

Spicer & Morley:

Will giant polar amphipods be first to fare badly in an oxygen-poor ocean? Testing hypotheses linking oxygen to body size.

Supplementary materials 4.

Coxal plates as putative extrabranchial exchange surfaces on *P. brevicornis*?

Coxal plates of *P. brevicornis*

Figure S4.1 shows the position and shapes of the coxal plates (CP) of *P. brevicornis*. They are noticeably more well developed than *P. miersi* or *S. gracilis* (cf. Fig.S1.2). They are not as well developed as *P. ovata*, but in the case of the latter, which is a tiny species the coxal plates are completely opaque and it is difficult see through or in the plate itself, even when shining light through.

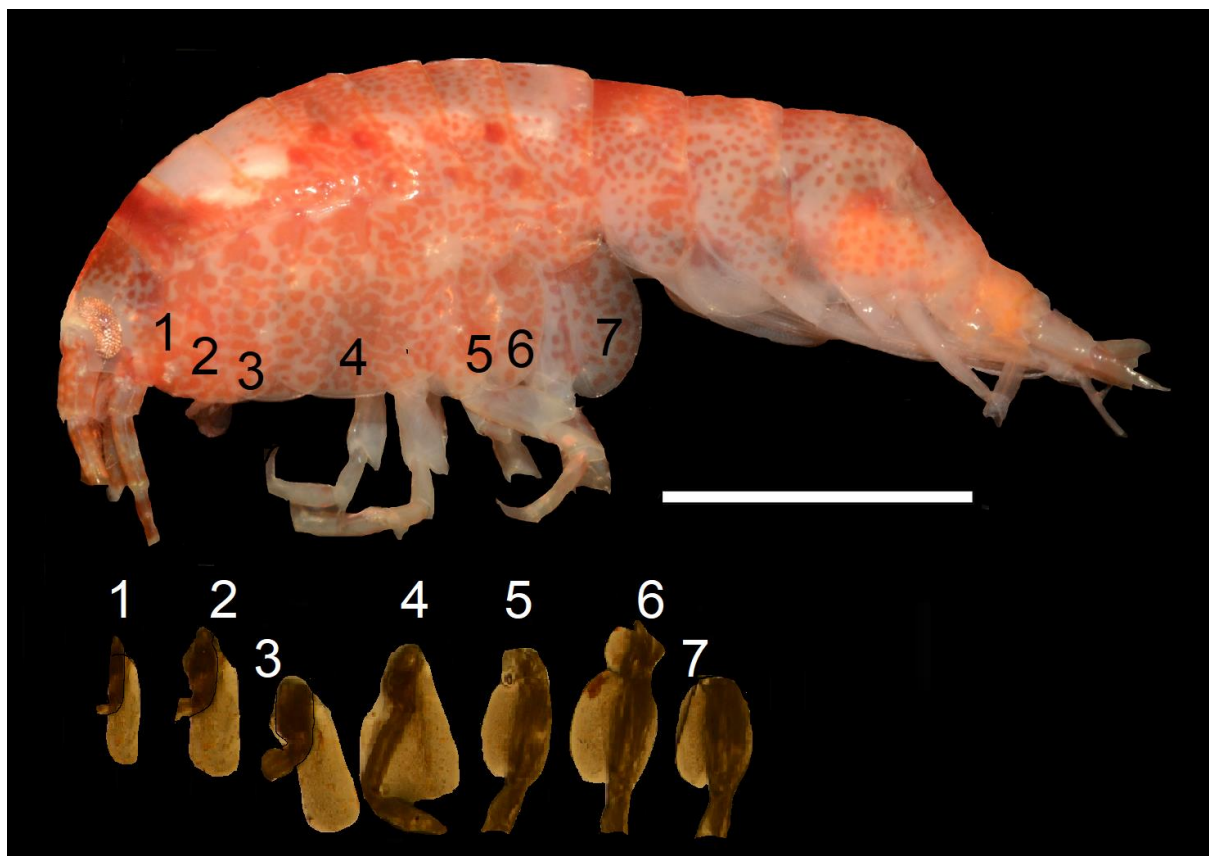


Figure S4.1 *Prostebbingia brevicornis* with coxal plates 1-7 marked. White line = 5 mm length. Below the main picture are the median (inside facing) surfaces of the excised coxal plate with the first couple of segments of the pereopods still attached. The numbers in white correspond directly with the numbers on the coxal plate marked on the photograph above

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Each coxae is broadly a rectangular (CP1-4) or rounded plate (CP5-7), and like all other amphipods, is the highly modified first article of each of the thoracic limbs [1]. Unlike many amphipod species CP 1-4 are, not much larger than CP5-7. For CP1-4 the anterior margin of each plate overlaps one in front of the other. In most amphipods, but particularly pronounced in the case of *P. brevicornis*, the coxal plates dramatically increase the compression of the body, forming a deep ventral groove, an extensive inner channel for respiratory and other water movements. As in other species this groove houses and protects the gills and (in females) oostegites, both of which are extensions of the thoracic limbs [1].

