Comparative Oxygen Consumption of Gastropod Holobionts from Deep-Sea Hydrothermal Vents in the Indian Ocean

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Abstract

Physiological traits are the foundation of an organism's success in a dynamic environment, yet basic measurements are unavailable for many taxa and even ecosystems. We measured routine metabolism in two hydrothermal vent gastropods, *Alviniconcha marisindica* (n=40) and the scaly-foot gastropod *Chrysomallon squamiferum* (n=18), from Kairei and Edmond vent fields on the Central Indian Ridge (23-25° S, approx. 3,000 m depth). No previous studies have measured metabolism in any Indian Ocean vent animals. After recovering healthy animals to the surface, we performed shipboard closed-chamber respirometry experiments to compare oxygen uptake at different temperatures (10, 16, 25 °C) at surface pressure (1 atm). The physiology of these species is driven by the demands of their chemoautotrophic symbionts. *Chrysomallon* has very enlarged respiratory and circulatory systems, and endosymbionts housed in its trophosome-like internal esophageal gland. By contrast, *Alviniconcha* has chemoautotrophic bacteria within the gill, and less extensive associated anatomical adaptations. Thus, we predicted that routine oxygen consumption of *Chrysomallon* might be higher than *Alviniconcha*. However, oxygen consumption of *Chrysomallon* was not higher than *Alviniconcha*, and further *Chrysomallon* maintained a steady metabolic demand in two widely separated experimental temperatures, while *Alviniconcha* does not. We interpret these findings to indicate that 1) the trophosome does not fundamentally increase oxygen requirement compared to other gastropod holobionts, and 2) cold temperatures (10 °C) induced a stress response in *Alviniconcha* resulting in aberrantly high uptake. While these two large gastropod species co-occur, differences in oxygen consumption may reflect the separate niches they occupy in the vent ecosystem.

Introduction

Hydrothermal vent ecosystems occur on geologically active tectonic margins on the seafloor, worldwide (Baker *et al.*, 2016; Beaulieu, 2017). While deep-sea vent systems are globally united by a suite of challenging abiotic conditions — no sunlight, highly acidic and toxic vent fluid emerging at superheated temperatures — most vent species are restricted to a particular biogeographic province (Rogers *et al.*, 2012). The region of the East Pacific Rise included the site of the first vent system ever discovered (Corliss *et al.*, 1979), and its fauna remains by far the best studied and most familiar (Mullineaux, 2014). The fast-spreading EPR is characterized by high turnover and geologically unstable chimney structures (Shank *et al*., 1998; Govenar *et al*., 2004). By contrast, the Indian Ocean has a markedly different geology, and different fauna, and is so far still relatively unexplored. The slow-to-intermediate-spreading Central Indian Ridge vent fields are characterized by highly stable, complex chimney structures with very little accumulation of mineral material or change (Van Dover *et al*., 2001; Nakamura *et al.*, 2012; Chen *et al.*, 2015a; Watanabe and Beedessee, 2015). This contrast in abiotic environmental context, and its interplay with the evolutionary history of various clades, underlies the diverse and non-overlapping faunas of different regional vent systems (Ramirez-Llodra *et al.*, 2007; Rogers *et al*., 2012).

Hydrothermal vent ecosystems are universally driven by chemosynthesis; in absence of sunlight, primary productivity is drawn from chemoautotrophic microbes that derive energy from the oxidation of hydrogen sulfide, methane, or a variety of other inorganic reducing agents (Stewart *et al*., 2005). While chemoautotrophic microbes were quickly recognized as the foundation of vent ecosystems, early interpretations clearly assumed that the only way for animals to engage in a bacterially-based food chain was through direct consumption (Jannasch and Wirsen, 1979). The discovery of symbionts within the internal trophosome tissue of the giant tubeworm *Riftia pachyptila* Jones, 1981 solved the mystery of how this and many other animal species function with reduced or absent digestive systems (Cavanaugh *et al.*, 1981). Dependency on microbes for energy production has led to the evolution of symbiotic relationships in many vent-endemic

animals that directly harness energy production by bacteria (Dubilier *et al*., 2008). These symbiotic relationships underpin major anatomical and physiological adaptations in these lineages.

