Morphological adaptations to chronic hypoxia in deep-sea decapod crustaceans from hydrothermal vents and cold seeps

Johan Decelle[1], Ann Andersen[1] and Stéphane Hourdez[2*]

[1] Equipe Ecophysiologie des Invertébrés des Milieux Extrêmes, Station Biologique de Roscoff, CNRS-UPMC, 29680 Roscoff, FRANCE

[2] Equipe Génétique de l'Adaptation en Milieux Extrêmes, Station Biologique de Roscoff, CNRS-UPMC, 29680 Roscoff, FRANCE

* corresponding author:

Stéphane Hourdez

Equipe Génétique de l'Adaptation en Milieux Extrêmes, Station Biologique de Roscoff, CNRS-UPMC, 29680 Roscoff, FRANCE

hourdez@sb-roscoff.fr

Phone: +33-298-29-2340

Fax: +33-298-29-2324

Mar Biol (2010) 157:1259–1269
DOI 10.1007/s00227-010-1406-8

Received: 20 October 2009 / Accepted: 2 February 2010 / Published online: 6 March 2010

Abstract

Animals inhabiting hydrothermal vents and cold seeps face conditions that are challenging for survival. In particular these two habitats are characterized by chronic hypoxia, sometimes reaching complete anoxia. The characteristics of the scaphognathite and gills were studied in 4 species of shrimp and 3 species of crabs from hydrothermal vents and cold seeps, in order to highlight potential adaptations that could enhance oxygen acquisition in comparison to shallow-water relatives. All the vent and seep species studied here exhibit significantly larger scaphognathites, likely allowing more water to flow over their gills per stroke of this appendage. This is probably more energetically efficient that prolonged hyperventilation. In contrast to annelids, vent and seep decapods usually do not possess enlarged gills, a phenomenon likely due to the physical limitations imposed by the size of the gill chamber. In the vent shrimp *Rimicaris exoculata* and the vent crab *Bythograea thermydron*, however, there is a significantly higher specific gill surface area linked to a higher number of lamellae per gram of gill. Again in contrast to annelids, the diffusion distance through the gills is not strikingly different between the vent shrimp *Alvinocaris komaii* and the shallow-water species *Palaemon* spp.. This may indicate that the epithelium and cuticle of the decapod gills are already optimized for oxygen uptake and that reducing the thickness of these compartments is not physically possible without affecting the physical integrity of the gills.

Key words: hydrothermal vents, cold seeps, *Bythograea*, *Austinograea*, *Segonzacia*, *Xantho*, *Alvinocaris*, *Lebbeus*, *Rimicaris*, *Palaemon*, scaphognathite, gill surface area.

Introduction

Decapod crustaceans have colonized many marine ecosystems including hydrothermal vents and cold seeps, respectively discovered in 1976 (Lonsdale et al. 1977) and in 1984 (Paull et al. 1984). With more than 125 species in 33 families, decapods are well-represented in these two environments (Martin and Haney 2005). Yet, these deep-sea habitats are inhospitable for metazoans because of their peculiar physical and chemical environmental conditions, and they represent serious challenges for essential physiological functions such as respiration. Although they are mobile, decapod crustaceans are exposed to harsh conditions similar to those experienced by the more sessile species such as tubeworms and mollusks.

Hydrothermal vents are mainly characterized by the very hot (up to 350˚C), anoxic fluid, rich in carbon dioxide and sulfide, and laden with toxic chemicals and heavy metals (Edmond et al. 1982). Living in a chaotic mixing zone between the hydrothermal fluid and seawater, the fauna constantly experiences rapid shifts in temperature and changes in sulfide and carbon dioxide concentration (Childress and Fisher 1992). Oxygen partial pressure varies inversely with sulfide concentration and temperature, and can fluctuate widely, down to very low values. In addition, the high concentration of carbon dioxide (8 mM; Edmond et al. 1982) affects respiration, as it must be eliminated by diffusion through the exchange surfaces.

Although more passive and less chaotic, hydrocarbon seeps also constitute a reduced environment. Sulfide slowly diffuses from the sediment to the ambient water and spontaneously reacts with the free oxygen. As a result, oxygen concentration decreases with proximity to the sediment (Kennicut et al. 1989), averaging 39 µM in a mussel bed, but it can be sometimes non-detectable (detection limit 10 µM; Smith et al. 2000). The temperature is very stable, with 8°C on average for the best-known sites in the Gulf of Mexico, in sharp contrast to the highly variable temperature at vents.

In addition, sulfide, common in both habitats, is a metabolic poison that can affect the mitochondrial electron transfer chain and consequently disrupt aerobic metabolism (Grieshaber and Völkel 1998). Interestingly, despite all these combined constraints, oxygen consumption rates of invertebrates from such reduced environments are similar to the ones of relatives living at higher environmental oxygen tensions (Childress and Mickel 1985; Hourdez et al. 2002; Fisher et al. 2000). These invertebrates must therefore possess specific adaptations of the respiratory system to extract enough oxygen from the hypoxic environment to meet their metabolic requirements, and to avoid having to rely on anaerobic metabolism. In crustaceans, physiological regulation is possible at different levels. Very limited data are available on the respiratory adaptations of the deep-sea hydrothermal-vent and cold-seep crustaceans to understand how these organisms can survive -and thrive- in such harsh habitats.

The first way to improve oxygen extraction from the environment is to increase ventilatory convection. In decapod crustaceans, the gills are ventilated by the rhythmic beating of the paddle-shaped scaphognathite, epipodites of the second maxillae, located in a narrow channel, just anterior to the branchial chambers (Borradaile et al. 1958). During a normal cycle, the downward movement of the scaphognathite creates a depression inside the branchial chamber, so water flows in through the limb bases, supplying oxygen to the chamber. The ventilation depends on both the frequency and the physical force of the scaphognathite beating. Hyperventilation, an increase in beating frequency, is a common behavior found in response to acute hypoxia (Taylor 1982). However, this immediate change represents only a short-term response as it ceases during chronic exposure, probably due to the high energetic costs (McMahon 2001). Ventilation can also be improved by increasing the stroke volume, a product of force of the scaphognathite beating. The mechanisms underlying this higher beating performance is still unclear.

In crustaceans, the thickness, calcification and sclerotization of the general body surface all represent an effective limitation to the diffusion of gases. Diffusion can only occur across thin and uncalcified permeable areas such as the gills in the branchial chambers. Oxygen diffusion is directly proportional to gill surface area, and inversely proportional to diffusion distance (Fick's law). A study in several vent and seep annelid species revealed that they have larger gills and shorter diffusion distances compared to their littoral relatives (Jouin and Gaill 1990; Hourdez et al. 2001; Hourdez and Lallier 2007). To date, no studies have addressed similar adaptations in crustaceans inhabiting these deep-sea environments.

This study investigated the respiratory anatomy in vent and seep decapod species to seek potential morphological adaptations that could enhance oxygen transfer efficiency. We focused on the first two levels of oxygen transfer. We measured scaphognathite surface area, gill surface area and diffusion distance in several crab and shrimp species, over a range of sizes for each species. We compared species that live at hydrothermal vents, cold seeps, and in the littoral zone as a reference. This allowed us to shed light on shared and on specific adaptations in species that live under chronic hypoxia.

Material and methods

Crustacean collections

The deep-sea species were collected during various oceanographic cruises to hydrothermal and cold-seep sites with remotely operated vehicles (ROVs) or manned submersibles (see Table 1 for details). Shallow-water crabs and shrimp were collected near the marine laboratory in Roscoff, France. All specimens were fixed in 4% formaldehyde in filtered seawater for 24 h, rinsed in fresh water, and transferred to 70% ethanol until used. Before dissection for the measurements, each individual was rinsed in fresh water, and its wet body weight (g) measured after removing excess water.

Scaphognathite surface area

First, we removed the scaphognathites of the shrimp and crabs from one of the branchial chambers by cutting them at their base. Photographs of the two scaphognathite faces were taken through a binocular microscope, and then their surface was measured with the software Image J (version 1. 36 B, developed by Wayne Rasband, National Institutes of Health, USA). A known reference surface was photographed at the same magnification to convert scaphognathite pixel areas into mm^2. We calculated the surface area of the scaphognathite only considering the chitinous paddle-shape area. The fine expansions of the scaphognathite (setae and setules) were not taken into account because their mechanical contribution to ventilation is probably small. The scaphognathite surface area, corresponding to the mean surface of the two faces (mm^2), was determined for all shrimp and crab species.

