Marine Environmental Research xxx (2015) 1–13

Contents lists available at ScienceDirect

Marine Environmental Research

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

Adapted to change: The rapid development of symbiosis in newly settled, fast-maturing chemosymbiotic mussels in the deep sea

Sven R. Laming [a, b, *], Sébastien Duperron [a, c], Sylvie M. Gaudron [a, 1], Ana Hilário [b], Marina R. Cunha [b]

[a] Sorbonne Universités, Université Paris 06, UMR7208 Laboratoire biologie des organismes et écosystèmes aquatiques (UPMC CNRS MNHM IRD CAEN), 7 quai St Bernard, 75005 Paris, France
[b] Departamento de Biologia and CESAM, Universidade de Aveiro, Campus Universitario de Santiago, 3810-193 Aveiro, Portugal
[c] Institut Universitaire de France, Paris, France

ARTICLE INFO

Article history:
Received 1 December 2014
Received in revised form
24 July 2015
Accepted 25 July 2015
Available online xxx

Keywords:
Bathymodiolinae
Bone
Cow
Marine
Symbiosis
Mussels
Development
Canyon
Colonisation
Ephemeral
Chemosynthesis

ABSTRACT

Symbioses between microbiota and marine metazoa occur globally at chemosynthetic habitats facing imminent threat from anthropogenic disturbance, yet little is known concerning the role of symbiosis during early development in chemosymbiotic metazoans: a critical period in any benthic species' life-cycle. The emerging symbiosis of *Idas* (*sensu lato*) *simpsoni* mussels undergoing development is assessed over a post-larval-to-adult size spectrum using histology and fluorescence *in situ* hybridisation (FISH). Post-larval development shows similarities to that of both heterotrophic and chemosymbiotic mussels. Data from newly settled specimens confirm aposymbiotic, planktotrophic larval development. Sulphur-oxidising (SOX) symbionts subsequently colonise multiple exposed, non-ciliated epithelia shortly after metamorphosis, but only become abundant on gills as these expand with greater host size. This wide-spread bathymodiolin recorded from sulphidic wood, bone and cold-seep habitats, displays a suite of adaptive traits that could buffer against anthropogenic disturbance.

1. Introduction

The growing scarcity and increasing extraction costs of consumable resources both on land and in shelf seas, has generated renewed commercial interest in the exploration and exploitation of deeper marine resources (Ramirez-Llodra et al., 2011). Communities identified to be under imminent threat include submarine benthos, which often include an organic-fall component. Organic falls provide localised influxes of labile organic matter and nascent substrata to the seabed that develop into highly ephemeral, ecologically complex habitats (e.g. megafaunal remains, vegetative debris, Smith, 2006; Bernardino et al., 2010; Laurent et al., 2013), however the conservation status of large-sized organic falls is one of the most poorly defined. In order to better understand the risks that human activities might pose to organic-fall habitats, there is an immediate need to first assess the degree of adaptability displayed by their associated communities.

Organic falls undergo physical and chemical transitions during degradation, culminating in a 'sulphidic stage', where the activity of sulphate-reducing bacteria drives the net efflux of sulphides (Treude et al., 2009; Laurent et al., 2013). In carcasses, the transitional process includes the breakdown of flesh by a combination of microbial degradation, microphages and mobile scavengers, the release of sulphides during the microbial decomposition of lipid-rich marrow, and the colonisation of exposed skeleton by sedentary symbiotic metazoans (reviewed in Smith, 2006). These can host heterotrophic symbionts, as in the posterior, root-like

---

* Corresponding author. Sorbonne Universités, Université Paris 06, UMR7208 Laboratoire biologie des organismes et écosystèmes aquatiques (UPMC CNRS MNHM IRD CAEN), 7 quai St Bernard, 75005 Paris, France.

E-mail address: svenlaming@googlemail.com (S.R. Laming).