Vent animals that acquire energy through chemosymbiosis have higher oxygen demands than other non-symbiotic species in the same environment (Girguis and Childress, 2006). Variation in metabolic rates among holobionts are generally correlated to growth rates, and to the availability of reducing agents for biochemical pathways (Childress and Girguis, 2011). A broad range of animals, in many ecosystems, have chemosymbiotic relationships with microbes, but in hydrothermal vent ecosystems only siboglinid annelids, and mollusks (gastropods and bivalves) have intracellular sulfur-oxidizing symbionts (Childress and Girguis, 2011).

Among vent mollusks, almost all species that house chemoautotrophic endosymbionts do so within the tissue of the gill (Dubilier *et al*., 2008). The only mollusks so far described with internal endosymbionts are two lineages of Indian Ocean vent gastropods, which house endosymbionts in an internal trophosome-like organ that is a hypertrophied oesophageal gland (Chen *et al.*, 2015b; Chen *et al.*, 2017). These two genera, *Chrysomallon*, and *Gigantopelta*, are both unusually large (~5 cm adult shell length) compared to the majority of vent gastropod taxa (~1 cm). Large body sizes are related to their housing endosymbiosis (Vermeij, 2016) but not uniquely associated with the 'trophosome'. There are other, similarly sized large-bodied vent gastropods, in the provannid genera *Alviniconcha* and *Ifremeria*. Species in these two genera have chemoautotrophic endosymbionts in the gill that contribute to most of the host's metabolic requirements (Warén and Bouchet, 1993; Beinart *et al*., 2014); dependency on symbionts apparently enables the animals to reach large adult sizes (Henry *et al.*, 2008). While the 'outcome' of large adult size in the holobiont is similar, these lineages represent two profoundly different strategies to house endosymbionts: on gill tissue in contact with the water, or in internal tissue within the visceral mass.

Trophosome structures in the genera *Chrysomallon* (the scaly-foot gastropod) and *Gigantopelta* are accompanied by other substantial anatomical adaptations, similar to those in siboglinid tubeworms, which alter the configuration and life history of the host to create an optimized environment for the microbes. These adaptations include enlarged circulatory systems with a muscular ventricle that acts as a pumping heart, unlike any other gastropod mollusk (Supplementary Video 1). Like siboglinids, the gastropod trophosome tissues are highly vascularized (Chen *et al*., 2015b), which anatomically suggests the circulatory system is adapted to supply oxygen and potentially hydrogen sulfide to the bacteria. There are clear parallels to the well-studied anatomy and physiology of *Riftia* and other tubeworms; however, the physiology and even growth rates of *Chrysomallon* and *Gigantopelta* remain undescribed.

Alviniconcha marisindica Okutani in Johnson *et al*., 2014 (with symbionts in its gill) and the scaly-foot gastropod *Chrysomallon squamiferum* Chen *et al*., 2015 (with symbionts in an internal trophosome) co-occur at vent sites in the Central Indian Ocean (Johnson *et al*., 2014; Watanabe and Beedessee, 2015). This presents an interesting opportunity for comparative physiology, to test the metabolic effects of these two different evolutionary strategies to harness energy from vents through endosymbiosis, in the gill (in *Alviniconcha*) or within a trophosome (in *Chrysomallon*). In the present study, we conducted experiments to measure the oxygen uptake rates of these two species in a range of temperatures. This is the first study of metabolism in Indian Ocean hydrothermal vent animals. We predicted that the oxygen demand of *Chrysomallon* might be higher than that for *Alviniconcha*. Chemosymbiosis, used by both taxa, is related to increased oxygen requirements (Childress and Girguis, 2011), but the trophosome may represent a more extreme dependency on microbial symbionts and thus further increased oxygen demand.

