

ADAPTATION TO TEMPERATURE STRESS AND AERIAL EXPOSURE IN CONGENERIC SPECIES OF INTERTIDAL PORCELAIN CRABS (GENUS *PETROLISTHES*): CORRELATION OF PHYSIOLOGY, BIOCHEMISTRY AND MORPHOLOGY WITH VERTICAL DISTRIBUTION

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Summary

We examined physiological and biochemical responses to temperature and aerial exposure in two species of intertidal porcelain crabs (genus *Petrolisthes*) that inhabit discrete vertical zones. On the shores of the Northeastern Pacific, *P. cinctipes* (Randall) occurs under rocks and in mussel beds in the mid to high intertidal zone and *P. eriomerus* (Stimpson) occurs under rocks in the low intertidal zone and subtidally to 80 m. Because of their different vertical distributions, these two species experience very different levels of abiotic stress. Individuals of *P. cinctipes* can be emersed during every low tide, but *P. eriomerus* is only emersed during the lowest spring tides and on most days is not emersed at all. Temperatures measured underneath rocks in the mid intertidal zone were as high as 31 °C, 15 °C higher than maximal temperatures measured under rocks in the low intertidal zone. In air, at 25 °C, large specimens of *P. cinctipes* were able to maintain a higher respiration rate than similarly sized *P. eriomerus*. No interspecific differences in the respiratory response to emersion were seen in small specimens. Examination of the response of heart rate to temperature revealed that *P. cinctipes* has a 5 °C higher Arrhenius break temperature (ABT, the temperature at which there is a discontinuity in the slope of an Arrhenius plot) than its congener (31.5 °C versus 26.6 °C). The heart rate of *P. cinctipes* recovered fully after exposure to cold (1.5 °C), but the heart rate of *P. eriomerus* did not recover after exposure to 2 °C or cooler. The ABT

of heart rate in *P. cinctipes* was very close to maximal microhabitat temperatures; thus, individuals of this species may be living at or near their thermal tolerance limits. *P. cinctipes* were able to maintain aerobic metabolism during emersion, whereas *P. eriomerus* shifted to anaerobic metabolism. A pronounced accumulation of whole-body lactate was found in specimens of *P. eriomerus* incubated in air at 25 °C over a 5 h period, but not in *P. cinctipes* similarly treated. *P. cinctipes* possesses a membranous structure on the ventral merus of each walking leg, but this structure is not found in *P. eriomerus*. To test the function of the leg membrane, we measured the aerial respiration rates and the lactate accumulation of *P. cinctipes* with their leg membranes obscured. These individuals had significantly lower aerial respiration rates at 30 °C than control crabs. Crabs with leg membranes obscured also accumulated a considerable amount of lactate during a 5 h period of emersion at 28 °C, but control crabs showed no accumulation under the same conditions. These data suggest that the leg membrane functions as a respiratory structure. The results of this study illustrate that a suite of morphological, physiological and biochemical features allows *P. cinctipes* to live higher in the intertidal region than *P. eriomerus*.

Key words: crabs, heart rate, intertidal zone, *Petrolisthes*, respiration, temperature.

Introduction

One predominant feature of the rocky intertidal zone is the distribution pattern of organisms in discrete vertical zones (Connell, 1961). Zonation patterns have been shown to be due both to biotic factors, such as competition and predation, and to abiotic factors, such as temperature, wave exposure and desiccation stress (Connell, 1961; Edney, 1961). Studies of abiotic stress have shown that species living higher in the

intertidal zone generally have a greater resistance to abiotic factors than do species living in the lower intertidal zone. Often, however, species found lower in the intertidal zone are competitively dominant and exclude the upper species from lower zones (Connell, 1961). Like animals and plants living on the surfaces of rocks, animals found beneath rocks (rupestrine species) have been shown to live in discrete vertical zones

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(Jones, 1976; Willason, 1981; Pellegrino, 1984; Menendez, 1987; Gherardi, 1990; Jensen and Armstrong, 1991). However, the levels of abiotic stress experienced by rupestrine fauna and the responses of those fauna to abiotic stress have not been well characterized.

Porcelain crabs of the genus *Petrolisthes* (Anomura: Porcellanidae) are a rupestrine taxon with a broad geographic distribution (Haig, 1960). Two species of porcelain crabs are common along rocky shores of the Northeastern Pacific, *P. cinctipes* and *P. eriomerus*. *P. cinctipes* is found in the mid to high intertidal region, but *P. eriomerus* is found from the bottom of the *P. cinctipes* distribution range to a depth of approximately 80 m (Morris *et al.* 1980; Jensen and Armstrong, 1991). Both species live under stones and in crevices, and *P. cinctipes* is one of the most abundant members of the mussel (*Mytilus californianus*) bed fauna, where it has been reported in densities nearing 4000 individuals m⁻² of mussel bed (Jensen and Armstrong, 1991).

Because of the difference in vertical distribution, these congeners may experience very different patterns of emersion during each tidal cycle (see Fig. 1). Individuals of *Petrolisthes cinctipes*, living near the top of their vertical distribution range, experience emersion twice per day, every day, but *P. eriomerus* at the top of their distribution only experience emersion once per day during periods of low spring tides and are not emersed during neap tides. The differences in frequency and duration of emersion are important in shaping the thermal characteristics of the vertical zones; initial studies described temperatures during low tide in *P. cinctipes* zones that were above the lethal limit of *P. eriomerus* (Jensen, 1989). Here we report a more detailed examination of the thermal microhabitats of *P. cinctipes* and *P. eriomerus*. Jensen and Armstrong (1991) showed differences in tolerance of high temperature between these two species under conditions of emersion. To investigate mechanisms that could account for the differences in thermal tolerance maxima between *P. cinctipes* and *P. eriomerus*, we examined the thermal sensitivity of heart rate during extreme, but environmentally realistic, temperature fluctuations.

