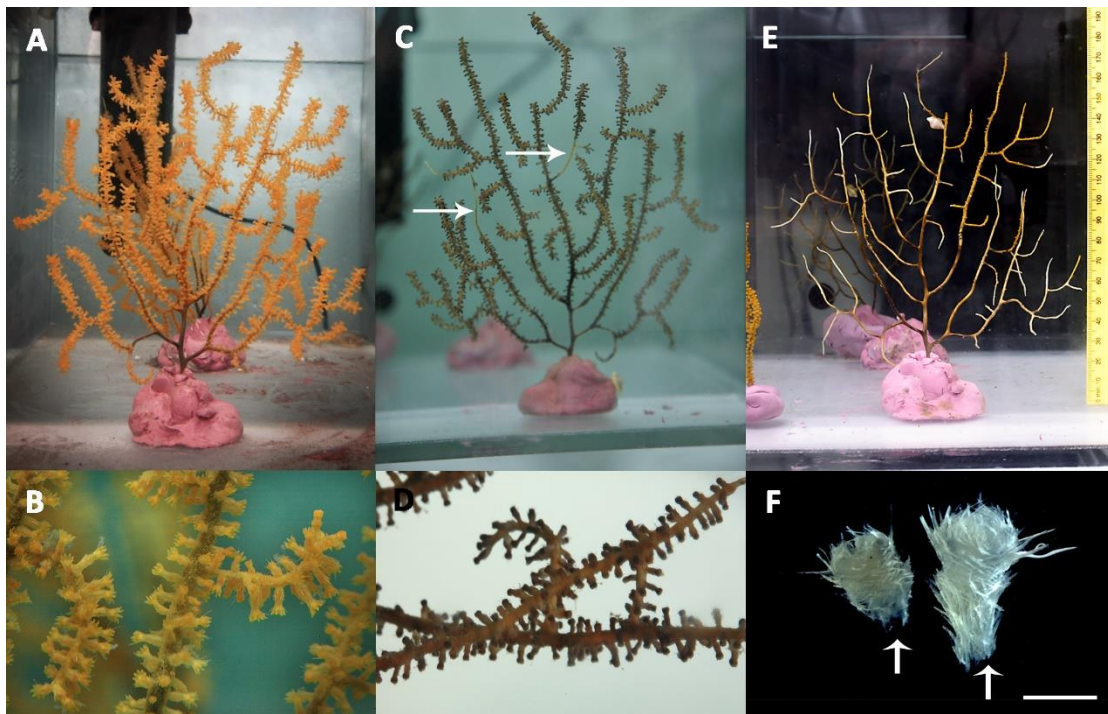


Figure 1: Full frame and close-up perspective of *Acanthogorgia armata* colonies during maintenance period: **a, b** colonies during the first 15 d, with vibrant colour and extended polyps; **c, d** colonies after 3 months, coloured dark brown with retracted polyps. Arrows indicate sporadic withdrawal of tissue; **e** colony after 5 months in the aquarium, with no visible tissue; **f** close-up image of detached polyps with exposed aboral sides (arrows). Scale bar: 1 mm

Colonies of *A. arbuscula* displayed increased mucus production immediately after collection which persisted during the whole period of maintenance (Fig. 2A). Twelve hours after transfer to the aquaria, polyps started to detach from mother colonies. The detached polyps had negative buoyancy and accumulated at the bottom of the tank or on rocks, either solitary or in small aggregations (Fig. 2A). Examination of the coral tissue revealed that polyps were released through the detachment of sclerites and tissue

from the lateral polyp side, which allowed a gradual separation of polyps from the skeleton (Fig. 2B). Despite polyp detachment, areas with open polyps were encountered throughout the colonies until the end of day 3. During day 4 and 5 colonies lost most of their polyps and tissue. Tissue detachment also led to the release of oocytes (Fig. 3) which are usually found at the polyp base (Beazley and Kenchington, 2012). Oocytes were subsequently encountered attached to mucus or floating at various levels of the water column. Although many of the released oocytes were most probably mature (diameter: 350-470 μm , Fig. 3) (Beazley and Kenchington, 2012), oocyte release seemed an inevitable result of polyp detachment rather than spawning. Collection and examination of free polyps during the first 48 hours of polyp detachment revealed mostly intact polyps with exposed aboral sides, covered with sclerites (Fig. 2C). However, most polyps collected on the third day were smaller and more spherical with a closed aboral side, discrete oral side and only a few incorporated sclerites, resembling sclerite-free spheres (Fig. 2D).