Gill surface area

In crustacean decapods, the gills are located inside two symmetrical branchial chambers, enclosed by the branchiostegite, an expansion of the cephalothorax. In this study, all the species have phylobranchiate gills, the leaf-like lamellae being attached in two rows along the raphe. First of all, for each species, we established a ratio between the surface area and the weight of a single gill ($mm^2\ g^{-1}$). To do so, different anterior and posterior gills from several individuals were excised, dabbed on a filter paper to remove excess water, and weighed on a high-precision balance. Under a binocular microscope, we then divided each gill with a scalpel blade into uniform sections containing 5 to 20 equally-sized lamellae, and the number was recorded. For each section of gill, we took photos of the first and last pair of lamellae.

Using the software Image J, we then calculated the surface area of each section by multiplying the mean areas of the first and last pair of lamellae by the number of lamellae in the section, and finally by doubling this result to take into account both faces of the lamellae. The surface areas of the different sections from the same gill, were obtained and added up to determine the total surface area of the whole gill. As a result, the ratio between the surface area and the weight of a single gill was obtained ($mm^2\ mg^{-1}$). Once this relationship was established for each species, the total gill surface area for a shrimp or a crab could be determined by simply excising all the gills, carefully removing excess water, weighing them and converting this weight into a surface area in mm^2 with the species-specific ratio calculated previously.

Diffusion distance

For this part of the study, we investigated the shrimp species *Palaemon elegans*, *Palaemon serratus* and *Alvinocaris komaii*. We preserved one anterior and one posterior gill from each individual in 4% glutaraldehyde in cacodylate buffer at 0.2 M, pH 7.4. The gills were then rinsed in 0.2 M sodium cacodylate buffer and post-fixed in 1% osmium. They were then dehydrated in a series of graded ethanol and finally embedded in Epon resin for 48 h at 60°C. Semithin (1 µm) and ultrathin (60 nm) sections were made with a LEICA UCT ultramicrotome. The semithin sections were stained with 1% toluidin blue and observed with a light microscope. The ultrathin sections were contrasted with 2% uranyl acetate in alcoholic solution and lead citrate before their observation on a JEOL JEM 1200EX transmission electron microscope. The molt stage was checked on the ultrathin sections, and all shrimp were at the C stage, corresponding to the intermolt or anecdysis (Drach et al. 1967). The diffusion distance, which corresponds to the combined thickness of the cuticle and the epithelium, was measured as the orthogonal distance between the surface of a gill and a hemolymphatic lacuna (where the distance appeared to be the shortest). This was carried out on several electron micrographs for each gill with the Image J software.

Ventilatory behavior

We used video recordings filmed by the ROV *Jason II* at the Kilo Moana (2650 m) and ABE (2140 m) hydrothermal sites in the Lau Back-Arc Basin to analyze the ventilatory behavior of *Alvinocaris komaii*. The scaphognathite was clearly visible inside the branchial chamber through the branchiostegite, so that we were able to measure the beating frequency of the scaphognathite (ventilation rate). To do so, we counted the number of scaphognathite beats over different 10-s periods for each individual, and the mean corresponds to the beat frequency (beat min^{-1}) of a single individual. We carried this out on several individuals and at two different locations: among the mussels *Bathymodiolus brevior* (n=25), and among the gastropods *Ifremeria nautilei* (n=8), where the chemistry of the water is different.

Statistical analysis

To study the allometric relationship between two characters, all the variables were log_{10} transformed (Teissier 1948). To compare the surface areas of the scaphognathite and the gills between the species, we used an analysis of covariance (ANCOVA, software R, version 2.6.1, Copyright © 2007. The R Foundation for Statistical Computing). We examined possible interactions between the factor “species” and the body weight, by testing the homogeneity of the slopes. If the slopes were parallel, we calculated the intercepts of the regression lines for each crab and shrimp species, which could reveal differences in the surface areas of the scaphognathite or gills. The diffusion distance values were first analyzed by a Normality Test (Shapiro-Wilk), and then by an analysis of variance (ANOVA) with the software Sigmaplot (version 11.0.1, Copyright © 2009 Systat Software Inc.). When the normality test failed (P <

0.05), we carried out a Kruskal-Wallis one-way analysis of variance based on ranks. Comparison between species was possible via the Multiple Comparison Procedures (Holm-Sidak method). With the same software, we used a T-test to compare the beating frequency means.

Results

Scaphognathite surface area

Shrimp

The surface area of the scaphognathite increases with body weight (Fig. 1A). For most species, the allometric coefficient (*a*), represented by the slopes of the linear regressions, ranged between 0.61 and 0.79, indicating that the scaphognathite of smaller individuals tends to have a higher specific surface area (per body weight unit, $mm^2\ g^{-1}$), than larger individuals. However, this is not the case for *Rimicaris exoculata*, for which the specific surface area of the scaphognathite seems independent on the size (a = 1.05).

The littoral shrimp *Palaemon elegans* and *P. serratus* have the same scaphognathite surface area (ANCOVA, P = 0.639). Another group is formed by *Alvinocaris muricola* and *A. komaii* (P = 0.071). However, the small number of *A. komaii*, and the limited size range of that vent species make it difficult to truly compare the two *Alvinocaris* species. As for *Lebbeus* sp., only two individuals were sampled, and their scaphognathite surface area does not belong to either the expected distribution of *A. komaii*, or to that of the littoral species at a 95% confidence interval. There is an overlap between *R. exoculata* and *A. muricola* for small individuals (about 0.15 g), indicating that these two species have a similar scaphognathite surface area in this size range. Nevertheless, due to the different allometric relationships, the scaphognathite surface areas in larger individuals markedly differ. When comparing shrimp of similar weight (e.g. 2.45 g, represented by the dotted line in Fig. 1A), the scaphognathite of the vent *R. exoculata* (57.5 mm^2) is about 11 times larger than that of the littoral species (5.1 mm^2), 3 times larger than that of the seep *A. muricola* (19.6 mm^2), and 4 times larger than the scaphognathite of the vent species *A. komaii* (14.5 mm^2).

Crabs

The scaphognathite surface areas also increase with body weight, and the allometric coefficients range from 0.57 to 0.70 in the five crab species (Fig. 1B). The scaphognathite of smaller crabs therefore has a greater specific surface area (mm^2 g^{-1}) in all five species. The vent crab *Bythograea thermydron* has a significantly larger scaphognathite surface area ($P = 0.000$) than the littoral species, *Carcinus maenas* and *Xantho pilipes,* and than the two other vent species *Austinograea alaysae* and *Segonzacia mesatlantica*. The two littoral species and *A. alaysae* have a comparable scaphognathite surface area ($P > 0.100$). When comparing crabs of similar weight (e.g. 14 g, represented by the dotted line in Fig. 1B), the scaphognathite from *B. thermydron* (41.9 mm^2) is twice as large as the one from *A. alaysae* (25 mm^2), *C. maenas* (21.2 mm^2) and *X. pilipes* (23 mm^2). If we extrapolate the correlation to this weight for *S. mesatlantica*, this species has almost the same scaphognathite surface area (37.3 mm^2) as *B. thermydron*.

Gill surface area

Shrimp

The littoral shrimp species, *Palaemon elegans* and *P. serratus*, each have 8 pairs of gills (5 pleurobranchs, 2 arthrobranchs and 1 podobranch). *Alvinocaris muricola*, *A. komai,* and *Rimicaris exoculata* all bear 10 pairs of gills (5 pleurobranchs and 5 arthrobranchs) whereas *Lebbeus* sp. only has 5 pairs (all pleurobranchs). Several different gills, dissected from two *P.*

elegans (*n*=4 gills), two *P. serratus* (*n*=4 gills), three *A. muricola* (*n*=7 gills) and one *A. komaii* (*n*=2 gills), have similar surface area/weight ratios, regardless of the type of gill and the species (ratios ranging from 37.6 to 44.9 $mm^2\ g^{-1}$). The mean of these ratios, 41.0 ± 3.0 $mm^2\ g^{-1}$ of gill (*n*=17), was used for calculating the gill surface area in these four species. Higher ratios were found for *R. exoculata*, (63.7 ± 3.0 $mm^2\ g^{-1}$; *n*=4 gills), and *Lebbeus* sp. (57.5 ± 9.1 $mm^2\ g^{-1}$; *n*=5 gills), and were consequently used for their respective species.