Materials and Methods

Gastropods were collected at two hydrothermal vents sites on the Central Indian Ridge by the human-occupied submersible (HOV) *Shinkai 6500* using a suction slurp gun during the R/V *Yokosuka* cruise YK16-E02. Most specimens were collected at the Kairei vent

field (25°19.2253’ S, 70°2.4217’ E, depth 2420 m) and a smaller population from the Edmond vent field (23°52.6823’ S, 69°35.8013’ E, depth 3280 m). The scaly-foot gastropod *Chrysomallon squamiferum* was found only at the Kairei vent field, co-occurring with *A. marisindica* (Table 1). Specimens were allowed one to two days of acclimation to lab conditions in aquaria at surface pressure and light.

Animals were housed in static aquaria in a constant temperature room in filtered seawater that was changed several times daily (more frequently in higher temperature groups). Two experimental temperatures were used: 25 °C (24.58 ± 0.150 s.d. °C over all experiments), 16 °C (16.49 ± 0.128 s.d. °C over all experiments), and 10 °C (10.51 ± 0.300 s.d. °C over all experiments). These were selected as relevant to the reported range of habitat temperatures particularly for *Alviniconcha*, 5–33 °C (*fide* Warén and Bouchet, 1993) and data measured *in situ* from the water of gastropod colonies during sampling for this study, mainly from 10–21 °C but up to a maximum of 38 °C (Table 1; Takai *et al*., 2016).

Closed-chamber respirometry experiments at surface pressure (1 atm) were used to measure oxygen consumption as a proxy for metabolic rate, following the methods of Carey *et al.* (2013). Animals were placed into individual Perspex chambers filled with filtered seawater at the experimental temperature; chambers were sealed with a rubber-gasket stopper and fitted with a fiber-optic oxygen probe (FOXY systems, Ocean Optics, Dunedin, Florida). Oxygen and temperature data were recorded continuously at intervals of 1 s. Probes were calibrated using a two-point calibration to air-saturated experimental filtered seawater (100% oxygen saturation) and 5% Na_2SO_3 in seawater (0% oxygen saturation). Calibrations were re-set every 24 hours, and checked at the start and end of each experiment, but there was no drift requiring correction. Six trials were run in parallel with one empty chamber in each set serving as a control to measure potential microbial consumption of oxygen in the experimental seawater. Experiments were run for up to 7 hours depending on specimen size and activity; most experiments took 3–4 hours to decrease to 60% of air-saturated conditions (with shorter times at higher temperatures).

Specimens were not re-used at different temperatures; each animal was used in only one experiment.

Respiratory rates (VO_2, $mgO_2 \cdot h^{-1}$) were calculated for each specimen from the average rate at which oxygen tension decreased, to measure routine oxygen consumption rate. These measurements were taken below 95% air-saturation and as early in the trial as possible, selected for periods with the smallest possible fluctuation in temperature and using the same time period for each set of six parallel trials. Recordings with substantial fluctuations and aberrant measurements were discarded. Thus the number of specimens used for analysis Air-saturated O_2 concentration ($mg \cdot L^{-1}$) was calculated according to Benson and Krause (1984) using the surface salinity measured by the Conductivity-Temperature-Depth profiler of the HOV *Shinkai 6500* on the date of collection (31.3) and average temperature in each experiment (e.g. 8.08 $mg \cdot L^{-1}$, for 16.53 °C and 31.3 salinity). Recordings from empty chambers were used as a control, to estimate a background rate of microbial activity. The rate of background oxygen consumption in the control apparatus was subtracted from each experimentally trial rate to determine VO_2 for each subject. As these experiments were conducted at sea, it was not possible to determine precise wet weights of the live animals; at termination of the experiments, subjects were immediately frozen at -80° for preservation and later weighed. Mass-specific oxygen uptake (MO_2, $\mu mol\ O_2 \cdot g^{-1} \cdot h^{-1}$) was calculated for each subject from the individual VO_2 and wet weight. We calculated a linear ordinary least squares regression from log-log transformed data to determine an approximate metabolic scaling exponent for *Alviniconcha*, finding *b* in a standard equation for metabolic rate (r) scaling with animal mass (M) of the form $r = aM^b$. Because there were no juvenile specimens of *Chrysomallon*, and the range of metabolic response measurements was similar to that in adult *Alviniconcha*, the same scaling calculation was not possible for the second species.