In both octocoral species, observations included tissue contraction and loss of sclerites, followed by polyp detachment. While free polyps of *A. armata* were covered by calcareous material throughout the observation period, spherical forms with fewer sclerites were found in *A. arbuscula*, indicating the existence of a process that leads to the transformation of intact free polyps to sclerite-free spheres. These observations are consistent with the description of polyp bail-out in scleractinians, including partial mortality of the coenosarc before polyp dissociation resulting in free, usually negatively buoyant polyps devoid of calcareous material (e.g. *Astroides calycularis*, Serrano et al., 2017; *Tubastraea coccinea*, Capel et al., 2014).

Free ball-like polyp forms have been reported by several studies on polyp bail-out in scleractinians (e.g. Domart-Coulon et al., 2004; Kvitt et al., 2015; Serrano et al., 2017).

Some of these studies reported subsequent settlement (e.g. Kvitt et al., 2015) which was not observed in the present study. However, maintenance of cold-water octocorals in aquaria has been proven demanding, contrary to the widespread husbandry of tropical and more recently cold-water scleractinians (Orejas et al., in press). The difficulties in

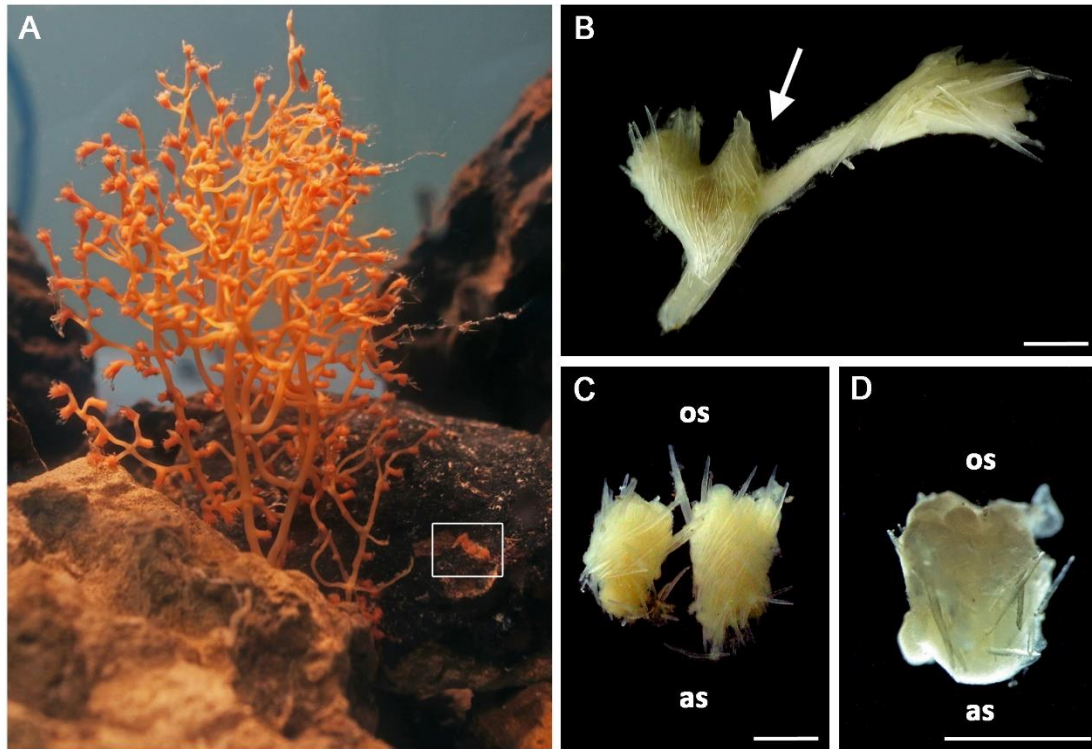


Figure 2: Polyp bailout in specimens of *Acanella arbuscula*: **a** live colony with mucus nets and expelled polyps accumulating on adjacent rocks (white rectangle) on the second day of maintenance; **b** polyp in the procedure of detachment, with detached tissue and sclerites on the lateral side (arrow); **c** expelled polyps covered with sclerites, open oral sides and exposed aboral sides, collected during the first 2 d of maintenance; **d** sphere-like form of an expelled polyp with a few sclerites attached, collected on the third day, *os* oral side, *as* aboral side. Scale bars: 1 mm

maintaining cold-water octocorals are further highlighted by the fact that larvae settlement in aquaria has been only observed in very few cold-water octocoral species so far (Sun et al., 2010; 2011). Furthermore, studies on larval behaviour of the

scleractinian cold-water coral *Lophelia pertusa*, more commonly used in aquaria experiments, reported planulae not settling after 10 months of aquaria maintenance (Strömberg et al., 2017). Such scarcity of information limits our understanding regarding settlement and maintenance requirements of these species, making it possible that the created aquaria conditions were not ideal for resettlement of polyps after polyp bail-out.

