

# Climate Change and Shell-Boring Polychaetes (Annelida: Spionidae): Current State of Knowledge and the Need for More Experimental Research

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**Abstract.** Anthropogenic climate change is considered to be one of the greatest threats facing marine biodiversity. The vast majority of experimental work investigating the effects of climate change stressors on marine organisms has focused on calcifying organisms, such as corals and molluscs, where cross-generational phenotypic changes can be easily quantified. Bivalves in particular have been the subject of numerous climate change studies, in part because of their economic value in the aquaculture industry and their important roles as ecosystem engineers. However, there has been little to no work investigating the effects of these stressors on the symbionts associated with these bivalves, specifically, their shell-boring polychaete parasites. This is important to understand because climate change may shift the synergistic relationship between parasite and host based on the individual responses of each. If such a shift favors proliferation of the polychaete, it may very well facilitate extinction of host bivalve populations. In this review I will (i) provide an overview of research completed thus far on the effects of climate change stressors on shell-boring polychaetes, (ii) discuss the technical challenges of studying these parasites in the laboratory, and (iii) propose a standardized framework for carrying out future *in vitro* and *in vivo* climate change experiments on shell-boring polychaetes.

## Introduction

Marine ecosystems face numerous threats, such as pollution, invasive species, and anthropogenically driven climate change. Of these, climate change, in particular, is unique be-

cause it can alter all levels of biological life, from genes to ecosystems (Brierley and Kingsford, 2009; Henson *et al.*, 2017). The most critical environmental perturbations that are expected to impact biotic communities are elevated water temperatures and more acidic environments, driven by increases in  $p\text{CO}_2$  (partial pressure of carbon dioxide) concentrations (Henson *et al.*, 2017). Experimental studies on marine invertebrates have played a central role in understanding the biological impacts of these stressors on marine life (Byrne and Przeslawski, 2013; Byrne *et al.*, 2020). This is partly due to the wide variety of calcifying species that exist within this diverse group of animals, whose impacts can be measured directly through experimental manipulation of abiotic factors (Rodolfo-Metalpa *et al.*, 2011). Studies on a number of calcifying species have all shown that experimentally induced  $\text{CO}_2$  and temperature increases can elicit both species-specific and life-history stage-specific effects (Byrne and Przeslawski, 2013). For example, in molluscs, multifactorial studies incorporating elevated temperature and  $p\text{CO}_2$  concentrations have shown disruption of the biomineralization process of adult and juvenile oysters, resulting in reduced hardness and fracture toughness of the shells, ultimately making them more vulnerable to predation (Kurihara, 2008). Larval stages are even more vulnerable to these stressors, and a number of studies have found lower survivorship and suppressed growth rates of larvae reared under near-future warming and acidified conditions (Kurihara *et al.*, 2007; Kurihara, 2008; Byrne, 2010; Gazeau *et al.*, 2010; Talmage and Gobler, 2011; Byrne and Przeslawski, 2013). Because of the dynamic nature of climate change, for some species interactive effects between lower pH and elevated temperatures have also been detected, where one stressor inhibits the impacts of another (Brennan *et al.*, 2010). For example, Davis *et al.* (2013) found that embryos of two species of gastropods reached mortality rates of 100% under acidified conditions and low

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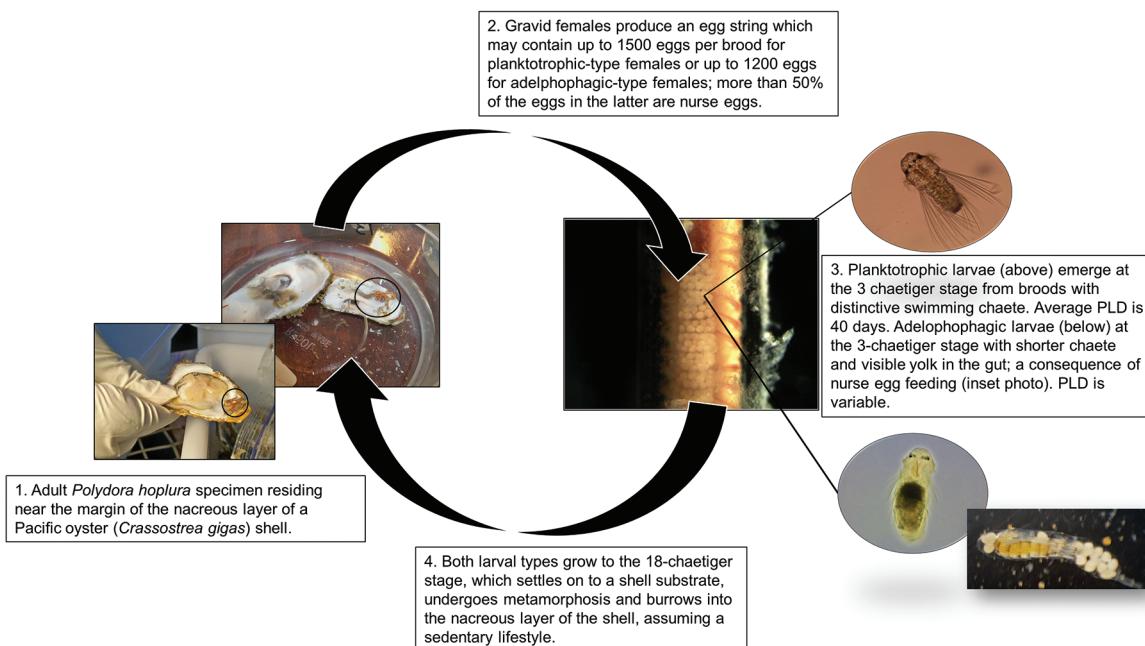
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Abbreviations:  $p\text{CO}_2$ , partial pressure of carbon dioxide; PLD, planktonic larval duration.