Because sample sizes were relatively small, and not balanced among experimental groups (species and temperatures), a generalized linear model approach was used to compare central tendency among groups implemented in R (R Core Team, 2017). The GLM compared MO_2, as the response variable, and species and temperature as factors, using a

Gaussian link function (function 'glm' in R). Additional pairwise comparisons among groups were performed using the Mann-Whitney U test.

Results

Experimental animals of both species appeared to be in good condition at surface pressure, readily attached to the side of respirometry chambers, and remained responsive (Fig. 1). *Chrysomallon squamiferum* specimens were active and explored their aquarium environment and did not show any behaviorally obvious signs of stress from transitions in pressure and temperature. A total of 55 *Chrysomallon* specimens were collected in one sampling event at Kairei field, some of which were used for respirometry. Because *Chrysomallon* was collected in only one event, at the end of the cruise, only two temperature treatments could be accommodated. *Alviniconcha marisindica* were collected in much larger numbers, from two sites (approximately 300 specimens from each of Kairei and Edmond fields, among several sampling events), though subsets of animals in good condition were used for these experiments (Table 1; Table 2). Some *Alviniconcha* specimens were observed to purge material from the pallial chamber, which had the appearance of a mixture of mucous, and bacterial mats; this was particularly observed in animals at 10° C (Fig. 1C, note condensation on the respirometry chamber surface from chilled seawater).

An analysis of deviance, using chi-squared goodness of fit, indicated significant effects of both species ($p = 0.0088$) and temperature ($p = 0.027$) for mass-specific oxygen uptake (MO_2, μmol $O_2 \cdot g^{-1} \cdot h^{-1}$). The metabolic rates of *Chrysomallon* were not significantly different between high (25 °C: mean MO_2 0.809 ± 0.158 s.d. $\mu mol \cdot g^{-1} \cdot h^{-1}$) and low (10 °C: 0.854 ± 0.113 $\mu mol \cdot g^{-1} \cdot h^{-1}$) temperature trials ($W = 44$, $p = 0.76$; Fig. 2A). Metabolic rates in *Alviniconcha* were similar at 25 °C and 16 °C, and not significantly different between those two temperature treatments ($W = 86$, $p = 0.61$) or to the *Chrysomallon* rates ($W = 289$, $p = 0.12$). That is, among data recorded for the five treatment groups (two temperatures for *Chrysomallon* and three for *Alviniconcha*), the metabolic rates were not significantly different among four out of five in pairwise comparisons (Fig 2A).

The metabolic rate in *Alviniconcha* in the low temperature treatment (10 °C) was much higher than any other experimental group (mean MO_2 2.108 ± 0.287 $\mu mol \cdot g^{-1} \cdot h^{-1}$; Fig. 2A). This is substantially higher than the 16 °C treatment (W = 43, $p = 0.066$) and significantly higher compared to the 25 °C treatment for *Alviniconcha* (W = 187, $p = 0.0026$). It is also significantly higher than the *Chrysomallon* rates (W = 153, $p = 4 \cdot 10^{-5}$).

The 25 °C temperature treatment included data from two separate populations of *Alviniconcha*, collected at the two vent fields. However, there was no significant difference in the mean values for data from the two fields (W = 86, $p = 0.6475$) and no evident trend that separated their metabolic responses (Fig. 2B). The regression results indicated an approximate scaling exponent of 0.25 for *Alviniconcha* in the form $r = 1.7 \cdot M^{-0.25}$ ($R^2 = 0.31$).