Because *Petrolisthes cinctipes* is emersed for a much greater percentage of time than is *P. eriomerus*, it is possible that this species possesses adaptations for a semi-terrestrial existence, such as those that have been shown for other terrestrial and semi-terrestrial crabs (Burggren and McMahon, 1981; DeFur *et al.* 1983; Burggren, 1992; Burnett, 1992; Wolcott, 1992). Terrestrial crabs can usually support higher rates of aerial respiration than can aquatic crabs (Wallace, 1972; Hawkins *et al.* 1982; Houlihan and Innes, 1984; Santos and Costa, 1993). Adaptations at the morphological, physiological and biochemical levels are responsible for the increased ability for aerial gas exchange in land crabs (Burggren, 1992). Here we examine differences between species of *Petrolisthes* in their ability to respire aerially at each of these three levels. Aerial and aquatic whole-animal oxygen consumption rates at a range of temperatures are measured, and poise of metabolism between aerobic and anaerobic pathways is examined by

measuring the amount of lactate accumulation during emersion.

Morphological adaptations of crabs that breathe air include a general reduction in gill number and gill surface area (Gray, 1957; Hawkins and Jones, 1982) as well as the formation of a functional lung by an increased vascularization of the inner lining of the branchiostegites (Farrelly and Greenaway, 1994). A different morphological strategy to facilitate aerial respiration has been described in two Scopimerinae ghost crabs endemic to Australia [*Scopimera inflata* and *Dotilla myctiroides* (Brachyura: Ocypodidae)]. These crabs have no lungs and, while they are emersed, they support their high metabolic rate using membranous gas-exchange surfaces ('gas windows') on the dorsal surface of the meral segments of each walking leg (Maitland, 1986). *Petrolisthes cinctipes* possesses an analogous uncalcified membranous area on the ventral surface of the meral segments of each walking leg, but every meral segment of *P. eriomerus* is fully calcified (Jensen and Armstrong, 1991). To assess the possible function of the leg membrane structures in *P. cinctipes* as respiratory structures, we have measured aerial and aquatic respiration rates and lactate accumulation (metabolic poise) of *P. cinctipes* with their leg membranes obscured.

The morphological, physiological and biochemical differences we describe between these two species suggest that *Petrolisthes cinctipes* possesses adaptations allowing it to live higher in the intertidal region than its congener.

Materials and methods

Habitat characteristics

Emersion patterns (Fig. 1A) were generated using Harbor Master software (Zihua, Monterey, CA, USA). Heights of the top of the *Petrolisthes cinctipes* (1.24 m above mean low tide) and *P. eriomerus* (0.15 m above mean low tide) zones at Cape Arago, OR, USA (43°21' N; 124°19' W) were empirically determined by noting the time when the tides covered and uncovered the tops of each zone. These times were used to determine the height of each zone using tidal prediction curves in Harbor Master. Measurements were made on four consecutive days, and the mean height of each zone was used for the generation of the emersion patterns (Fig. 1A). Tidal predictions for Bandon, OR, USA (43°07' N; 125°25' W) were selected as an approximation for the tidal patterns at Cape Arago, OR, USA.

Rocks that had the appropriate morphology to attain high temperatures (flat and thin, and with a horizontal surface fully exposed to sunlight) and that supported natural assemblages of *Petrolisthes* were selected in the upper and lower intertidal zones. Thermocouple probes (Omega Inst., K-type wire probes) were placed under the rocks just as they were uncovered by the receding tide and left in place throughout the low tide period. At 30 min intervals, each probe was connected to a digital thermometer (Omega Inst., HH 82) and the temperature was recorded. Air and ocean water temperatures were also monitored. Cloud cover and wind speed were

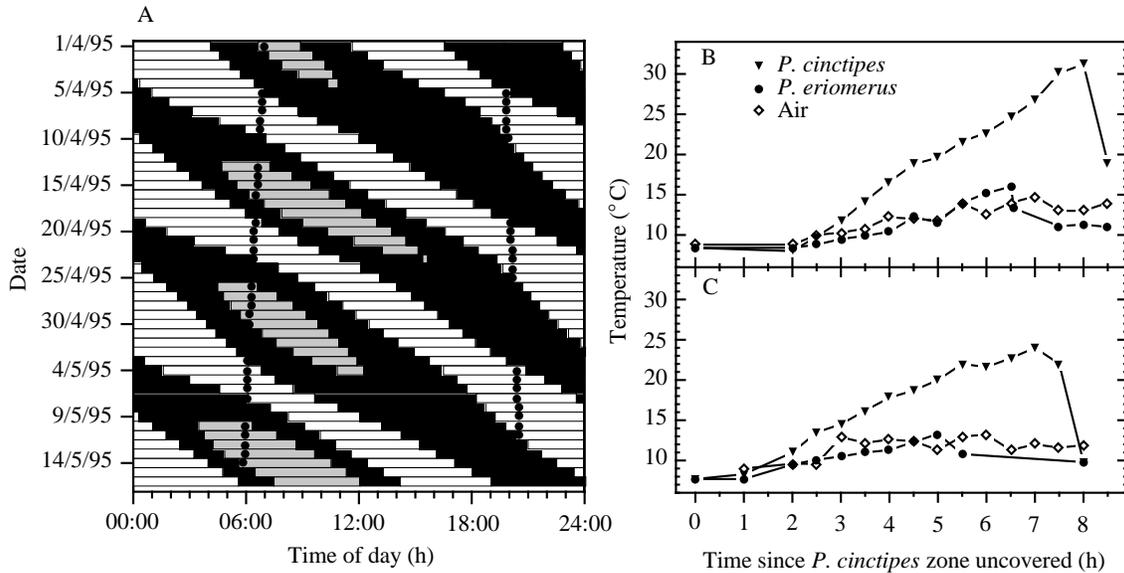


Fig. 1. (A) Coverage patterns of *Petrolisthes cinctipes* and *P. eriomeres* living at the tops of their vertical distributions. Areas enclosed by black represent times during which *P. cinctipes* are emersed, and areas enclosed by stippled markings indicate times during which both *P. cinctipes* and *P. eriomeres* are emersed. White areas represent times when both species are immersed. Black dots represent the times of sunrise and sunset for each solar day. For example, on 1 April 1995, *P. eriomeres* were emersed from 06:30 to 08:45 h, and *P. cinctipes* were emersed from 04:00 to 11:30 h and from 16:30 to 22:45 h. The methods used for the generation of coverage patterns are described in Materials and methods. (B,C) Habitat temperatures during low tide on 18 May 1995 (B) and 19 May 1995 (C). Environmental conditions on 18 May were no clouds, no wind, no waves and a low tide of -0.61 m at 09:50 h. Conditions on 19 May were no clouds, but high winds, with a low tide of -0.43 m at 10:45 h. Maximum temperatures in the *P. cinctipes* zone were 31.2°C on 18 May (B) and 24°C on 19 May (C). Ambient seawater temperature was $8\text{--}9^{\circ}\text{C}$ on both days.

qualitatively determined. Temperatures were monitored during the lowest tide on consecutive days in May 1995 at Cape Arago, OR, USA (Fig. 1B,C).