In *Palaemon* spp., *A. muricola*, *A. komaii*, and *R. exoculata*, gill surface areas increase with body weight (Fig. 2A). The allometry coefficients, ranging from 0.92 to 1.17 indicate that the specific gill surface area remains relatively constant throughout growth in these shrimp. *Rimicaris exoculata* clearly has a larger gill surface area (P = 0.000) than the other species. For shrimps of 2 g wet weight (dotted line in Fig. 2A), the gill surface area of *R. exoculata* is nearly twice that of the other species. This increase correlates with a higher number of lamellae per milligram of gill. The gills of *R. exoculata* have roughly twice as many lamellae (82 lamellae mg^{-1}) as *A. muricola* and *A. komaii,* which have 44 lamellae.mg^{-1}. Although the ANCOVA indicates that *P. elegans* has a larger gill surface area (P = 0.000) than *A. muricola* and *P. serratus*, and a similar one to that of *A. komaii* (P = 0.400), all these species seem to form a single group, distinctly different from *R. exoculata*.

Crabs

Bythograea thermydron, *Austinograea alayseae*, *Carcinus maenas*, *Xantho pilipes and Segonzacia mesatlantica* all have nine pairs of phyllobranchiate gills. The surface area/weight ratios for gills from several individuals are different for each species: *X. pilipes* 33.7 ± 3.6 $mm^2\ g^{-1}$ (*n* = 8 gills), *C. maenas*, 24.8 ± 3.2 $mm^2\ g^{-1}$ (*n* = 4 gills), *A. alayseae* 128.1 ± 33.6 $mm^2\ g^{-1}$ (*n* = 4 gills), *B. thermydron* 74.2 ± 12.2 $mm^2\ g^{-1}$ (*n* = 4 gills), and *S. mesatlantica* 67 ± 18.7 $mm^2\ g^{-1}$ (*n* = 4 gills).

The allometric relationship between the surface area and the body weight is similar in all crabs, ranging from 0.88 to 1.10 (Fig. 2B). The crab *B. thermydron* clearly has a higher specific gill surface area than the other species (P = 0.000). There are nearly twice as many lamellae per unit gill weight in *B. thermydron* (28 lamellae mg^{-1}) compared with *C. maenas* (10.5 lamellae mg^{-1}) and *X. pilipes* (13 lamellae.mg^{-1}). The Mid-Atlantic Ridge vent species *S. mesatlantica* has a ratio of 23.6 lamellae mg^{-1}, although this does not translate into a markedly increased gill surface area. *Xantho pilipes* and *A. alaysae* form a group (P = 0.270), and have a smaller gill surface area than *C. maenas* (P = 0.044 and P = 0.015, respectively). Although the difference is not great, the gill surface areas of *S. mesatlantica* and *C. maenas* are significantly different (P = 0.015).

Diffusion distance

The gill structure of *Palaemon elegans*, *Palaemon serratus* and *Alvinocaris komaii* was compared, with an emphasis on the proximal zone. The branchial lamellae of these shrimp have a similar structure, with a hemocoelic space, enclosed by an epithelium and a cuticle (Fig. 3). The axial tissue zone is formed by H-shaped epithelial cells, which spread their thin lateral expansions beneath the cuticle. We measured the thickness of the epithelium and the cuticle, which form the barrier between the water and the hemolymph. The epithelium thickness is similar for all the species, for both anterior and posterior gills, with a mean ranging from 0.41 to 0.54 µm (Fig. 4C, P = 0.217). Differences in the overall diffusion distance could then only be due to the thickness of the cuticle. In the littoral shrimp *P. elegans* and *P. serratus*, the cuticle is thinner in the posterior gills than that in the anterior ones (Fig. 4B, P < 0.001), while the cuticle thickness remains constant for the vent species *A. komaii* (Fig. 4B, P = 0.224). As a result, in the littoral shrimp, the posterior gills have a significantly shorter diffusion distance than the anterior ones (Fig. 4A, P < 0.001), while in *A. komaii* the diffusion distance is the same regardless of the position of the gill (Fig. 4A, P = 0.102). The diffusion distance of the latter species is comparable to the posterior gills but shorter than the anterior gills of the littoral species *P. serratus* and *P. elegans*.

Ventilatory behavior

The vent shrimp *Alvinocaris komaii* were mainly found among the mussels *Bathymodiolus. brevior* (*n*=25), but a few were observed among the gastropods *Ifremeria nautilei* (*n*=8), where the temperature usually is higher and the oxygen concentration lower (Podowski et al. submitted). The scaphognathite beating frequency was higher (P = 0.033) among gastropods than among mussels (Fig. 5). In addition, the ventilatory activity showed great variability in shrimp living among mussels, ranging from 30 to 156 beat min^{-1} (represented by the box plot extremes), whereas when among gastropods, the scaphognathite always had a high beating frequency (144 to 168 beats min^{-1}).

Discussion

This study examined potential morphological adaptations to chronic hypoxia in decapods by comparing hydrothermal-vent and cold-seep species of crabs and shrimp to intertidal relatives.

Under hypoxia, the oxygen-depleted water must be renewed very rapidly inside the branchial chambers to maintain an optimal difference of oxygen partial pressure between the two sides of the diffusion barrier (i.e. the environment and the hemolymph in the lacunae). During such acute hypoxic exposures, there usually is hyperventilation, a higher beating frequency of the scaphognathite (Taylor 1982). This increases the volume of water flowing through the branchial chambers, and thus improves oxygen supply (McMahon and Wilkens

1975; Burggren and McMahon 1983). However, as the scaphognathite muscles in decapod crustaceans are highly aerobic (Wilkens et al. 1984), hyperventilation itself increases the oxygen demand. As a consequence, this high pumping activity does not last long under chronic hypoxia (McMahon 2001), and the organism must rely on other compensatory mechanisms. Two earlier studies (Cumberlidge and Uglow 1977; Pilkington and Simmers 1973) showed that the ventilation volume can be modulated, not only through changes in scaphognathite beating rate, but also through variations in the force per scaphognathite stroke.

In *Carcinus maenas*, Cumberlidge and Uglow (1977) found that the performance of the scaphognathite decreases throughout growth: smaller crabs pump proportionally more water per scaphognathite stroke than larger crabs do. Our morphological investigation on the scaphognathite revealed that smaller individuals clearly have a proportionately bigger scaphognathite than larger ones, in all crab and all but one shrimp species (*Rimicaris exoculata*). Thus, the better performance in smaller crabs observed by Cumberlidge and Uglow may be explained by the comparatively larger surface area in younger specimens that allows them to move more water per stroke. Interestingly, the allometric growth of the scaphognathite ($a \approx 0.75$) parallels the typical allometric relationship of metabolic oxygen demand (Schmidt-Nielsen 1984). A larger scaphognathite would thus be advantageous for smaller crab and shrimp individuals because it would improve the ventilation volume over their gills per stroke, thus meeting their higher metabolic requirements. *Rimicaris exoculata* is a noticeable exception to this, with a specific scaphognathite surface area that remains relatively constant regardless of the size of the shrimp. This may be related to the unique biology of this shrimp that lives in symbiosis with bacteria that mainly grow on the branchiostegite inside the gill chamber, and on the scaphognathite (Van Dover et al. 1988). This gill chamber increases in size as the shrimp grows and accommodates more symbiotic bacteria.

In addition to these allometric differences within species, we observed between-species differences. The scaphognathites of crab and shrimp species that inhabit hydrothermal vents and cold seeps clearly have a larger surface area when compared to the littoral species for a similar wet weight. Moving a larger scaphognathite undoubtedly requires more strength but the elasticity and whip-like motion of this appendage suggest that this higher energy does not scale linearly with the surface area of the scaphognathite. These deep-sea species most likely have an improved effectiveness of scaphognathite beating and, as a consequence, a better ventilation capacity than the littoral species. Hydrothermal-vent and cold-seep species nonetheless retain their ability to adjust ventilation rate, as observed for the shrimp *Alvinocaris komaii* found among the mussels (*Bathymodiolus brevior*) and gastropods (*Ifremeria nautilei*). These two species live in environments that differ in oxygen partial pressure, which likely influences the ventilation rate. This rate can also be influenced by temperature, which affects the metabolic rate, and therefore the oxygen requirements.