Discussion

The thermal context of vent habitats

Hydrothermal vent ecosystems are characterized by steep temperature gradients, from near freezing ambient temperatures on the sea floor to superheated vent effluent temperatures measured at 361.4°C (Kairei field) and 375.4°C (Edmond field) during the dives that sampled specimens used here (Takai *et al*., 2016). The continuous eruption of vent fluid creates a turbid environment with fine scale but extreme fluctuations in chemistry and temperature (Johnson *et al*., 1988). In contrast to other deep-sea habitats the chimney surface is typically hot, with water temperatures more similar to tropical shallow water environments than abyssal seas (Childress and Mickel, 1985). Yet turbid changes in temperature and the steep thermal gradient to ambient water mean that exposure to temperatures over 40-50 °C is probably relatively rare and transient (Girguis and Lee, 2006).

Certain patches within vent habitats experience high amplitude fluctuations but this does

not imply homogenization of the abiotic conditions. Local characteristics of temperature, chemistry, and hydrodynamics within vent sites create microhabitats and some species are potentially adapted to narrowly defined niches (Bates *et al*., 2005; Bates *et al.*, 2010; Podowski *et al*., 2010; Beinart *et al*., 2015). Based on the patchy distribution and high local biomass in localized colonies, vent endemic animals like *Chrysomallon* and *Alviniconcha* are inferred to be constrained to a niche with characteristic chemistry and temperature. During the cruise that obtained the animals used in these experiments, samples were taken from within the gastropod colony masses to measure water temperature and chemistry *in situ*. The temperatures taken on these dives are as accurate as possible but still gave a very broad range, from 5 to 20°C in a single *Alviniconcha* colony (Takai *et al*., 2016).

Previous studies at Kairei vent field reported a temperature of 2–4 °C around *Alviniconcha* (Suzuki *et al*., 2006), but those data reflect ambient conditions in the vicinity of the chimney surface, and not directly measured *in situ* water temperature of the gastropod colony. Additional specimens of *A. marisindica* kept at 4 °C in our laboratory were noticeably lethargic and unresponsive. Only specimens from the Kairei field were tested at low temperatures, but we were able to complete experiments with large sample sizes in two temperatures for both fields, and found no site-specific differences in metabolic rates.

Stress effects and thermal optima

Both experimental species survived the transition to surface pressure, and behaved apparently normally in laboratory conditions in the shipboard laboratory. *Chrysomallon squamiferum* suffered no mortalities, and appeared much more robust to captivity but were kept for a shorter period than *Alviniconcha marisindica*. Other work on *Alviniconcha* sp. found the animals unable to tolerate laboratory maintenance (pressurized) for more than a few days (Henry *et al.*, 2008). Among our samples of *Alviniconcha marisindica* (several hundred individuals) the smaller juvenile animals had higher levels of activity as observed in aquaria, as well as higher metabolic rates in line

289 with expectations from allometric scaling of metabolism. These smaller individuals were
290 perhaps more able to cope with the stress of captivity.
291
292 Despite short-term survivorship, the animals we used were certainly compromised during
293 experimental trials, especially at lower temperatures. Several *Alviniconcha* specimens
294 were excluded from experimental trials because they appeared to be stressed. We
295 frequently observed larger individuals of *Alviniconcha marisindica* purging what
296 appeared to be bacterial mats from the pallial cavity. This may speculatively have been
297 symptomatic of bleaching of the microbial symbionts associated with the gill; if true,
298 would be a clear indication of environmental stress for host and symbiont. We are not
299 certain whether the behavior observed was bleaching but it is certainly possible, a
300 comparable experience to coral bleaching under acute thermal stress (e.g., Fujise *et al.*,
301 2014). Bleaching also occurs among other mollusks: some bivalves host exocellular
302 photosymbiotic dinoflagellates (Vermeij, 2013), and can also experience bleaching under
303 thermal stress, reduced salinity, or excessive light exposure (Norton *et al.,* 1995; Buck *et*
304 *al.*, 2002; Maboloc *et al*., 2015). Bleaching damages the metabolic capacity of the host
305 and reduces fitness, fecundity, and survival, but is not lethal. By comparison with energy-
306 harnessing photo-endosymbionts in other systems, vent mollusks could also potentially
307 lose their symbiotic microbes when stressed.
308
309 In general, higher temperatures lead to higher metabolic rates in ectotherms like
310 gastropod mollusks. But gastropods can respond to transient environmental stress either
311 through increasing oxygen consumption or a hypometabolic dormant state. Previous
312 studies have shown that hydrothermal vent organisms do not necessarily demonstrate a
313 straightforward relationship between ambient temperature and metabolic rate (Girguis *et*
314 *al.*, 2002; Henry *et al.*, 2008). In several cases, other vent animals demonstrated an
315 apparent optimum temperature with peak metabolic rate, with relatively depressed
316 activity at higher and lower temperatures (e.g., *Riftia pachyptila* in Girguis *et al.*, 2002).
317
318 Temperature has a strong control over metabolic rate in vent gastropods (Childress and
319 Mickel, 1985), but based on available data for other species of vent invertebrates