Collection and maintenance of specimens

Specimens were collected from intertidal locations as specified for each experiment (below). All specimens were collected at low tide and immediately transported to Oregon State University (OSU) in Corvallis, Oregon, where the crabs were held in temperature-controlled, recirculating aquaria at the temperature of the water at the collection site. Every third day, crabs were fed a diet composed of a unicellular algal culture (Algal diet C, Coast Seafood, Inc., Bellevue, WA, USA) and a homogenized mixture of algae (mostly *Ulva* spp., *Laminaria* spp. and *Macrocystis* spp.), mussels and fish pellets. Crabs were not fed on any of the 3 days immediately preceding experimentation.

Respirometry

Specimens used for measurement of whole-animal oxygen consumption rate (\dot{V}_{O_2}) were collected on 1 August 1993 at Clallam Bay, Washington ($48^{\circ}16' \text{N}$; $124^{\circ}18' \text{W}$). Crabs were held at the collection temperature, $14 \pm 1^{\circ}\text{C}$, for 2 weeks preceding measurement of the oxygen consumption rate. Aquatic and aerial \dot{V}_{O_2} were determined using a Gilson differential respirometer. For measurement of aquatic \dot{V}_{O_2} ($\dot{V}_{\text{O}_{2w}}$), crabs were placed in glass chambers containing 20 ml

of sea water that had been filtered to remove particulate matter, and with a piece of Whatman filter paper was added for traction. The chambers were connected to the respirometer and adjusted to the experimental temperature. The change from the aquarium temperature to the experimental temperature was made at a rate intended to mimic the rate of temperature change in the natural environment during a low tide period. After the experimental temperature had been reached, the chambers were allowed to equilibrate for 1 h before the respirometer was closed and measurements of oxygen consumption were initiated. Every measurement was conducted in triplicate 30–60 min periods, with 10 min between each period, during which time the respirometer was opened to allow flushing of the system with fresh air; the water was not changed between replicates. Following measurement of $\dot{V}_{\text{O}_{2w}}$, each chamber was removed from the apparatus, the water was removed, and the chambers were reconnected to the respirometer. Then, triplicate measurements of aerial \dot{V}_{O_2} ($\dot{V}_{\text{O}_{2a}}$) were made on the same specimen in the same manner as for $\dot{V}_{\text{O}_{2w}}$. On a given day, \dot{V}_{O_2} for each animal was measured at only one temperature, first in water and then in air. Following measurement of $\dot{V}_{\text{O}_{2a}}$, each crab was uniformly blotted and weighed to the nearest 0.01 g. On successive days, temperatures were increased. This protocol was used to minimize the likelihood of damage to the organisms from sublethal levels of thermal stress. \dot{V}_{O_2} values were measured for large (carapace width, cw, 15–20 mm, wet mass 3.5–5 g)

and small (cw 5–8 mm, wet mass 0.5–1.2 g) specimens to examine the effect of size on the response of \dot{V}_{O_2} at different temperatures.

To calculate \dot{V}_{O_2} , the change in gas volume (corrected against a blank) was plotted against time. The consumption of oxygen was always linear with time, suggesting that the specimens did not experience P_{O_2} values below the critical O_2 concentration. The slope of the linear relationship between O_2 consumption and time was used to calculate mass-specific O_2 consumption rate.

Thermal tolerance limits of heart rate

The thermal tolerance limits of both species were indexed by the response of heart rate to changes in temperature. Specimens were collected at Cape Arago, OR, USA, on 4 October 1994 and held at $11 \pm 1^\circ\text{C}$ for 4 weeks preceding experimentation. Heart rates of *Petrolisthes cinctipes* and *P. eriomerus* were monitored by impedance. Specimens were immobilized by lashing them to a piece of plastic mesh (Vexar), which was then secured to a small rock. The specimen was immersed in an aerated, temperature-controlled water bath containing sea water at the acclimation temperature. Pinholes through the carapace were made laterally on either side of the heart, and an impedance electrode, consisting of 0.025 mm diameter ceramic-coated copper wire, with the ceramic insulation removed over the last 1 mm of the tip, was inserted into each hole. Because the specimens were immobilized, the wires did not need to be secured to the carapace or held in place by any device other than by bending the wire at the hole. Wires were connected to an impedance pneumograph (Narco Bio-systems, Houston, TX, USA). The impedance signal was amplified and individual heart beats were monitored and recorded on a strip chart recorder (Gould, Inc., Cleveland, OH, USA). The pinholes had no deleterious effects on crab survival.

Temperatures were either increased or decreased at a rate that was determined to be environmentally realistic (1°C every 15 min, see Fig. 1) for a thermally extreme day. Heart rate was monitored for 1–2 min intervals every 15–30 min. Heart beats were counted if the amplitude of a peak on the chart was at least three times as large as the background noise. Heart rates were expressed as beats min^{-1} or transformed to the natural logarithm of beats min^{-1} for Arrhenius plots. Arrhenius break temperatures (ABTs) were determined using regression analyses to generate the best fitting line on both sides of a putative break point (where there was an inflection in the slope of data points) on Arrhenius plots. The temperature at which these two lines intersected was taken as the ABT (see Dahlhoff *et al.* 1991).

Lactate accumulation experiments

Specimens used for interspecific comparisons of lactate production were collected from Cape Arago, OR, USA, on 29 January 1995.

Lactate production was measured in crabs kept in air at two different temperatures over a 5 h period. Specimens were blotted and weighed to the nearest 0.1 g and then placed into

individual containers containing a piece of seawater-soaked filter paper. The glass containers were partially immersed into a circulating water bath for temperature control, and the air was humidified by vigorous aeration of the water in the bath. Individuals were removed after incubation periods of 1, 3 and 5 h, and immediately frozen by freeze-clamping in liquid N_2 . Specimens were stored at -70°C for subsequent analysis of lactate concentration.