Diffusion of gases through gills depends on two limiting factors: the gill surface area and the diffusion distance. There usually is a separation of respiratory and ion transport functions in the gills of many aquatic crustaceans, with the anterior gills mainly involved in respiratory gas exchange, and the posterior gills being specialized for ion transport, where the respiratory lamellae are relatively thick (Copeland and Fitzjarrell 1968; Aldridge and Cameron 1979; Neufeld et al. 1980; Henry and Cameron 1982). In littoral and vent shrimp, we observed that the diffusion distance varies depending solely on cuticle thickness, and not on that of the epithelium (which has the same thickness regardless of the species or gill type). Unexpectedly, in *Palaemon elegans* and *Palaemon serratus*, the posterior gills have a thinner cuticle, and thus a shorter diffusion distance than the anterior ones. In contrast, the diffusion distance in the vent *A. komaii* is the same in anterior and posterior gills. The diffusion barrier measured in *A. komaii* is smaller than that found in the lophogastrid shrimp, *Gnathophausia* *ingens* (1.1 *vs.* 1.5 - 2.5 µm for the latter species) that lives in the minimum oxygen layer (Belman and Childress 1976), and in *Rimicaris exoculata* (2.8 - 3 µm; Martinez et al. 2004). This greater diffusion distance in *R. exoculata* is explained by a thicker epithelium, as the cuticle thickness (0.3 - 0.5 µm) is comparable to that of *A. komaii* (0.3 - 0.8 µm). There are unfortunately no data for vent and seep species of crabs.

The observed difference between the vent and non-vent species of shrimp is small in comparison to what occurs in annelids, where the diffusion distance in hydrothermal-vent and cold-seep species may be only half of that in their littoral relatives (Hourdez et al. 2001). This large difference is mainly because in annelids, shorter diffusion distances are achieved by the development of intraepidermal vascular loops (see Hourdez and Lallier 2007) while in decapods the distance is already short, with a thin epithelium and cuticle. In addition, the cuticle has to maintain the structural and functional integrity of the gills in a rapid flow of water, thereby imposing a lower limit on cuticle thickness. The slight diffusion distance differences between the littoral and vent species are not sufficient to represent a true respiratory adaptation, especially considering the limited number of individuals studied, and biases could have resulted from possible slight variations in the section angle.

Various studies on crabs found some variations in gill surface areas in relation to lifestyle in littoral species of decapods (Gray 1957; Johnson and Rees 1988). Our work shows that two of the vent species, *R. exoculata* and *Bythograea thermydron*, clearly have a greater gill surface area than the other species do. This trend was also observed in several cold-seep and hydrothermal-vent polychaetes (reviewed by Hourdez and Lallier 2007). In the shrimp and crab species studied here, the increase of gill surface area is due to a larger number of lamellae per mass of gill (ca. twofold, as also reported by Gray (1957), and Johnson and Rees (1988) for littoral crabs. The higher gill surface area, the oxygen uptake from the environment in *R. exoculata* and *B. thermydron* is consequently enhanced (assuming the diffusion distance remains similar, as we found for the shrimp). This morphological adaptation was however not observed in all the vent species. This can be explained by the fact that the shrimp *A. komaii* and *Lebbeus* sp. are usually found in somewhat colder niches, where hypoxia should be less pronounced. *R. exoculata* and *B. thermydron* on the other hand are commonly found along the chimney-walls close to the hot and anoxic hydrothermal fluid, or inside *Riftia* thickets for the crab (Segonzac 1992; Gebruk et al. 1993), where access to oxygenated deep-sea water is likely more limited than for the other species of crabs.

The surface areas measured in *R. exoculata* and *B. thermydron* are similar to those measured in fish with high metabolic activities (Wegner et al. 2009). Their metabolic activities are however very similar to their shallow-water relatives. The oxygen uptake per unit area of gills (VO_2/SGA) differs markedly between the shallow-water species on one hand, and *R. exoculata* and *B. thermydron* on the other hand. Shallow-water species have a high flux per unit area, which fits with the large O_2 gradients they experience, whereas the two vent species are characterized by large gills that are not meant to support high fluxes per se, but modest fluxes at very small oxygen gradients. This is similar to what was observed in *Gnathophausia ingens* (Belman and Childress 1976).

In contrast to annelids, the gills of decapods are enclosed in a chamber, necessarily limiting the possible development of gills. This probably represents a physical limit to developing larger gills in decapods, with the noticeable exception of *R. exoculata* that has enlarged gill chambers containing epibiotic bacteria. There may also be physiological constraints to developing larger gills. The environment in which these species live is not only hypoxic but also laden with toxic compounds and heavy metals (which could also then be taken up in larger amounts). Besides, crustacean gills have been shown to be the organ in which accumulation of some toxics, such as cadmium (Papathanassiou and King 1983; Soegianto et al. 1999), and even sulfide (Compère et al. 2002), can occur.

In the particular case of *R. exculata*, it is hard to evaluate whether the increase in scaphognathite and gill surface areas are truly respiratory adaptations or simply due to its bacterial epibiosis relationship, which develops on different parts of the branchial chamber (Van Dover et al. 1988; Casanova et al. 1993; Gebruk et al. 1993; Segonzac et al. 1993). No epibiont grows on gill lamellae, keeping the exchange surface free, but the inner faces of the branchiostegites and the scaphognathites bear long bacteriophore setae (Zbinden et al. 2004). The latter have likely expanded to host and/or compensate for the presence of the epibiont community, which may affect gill ventilation. With an improved ventilatory convection and oxygen diffusion, *R. exoculata* has a better tolerance to the warmer and more hypoxic conditions around the chimneys, and thus, as a host, this species can come close to the sulfide-containing fluid in order to fuel its epibionts.

The gills may not represent the only respiratory surface in the organisms studied here. Additional respiratory structures, such as the branchiostegite, were found in some littoral crabs, acting as lungs (Henry 1994). Supplementary studies would be interesting to carry out in order to find out if this occurs in hydrothermal vent and cold seep crustaceans.

Finally, this study focused on the first two levels in the oxygen transfer system. Once past the branchial epithelium, the oxygen is reversibly bound by hemocyanins. Earlier studies of the functional properties of these hemocyanins in various vent species revealed that they possess a very high affinity for oxygen, binding it even when the environmental concentration is low (reviewed by Hourdez and Lallier 2007). This property also favors the inward flow of oxygen as the resulting amount of free oxygen remains low and the difference of partial pressure is maximized. These hemocyanins are also characterized by a pronounced Bohr effect (decreased affinity for oxygen at lower pH), allowing the release of oxygen near metabolically active tissues. Interestingly, hemocyanins from hydrothermal vent species studied to date are insensitive to temperature variations within the physiological range of pH and temperature. This may also represent an adaptation in the highly variable vent environment.

Acknowledgments The authors would like to thank François Lallier, Matthieu Bruneaux for his help in the laboratory, and Lucia Di Iorio for her assistance for the statistical analyses. We would like to thank the chief scientists, and ship and submersible crews involved in the cruises during which the crustaceans were collected. We would like to thank Jim Childress who prompted very stimulating discussions. The mid-Atlantic cruise during which *S. mesatlantica* were sampled was supported by the Priority Program 1144 (From Mantle to Ocean: Energy- Material- and Life-Cycle at Spreading Axes; contribution number 45) of the German Research Foundation (DFG), and the DFG Cluster of Excellence at MARUM, Bremen. The Lau Basin samples were collected during a project funded by a NSF grant (OCE-0240985) to C. R. Fisher and S. Hourdez. The cruise during which *R. exoculata* were collected was supporter by IFREMER (Serpentine cruise grant). *B. thermydron* were collected on a project funded by an NSF grant to C.R. Fisher (NSF OCE-0002729), and *A. muricola* were collected on a project funded by a Mineral Management Service (Investigations of chemosynthetic communities on the lower continental slope of the Gulf of Mexico) grant to C.R. Fisher. Some of the research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the HERMIONE project, grant agreement n° 226354.