including gastropods, those responses are evidentially difficult to predict *a priori*. We reviewed prior literature on physiology of vent endemic invertebrates to find comparative measurements obtained at the same temperatures as used herein (Fig. 2). We are able to make direct comparison to data for two other gastropods, *Ifremeria nautilei* and *Alviniconcha* sp. to our higher temperature treatment (Henry *et al.*, 2008). Across a range of temperatures, Henry and colleagues (2008) found that *Alviniconcha* sp. had variable metabolic rates, from a low of around 2 μmol g^{-1} hr^{-1} at 5 °C to rates over 7 μmol g^{-1} hr^{-1} with a maximum in a treatment at 19 °C and lower rates at higher temperatures; *Ifremaria nautilei*, by contrast, showed little variation with temperature, although these experiments had limited sample sizes. The rate of oxygen consumption recorded for *Alviniconcha* sp. at ~25 °C in those experiments is much higher than our results, while the mean rate they reported for *Ifremeria nautilei* is very similar to measurements we obtained for both *Alviniconcha marisindica* and *Chrysomallon squamiferum* (Figure 2B).

Data in Henry *et al*. (2008) were based on animals collected from Lau Basin, where there are three *Alviniconcha* species, which were taxonomically revised after those experiments were published (Johnson *et al*., 2014). It is not possible to determine which species (whether singular or perhaps plural) contributed to those data. Species determination is important, since superficially similar species can have distinctly different metabolic rates related to what might seem to be minor differences in life history (e.g. Carey et al., 2013). Although the congeneric *Alviniconcha* sp. is clearly closely related to the species we examined, unfortunately it is unclear how much variation should be expected within species or among similar and congeneric species.

While a thermal optimum measured for individuals of a species is likely connected to the thermal regime in their niche within the vent system, laboratory effects also impact measurements of metabolic activity. *Alviniconcha* sp. experienced lethal effects above 35 °C, but freshly collected specimens were more tolerant to higher temperatures (Henry *et al.*, 2008). This is reflective of a broader pattern, that vent animals under additional stress, including decreased pressure, are tolerant to a narrower thermal range (e.g., Mickel and Childress, 1982a; Mickel and Childress, 1982b). Early measurements in situ at 2,600 m

found that the vent bivalve *Calyptogena magnifica* Boss & Turner, 1980 was a strong oxyregulator, but that metabolic rates of captive animals were relatively depressed (Arp *et al.*, 1984). Smaller vesicomyid species, *Calyptogena elongata* Dall, 1916 and *Calyptogena pacifica* Dall, 1891, also showed much lower mass specific metabolic rates (Childress and Mickel, 1985). A number of vent species show a narrow temperature band with maximum metabolic rate, and rapidly decreasing oxygen consumption at higher or lower temperatures outside that optimum. For example, *Alviniconcha* sp. from the Western Pacific had a thermal optimum at between 20-25 °C where they had the highest oxygen consumption, and gradually decrease either side of that temperature (Henry *et al.*, 2008). *Riftia* has a higher thermal optimum at 25-27 °C, represented by a peak in oxygen, CO_2, and H_2S consumption, compared to a gradual decline in uptake at lower temperatures or a sharp drop at temperatures over 30 °C (Girguis *et al*., 2002).