To assay whole-body lactate levels, frozen crabs were ground with a mortar and pestle under liquid N_2 and immediately placed into 2 body-mass-equivalent volumes (2 ml g^{-1}) of $0.75 \text{ mol l}^{-1} \text{ HClO}_4$. The mixture was incubated on ice for 10 min and then centrifuged at $10\,000g$ for 10 min. The supernatant was neutralized to pH 7.0 by addition of $3 \text{ mol l}^{-1} \text{ KOH}$, incubated on ice for 10 min and then centrifuged at $10\,000g$ for 10 min to remove the KClO_4 precipitate. The resulting supernatant was held on ice and used within 1–2 h for enzymatic determination of lactate concentration using the method described by Noll (1984), with a commercially purchased L-lactic acid kit (Boehringer Mannheim).

Leg membrane studies

Dissection microscopy was used to visualize the morphological features of the meral segment of walking legs. The legs were cut along the dorsal–ventral axis (the thinnest axis), and the muscle tissue was removed from the inner surface of the ventral merus.

Examination of the physiological function of the leg membrane of *Petrolisthes cinctipes* was conducted by obscuring the leg membrane and measuring (in large and small specimens) the respiratory response to emersion (as defined above) and (in large specimens) the metabolic poise during emersion. Leg membranes were obscured by the application of two thin coats of nail polish (Revlon creme). Only a few legs were painted at a time to minimize the period that the animals spent out of water. Crabs had all of their legs painted, half of their legs painted (control for paint effects) or none of their legs painted. Crab legs were painted 2 days before experimentation began. Respirometry was conducted as described above. Specimens used for respirometry were collected at Cape Arago, OR, USA, on 4 October 1994 and held at $11 \pm 1^\circ\text{C}$ for 2 weeks preceding experimentation. Specimens used to examine lactate production of *P. cinctipes* with their leg membranes obscured were collected from Cape Arago, OR, USA, on 18 May 1995 and held at $11 \pm 1^\circ\text{C}$ for 2 weeks as above.

A taxonomic survey was completed at the Los Angeles County Museum of Natural History to determine the prevalence of the leg membrane in the genus *Petrolisthes*.

Results

Habitat characteristics

Differences in frequency and duration of emersion of crabs living at the vertical maxima of their distributions show that

Petrolisthes cinctipes may be emersed during every low tide, but that *P. eriomerus* are emersed only during low spring tides (Fig. 1A). These emersion patterns indicate that individuals of *P. cinctipes* may spend as much as 50% of their time out of water. While some rocks have small puddles of water beneath them, most have only wet substratum or other rocks beneath, and thus the crabs cannot seek refuge from emersion (J. H. Stillman and G. N. Somero, personal observation).

Measurements of habitat temperatures on two consecutive days during May 1995 illustrate the importance of weather conditions to microhabitat temperatures as well as the large differences between the thermal microhabitats of *Petrolisthes cinctipes* and *P. eriomerus* on hot days (Fig. 1B,C). When low spring tides occur towards the middle of the solar day, as on 18 May 1995, and the weather is clear and calm (Fig. 1B), microhabitat temperatures increase rapidly, and dramatic differences can be seen between conditions under rocks in the upper intertidal (*P. cinctipes*, Fig. 1B,C) versus the lower intertidal zone (*P. eriomerus*, Fig. 1B,C). With an increase in wind, the maximal temperature achieved is decreased, and on 19 May 1995 (Fig. 1C), a windy but sunny day, the maximal temperature (24°C) was approximately 7°C lower than on 18 May 1995 (31.2°C), a calm and sunny day (Fig. 1B). Upper intertidal rocks are for the most part bare on the upper surface, and the temperature under flat thin rocks in this zone increases at a rate of approximately 4°C h⁻¹ during the most rapid heating conditions. Temperatures under rocks in the lower intertidal do not increase to the same degree as those in the upper intertidal zone (Fig. 1B,C). In the spring and summer, rocks in the lower intertidal zone usually harbor an abundance of algae. This minimizes increases in the under-rock temperatures by insulating the rock and by providing a source of water for evaporative cooling, which removes thermal energy from the rock.

Respiratory responses to abiotic stress

Respiratory responses to temperature and emersion differed as functions of species and body size. The ability to respire aerielly was different between species, as well as between large and small individuals within a species. Large specimens showed little interspecific difference in $\dot{V}_{O_{2w}}$ at 15, 20 and 25°C (Fig. 2A). However, $\dot{V}_{O_{2a}}$ of *P. eriomerus* was 40% lower than that of *P. cinctipes* at 25°C [81.7±29.1 versus 49.0±10.3 μl O₂ h⁻¹ g⁻¹; analysis of variance (ANOVA) of log₁₀-transformed data; $P=0.012$] (Fig. 2A). In addition, all large specimens of *P. eriomerus* died during measurement of $\dot{V}_{O_{2w}}$ at 30°C (Fig. 2A). For small specimens, there were no differences between $\dot{V}_{O_{2w}}$ and $\dot{V}_{O_{2a}}$ within species, except at 30°C for *P. eriomerus*; most of the variation was interspecific and not dependent on emersion state (Fig. 2B). Small specimens of *P. eriomerus* had a very high $\dot{V}_{O_{2w}}$ at 30°C (Fig. 2B); however, all specimens were found to be moribund at the end of the experiment and did not recover after they were returned to ambient temperature water. The only mortality observed during the experiment involved *P. eriomerus* at 30°C.