It is a straight-forward process to remove the coxal plates and measure their surface area using exactly the same procedure as used for the gills (Fig S4.1). For *P. brevicornis* the coxal plates are so thin they are translucent and under low power magnification it is possible to see that they are highly perfused with haemolymph in a similar fashion to that observed in the gills. A separate (from the one that supplies the gill) afferent haemolymph vessel which runs from the sternal sinus enters the middle of each coxal plate. It runs down to the bottom of the plate. Here it bifurcates. Each branch follows the edge of the plate but the haemolymph also seems to perfuse the whole plate. The efferent branches which have followed the edge of the plate, and the haemolymph perfusing the plates themselves reunite at the top of the plate and empty into the efferent haemolymph vessel of the limb.

Lloyd Peck is quite correct in pointing out (pers. comm.), that in what we do here (direct addition of extrabranchial area to gill area) there are likely to be errors. Essentially they are associated with, (i) the unlikely assumption that such extrabranchial gas exchange surfaces are unlikely to be perfused at the same rate as the gills and, (ii) not knowing the diffusion distances (sea water to hemolymph, i.e. cuticle and cellular thicknesses) - across both the gills and across the extrabranchial areas.

Although not formally investigated the thickness of the medial (inside facing) surface of the coxal plate seems particularly thin, as thin as the gills. Haemolymph cells are readily observable immediately beneath the cuticle of both. If it turns out they are different then we will need to incorporate a multiplier which takes into account the ratio of gill/extrabranchial thickness, when we estimate total functional gas exchange surface.

Coxal plates as extrabranchial exchange surfaces?

Cossans [2] was first to note that in *Gammarus* species, first four walking legs, were attached to enlarged coxal plates, and these were larger in *G. locusta* compared with *G. pulex*. She stated that these coxal plates functioned as 'accessory respiratory

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organs, as their inner surface was covered by very thin cuticle (p.5) but did not support this with physiological measures.

Hudson and Maitland [3] presented more detailed anatomical evidence to support the idea that amphipod coxal plates were involved in extrabranial gas exchange. They reported that the distance across the medial (i.e. inside facing) surface of coxal plates of the semi-terrestrial talitrid amphipod *Orchestia gammarellus* was about a third of the equivalent distance across both the coxal gills and the lateral (external) surface of the coxal plates. Spicer and McMahon [4] and Spicer [5] demonstrated that gill excision or gill disease (necrosis) respectively did not alter the rate of O₂ uptake in two related talitrid amphipods. They put forward the idea that at least some of the oxygen uptake could be accounted for by gas exchange across the inside of the coxal plates.

References

- [1] Lincoln RJ 1979. *British Marine Amphipoda: Gammaridea*. British Museum (Natural History). London
- [2] Cossans M 1904. Gammarus XII. *L.M.B.C. Memoirs on typical British marine plants and animals* (WA Herdman ed) London, Williams and Norgate 47 pages.
- [3] Hudson LJ, Maitland DP 1996. "Anatomy of structures associated with air-breathing in *Orchestia gammarellus* (Crustacea: Amphipoda: Talitridae): coxal plates and gills. *Mar. Biol.* **125**, 287-295.
- [4] Spicer J.I., and McMahon BR, 1994. Gill function in the amphipod *Megalorchestia (Orchestoidea) californiana* (Brandt, 1851) (Crustacea). *Can. J. Zool.* **72**, 1155-1158.
- [5] Spicer JI 2013. Physiological changes accompanying the presence of black gill syndrome in the high shore amphipod *Traskorchestia traskiana*. *J. Exp. Mar. Biol. Ecol.* **446**, 131-138.

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Supplementary materials 5.

Putative respiratory pigment in the haemolymph of *P. brevicornis*.

The absorbance peak detected at $\lambda = 332$ nm in oxygenated, but not deoxygenated, hemolymph from *Prostebbingia brevicornis in vitro* we interpret as indicating the presence of hemocyanin (Hc), a respiratory pigment that reversibly and cooperatively binds oxygen, and is used in oxygen transport in this species, for the following reasons.

- (1) In all crustaceans examined to date oxygenated Hc is detected by an intense absorption peak at, or around, $\lambda = 340$ nm [1,2,14]. This is close to the absorbance of the peak of $\lambda = 332$ nm detected for hemolymph from *P. brevicornis*.
- (2) In all crustaceans examined to date, this oxygenated Hc peak disappears when haemolymph is deoxygenated [1,2] and the same happens in hemolymph from *P. brevicornis*.
- (3) Haemocyanin that binds oxygen reversibly and cooperatively has been found in the haemolymph of most gammaridean amphipods in which it has been sought [3-10] including giant amphipods in Lake Baikal [11], (exceptions being *Paraceradocus miersi* and *Shraderia gracilis* in this present study – see below for implications of this finding). Non gammaridean groups such as the cyamid whale lice possess haemoglobin and not Hc [12] and the planktonic hyperiids possess neither [13]. This makes our interpretations in points 1 and 2 more likely for *P. brevicornis*.
- (4) If we use the absorbance measures obtained at $\lambda = 332$ nm, and assume that they are associated with the presence of a haemocyanin subunit of 74 kD in mass [3] we can use the extinction coefficients for Hc produced by Nickerson and Van Holde [14] ($E^{1\%_{cm}} = 2.83$, equating to $E^{mmol.l^{-1}cm} = 17.26$ at $\lambda = 340$ nm) to calculate putative Hc concentration. For *P. brevicornis* the calculated putative Hc concentration ranges between 0.33 and 0.39 $mmol.l^{-1}$ at the upper end of the range reported for other amphipod species, 0.14 – 0.41 $mmol.l^{-1}$ [3]. This congruence strengthens the suggestion that there is Hc in the hemolymph of *P. brevicornis*.