to moderate temperatures. However, this rate was significantly lowered at elevated temperatures, indicating a suppressive effect of temperature on pH, which, in the most extreme case, could be the difference between the persistence or extirpation of a population. Finally, long-term experimental studies that investigate transgenerational effects (also known as transgenerational plasticity) of these stressors can introduce an added and more realistic layer of complexity in understanding the impacts of climate change on marine organisms (Ross *et al.*, 2016). Transgenerational effects are important because the responses of early life-history stages (*e.g.*, embryos and larvae) may influence the success of adults both within a single generation and across multiple generations (Ross *et al.*, 2016; Byrne *et al.*, 2020), which in turn would determine long-term acclimatization and adaptation of the species.

The scientific literature is now filled with more than 20 years' worth of important experimental research on multi-factorial stressors and their impacts on marine molluscs. However, one overlooked issue that has garnered virtually zero attention is the impacts of climate change on molluscan symbionts, specifically, shell-boring polychaetes. These polychaetes are known to burrow into the nacreous layer of molluscan shells, such as bivalves and gastropods, where they assume a sedentary lifestyle as adults (Blake, 1969; Marshall and Day, 2001; Simon and Sato-Okoshi, 2015; Waser *et al.*, 2020). Early studies on *Polydora websteri* found that worms initiated the boring process by secreting an acidic mucus (Haigler, 1969), although the exact mechano-chemical mechanism is still unknown (Sato-Okoshi, 1997). As the primary dispersal agent they utilize a free-living larval stage, which

seeks out other molluscan hosts; then they settle on their shell, metamorphose, and burrow into the shell (David *et al.*, 2014) (Fig. 1). Alternatively, some larvae—specifically, short-lived lecithotrophic and adelphophagous larvae—may settle in their natal habitat, resulting in multiple generations of polychaetes infecting the same oyster population (Blake and Arnofsky, 1999; David *et al.*, 2014). Heavy infection stimulates shell regeneration, which takes the form of mud blisters, and this prevents the worm from burrowing deeper into the shell near the mantle. Since additional calcium carbonate secretion requires an input of energy from the mollusc, there is an energetic trade-off, where growth and tissue condition are compromised. Like all marine invertebrates, shell-boring polychaete worms are expected to be affected by elevated temperature and  $p\text{CO}_2$  levels. However, there are virtually no studies that have explicitly explored how such stressors will influence the life-history patterns of these symbiotic polychaetes, both as an individual unit and as a symbiotic unit living within the molluscan shell. This is important to consider because if these shell borers exhibit greater adaptive potential than their molluscan hosts under climate change stressors, there could be a two-pronged attack, where the molluscan host will have to confront not only the direct effects of climate change but also the indirect pathogenic effects associated with a more physiologically tolerant parasite. For example, elevated  $p\text{CO}_2$  levels are expected to reduce aragonite saturation state ( $\Omega$ ) to less than 3 in surface waters (IPCC, 2013), causing disruptions in the biomineralization process and ultimately resulting in dissolution of molluscan shells. If infestation rates by shell borers were to remain unchanged under such conditions, this



**Figure 1.** Simplified life cycle of a representative shell-boring polydorid, *Polydora hoplura*. PLD, planktonic larval duration.

will have severe implications for both the global aquaculture industry (worth more than 20 billion USD) and marine ecosystems in general, because bivalves serve as important ecosystem engineers and, therefore, are crucial for maintaining marine biodiversity (van der Schatte Olivier *et al.*, 2020). The negative effects of shell-boring polychaetes on commercial molluscs include decreased growth (resulting in smaller sizes at maturity) and poor tissue condition, both of which result in commercial losses for farmers and decreased profitability of farms (Nel, 1996; McDiarmid *et al.*, 2004; Simon and Booth, 2007; Read, 2010; Williams *et al.*, 2016). For example, in South Africa, a single farm was forced to cull 500,000 highly prized abalone (*Haliotis midae*) as a result of polydorid infestation (Simon *et al.*, 2009). In the wild, heavy infestation by shell borers can significantly weaken the structural integrity of the calcareous matrix, leading to increased mortalities, which in turn may threaten valuable oyster reef habitats that help enhance biodiversity and water quality in critical hotspots (David, 2020). Therefore, a thorough understanding of how this parasitic interaction will fare in a warmer and more acidic ocean is crucial from both an economic standpoint and an ecological standpoint. To address these issues, experimental studies on shell-boring polychaetes under near-future climate change scenarios are desperately needed. Indeed, a recent review by Byrne *et al.* (2020) echoed this sentiment by commenting on how poorly studied polychaetes are in general, with regard to understanding multigenerational plasticity in response to climate change stressors.

The overarching goal of this review is to propose a standardized framework for carrying out such investigations. To accomplish this goal, I will first provide a review of the current state of knowledge on shell-boring polychaetes and their response to changes in abiotic stressors associated with climate change, specifically, temperature and CO<sub>2</sub>-induced acidification. I will then discuss specific examples of other symbioses and their responses to climate stressors; these can provide some insights into what we could expect from the polydorid-mollusc system. Finally, I will discuss the challenges of culturing polydorid parasites in a laboratory setting and will propose an experimental framework using a combined *in vitro* and *in vivo* approach for overcoming these challenges.