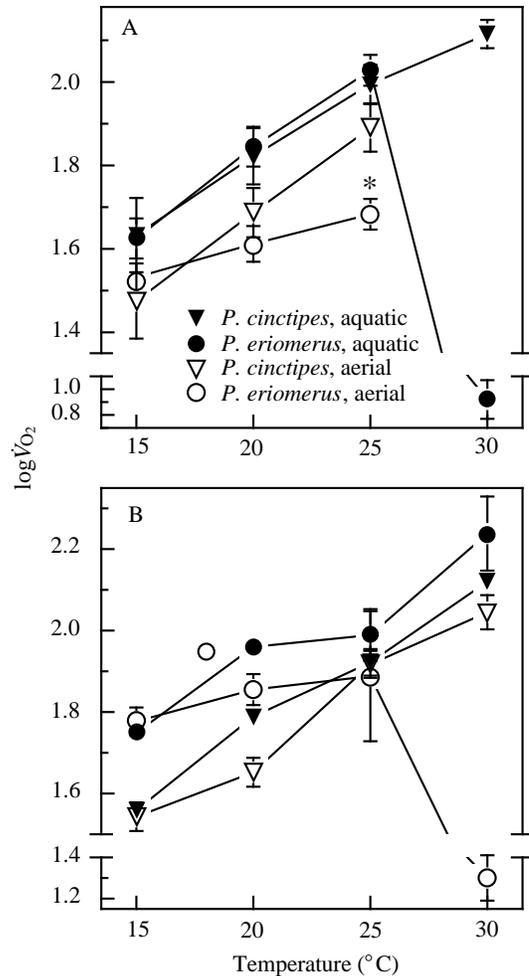


Fig. 2. Whole-animal oxygen consumption rates (\dot{V}_{O_2}) ($\mu\text{l O}_2 \text{ h}^{-1} \text{ g}^{-1}$) of crabs at different temperatures and aerial exposures in (A) large (carapace width, cw, 15–20 mm, wet mass 3.5–5 g) and (B) small (cw 5–8 mm, wet mass 0.5–1.2 g) specimens of *Petrolisthes cinctipes* and *P. eriomerus*. Each point represents the mean \pm S.E.M. In A, $N=6$ individuals. In B, $N=6$ for all points except the following: $N=12$ for immersed and emersed *P. cinctipes* at 15°C, $N=11$ for immersed *P. eriomerus* at 15°C, $N=10$ for emersed *P. eriomerus* at 15°C, $N=5$ for immersed and emersed *P. eriomerus* at 20 and 25°C, $N=4$ for immersed *P. eriomerus* at 30°C, and $N=3$ for emersed *P. eriomerus* at 30°C. * denotes a significant difference between aerial \dot{V}_{O_2} of *P. cinctipes* and *P. eriomerus* at 25°C (ANOVA; $P<0.05$).

Thermal tolerance limits of heart rate

The thermal tolerance limits of *Petrolisthes cinctipes* and *P. eriomerus*, as indexed by the ABT and post-cold recovery of heart rate, are shown in Fig. 3. ABTs of heart rate in *P. cinctipes* were approximately 5°C higher than in *P. eriomerus* (31.5±0.5°C versus 26.6±0.3°C, ANOVA; $P<0.0001$, $N=6$ for each species) (Fig. 3A). Differences were also seen after cold exposure (Fig. 3B). The heart rate of *P. cinctipes* exhibited complete recovery after exposure to 1.5°C, whereas the lower critical temperature of *P. eriomerus* was 2°C, and recovery after exposure to this temperature was never observed ($N=6$ for each species). It is worth noting that the Arrhenius plots of *P.*

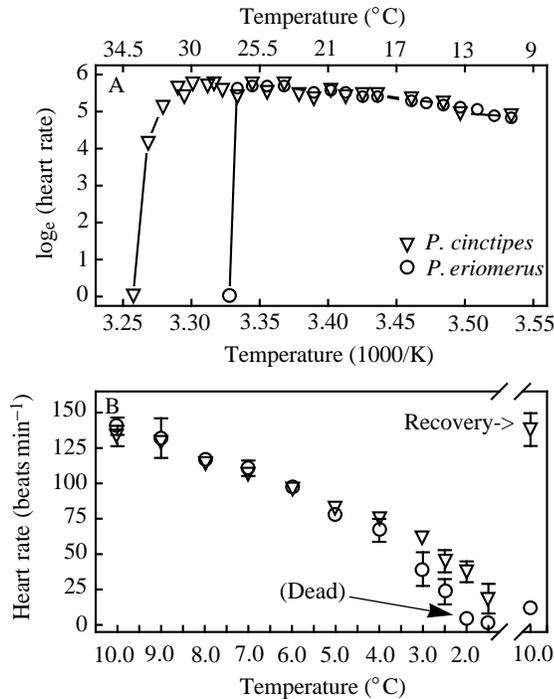


Fig. 3. Thermal limits of *in vivo* heart rate in *Petrolisthes cinctipes* (open triangles) and *P. eriomerus* (open circles). Temperatures were increased (A) or decreased (B) at a rate selected to mimic extreme field conditions (1 $^{\circ}\text{C}$ per 15 min). (A) Arrhenius break temperatures (ABT) of heart rates of single individuals of *P. eriomerus* and *P. cinctipes*. The mean ABT for *P. eriomerus* was 26.6 ± 0.3 $^{\circ}\text{C}$ and that for *P. cinctipes* was 31.5 ± 0.5 $^{\circ}\text{C}$ (mean \pm S.E.M.), a highly significant difference (ANOVA; $P < 0.0001$, $N = 6$ for each species). (B) Heart rate of *P. cinctipes* showed a complete recovery after cold exposure (1.5 $^{\circ}\text{C}$), but all *P. eriomerus* died at 2 $^{\circ}\text{C}$ (each point represents the mean \pm S.E.M. for six specimens).

cinctipes and *P. eriomerus* heart rate are qualitatively different. The heart rate of *P. cinctipes* decreased more gradually above the ABT than did the heart rate of *P. eriomerus*.

Lactate accumulation

Whole-body lactate accumulation shows that large specimens of *P. eriomerus* in air at 25 $^{\circ}\text{C}$ undergo a shift in metabolic poise towards anaerobiosis (Figs 2A, 4). Over a 5 h incubation period at 25 $^{\circ}\text{C}$, the lactate concentrations in whole crabs increased for *P. eriomerus* and decreased for *P. cinctipes* (Fig. 4). *Petrolisthes eriomerus* incubated in air for 5 h at 25 $^{\circ}\text{C}$ had an 808% higher concentration of lactate (7.827 ± 1.58 versus 0.968 ± 0.15 mmol g^{-1}) than *P. cinctipes* at the same temperature, a significantly higher level (ANOVA; $P < 0.0005$, $N = 5$ for each species) (Fig. 4). In addition, *P. eriomerus* held at 25 $^{\circ}\text{C}$ for 5 h accumulated 173% more lactate than conspecifics held at 10 $^{\circ}\text{C}$ (4.52 ± 1.04 mmol g^{-1}), but the difference was not significant (ANOVA; $P = 0.083$, $N = 5$ for each group). *P. cinctipes* at 10 and 25 $^{\circ}\text{C}$ did not accumulate significantly different levels of lactate at any individual time point (Fig. 4). In *P. cinctipes* incubated at 10 and 25 $^{\circ}\text{C}$, lactate levels decreased significantly between hours 1 and 5 (one-