References

Aldridge JB, Cameron JN (1979) CO_2 exchange in the blue crab, *Callinectes sapidus* (Rathbun). J Exp Zool 207:321–328

Borradaile LA, Potts FA, Eastman LES, Saunders JT (1958) Chapter XI. The class Crustacea. The Invertebrata, Third Edition, (revised by G. A. Kerkut), pp. 340–419. London: Cambridge University Press

Belman BW, Childress JJ (1976) Circulatory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. Biol Bull 150:15–37

Burggren WW, McMahon BR (1983) An analysis of scaphognathite pumping in the crayfish *Orconectes virilis*: compensatory changes to acute and chronic hypoxia. Physiol Zool 56:309–318

Casanova B, Brunet M, Segonzac M (1993) L'impact d'une épibiose bactérienne sur la morphologie fonctionnelle de crevettes associées à l'hydrothermalisme médio–Atlantique. Cah Biol Mar 34:573–588

Copeland DE, Fitzjarrell AT (1968) The salt absorbing cells in the gills of the blue crab (*Callinectes sapidus* Rathbun) with notes on modified mitochondria. Z Zellforsch Mikrosk Anat 92:1–22

Childress JJ, Mickel TJ (1985) Metabolic rates of animals from hydrothermal vents and other deep-sea habitats. Biol Soc Wash Bull 6:249–260

Childress JJ, Fisher CR (1992) The biology of hydrothermal vent animals: Physiology, biochemistry and autotrophic symbioses. Oceanogr Mar Biol Annu Rev 30: 337–441

Compère P, Martinez AS, Charmantier-Daures M, Toullec JY, Goffinet G, Gaill F (2002) Does sulphide detoxification occur in the gills of the hydrothermal vent shrimp *Rimicaris exoculata*. C R Biologies 325:591–596

Cumberlidge N, Uglow RF (1977) Heart and scaphognathite activity of the shore crab *Carcinus maenas* (L.). J Exp Mar Biol Ecol 28:117–124

Dalla Via J (1985) Oxygen consumption and temperature change in the shrimp *Palaemon elegans*. Mar Ecol Prog Ser 26:199–202

Drach P, Tchernigovtzeff C (1967) Sur la méthode de détermination des stades d'intermue et son application générale aux crustacés. Vie et Milieu 18A:595–610

Edmond JM, Van Damm KL, McDuff RE, Measures CI (1982) Chemistry of hot springs on the EPR and their effluent dispersal. Nature 297:187–191

Fisher CR, MacDonald IR, Sassen R, Young CM, Macko S, Hourdez S, Carney R, Joy S, McMullin E (2000) Methane ice worms: *Hesiocaeca methanicola* colonizing fossil fuel reserves. Naturwissenschaften 87:184–187

Gebruk A, Pimenov N, Savvichev A (1993) Feeding specialization of bresiliid shrimps in the TAG site hydrothermal community. Mar Ecol Prog Ser 98:247–253

Gray IE (1957) Comparative study of the gill area of crabs. Biol Bull 112:34–42

Grieshaber MK, Völkel S (1998) Animal adaptations for tolerance and exploitation of poisonous sulfide. Ann Rev Physiol 60:33–53

Henry RP (1994) Morphological, behavioral, and physiological characterization of bimodal breathing crustaceans. Amer Zool 34:205–215

Henry RP, Cameron JN (1982) Acid/base balance in the euryhaline blue crab, *Callinectes sapidus* , during acclimation from high to low salinity. J Exp Biol 101:255

Hourdez S, Lallier FH (2007) Adaptations to hypoxia in hydrothermal-vent and cold-seep invertebrates. Rev Environ Sci Biotech 6:143–159

Hourdez S, Frederick LA, Schernecke A, Fisher CR (2001) Functional respiratory anatomy of a deep-sea Orbiniid Polychaete from the Brine Pool NR-1 in the Gulf of Mexico. Inv Biol 120:29–40

Hourdez S, Weber RE, Green BN, Kenney JM, Fisher CR (2002) Respiratory adaptations in a deep-sea orbiniid Polychaete from Gulf of Mexico Brine Pool NR-1: metabolic rates and hemoglobin structure/function relationships. J Exp Biol 205:1669–1681

Johnson L, Rees CJC (1988) Oxygen consumption and gill surface area in relation to habitat and lifestyle of four crab species. Comp Biochem Physiol A 89:243–246

Jouin C, Gaill F (1990) Gills of hydrothermal vent annelids: structure, ultrastructure and functional implications in two alvinellids species. Prog Oceanogr 24:59–69

Kennicutt MC, II Brooks JM, Burke RA Jr (1989) Hydrocarbon seepage, gas hydrates, and authigenic carbonate in the northwestern Gulf of Mexico. Offshore Technology Conference 5952:649-654

Lonsdale PF (1977) Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at oceanic spreading centers. Deep-Sea Res 24:875

Martin JW, Haney TA (2005) Decapod crustaceans from hydrothermal vents and cold seeps: a review through 2005. Zool J Linn Soc 145:445–522

Martinez AS, Charmantier G, Compère P, Charmantier-Daures M (2005) Branchial chamber tissues in two caridean shrimps: the epibenthic *Palaemon adspersus* and the deep-sea hydrothermal *Rimicaris exoculata*. Tissue and Cell 37:153–165

McMahon BR (2001) Respiratory and circulatory compensation to hypoxia in crustaceans. Respir Physiol 128:349–364

McMahon BR, Wilkens JL (1975) Respiratory and circulatory responses to hypoxia in the lobster *Homarus americanus*. J Exp Biol 62:637–655

Neufeld GJ, Holliday CW, Pritchard JB (1980) Salinity adaptation of gill Na,K-ATPase in the blue crab, *Callinectes sapidus.* J Exp Zool 211:215–224

Papathanassiou E, King PE (1983) Ultrastructural studies on the gills of *Palaemon serratus* (Pennant) in relation to cadmium accumulation. Aquat Tox 3:273–284

Paull CK (1984) Biological communities at the Florida escarpment resemble hydrothermal vent taxa. Science 226:965

Pilkington JB, Simmers AJ (1973) An analysis of bailer movements responsible for gill ventilation in the crab, *Cancer nova-zelandiae*. Mar Behav Physiol 2:73–95

Ravaux J, Gaill F, Le Bris N, Sarradin P-M, Jollivet D, Shillito B (2003) Heat-shock response and temperature resistance in the deep-sea vent shrimp *Rimicaris exoculata*. J Exp Biol 206:2345–2354

Schmidt-Nielsen K (1984) Scaling: why is animal size so important? Cambridge: Cambridge University Press.

Segonzac M (1992) The hydrothermal vent communities of the Snake Pit area (Mid Atlantic Ridge; 23°N. 3480 m). Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, Paris 314:593-600

Segonzac M, De Saint Laurent M, Casanova B (1993) L’énigme du comportement trophique des crevettes Alvinocarididae des sites hydrothermaux de la dorsale médio-atlantique. Cah Biol Mar 34:535–571

Smith EB, Scott KM, Nix ER, Korte C, Fisher CR (2000) Growth and condition of seep mussels (*Bathymodiolus childressi*) at a Gulf of Mexico brine pool. Ecology 81:2392–2403

Soegianto A, Charmantier-Daures M, Trilles JP, Charmantier G (1999) Impact of copper on the structure of gills and epipodites of the shrimp *Penaeus japonicus* (Decapoda). J Crustac Biol 19:209–223

Taylor EW (1976) The respiratory responses of *Carcinus maenas* to declining oxygen tension. J Exp Biol 65:309–322

Taylor EW (1982) Control and co-ordination of ventilation and circulation in crustaceans: responses to hypoxia and exercise. J Exp Biol 100:289–319

Taylor EW, Butler PJ (1978) Aquatic and aerial respiration in the shore crab, *Carcinus maenas* (L.), acclimated to 15˚C. J Comp Physiol 127:315–323

Teissier G (1948) La relation d’allométrie: sa signification statistiques et biologiques. Biometrics 4:14–53

Van Dover CL, Fry B, Grassle JF, Humphris S, Rona PA (1988) Feeding biology of the shrimp *Rimicaris exoculata* at hydrothermal vents on the Mid-Atlantic Ridge. Mar Biol 98:209–216

Wegner NC, Sepulveda CA, Bull KB, Graham JB (2009) Gill morphometrics in relation to gas transfer and ram ventilation in high-energy demand teleosts: Scombrids and billfishes. J Morphol 271:36–49

Wilkens JL, Wilkes PRH, Evans J (1984) Analysis of the scaphognathite ventilatory pump in the shore crab *Carcinus maenas*: Pumping efficiency and metabolic cost. J Exp Biol 113:69–81

Zbinden M, Le Bris N, Gaill F, Compère P (2004) Distribution of bacteria and associated minerals in the gill chamber of the vent shrimp *Rimicaris exoculata* and related biogeochemical processes. Mar Ecol Prog Ser 284:237–251

Table 1: Collection information for the specimens used in the present study.