### Biology of Shell-Boring Polychaetes

With more than 20,000 species described thus far, polychaetes are by far one of the largest groups of marine animals (Read and Fauchald, 2018). While many groups serve as important members of infaunal communities, where they play key ecological roles in benthic food webs (Levin, 1984; Ambrose, 1991; Hutchings, 1998), some species have adopted an interesting symbiotic lifestyle that can be best thought of as parasitic *sensu lato* (Radashevsky and Pankova, 2013). At least 95% of these parasitic polychaetes can be found within a single family, the Spionidae, and, more specifically, within a

species complex known as the *Polydora* complex (Radashevsky *et al.*, 2006; Simon *et al.*, 2006; Simon, 2011). The *Polydora* complex consists of the genus *Polydora* and nine other genera, also known collectively as the polydorids (Blake, 1996). In this review, the terms “shell borer” and “polydorid” will be used synonymously while recognizing that there are species within this group that are not *bona fide* shell borers. Polydorids are distinguished from other polychaetes by possessing modified chaetal spines on an enlarged fifth chaetiger (the most recent taxonomic revision of the group is provided by Blake, 1996). For shell borers, these fifth chaetiger spines are used to maintain the diameter of the worm’s burrow as it tunnels farther into the shell. Despite the characteristic fifth chaetiger, it is not demonstrably synapomorphic because there has yet to be a molecular phylogenetic study to investigate the monophyly of the complex (Radashevsky and Fauchald, 2000). Consequentially, polydorids do not have an official taxonomic rank, and the use of the term “polydorid” in this review does not allude to evolutionarily distinct ranking.

Moderate infestation by polydorids often results in the formation of lesions, or mud blisters, on the nacreous layer of the shell; and under heavy infestation, extensive tunnels are visible on the periostracum (Diez *et al.*, 2013; Morse *et al.*, 2015; Williams, 2015). These mud blisters are the direct consequence of shell thickening, which the mollusc employs as a shell repair mechanism. It has been suggested that the shell thickening and mud blister formation also prevent the worm from burrowing farther into the inner layer of the shell (Marshall and Day, 2001; McDiarmid *et al.*, 2004). The result is a decrease in growth and health of the adult mollusc, because the animal must now divert energy from basic maintenance to shell repair (McDiarmid *et al.*, 2004; Williams, 2015). Under heavy infestation, the structural integrity of the shell can become weakened; this makes the animal vulnerable to predation in its natural environment (Zottoli and Carriker, 1974; Riascos *et al.*, 2008). Some studies have shown that, in some cases, tissue quality is also compromised, resulting in low-quality shellfish meat (Mackin and Cauthron, 1952; Wargo and Ford, 1993; Spencer *et al.*, 2021), although this has been contested by other researchers who found no effect on meat quality (Loosanoff and Engle, 1943; Caceres-Martinez *et al.*, 1998; Cole *et al.*, 2020). The pathology associated with polydorids is most notably observed on aquaculture farms, and recent studies have shown that their ranges are expanding, with reports of increased infestation. For example, a genetic study of Pacific oyster symbionts on the west coast of North America confirmed the presence of *Polydora websteri* for the first time in the northwest Pacific (Martinelli *et al.*, 2020). Martinelli *et al.* surmised either that the species was accidentally introduced or, equally likely, that it was present all along but, due to changes in environmental conditions, proliferated rapidly until its realized range extended into oyster farms. Similar range expansions have also been recently recorded in Europe’s Wadden Sea (Waser *et al.*, 2020). Meanwhile, a two-year

survey of *P. websteri* infestation in the Gulf of Mexico found that the species reproduced year-round and exhibited the highest infestation rates during summer months (Cole *et al.*, 2020), while de Souza *et al.* (2017) found that in northeastern Brazil host size and age were the crucial factors driving infestation rates of *P. websteri* and *Boccardiella ligerica*.

Four notorious polydorids, *Polydora haswelli*, *Polydora websteri*, *Polydora hoplura*, and *Dipolydora armata*, are prolific parasites of commercially reared oysters and abalone in many parts of the world (Simon, 2011; Sato-Okoshi *et al.*, 2015; Simon and Sato-Okoshi, 2015). They are defined as parasites *sensu lato* because although they are obligated to reside in a molluscan shell to reach sexual maturity and reproduce, they do not actually feed on the mollusc itself but rather engage in suspension feeding by extending their tentacular appendages (known as palps) through openings in the shell (Blake, 1996). This contrasts with another polydorid, *Polydora colonia*, which exclusively burrows into sponges and has been shown to selectively feed on the sponge in combination with suspension feeding (David and Williams, 2012b). Perhaps the most dynamic feature of polydorids is their reproductive strategies (Blake, 1969; Blake and Arnofsky, 1999). All polydorids store sperm, produce broods within the form of egg strings, and ventilate their developing larvae, a rudimentary form of parental care. Some species produce only planktotrophic larvae, where embryos undergo development to the three-chaetiger stage, which are then released from the egg capsules and spend weeks feeding in the water column before settling on an appropriate substrate. Others produce lecithotrophic larvae, which are provisioned with yolk, released at a more advanced stage upon hatching, and settle close to the natal habitat. In a few species, females exhibit adelphophagy, a form of lecithotrophic development where the yolk is packaged into nurse eggs that are consumed by larvae (Mackay and Gibson, 1999; Simon, 2015). In adelphophagic broods, larvae have been observed to compete (sibling competition) and, in extreme cases, even consume each other (sibling cannibalism) (Gibson, 1997; David *et al.*, 2014). The latter often occurs when nurse eggs have been exhausted and the female has not yet liberated the brood (David and Simon, 2014; David *et al.*, 2014). An even smaller number of species exhibit poecilogeny, a rare reproductive strategy among marine invertebrates where a female produces a combination of different larval types (*e.g.*, planktotrophic and adelphophagic) either as a genetically distinct polymorphism or an environmentally induced polyphenism (see reviews by Hoagland and Robertson, 1988; Knott and McHugh, 2012). While polyphenistic shifts in development have been attributed to some poecilogenous animals, such as the saccoglossan sea slug *Aldera willowi* (Krug *et al.*, 2012), there are no studies that have experimentally demonstrated such environmental shifts in any polychaete (but for an interesting case where sperm availability induces adelphophagic development in *Polydora cornuta*, a non-boring polydorid, see Rice and Rice, 2009).