Chrysomallon squamiferum has very enlarged respiratory and circulatory systems, adaptations to provide oxygen to endosymbionts housed in its trophosome-like internal esophageal gland (Chen *et al*., 2015b). The fact that we found no significant difference in oxygen consumption at two very different temperatures in *C. squamiferum* may be evidence that species is more robust than *Alviniconcha* to a wider thermal range. However, it is also possible that *Chrysomallon* would have a higher metabolic rate between 10 and 25 °C. Given that the *in situ* point measurement for temperature was relatively low (as low as 10 °C; Table 1), it is unlikely that the thermal optimum for this species is any higher than 25 °C. The closest comparators available in the literature are the gastropods *Alviniconcha* and *Ifremeria*, and another organism with a large trophosome, the tubeworm *Riftia*; these organisms all have maximum uptake rates at relatively high temperatures, closer to our experiments at 25 °C. Based on comparison to data from other *Alviniconcha* sp., and the best available measurements of temperature within the colony mass, we infer that the elevated metabolic rate recorded for *Alviniconcha marisindica* at 10 °C is not a thermal optimum, but represents a response to stress from unusually low temperature. The gradation of temperatures available here is not fine enough to draw any further conclusions about thermal optima, and there is the further consideration of the interaction of temperature and pressure on metabolism.

To facilitate effective oxygen extraction and consumption, *Alviniconcha* spp. has an increased surface area of the gill and use a combination of high oxygen affinity hemocyanins (for most of the body) and hemoglobin (for gill where symbionts are located); the gill of of *Alviniconcha hessleri* contains hemoglobins at relatively high concentrations (Wittenberg and Stein, 1995). *Chrysomallon* also has much increased gill surface area for oxygen extraction and a very large volume of blue blood which indicates usage of hemocyanin for oxygen transportation (Chen *et al*., 2015b). These are convergent with the same features in vent annelids including *Riftia* (e.g. Andersen *et al.*, 2002). Substantial work remains to understand the similarities and differences of these aspects of respiration among vent holobionts.

The oxygen binding affinity of hemoglobin and hemocyanin — and hence oxygen metabolism — are directly affected by both temperature and pressure (Arp *et al*., 1984; Childress *et al.*, 1993; Girguis *et al.*, 2002; Girguis and Childress, 2006; Girguis & Lee, 2006). Much of the other past work on the physiology of vent organisms was conducted in pressurized aquaria; after the animals were recovered to the surface (exactly as in our experiments) they were secondarily transferred to a pressurized system and re-pressurized to match their natural depth before respirometry measurements were conducted (see schematics in e.g., Quetin and Childress, 1980; Kochevar *et al*., 1992). Conducting experimental measurements at surface pressure reduces the interference with the animal subjects, but diminishes comparability with past work and their natural environment. The available literature on the physiology of vent organisms is sparse, especially in comparison to the biodiversity of vent endemic species. A number of other studies conducted at surface pressure are not directly comparable because of differences in technique, for example oxygen consumption reported per individual organism rather than wet tissue mass limits the comparative power (Koike *et al*., 1988; Fujikura *et al*., 1993). There is clearly a metabolic response to shifting ambient temperature, as demonstrated in the crab *Bythograea thermydron* which increased oxygen consumption at surface pressure compared to experiments in a re-pressurized system at a range of temperature (Mickel and Childress, 1982a). That metabolic increase was not an effect of pressure on

oxygen metabolism *per se*, which would predict a decreased rate due to lower oxygen binding affinity, but rather the sublethal effects of the neuromuscular system that increase individual oxygen demand (Mickel and Childress, 1982b). Given the possibility of such stress responses, low-pressure effects do not necessarily account for relatively low metabolic rate in the gastropod species studied here.