Taken together we have presented a number of different lines of evidence that there is a Hc in the hemolymph of *P. brevicornis*. However, a number of puzzles, incongruities and alternative explanations also exist which need to be aired. They are as follows.

- (1) We have not directly measured oxygen-binding by the pigment, or isolated, identified, and verified the hemocyanin molecule itself. Even if there is oxygen binding present we have no evidence that it is cooperative.

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- (2) While the congruence between the Hc values we might expect and what we estimate for *P. brevicornis* is good when using the extinction coefficient for the copper peak at 340 nm, the congruence is not so good when we measure the protein peak at 280 nm and assume that it is likely to be 60-95% Hc in line with other amphipods investigated [3]. In that case the protein concentration is always lower than the value calculated based on how much Hc there seems to be. In *P. brevicornis* brought back to the UK from the Antarctic the haemolymph protein levels can be very low even though the animals are well fed and have sufficient copper in their food (JIS, pers obs). Therefore we have been unable to substantiate the claim of Hc being present in the haemolymph by (i) measuring the oxygen content of the hemolymph or (ii) replicating the disappearance of any peak at $\lambda = 332$ nm when the haemolymph is equilibrated with nitrogen gas.

Aside from oxygen transport, Hcs are also known to participate in homeostatic and physiological processes: moulting, hormone transport, osmoregulation and protein storage and as precursors of antimicrobial and antiviral peptides.

References

- [1] Mangum CP 1997. Invertebrate blood oxygen carriers. *Handbook of Physiology, Section, 13*, 1097-1131.
- [2] Giomi F, Beltramini M 2007. The molecular heterogeneity of hemocyanin: its role in the adaptive plasticity of Crustacea. *Gene* **398**, 192-201.
- [3] Spicer JI 1993. Oxygen binding by amphipod (Crustacea) haemocyanins. *Mar. Behav. Physiol.* **24**, 123-136.
- [4] Spicer JI, Taylor AC 1994. Oxygen-binding by haemocyanins from an ecological series of amphipod crustaceans. *Mar. Biol.* **120**, 231-237.
- [5] Maltby L 1995. Sensitivity of the crustaceans *Gammarus pulex* (L.) and *Asellus aquaticus* (L.) to short-term exposure to hypoxia and unionized ammonia: observations and possible mechanisms. *Water Res.* **29**, 781-787.
- [6] Spicer JI 1995. Ontogeny of respiratory function in crustaceans exhibiting either direct or indirect development. *J. Exp. Zool.* **272**, 413-418.
- [7] Sandberg-Kilpi E, Vismann B, Hagerman L 1999. Tolerance of the Baltic amphipod *Monoporeia affinis* to hypoxia, anoxia and hydrogen sulfide. *Ophelia* **50**, 61-68.
- [8] Hodgson E, Spicer JI 2001. Subunit compositions of crustacean haemocyanins are species-specific: evidence from non-decapod species. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **128**, 873-888.
- [9] Spicer JI, Hodgson E 2003. Structural basis for salinity-induced alteration in oxygen binding by haemocyanin from the estuarine amphipod *Chaetogammarus marinus* (L.). *Physiol. Biochem. Zool.* **76**, 843-849.
- [10] Hagner-Holler S, Kusche K, Hembach A, Burmester T 2005. Biochemical and molecular characterisation of hemocyanin from the amphipod *Gammarus roeseli*: complex pattern of hemocyanin subunit evolution in Crustacea. *J. Comp. Physiol. B*, **175**, 445-452.
- [11] Bedulina DS, Gurkov AN, Baduev BK, Borvinskaya EV, Dimova MD, Timofeyev, MA 2016. Preliminary analysis of hemocyanins in hemolymph plasma of Baikal endemic amphipods. *J. Stress Physiol. Biochem.* **12**, 74-86.

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[12] Terwilliger NB 1991. Arthropod (*Cyamus scammoni*, Amphipoda) hemoglobin structure and function. In: Structure and Function of Invertebrate Oxygen Carriers (ed. S Vinogradov and O Kapp), pp. 59–63. Berlin, Heidelberg, New York: Springer Verlag.

[13] Spicer JI, Morrill D 1995. Oxygen carriage by the haemolymph of hyperiid amphipods. *J. Mar. Biol. Assoc. UK* **75**, 997-998.

[14] Nickerson KW, Van Holde KE 1971. A comparison of molluscan and arthropod hemocyanin-I. Circular dichroism and absorption spectra. *Comp. Biochem. Physiol.*: **39B**, 855-872.