Considering that larval mode plays a crucial role in the dispersal and connectivity of sedentary polychaetes such as shell borers (David *et al.*, 2016), any climate change-induced effects on their development warrant experimental investigation. Because each larval type can exhibit different levels of tolerance to environmental stressors, poecilogenous shell borers may have a selective advantage over their monotypic cohorts. For example, in the poecilogenous polychaetes *Boccardia proboscidea* and *P. hoplura*, adelphohagic larvae exhibit a greater tolerance for elevated temperature than their planktotrophic siblings (David and Simon, 2014), opening up the possibility of epigenetic-mediated shifts in developmental modes. Finally, many polydorids are capable of autotomy, where they undergo fragmentation either due to an external environmental stimulus or as a defense mechanism against sublethal predation. In a few species, asexual reproduction occurs via fragmentation (architomy) and can allow for rapid colonization of a substrate in a short amount of time (David and Williams, 2012a).

Under near-future predicted climate change scenarios, it is obvious that the shell-boring activities of polydorids, in combination with an already fragile shell caused by more acidic environments, could trigger synergistic effects, leading to the extirpation of local shellfish populations, including highly productive oyster reefs. Some research has suggested that the innate immune responses of molluscs will be crucial in defending against the predicted increase in intensity of micro-parasites that cause shellfish diseases in warmer temperatures (Mackenzie *et al.*, 2014). Because polydorids reside within the nonliving shell, they can evade the effects of any such immune response mounted by the molluscan host; this makes this interaction unique among other host-parasite model systems. Consequently, understanding the performance and plasticity of polydorids under climate change stressors is crucial to mapping out the various adaptive scenarios that may unravel.

### Current State of Knowledge on Polydorids' Response to Climate Stressors

Despite the obvious importance of understanding the physiological performance of shell-boring polydorids under abiotic stressors, there is surprisingly little to no experimental data available for this group of marine invertebrates. One field study by Riascos *et al.* (2011) in northern Chile found that increased sea surface temperatures (SSTs) correlated with increased infestations (= prevalence) of *Polydora bioccipitalis* on the surf clam *Mesodesma donacium*. Riascos *et al.* hypothesized that the increased infestation rates were likely due to faster growth rates of offspring, earlier release of larvae from egg capsules, and activation of asexual reproduction. Other field studies by Mackin and Cauthron (1952), Blake (1969), Zajac (1991), and Cole *et al.* (2020) have also shown that higher summer temperatures are positively correlated with infestation rates. Pertinent to host-parasite dynamics, these

authors also found that warmer waters resulted in more physiologically stressed clams and, in turn, may also have contributed to the observably high rates of infestation. In addition, a shift to asexual reproduction due to increased water temperatures has been demonstrated in at least one other polydorid (David and Williams, 2012a, b) and is of ecological importance because this type of reproduction rapidly produces clones that are more efficient at colonizing new substrates and/or hosts.

One of the few laboratory experiments to investigate the effects of temperature on a shell borer was carried out by Oyarzun *et al.* (2011) on *Boccardia proboscidea*. *Boccardia proboscidea* is an interesting polydorid in that it has been classified by Simon *et al.* (2010) as a secondary borer, meaning that it does not actively burrow deep into the nacreous layer of the shell but rather creates its burrows on the surface of the shell and/or occupies existing burrows that were vacated by primary borers, such as *Polydora websteri* or *Polydora hoplura*. In addition, *B. proboscidea* is not an obligate borer; for example, in Argentina, populations of *B. proboscidea* assume a strictly free-living existence, where it is an auto-ecosystem engineer, creating massive biogenic reefs in the intertidal zone that support its proliferation but exclude other intertidal invertebrates (Jaubet *et al.*, 2011). In contrast, in South Africa, *B. proboscidea* has been regarded as a prolific pest of abalone (*Haliotis midae*) (Boonzaaier *et al.*, 2014) and was at one point found in large densities in sediment at the outflow of an abalone farm (David and Simon, 2014; David, 2015). Oyarzun and colleagues (2011), working on sediment-dwelling populations of *B. proboscidea* from the northwest Pacific, found that increased water temperatures resulted in shorter brooding times and increased variability in the larval stage at hatching. The larval stage at hatching is crucial because it determines planktonic larval duration (PLD) times, which, in turn, influence dispersal patterns and, ultimately, the demographic structure of the species (Levin, 2006; Shanks, 2009; Selkoe *et al.*, 2011). The researchers also observed that at warmer water temperatures, brooding females exhibited increased frequency of body oscillations, an activity that ventilates their burrows, expelling waste while also bringing in fresh seawater. Such activities clearly require an additional input of energy; and if we assume that such activities are sustained under higher than normal temperatures, there is an energetic cost involved that could result in trade-offs, for example, decreased reproductive output in future broods. Experimental studies by David and Simon (2014) on *Polydora hoplura* populations from oysters in South Africa also found that increased water temperatures decreased brooding time but also resulted in a combination of both larger-sized larvae at hatching and lower survivorship rates when compared to the control treatment. Unlike *B. proboscidea*, which produces an egg string consisting of individual capsules strung together (larval movement is confined to a single capsule), *P. hoplura* produces a continuous egg string containing compartments