Polyp bail-out, as a stress response, has been associated with a variety of inducing factors such as low food availability and use of closed aquaria systems (Serrano et al., 2017), temperature stress (Kružić, 2007; Fordyce et al., 2017), salinity (Shapiro, 2016) and pH changes (Kvitt et al., 2015) as well as competition with macroalgae (Lee et al., 2012). Although rapid temperature and oxygen changes most likely contributed to the stress response by *A. armata*, a combination of factors might have caused polyp bail-out in the case of *A. arbuscula*, including thermal stress during the long ascent of the ROV from deep grounds (1094-1127 m), rapid pressure change and maintenance in closed system which might have prevented oxygen circulation and clearance of metabolic products such as carbon dioxide, ammonia and nitrite (e.g. Parent and Morin, 2000; Crab et al., 2007).



Figure 3: Oocytes of the species *Acanella arbuscula*, encountered in the water column and/or attached to mucus nets released by colonies during maintenance in aquaria. Scale bar: 60 μm

To our knowledge, this is the first record of a polyp bail-out response in cold-water octocoral species. Similar responses such as fragment detachment (Dahan and Benayahu, 1997) and pinnitomy (Gohar, 1940), have been recorded in shallow octocoral species, however both studies report these as frequent phenomena and modes of asexual reproduction rather than stress induced responses. The existence of polyp bail-out in the two cold-water alcyonacean species described herein suggest that this behaviour might be more common than previously thought among coral species. Moreover, the transformation of sessile adult stages into ball-like forms in *A. arbuscula* can be considered a form of reverse development, highlighting the existence of such developmental pathways not only in scleractinian but also in octocoral species. However, it is unclear if these traits are also shared with deep-water scleractinian and shallow-water octocoral species. As unravelling the evolutionary relationships of classes within the phylum Cnidaria has been challenging (McFadden et al., 2006; Zapata et al., 2015, Kayal et al., 2018), further studies are needed to clarify the origin

of reverse development and polyp bail-out. Although understudied, such mechanisms might be important in possible unfavourable future conditions caused by increasing anthropogenic activities and climate change. More studies are essential to determine the extent to which such behaviours are present in coral species both in shallow and deep environments, describe the underlying physiological processes and investigate the triggering factors behind them.

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Conflict of interest statement and compliance with Ethical Standards

On behalf of all authors, the corresponding author states that there is no conflict of interest. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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