[1] Present address: UMR8187 Laboratoire d'Océanologie et de Géosciences, Station marine de Wimereux, 28 Avenue Foch, 62930 Wimereux, France.

http://dx.doi.org/10.1016/j.marenvres.2015.07.014

Please cite this article in press as: Laming, S.R., et al., Adapted to change: The rapid development of symbiosis in newly settled, fast-maturing chemosymbiotic mussels in the deep sea, Marine Environmental Research (2015), http://dx.doi.org/10.1016/j.marenvres.2015.07.014

trophosome of bone-eating polychaete worms (genus *Osedax*, Katz et al., 2011), or chemosynthetic symbionts, such as those associated with the gill filaments of bone-colonising mussels (e.g. Southward, 2008; Fujiwara et al., 2010).

The emission of sulphides, H_2, CO_2, and – at times – alkanes, are features of many reducing habitats. Consequently, at higher taxonomic levels certain chemosymbiotic organisms are pervasive. The mussel subfamily Bathymodiolinae is almost ubiquitous across vents, seeps and organic falls globally (Duperron, 2010) and thus represents a keystone taxon. The majority of adult Bathymodiolinae host single or dual symbioses within (i.e. intracellular) or upon (i.e. extracellular) their gill filaments, typically involving chemoautotrophic bacteria, methylotrophic bacteria, or both (Dubilier et al., 2008), with some rare exceptions (no symbionts: *Idas argenteus* Ockelmann and Dinesen, 2011; Rodrigues et al., 2015, multiple symbioses: *Bathymodiolus heckerae* and *Idas modiolaeformis*, Duperron et al., 2007; Duperron et al., 2008). In at least some species, these symbioses have been demonstrated to provide some or all of the host's carbon and energy requirements (e.g. Riou et al., 2008). Bathymodiolins that colonise decomposing bones and plants tend to be smaller in size than other mytilid species (Lorion et al., 2013) and almost invariably possess symbiotic bacteria in their gill tissues (see Duperron et al., 2013 for Atlantic and Mediterranean species). Although most symbioses in these small-sized mussels are extracellular, some evidence for active host assimilation exists (e.g. *Idas washingtonius*, Deming et al., 1997).

One such small-sized species, "*Idas*" *simpsoni* (quotations indicate *sensu lato*), demonstrates a remarkable capacity to colonise diverse substrates under varying reducing conditions, having first been discovered on megafaunal skeletons (earliest: Marshall, 1900), on sunken wood (earliest: Marshall, 1901), and more recently on oil-drill cuttings (Hartley and Watson, 1993) and carbonate crust at a hydrocarbon seep (Ritt et al., 2012: identified as "*I.*" *simpsoni* post-publication by Génio et al., 2014; based on molecular data from Thubaut et al., 2013b). These habitats range from the North Atlantic to the Marmara Sea (Mediterranean) and from 73 to 1120 m in depth. "*Idas*" *simpsoni* therefore appears to be well-adapted to the varying conditions found at reducing habitats, though limited evidence has been accumulated to confirm why this is the case. This level of habitat flexibility is very rare in larger bathymodiolin mussels and uncommon in smaller species, excluding *I. washingtonius* and *I. modiolaeformis* (Rodrigues et al., 2015). In *I. modiolaeformis*, a small-sized bathymodiolin that colonises sunken wood and cold seeps, multiple habitat use almost certainly relates to this species metabolically diverse extracellular symbioses (with up to six distinct bacterial phylotypes identified on the gill filaments of one individual, Duperron et al., 2008). In contrast, despite colonising several habitat types where reduced fluid composition are expected to differ considerably, "*I.*" *simpsoni* is only known to harbour extracellular, gill-associated SOX symbionts (e.g. at a seep, Ritt et al., 2012; on mammal bones, Génio et al., 2014; on wood, SR Laming unpublished data), nestled between microvilli that border the gill bacteriocytes (Southward, 2008). A single record of Bacteroidetes (CFB) bacterium also exists, however its localisation remains unknown (molecular data only, Génio et al., 2014). Despite lower symbiont diversity, "*I.*" *simpsoni* has recently been recorded at greater densities than *I. modiolaeformis* where both species co-occur on sunken wood (SR Laming, unpublished data), suggesting that different competitive factors must exist other than the metabolic diversity of symbioses.