separated by a thin membrane. Like *B. proboscidea*, *P. hoplura* also produces a combination of planktotrophic and adelphophagous larvae. Increased temperatures (3–5 °C) result in faster development of the adelphophagous larvae and increased competition for nurse eggs; in extreme cases, sibling cannibalism of smaller planktotrophs was found to be more frequent at higher temperatures. One long-term consequence of shorter PLD times would be the higher probability of local recruitment to the natal site and the subsequent contraction of the species' distributional range. In most polydorids, the brooding female has no control over the physiological processes of her offspring, but she does exert full control over the timing of hatching, larval sizes, and overall survival rate, because larvae are unable to escape capsules on their own (Blake, 1969; Blake and Arnofsky, 1999; David *et al.*, 2014). Investigating plastic responses of both larval interactions and female brooding activities can be achieved through multi-generational studies and will be crucial to understanding how increased water temperatures affect both the prevalence and intensity of polydorid parasites within their shellfish hosts.

If experimental studies on the effects of elevated water temperatures on life-history variation in shell-boring polychaetes are rare, studies investigating the effects of CO<sub>2</sub>-induced acidification are virtually nonexistent. Interestingly, there have been several studies that have explored acidification on free-living, mainly benthic polychaetes over the last decade, probably because of their role as important bioindicators. These studies have found mixed results with respect to tolerance, but key findings include elevated metabolic rate (Calosi *et al.*, 2013; Turner *et al.*, 2015), increased oxidative stress (Freitas *et al.*, 2016; De Marchi *et al.*, 2019), and reduced sperm motility (Lewis *et al.*, 2013; Campbell *et al.*, 2014; Schlegel *et al.*, 2014) in polychaetes that were subjected to hypercapnic conditions. In one of these studies, Calosi *et al.* (2013), using *in situ* transplant studies, found that *Platynereis dumerilii* (family Phyllodocidae) populations living in CO<sub>2</sub> vents were more physiologically tolerant to elevated pCO<sub>2</sub> levels than nearby populations adapted to significantly lower pCO<sub>2</sub> conditions. Most importantly, Calosi *et al.* (2013) suggested that such an adaptive event was relatively recent, considering that the vent system where the populations were sampled from was less than 2000 years old. In another study with three different species, *Arenicola marina* (family Arenicolidae), *Pomatoceros* (= *Spirobranchus*) *lamarcki* (family Serpulidae), and *Galeolaria caespitosa* (family Serpulidae), sperm motility was significantly reduced during short-term elevated pCO<sub>2</sub> exposure. This is concerning because sperm motility in marine invertebrates is an important factor influencing fertilization success and, should it be compromised because of climate change, would result in lower recruitment success. Finally, Díaz-Castaneda *et al.* (2019) found that the calcareous tubes of two serpulid polychaetes, *Spirorbis* sp. and *Spirobranchus triquetus*, were more fragile from habitats that had lower pH (~0.3 units below), as a result of dissolution

of the calcite and aragonite foundation (similar to what occurs in bivalves and shelled gastropods), providing the first evidence that the structural integrity of polychaete tubes can be compromised in a more acidic ocean. Until similar studies on spionids are carried out, the data on serpulids, which are more evolutionarily related to spionids than any other polychaete family (Struck *et al.*, 2011), provide the best insights into the responses of polydorids to acidification.

### Interactive Effects of Climate Change on Polychaete-Molluscan Symbiosis

As ocean temperatures rise, faster developmental rates and shorter PLD times (resulting in higher probability of local recruitment) of polydorid larvae, coupled with increases in asexually reproducing propagules, means that shellfish infestation will potentially increase. Indeed, a previous study by Simon and Booth (2007) found that water temperature was one of two major factors affecting population growth, recruitment, and infestation intensity of shell-boring polychaetes on farmed abalone (*Haliotis midae*) in South Africa. This problem is exacerbated by the fact that generational times are much shorter for polydorids, which can reach sexual maturity from egg to adult in fewer than six weeks for planktrophic brooders (David *et al.*, 2014) compared to *Crassostrea gigas*, whose initial maturation time has been reported as being from four to six months (Shpigel, 1989), although this can vary based on population and latitude (Gomes *et al.*, 2014). Increased ocean acidification is expected to negatively affect the reproductive capacity (*e.g.*, fecundity and sperm motility) of both annelids and molluscs (Parker *et al.*, 2013; Kroeker *et al.*, 2014; Verkaik *et al.*, 2017; but see Lucey *et al.*, 2015, who showed that brooding polychaetes are more tolerant of acidifying conditions than their broadcast-spawning cohorts). While the calcareous tubes of free-living polychaetes (*e.g.*, serpulids) exhibit increased fragility under acidified conditions, polydorids utilize the calcareous matrix of their shellfish hosts to form their tubes, meaning that the condition of their tubes is directly intertwined with shell condition. All previous studies that have explicitly explored the boring mechanism of polydorids have found that worms never burrow through the nacreous layer into the inner layer of the shell. This is due to a rapid calcification response by the mollusc, which adds extra layers of shell (Zottoli and Carriker, 1974; Marshall and Day, 2001; McDiarmid *et al.*, 2004). If shell dissolution overwhelms carbonate deposition, as has been reported in several studies (Rodolfo-Metalpa *et al.*, 2011; Gazeau *et al.*, 2013 and references therein), adding extra layers as a defense against infestation will become more difficult, allowing worms to burrow closer to the mantle than was previously possible. The overall result is increased physiological stress on shellfish, ultimately resulting in lower growth rates and higher mortalities in both farmed and wild stock. Interestingly, a recent study by Clements *et al.* (2017)