Conclusions

In the context of hydrothermal vent environments, which are complex, dynamic, and inaccessible, it is particularly difficult to measure temperature with sufficiently fine spatial detail to understand the abiotic environment as another species would experience it. Vent endemic species are often characteristically constrained to a very narrow environment in space and time. Hydrothermal vents on slower spreading ridges such as those in the Indian Ocean are much more stable in comparison to those on faster spreading systems such as the East Pacific Rise (Lalou *et al*., 1993; Copley *et al*., 2016). Gastropod colonies of the species we studied comprise hundreds or thousands of animals occupying 1 m^2 or less. The scaly-foot gastropod colony sampled herein was found in the same, previously recorded location from 2001 (see Suzuki *et al*., 2006), indicating that it has not moved or shifted on a scale of decades. There is evidentially a specific environment of temperature, flow, and water chemistry in that space that characterizes the niche of that species.

Further work on temperature tolerance would be beneficial to understand the boundaries of the niches of vent endemic species, both in terms of metabolic performance (e.g., Henry *et al.*, 2008; Beinart *et al.*, 2015) and behavioral choices (e.g., Bates *et al.*, 2005; Girguis and Lee, 2006). Previous research has mostly focused on species from the better studied East Pacific Rise (with some additional examples from the Western Pacific back-arc basins), and the Indian Ocean presents a very different geological context. Niche specificity limits the dispersal potential of organisms. If a species is constrained in a small niche but nonetheless need to disperse pelagically to maintain connectivity between

vent fields, then it is limited as not all vent fields may contain the specific niche required by that species.

We found different metabolic responses of two co-occurring vent gastropods with notably different symbiont-housing mechanisms. Interestingly, the trophosome anatomy of *Chrysomallon* does not fundamentally increase oxygen requirement compared to other gastropod holobionts. This fits with a general model that the scaly-foot gastropod adaptations — including a reduced, non-ganglionated nervous system as well as the large, well-developed trophosome — render it a vessel for its symbionts, and its physiology may buffer the bacteria from environmental fluctuations. By contrast, *Alviniconcha* has no way of protecting its symbionts housed in the gill epithelium which comes in direct contact with vent fluid, resulting in the speculative putative bleaching symptoms under stress.

Based on the evidence reported here, it is possible that the optimum metabolic performance of the scaly-foot gastropod would be found between the high and low temperatures we were able to use. However, based on the respirometry trials and the observed behavior of the captive animals, it is possible that *Chrysomallon* may simply be more robust to a wider range of environmental conditions. The limits of these species' tolerance remains an important question, because differential abiotic ranges could be taken as evidence that niches are defined as much by biotic competition as by physiological constraint.

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Figure captions

Figure 1. Indian Ocean hydrothermal vent gastropods. A. The scalyfoot gastropod, *Chrysomallon squamiferum*, inside a respirometry chamber. The insertion of the fiber optic oxygen probe is visible at bottom. The animal is attached to the chamber floor and its head is extended, apparently exploring its surroundings. B. *Alviniconcha marisindica*, in a shipboard aquarium. C. *Alviniconcha marisindica*, attached to the wall of a respirometry chamber at 10 °C.

Figure 2. Oxygen consumption rates of hydrothermal vent gastropods per gram wet tissue mass (μmol O_2 / g / hr), in *Chrysomallon squamiferum* and *Alviniconcha marisindica*. A. Mean mass-specific metabolic rates in each temperature group; error bars indicate standard deviation and numbers within the bars show sample size per group, grey bars are *Alviniconcha marisindica* (three temperature treatments), black bars are *Chrysomallon squamiferum* (two temperature treatments). B. Log-log plot of mass-specific metabolic rate of individual animals at 25 °C. Grey points are *Alviniconcha* (diamonds are individuals from Kairei field, circles are individuals from Edmond field; dashed line indicates the least-squares regression of a power scaling relationship for allometric scaling of metabolism using all samples, $r = 1.7M^{-0.25}$ for oxygen consumption rate *r* and mass *M*), black squares are *Chrysomallon squamiferum*, crosses are two additional data points for other vent gastropod species with metabolic rates inferred at the same experimental temperature (Henry *et al.*, 2008).

Supplementary Material

Supplementary Video 1. The scaly-foot gastropod, *Chrysomallon squamiferum*, has a large internal trophosome to house symbiotic bacteria, a large gill, and a large muscular heart that pumps, as shown in this video of the live heartbeat.