Species	Ecosystem	Collection site	Coordinates		Coll. year	Depth (m)	*n*
Shrimp			**Latitude**	**Longitude**			
Alvinocaris komaii	Hydrothermal vents	Kilo Moana (LBAB)	20°03.34’ S	176°08.55’ W	2006	2650	35
Alvinocaris muricola	Cold seeps	AC818 (GoM)	26˚10.87’ N	94˚37.35’ W	2006	2750	6
Lebbeus sp.	Hydrothermal vents	ABE (LBAB)	20°45.65’ S	176°11.45’ W	2006	2140	2
Palaemon elegans	Littoral	Roscoff	48°43.22’ N	3°59.25’ W	2007	0	47
Palaemon serratus	Littoral	Roscoff	48°43.22’ N	3°59.25’ W	2007	0	54
Rimicaris exoculata	Hydrothermal vents	Logatchev (MAR)	14˚45.18’ N	44˚58.75’ W	2007	3000	30
Crabs							
Austinograea alaysae	Hydrothermal vents	ABE site (LBAB)	20°45.65’ S	176°11.45’ W	2006	2140	6
Bythograea thermydron	Hydrothermal vents	Tica (EPR)	9˚50.41’ N	104˚17.50’ W	2001	2500	17
Carcinus maenas	Littoral	Roscoff	48°43.22’ N	3°59.25’ W	2007	0	23
Segonzacia mesatlantica	Hydrothermal vents	Logatchev (MAR)	14˚45.18’ N	44˚58.75’ W	2009	3000	21
Xantho pilipes	Littoral	Roscoff	48°43.22’ N	3°59.25’ W	2007	0	22

EPR: East Pacific Rise; GoM: Gulf of Mexico; LBAB: Lau Back-Arc Basin; MAR: Mid-Atlantic Ridge.

Table 2: Oxygen consumption rates, gill surface areas, and oxygen flow rates in species for which metabolic rates were available.

Species	**M O_2 (µmole O_2 g^{-1} h^{-1})** [a]	**Gill Surface Area (cm^2 g^{-1})** [b]	**Ratio M O_2/GSA (nmole O_2 cm^{-2} h^{-1})**
Crabs			
Bythograea thermydron	1.79[c]	13.1	137
Carcinus maenas	1.29[d]	5.7	226
	3.10[e]		544
Shrimp			
Rimicaris exoculata	5.44[f]	17.5	311
Palaemon elegans	5.90[g]	6.2	952
Palaemon serratus	3.59[h]	4.4	815

[a] Wet weight, measurements at 15°C; [b] All data this study, calculated for a 10-g specimen for crabs and a 1-g specimen for shrimp; [c] Childress and Mickel 1985; [d] Taylor 1976; [e] Taylor and Butler 1978; [f] Ravaux et al. 2003; [g] Dalla Via 1985; [h] Decelle, unpub. Data.

Figure legends

Fig. 1 Bilogarithmic representation of scaphognathite surface area as a function of body weight (W), in shrimp (A) and crab species (B).

Dotted lines: scaphognathite surface areas in specimens of a given body weight for comparison (2.45 g and 14 g for the shrimp (A) and crabs (B), respectively).

Regression lines equations: $\log(Area)=\log(a) + b\log(W)$, where a is the allometry coefficient and b a constant. **A.** *R. exoculata* (y = 1.050x + 1.433; R^2=0.928), *A. komaii* (y = 0.792x + 0.812; R^2=0.959), *P. serratus* (y = 0.612x + 0.405; R^2=0.968), *P. elegans* (y = 0.708x + 0.423; R^2=0.942), *A. muricola* (y = 0.766x + 1.057; R^2=0.910). **B.** *B. thermydron* (y = 0.573x + 0.984; R^2=0.9845), *A. alaysae* (y = 0.597x + 0.730; R^2=0.938), *X. pilipes* (y = 0.601x + 0.597; R^2=0.979), *C. maenas* (y = 0.645x + 0.564; R^2=0.994), *S. mesatlantica* (y = 0.700x + 0.764; R^2=0.984)

Fig. 2 Bilogarithmic representation of gill surface area as a function of body weight, in shrimp (A) and crab species (B).

Dotted line in (A): gill surface areas for shrimp of similar body weight (2 g)

Regression lines equations: $\log(Area)=\log(a) + b\log(W)$, where a is the allometry coefficient and b a constant. **A:** *R. exoculata* (y = 0.932x + 1.242; R^2=0.952), *A. komaii* (y = 0.922x + 0.892; R^2=0.945), *P. serratus* (y = 1.132x + 0.641; R^2=0.962), *P. elegans* (y = 1.049x + 0.790; R^2=0.898), *A. muricola* (y = 1.061x + 0.688; R^2=0.949). **B:** *B. thermydron* (y = 0.940x + 1.177; R^2=0.975), *A. alaysae* (y = 1.108x + 0.443; R^2=0.945), *X. pilipes* (y = 0.876x + 0.678; R^2=0.985), *C. maenas* (y = 0.994x + 0.767; R^2=0.986), *S. mesatlantica* (y = 0.968x + 0.841; R^2=0.965)

Fig. 3 Transmission electron micrographs of cross sections through gill lamellae. **A.** Anterior gill from *Palaemon elegans*. **B.** Anterior gill from *Alvinocaris komaii*. C: cuticle; EP: epithelium; LH: lacuna containing the hemolymph; N: Nucleus

Fig. 4 Total diffusion distance (A), cuticle thickness (B) and epithelium thickness (C) of anterior and posterior gills from the littoral shrimp *P. elegans* and *P. serratus*, and the vent shrimp *A. komaii*. Error bars: SD, number of observations indicated above. Significance determined at $P < 0.001$ level with the Holm-Sidak method

Fig. 5 Boxplot representation of beating frequencies measured in situ for *Alvinocaris komaii* among mussels *Bathymodiolus brevior*, and among snails *Ifremeria nautilei*. Difference statistically significant ($P < 0.001$)