found that when oyster shells were subjected to extreme drops in pH, they showed a reduced susceptibility to infection by *P. websteri*. The researchers hypothesized either that acidic conditions may have dissolved the softest parts of the shells, with the hardest layers being inaccessible to the worms, or that the worms preferentially avoided more brittle shells—the latter of which is very possible, considering that shell-boring polydorids do exhibit substrate preferences (David, 2015).

### Lessons from other marine invertebrate taxa

Obligate shell-boring polychaetes and their shellfish hosts represent a unique host-symbiont relationship with few comparable analogs in the marine environment. In many ways, expected alterations in life-history traits for polydorids should be similar to other soft-bodied invertebrates, though some traits, such as sibling cannibalism and tube construction mechanisms, will be specific to this group. In terms of the relationship between the polydorid and its molluscan host, we can turn to the literature to see how climate stressors affect other marine invertebrate symbioses and, by doing so, can gain some valuable insights into how polydorid parasites may respond under similar scenarios. One intriguing example comes from a coral reef study that showed that elevated water temperatures (sub-bleaching threshold) resulted in a mutualistic to parasitic transition, where the algal symbiont *Symbiodinium* exhibited increased assimilation rates of carbon and nitrogen, whereas the coral host failed to receive proportional benefits with regard to crucial metabolic products (Baker *et al.*, 2018). This was an indirect result of an overall increase in respiration rates of the holobiont and consequential decrease in net primary productivity (NPP). Another recent example involves the pea crab, *Afropinnotheres monodi*, a parasite of shellfish, such as cockles and mussels, that has expanded its range from the waters around Africa to the Mediterranean Sea, possibly as a result of anthropogenic factors (Drake *et al.*, 2014). *Afropinnotheres monodi* exhibits an autumnal decrease in reproductive capacity, which, in turn, provides a seasonal release of burden for host bivalves. However, Drake *et al.* (2014) suggested that if water temperatures in the Mediterranean continue to rise, this autumnal decrease may disappear or be significantly reduced, potentially resulting in increased prevalence of *A. monodi* infections and a subsequently decreased physiological performance of their host bivalves, as has been found on aquaculture farms (see Perez-Miguel *et al.*, 2018). Other case studies have shown that hosts themselves may derive different levels of benefits from these types of symbiotic shifts. For example, studies on endolithic cyanobacteria, which are known to erode the shells of their molluscan hosts, have shown that under climate change stressors, specifically, elevated temperatures, there appears to be a reversal from a parasitic relationship to one that confers benefits to the host (Zardi *et al.*, 2016; Gehman and Harley, 2019). After the dark-colored periostracum is degraded due to the

boring activity of the endolith, the lighter-colored prismatic layer reflects rather than absorbs heat; and under experimentally induced thermal stress, infected shellfish exhibit higher survival rates than their uninfected congeners. Using a population-modeling approach, Gehman *et al.* (2018) used experimental data to build thermal performance curves (TPCs) for the marine crab *Eurypanopeus depressus* and its rhizocephalan barnacle parasite (*Loxothylacus panopaei*), then used that information to develop an epidemiological model to determine how future warming scenarios may alter the interactions between the two species. The model predicted extinction of the parasite in the southern United States when temperature was increased by only 2 °C, indicating that in some cases hosts may be released entirely from their parasitic burden. Together, these examples re-emphasize the plastic nature of invertebrate symbioses; but, more importantly, they show that subtle changes in life-history traits and behavior could have wider adaptive consequences for whole species and communities. For shell-boring polydorids and their molluscan hosts, similar thermal performance curves and epidemiological models would be extremely valuable, not only for predicting the long-term viability of important habitats, such as oyster reefs, but also for risk assessment of commercially important shellfish farms that are vulnerable to polydorid infestation. In addition, because of the combined effects of shell dissolution and the burrowing activities of polydorids, constructing analogous pH performance curves would produce a more robust epidemiological model, relevant to the complexity of the polydorid-bivalve model system.