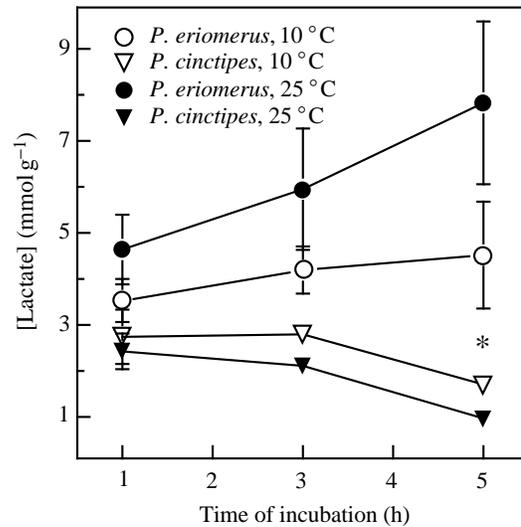


Fig. 4. Lactic acid accumulation in large specimens of *Petrolisthes cinctipes* and *P. eriomerus* after emersion at two different temperatures for 1, 3 or 5 h. Each point represents the mean \pm S.E.M. for five individuals. * denotes a significant difference in lactate concentration between *P. cinctipes* and *P. eriomerus* at 25 $^{\circ}\text{C}$ (ANOVA; $P < 0.0005$).

tailed *t*-test; $P < 0.05$), but there was no effect of temperature on lactate levels (Fig. 4).

Leg membrane studies

Photographs of the ventral merus with muscle fibers removed indicate that there is a gross morphological difference between the exoskeletons of *Petrolisthes cinctipes* and *P. eriomerus* (Fig. 5A,B). *P. cinctipes* possesses an uncalcified exoskeleton over a portion of the ventral surface of the meral segment (Fig. 5A), but *P. eriomerus* has a solid, thick exoskeleton over the entire meral segment (Fig. 5B).

Measurement of $\dot{V}_{\text{O}_{2a}}$ in large specimens of *Petrolisthes cinctipes* with their leg membranes obscured revealed a response to aerial exposure similar to that seen in *P. eriomerus* (Fig. 6A; compare with Fig. 2A). Crabs with their leg membranes obscured had a 28% lower $\dot{V}_{\text{O}_{2a}}$ at 30 $^{\circ}\text{C}$ (44.0 ± 7.47 versus 61.1 ± 1.39 $\mu\text{l O}_2 \text{h}^{-1} \text{g}^{-1}$; ANOVA of \log_{10} -transformed data; $P = 0.004$, $N = 8$ for obscured crabs and $N = 10$ for control crabs), but rates were not significantly different at 15, 20 or 25 $^{\circ}\text{C}$. Control crabs with half of their leg membranes obscured did not have different $\dot{V}_{\text{O}_{2a}}$ values from those crabs with no leg membranes obscured (Fig. 6A,B). Small specimens of *P. cinctipes* with their leg membranes obscured did not show a reduction in $\dot{V}_{\text{O}_{2a}}$ at 30 $^{\circ}\text{C}$, and rates were similar for all three groups of crabs at each temperature measured (Fig. 6B). Consistent with the effects of obscuring the leg membranes on respiration rate, *P. cinctipes* with their leg membranes obscured had a 188% higher lactate concentration after incubation for 5 h at 28 $^{\circ}\text{C}$ than did control crabs (12.54 ± 2.06 versus 6.64 ± 1.43 mmol g^{-1} ; ANOVA; $P = 0.003$, $N = 6$ for each group) (Fig. 7).

Sixteen out of 79 species of *Petrolisthes* surveyed possessed

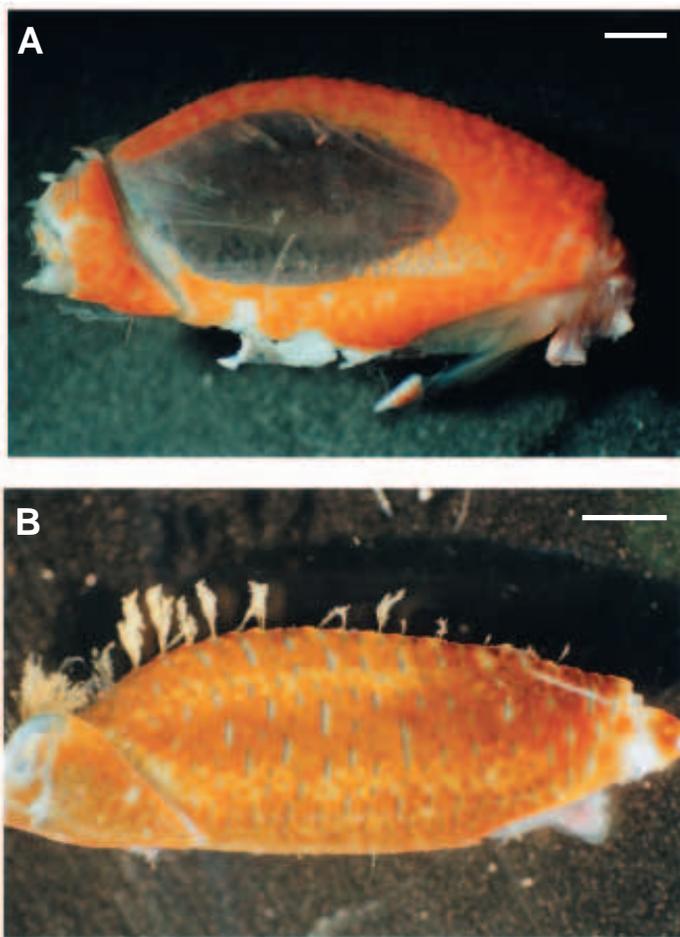


Fig. 5. Light microscope images of the ventral surfaces of meral segments of the second walking leg in (A) *Petrolisthes cinctipes* and (B) *P. eriomerus*. White scale bars represent 1 mm.

a leg membrane structure similar to that found on *P. cinctipes* (data not shown). From the collection data and the data given in Haig (1960), we could find no statistically significant relationship between the maximal size of the species and leg membrane occurrence. Owing to inadequate collection records, we are unable to ascertain whether the leg membrane appeared predominantly in species that occur higher in the intertidal zone.