The acquisition of symbionts in many bathymodiolins appears to be horizontal (i.e. not inherited vertically from the parental germ-line, Bright and Bulgheresi, 2010), based on an overall lack of symbiont-host co-speciation (Won et al., 2003), the absence of symbionts in adult gonads of at least one species (*I. modiolaeformis*, Gaudron et al., 2012) and the ongoing incorporation of symbionts from external sources (Le Pennec et al., 1988; Wentrup et al., 2014). Accordingly, infection is believed to be environmental via 'free-living' bacteria, lateral from a proximal host (including conspecifics), or both. Identifying the moment at which symbionts first appear in the lifecycle remains challenging however (e.g. Salerno et al., 2005; Wentrup et al., 2013), though the initial symbiont-colonisation phase has been demonstrated in juvenile *I. modiolaeformis* (Laming et al., 2014). Even though early symbiont acquisition and development might provide a competitive angle for "*I.*" *simpsoni* over *I. modiolaeformis*, it is not known when this species' symbiotic association begins – be it before, during, or following settlement and metamorphosis (the latter being true of *I. modiolaeformis*, Laming et al., 2014) – and whether these associations develop further with host growth, as in most other bathymodiolins examined (Salerno et al., 2005; Wentrup et al., 2013; Laming et al., 2014), or remain unchanged (e.g. Streams et al., 1997). More generally, early life-history stages remain poorly understood in most chemosymbiotic bathymodiolin species save for a handful of studies (e.g. Berg, 1985; Arellano and Young, 2009; Génio et al., 2014; Laming et al., 2014). Limited data for "*I.*" *simpsoni* suggest a propensity for semi-continuous and abundant larval supply (Ritt et al., 2012; Génio et al., 2014) and sizes at first maturity equivalent to <5% of the species' maximum size (mature at shell length [SL] 1.8 mm, Génio et al., 2014; maximum SL 45 mm, Warén and Carrozza, 1990).

In light of current and future threats to reducing habitats and the general lack of biological data during the early life-history of bathymodiolins, the current study examined a size-spectrum of "*I.*" *simpsoni* specimens in order to 1) characterise the anatomical development of juvenile "*I.*" *simpsoni* up to maturity; 2) identify whether post-metamorphic "*I.*" *simpsoni* mussels are already symbiotic and if not, at what sizes bacteria first appear; 3) describe how bacterial distributions vary as the association develops with increasing host size, and ultimately; 4) establish whether features of early life-history and adult biology in "*I.*" *simpsoni* might influence its ongoing survival in increasingly disturbed environments.

2. Methodology

2.1. Sampling and processing

In 2011, five bovine carcasses attached to concrete ballast were deployed at 1000 m depth in the Setúbal Canyon, located on the western Portuguese margin (38°16.85′N, 09°06.68′W). Details of the experiment may be found in Hilário et al. (in press). Some intact bone remains, colonised by a diverse array of fauna including numerous "*I.*" *simpsoni*, were recovered 18 months later (Nov. 2012 by ROV *Luso*, details in Génio et al., 2014; Hilário et al., in press). A random, size-stratified selection of these mussels from post-settlement to adult was used in the current study, fixed in 4% formaldehyde (in filtered seawater) for 2–4 h and preserved in 96% ethanol following serial transfer (70%, 80%, 96%, stored at room temperature).

2.2. Shell and preliminary soft tissue analysis

Shell dimensions recorded were for the prodissoconch I – the earliest-formed larval shell (PdI, n = 24), the prodissoconch II – the larval shell of the settling pediveliger (PdII, n = 38), and for entire specimens (i.e. including any deposition of juvenile 'dissoconch' shell, *n* = 38). Individuals were measured and photographed using a calibrated, camera-mounted dissection microscope (Nikon Elements, Japan). Shell heights (SHs) x lengths (SLs) of PdII and whole shells were measured according to Laming et al. (2014). PI SL was measured parallel to the vestigial provinculum (SH not taken). Shell

Please cite this article in press as: Laming, S.R., et al., Adapted to change: The rapid development of symbiosis in newly settled, fast-maturing chemosymbiotic mussels in the deep sea, Marine Environmental Research (2015), http://dx.doi.org/10.1016/j.marenvres.2015.07.014