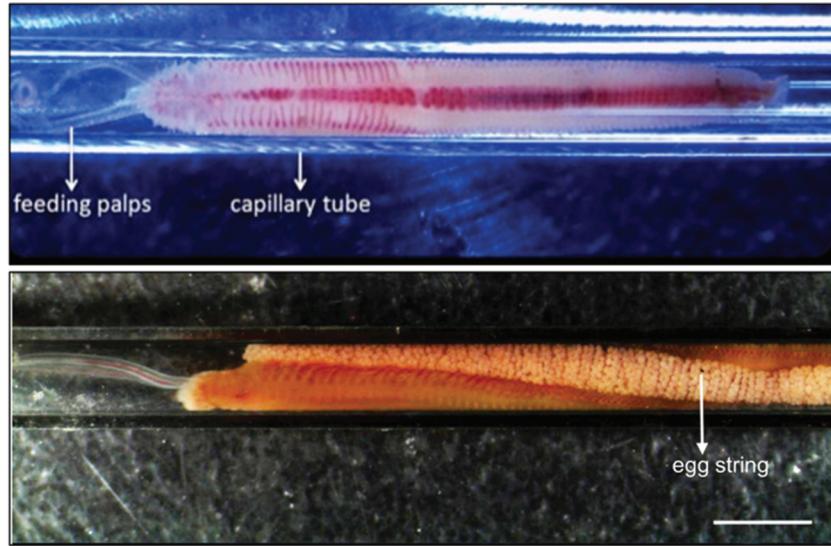
### Challenges for Experimental Studies in Polydorids

To experimentally investigate the responses of polydorids to climate change, standard experimental procedures must be established to allow for both reproducibility and comparison of results across studies. The biggest challenge in developing experimental protocols for shell-boring polydorids is survival of the worm outside of its molluscan host. Because most shell-boring polydorids are obligate symbionts, they are unable to survive once extracted from their tubes. One exception is *Boccardia proboscidea*, which can be cultured in sediment because of its dual role as both a secondary shell borer and a sediment dweller. Once a method for securing the adult worm *in vitro* has been established, the second challenge would be maintaining long-term cultures by determining the right type of food for the adults, appropriate settlement cues for larvae, and so on. Food should be a combination of different species of algae and/or phytoplankton that closely mirrors what the worms would encounter while in their hosts' shells. To track transgenerational effects, any larvae produced would have to be kept alive in separate culture chambers and should be stimulated to settle and metamorphose. This is a challenge because many polychaete larvae are particularly sensitive to water stagnation, and this can lead to bacterial in-

fection or predation by ciliates at the earliest stages of development. In previous studies, the use of antibiotics, such as penicillin G and streptomycin, has been applied to polychaete larval cultures to deal with pathogens (Qian and Chia, 1991; Gibson and Paterson, 2003; Doherty-Weason *et al.*, 2019). However, when applying abiotic stressors to study plasticity across generations, antibiotics could introduce additional variables that may confound results. Once a consistent culture method that allows for replication has been established, water-quality parameters, such as temperature and pH, can be modulated to track variation in physiological performance and various life-history traits.

### Culture methods for polydorids

Non-heparinized glass capillary tubes have been used in previous studies to examine reproduction, larval development, feeding behavior, and regenerative capabilities of polydorids, such as *Dipolydora commensalis* and *Polydora robi* (Williams and McDermott, 1997; Williams, 2001; Dualan and Williams, 2011). More recently, David and Simon (2014) used this method to track the development of *Polydora hoplura* from oviposition to larval release, as well as to document behavior, such as sibling competition and cannibalism, under varying temperature regimes. Using this capillary-house method, the polychaete is coaxed into an open-ended glass tube that should ideally be 0.02–0.05 mm wider than the diameter of the worm (Fig. 2). If the tube diameter is too wide, the worm will simply crawl out through the opposite end; and if it is too narrow, it may undergo autotomy (fragmentation) and/or there may be insufficient space for gravid females to lay their egg strings. Often, it may take several attempts before a worm decides to settle within a tube; but when it finally does, it will clog both open ends with mucus and begin ventilating the tube through repeated body oscillations. Once this has occurred, the tube can then be placed in a glass petri dish with sediment covered with a micromesh (10–35 µm), placed in a larger holding chamber, and weighted down into a small aquarium tank connected to a flow-through system (Fig. 3). The use of a flow-through system is crucial because it eliminates the need for manual water changes and, thus, minimizes disturbance of the animals during the experiment. Furthermore, such systems come with flow sensors that can be used to ensure that a constant flow is occurring in all the tanks. Using this method, multiple batches of adult worms can be reared under varying temperature and pH regimes with appropriate controls and replicates. For maintaining long-term cultures of shell borers, worms should be fed *ad libitum* on exclusively lab-grown algal cultures (*e.g.*, *Isochrysis*), as opposed to dry food, such as Tetramin. While the latter has been successfully used for culturing adult polychaetes (Marsh and Pasqualone, 2014), dry flakes tend to promote high ciliate activity, which may overwhelm egg strings and developing embryos (David *et al.*, 2014); and females