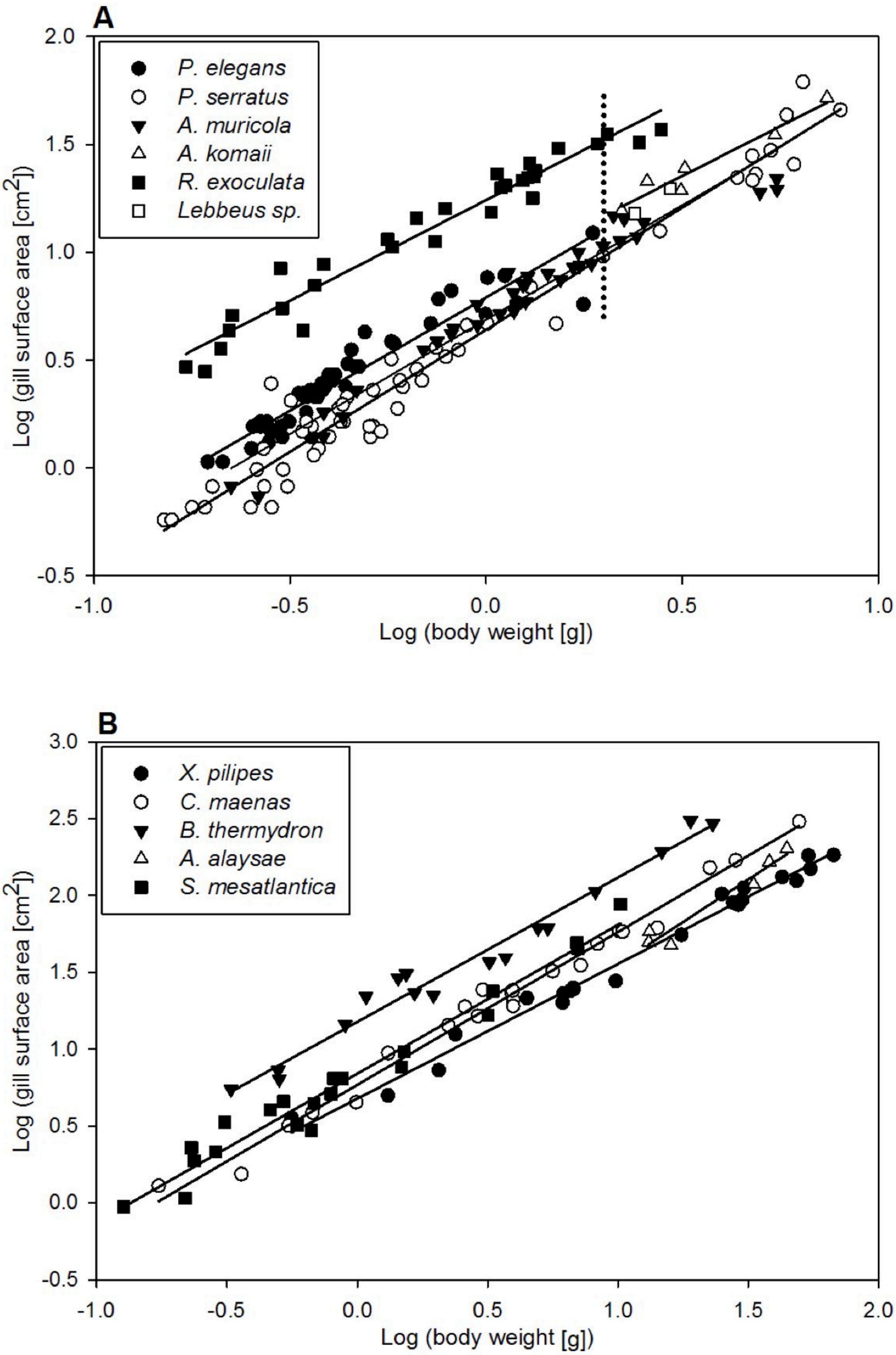

A
P. elegans
P. serratus
A. muricola
A. komaii
R. exoculata
Lebbeus sp.
Log (gill surface area [cm2])
Log (body weight [g])
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-0.5
0.0
0.5
1.0
B
X. pilipes
C. maenas
B. thermydron
A. alaysae
S. mesatlantica
Log (gill surface area [cm2])
Log (body weight [g])
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0

Figure 1

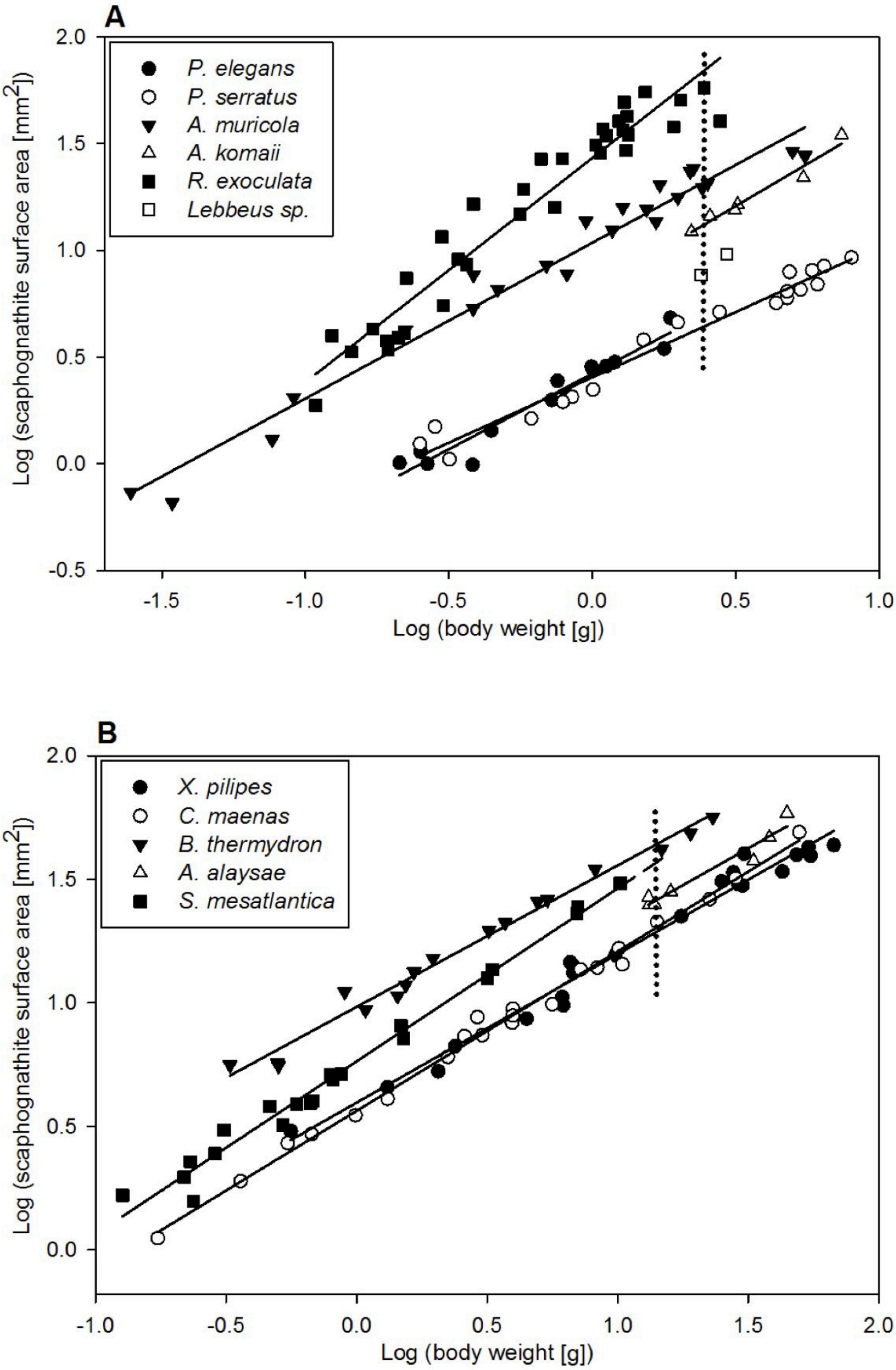

A
Log (scaphognathite surface area [mm2])
Log (body weight [g])
P. elegans
P. serratus
A. muricola
A. komaii
R. exoculata
Lebbeus sp.
2.0
1.5
1.0
0.5
0.0
-0.5
-1.5
-1.0
-0.5
0.0
0.5
1.0
B
Log (scaphognathite surface area [mm2])
Log (body weight [g])
X. pilipes
C. maenas
B. thermydron
A. alaysae
S. mesatlantica
2.0
1.5
1.0
0.5
0.0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0