Discussion

Our results indicate that *Petrolisthes cinctipes* experiences much longer periods of emersion, as well as much higher microhabitat temperatures, than does *P. eriomerus*. *P. cinctipes* possesses physiological, biochemical and morphological characteristics that may facilitate its survival in the mid to high intertidal zone and, by the same token, the lack of these characteristics may play a role in restricting *P. eriomerus* to the lower intertidal and subtidal zones. These characteristics include the ability of *P. cinctipes* to respire effectively in air, the higher thermotolerance of heart rate of *P. cinctipes* and the

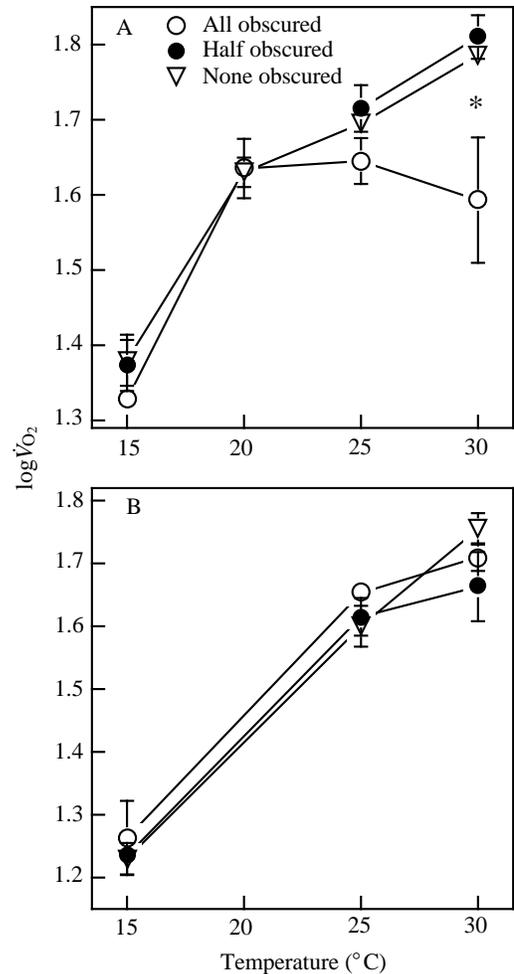


Fig. 6. Whole-animal aerial oxygen consumption rates ($\dot{V}O_2$) ($\mu\text{l O}_2\text{ h}^{-1}\text{ g}^{-1}$) of *Petrolisthes cinctipes* with their leg membranes obscured, at different temperatures and aerial exposures. Points represent mean \pm S.E.M. In A (large specimens), $N=4$ for all points except for the following: $N=6$ for no membranes obscured at 20 °C, $N=8$ for all leg membranes obscured at 30 °C, and $N=10$ for no membranes obscured at 30 °C. In B (small specimens), $N=4$ for all groups except for the following: $N=3$ for all leg membranes obscured at 25 and 30 °C, for half leg membranes obscured at 30 °C, and $N=2$ for no leg membranes obscured at 30 °C. * denotes a significant difference between the aerial $\dot{V}O_2$ of large specimens of *P. cinctipes* with and without their leg membranes obscured at 30 °C (ANOVA; $P=0.004$).

morphological differences that confer the ability for aerial respiration in *P. cinctipes*.

Habitat differences

Differences in the duration and frequency of emersion (Fig. 1A) and the vertical distribution of macrophytes contribute to the large differences in thermal microhabitats of *Petrolisthes cinctipes* and *P. eriomerus* (Fig. 1B,C). At the extremes, *P. cinctipes* can experience emersion for a total of over 12 h per day, divided between two low-tide periods (Fig. 1A), and can experience temperature fluctuations of over

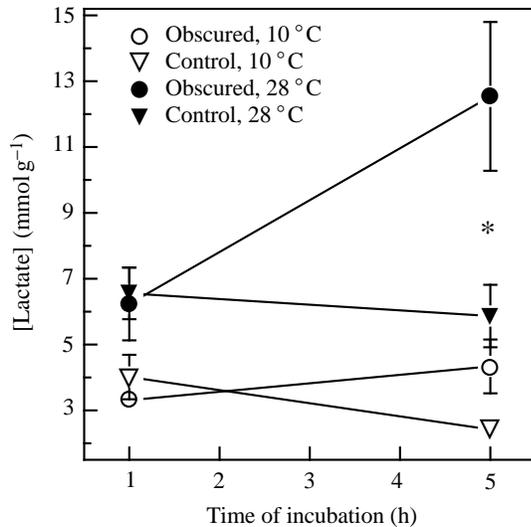


Fig. 7. Lactic acid accumulation in large specimens of *Petrolisthes cinctipes* with their leg membranes obscured after emersion at 10 and 28 °C for 1, 3 or 5 h. Each point represents the mean \pm S.E.M. for six individuals. * denotes a significant difference between crabs with leg membranes obscured and control crabs at 28 °C (ANOVA; $P=0.003$).

20 °C during a low tide (Fig. 1B) or, as shown for mussel beds, over 30 °C annually (Elvin and Gonor, 1979). Although the temperatures that we measured were not crab body temperatures, because the air underneath rocks is likely to be saturated with water, crab body temperatures and air temperatures are likely to be equivalent (Weinstein, 1995). It is worth noting that the temperatures seen on 18 May 1995 (Fig. 1B,C) represent extreme, infrequently occurring temperatures, as the prevailing weather conditions on the Oregon coast are not conducive to creating these extreme temperatures. The infrequency of thermally stressful days, however, does not negate their selective importance; only one day such as that observed on 18 May 1995 would be necessary to subject *P. eriomerus* living above their normal distribution limit to a lethal thermal stress. In addition, although all individuals of *P. cinctipes* are subjected to varying degrees of thermal fluctuation, subtidal *P. eriomerus* never experience emersion and only experience a 5 °C annual temperature fluctuation, associated with upwelling (Barry *et al.* 1995). The rocks in the low intertidal zone where *P. eriomerus* are found usually harbor large amounts of foliose algae. The algae act as a thermal buffer for rupestrine fauna owing to the thermal inertia of the algae as well as the large amount of algae-associated water (both intracellular and extraorganismal) that can support evaporative cooling, hence removing thermal energy from the rock. On 18 May 1995 (the day on which the data in Fig. 1B were collected), the algae dried out extensively during low tide, turning black and brittle (J. H. Stillman and G. N. Somero, personal observation).