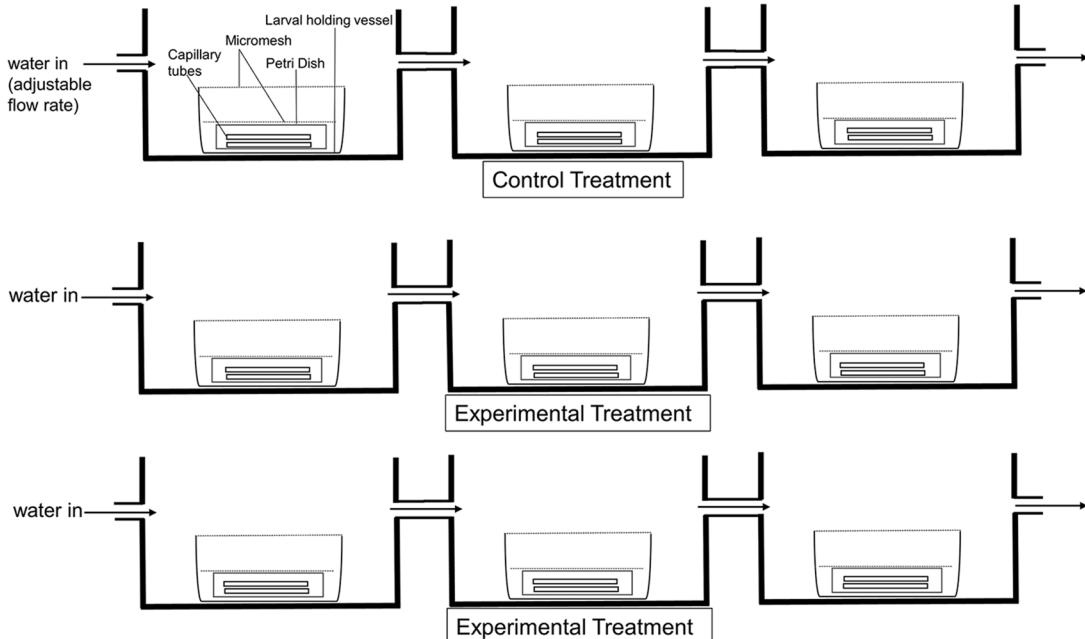


**Figure 2.** *In vitro* culture procedure for culturing shell-boring polydorids. Specimen shown is *Polydora hoplura*. Scale bar = 1 mm.

have been observed to abandon such broods (AAD, pers. obs.). Any gravid females fitted into tubes will eventually lay their egg strings or capsules ventrally, with the stalks attached to the base of the capillary tube.

Once the female has ruptured her egg string, larvae are capable of exiting the tube on their own, and at this point they can be released from the petri dish into the larger containment vessel. Larval growth of a specific cohort can then be tracked

daily until it settles. For tubicolous non-boring polydorids, such as *Polydora cornuta*, natural sediment is sufficient for inducing settlement and metamorphosis of advanced larvae, presumably because of the presence of specific bacterial species that reside within the sediment (Sebesvari *et al.*, 2006, 2013), although other cues, such as food type and the presence of sympatric species, may also play a role in settlement (Qian, 1999). For shell-boring polydorids, crushed, heat-sterilized



**Figure 3.** Schematic diagram of a proposed flow-through experimental setup for culturing shell-boring polydorids. Setup can be retrofitted into a typical ocean acidification laboratory where flow velocity, oxygen concentration,  $p\text{CO}_2$  (partial pressure of carbon dioxide), and temperature can all be modulated from a master control. The size of tanks and holding chambers can be variable, depending on sample size and replicate number (for the purpose of simplicity and clarity, only one larval holding tank per aquarium is depicted).

oyster shells have been successfully used in a previous project to induce settlement for *Polydora hoplura* (David, 2015), but the method has yet to be applied to other polydorids. This method is ideal for laboratory experiments because it allows for the development of a specific genetic lineage that can be tracked across multiple generations to determine transgenerational effects of climate change stressors. In addition, this method can also provide *in vivo* insights into the synergistic impacts of polydorid infestation on shell architecture, integrity, and, most importantly, resilience. Indeed, all published studies investigating the impacts of polydorids on molluscan shells thus far have focused on field animals where the polydorids are at various stages of development inside of the shell, have different levels of nutrition (because of intraspecific competition), and may also consist of sibling species and or other non-polydorid shell borers (e.g., *Terebrasabella* sp.); all of these factors may contribute to an overall negative impact, making it impossible to tease apart the effects of the polydorid itself.

A rudimentary setup of the aforementioned procedure was developed and adopted by David *et al.* (2014) to track early life-history features of the primary and secondary borers *P. hoplura* and *B. proboscidea* under various temperature treatments (Fig. 3). Traits, such as brooding time, embryo and larval morphogenesis, PLD, and larval behavior and developmental rate, were all quantified and statistically compared for a single generation. A point of contention in using this capillary-house method is that inherent biases in data can emerge from such an artificial setup. Larval developmental traits, such as PLD and settlement times, would probably not be any different from larvae grown *in vitro* versus in the wild, with the exception that those in the wild are subjected to abiotic (e.g., oceanographic features) and biotic (e.g., predation) pressures. One solution would be to assess discrepancies by comparing data, such as fecundity and egg sizes, from wild broods with those produced by lab-reared specimens in pilot studies.

### Summary and Concluding Thoughts

In conclusion, the number of studies addressing the effects of climate change stressors on marine organisms has risen significantly over the past 2 decades (Wernberg *et al.*, 2012; Baumann, 2019), with more than 80 papers published per year since 2000 (Hoegh-Guldberg and Bruno, 2010). A vast majority of these studies have focused on calcium carbonate-secreting organisms, such as molluscs, corals, and some arthropods. The role of molluscs as ecosystem engineers, and their importance in the aquaculture industry, makes them prime candidates for experimental research into climate change adaptability. However, any holistic approach to understanding impacts on molluscs must also include assessing the performance of their symbionts, especially parasites. Until now, there seem to have been no studies or reviews that have explicitly addressed the effects of climate change stressors on shell-boring polychaetes, which parasitize both wild and farmed molluscs. In this

review, I presented the data available on temperature and pH effects on shell borers and their close polychaete relatives while also proposing a standardized experimental framework for studying shell-boring polydorids. This framework involves maintaining *in vitro* and *in vivo* cultures that can be tracked across multiple generations in the lab. Perhaps more importantly, this framework lends itself to rigid reproducibility, which is a prerequisite for any serious long-term laboratory research into climate change stressors. This work not only is important to polychaetologists but also should be a call to action to malacologists because the long-term viability of the most ecologically and commercially important groups of molluscs is now at risk.

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