Figure 2

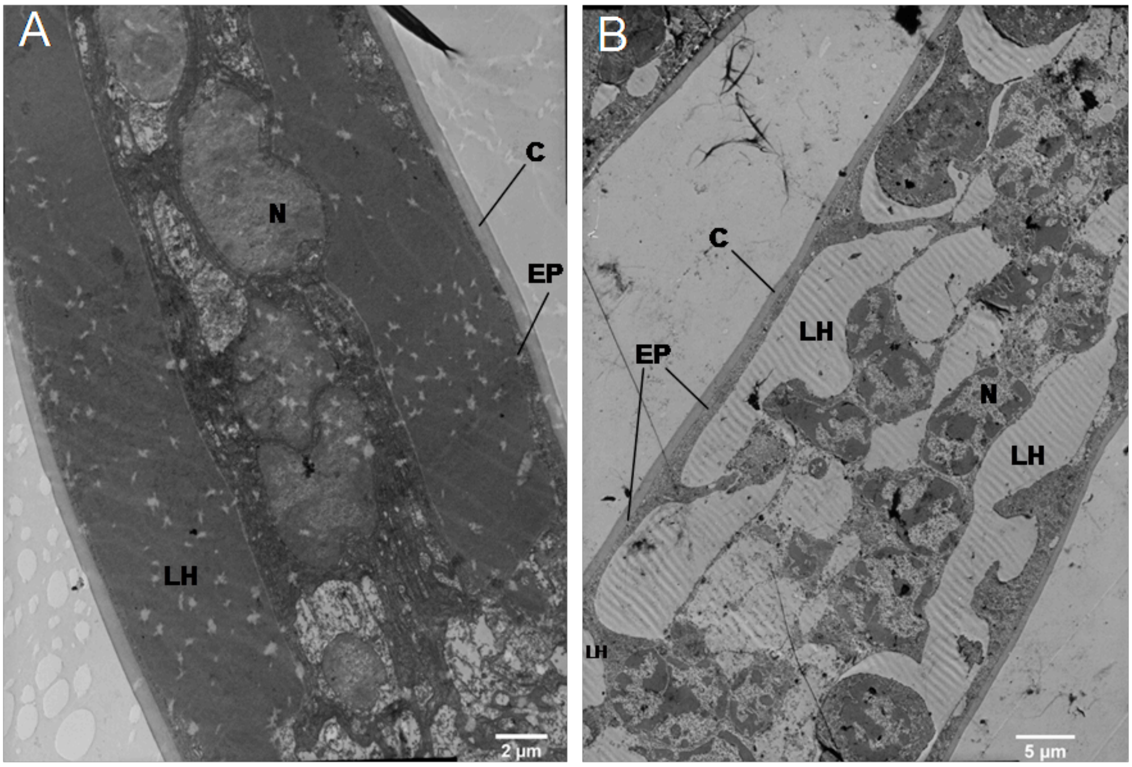


Figure 3

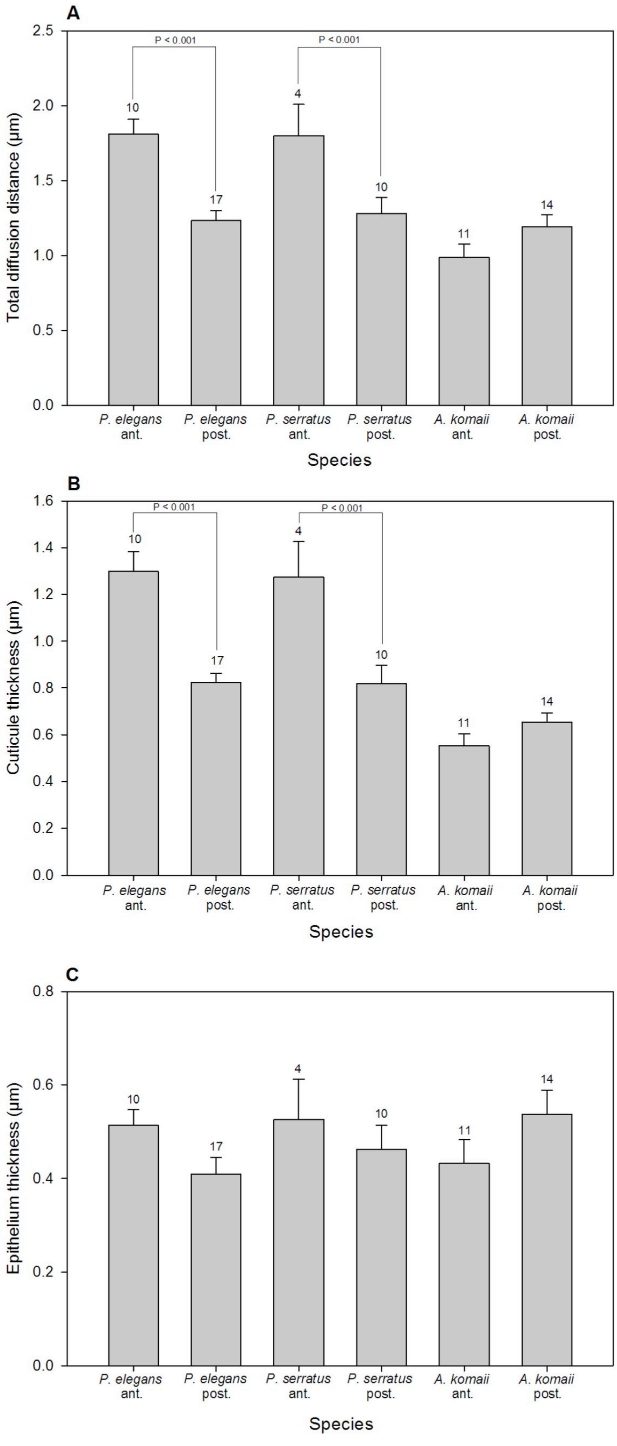
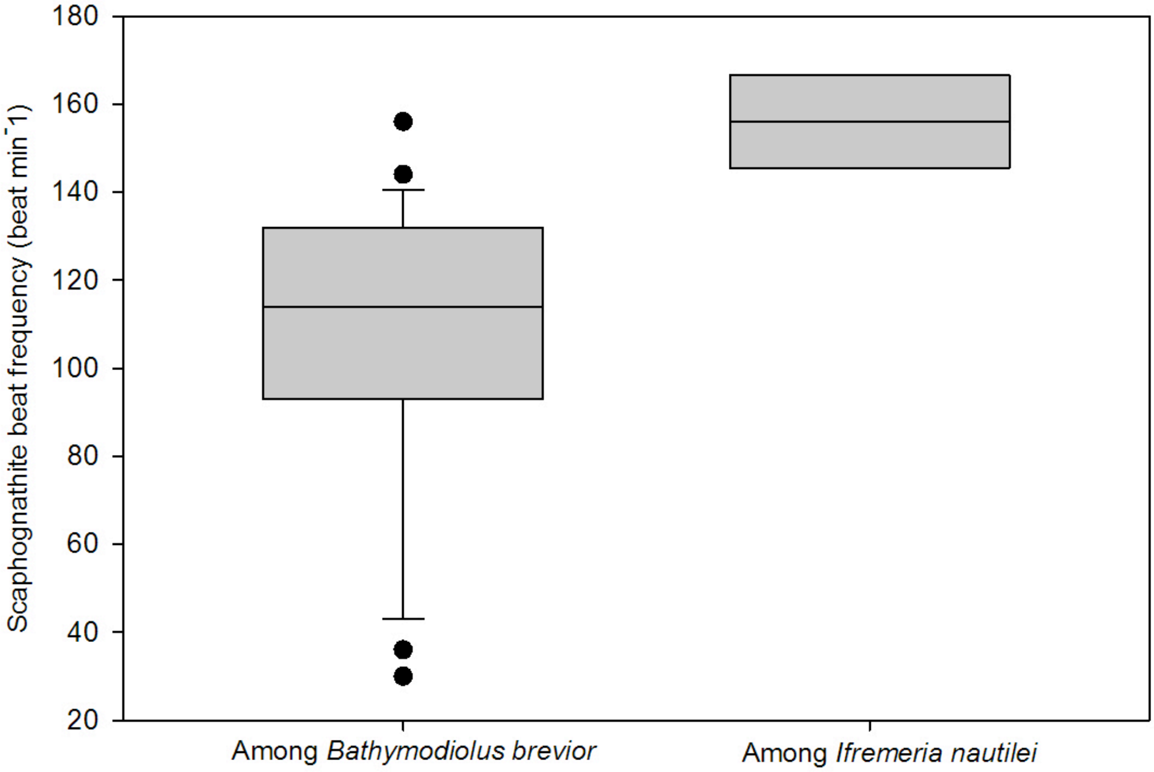

A
Total diffusion distance (µm)
P < 0.001
P < 0.001
B
Cuticule thickness (µm)
P < 0.001
P < 0.001
C
Epithelium thickness (µm)
P. elegans ant.
P. elegans post.
P. serratus ant.
P. serratus post.
A. komaii ant.
A. komaii post.
Species

Figure 4



Scaphognathite beat frequency (beat min-1)
180
160
140
120
100
80
60
40
20
Among *Bathymodiolus brevior*
Among *Ifremeria nautilei*

Figure 5