Rock morphology affects the under-rock temperature, as massive stones, stones without air spaces underneath or stones with flowing water or air circulation will not show under-rock

temperatures as high as those for flat, thin stones with a small dead air space below. Thus, because most rocks do not meet the above criteria for the thermally maximal microhabitat, not all of the individuals of *P. cinctipes* experience temperatures as high as those shown in Fig. 1B. However, the fact that some individuals do experience thermally stressful microhabitats maintains the potential selective importance of a high thermal tolerance. The ability of crabs to select rocks with a particular morphology is not known, but the observation that large individuals are more common under large stones suggests that selection of, and competition for, shelter may occur. We have never observed *Petrolisthes* moving between rocks when emersed, so they may have a limited ability to thermoregulate behaviorally. Where *P. cinctipes* occur in beds of *Mytilus californianus*, the thermal microhabitat may be more uniform, and potentially as thermally stressful, as *M. californianus* body temperatures as high as 33 °C have been measured and have been estimated to be as high as 37 °C (Elvin and Gonor, 1979). We have measured temperatures among mussels as high as 28 °C (J. H. Stillman and G. N. Somero, unpublished observation).

Respiratory responses to abiotic stress

Large differences in respiration rate were observed between conspecifics of different size. The interspecific difference in $\dot{V}_{O_{2a}}$ of large specimens at 25 °C was not seen in small specimens (Fig. 2A,B). Hawkins *et al.* (1982) showed a similar size-dependence in respiratory responses to emersion in intertidal crabs. Large differences in $\dot{V}_{O_{2a}}$ between large specimens of *Helice crassa* (an inhabitant of the high intertidal and supratidal zones) and *Macrophthalmus hirtipes* (an inhabitant of the low intertidal and subtidal zones) were apparent at higher temperatures, but there were much smaller differences between small specimens of the same two species. Thus, adaptations facilitating aerial respiration may be most pronounced in terrestrial or semi-terrestrial species that attain a large body size.

Thermal tolerance ranges

Studies of the thermal tolerance limits of heart rate reveal that *Petrolisthes cinctipes* is able to tolerate both higher and lower temperatures than is *P. eriomerus*, and is therefore more eurythermal. This finding, together with the description of the thermal microhabitat that these crabs can experience (above), can explain in part the differential survival of these species when reciprocally transplanted to different vertical zones in the intertidal region. Such transplant experiments revealed that *P. eriomerus* did not survive transplantation to the *P. cinctipes* zone, but that *P. cinctipes* survived when transplanted to the *P. eriomerus* zone (Jensen and Armstrong, 1991).

Our studies of thermal tolerance ranges also reveal that *Petrolisthes cinctipes* may be living at or near the edge of its physiological tolerance limits, as field temperatures measured (Fig. 1B) during very hot days were very close to the ABT of heart rate in this species (Fig. 2A). Temperatures measured at the top of the *P. eriomerus* zone never exceeded 25 °C, and temperatures measured at the top of the *P. cinctipes* zone never

exceeded 32 °C. Thus, small increases in sea surface temperature, which might translate into small increases in habitat temperature, might profoundly affect the distribution patterns of the species at the latitudinal and vertical limits of their range. A recent comparison of species assemblages in 1931–1933 (Hewatt, 1937) and 1993–1994 (Barry *et al.* 1995) has shown a decrease in the abundance of *P. cinctipes* from the intertidal zone in Pacific Grove, CA, USA, at the Hopkins Marine Station of Stanford University (Barry *et al.* 1995). Barry *et al.* (1995) also showed that there was an increase in mean sea surface temperature and in maximal sea surface temperature between 1933 and 1993. Whether the change in abundance of *P. cinctipes* over this 60 year period is a direct result of increases in global temperature or is a result of other factors, such as microhabitat rearrangement and the settlement characteristics of *Petrolisthes* (Jensen, 1989), remains to be tested. Although thermally induced mortality of *Petrolisthes* has not been observed in the field, we have observed high levels of mortality among *Hemigrapsus oregonensis* during low tide near Seattle, in the Puget Sound, on days when under-rock temperatures exceeded 33 °C. Mass mortality of *Mytilus edulis* has been reported during extremely stressful intertidal periods (Tsuchiya, 1983).

Petrolisthes eriomerus did not survive temperatures at or below 2 °C (Fig. 3B), but *P. cinctipes* showed complete recovery from 1.5 °C (Fig. 3B). Tissue temperatures of *Mytilus californianus* as low as –10 °C have been estimated (Elvin and Gonor, 1979), and it is likely that during these periods *P. cinctipes* living among mussels would benefit from having hearts that could remain functional during exposure to low temperatures. Thus, tolerance to cold temperatures could be selectively advantageous in *P. cinctipes*.

Lactate accumulation

Compared with its congener, *Petrolisthes eriomerus* exhibited an elevated dependence on anaerobic glycolysis when emersed at 25 °C, as shown by a significant rise in total body lactate concentration (Fig. 4) and a smaller rise in $\dot{V}_{O_{2a}}$ with rising temperature than those seen in *P. cinctipes* (Fig. 2). The latter species showed a decrease in total body lactate concentration with time at both 10 and 25 °C, but no effect of temperature on lactate concentration (Fig. 4). Levels of lactate seen in *P. eriomerus* after aerial incubation were of the same magnitude as those found in the crab *Leptograpsus variegatus* (Forster *et al.* 1989; Greenaway *et al.* 1992). Although porcelain crabs are for the most part quiescent during periods of emersion, because they are poikilothermic, rising body temperatures will elevate metabolic rates. Unlike its congener, *P. cinctipes* appears to rely largely on aerobic ATP-generating pathways during heating, so it is better able to withstand emersion at high temperatures without suffering losses in metabolic efficiency due to shifts from aerobic to anaerobic generation of ATP.

Leg membrane studies

The data presented here support the hypothesis that the leg

membrane structures in *Petrolisthes cinctipes* are functional respiratory structures and are utilized during periods of high metabolic demand while emersed (Figs 6, 7). *P. cinctipes* with their leg membranes obscured had a significantly lower $\dot{V}_{O_{2a}}$ at 30 °C and a significantly higher lactate accumulation at 28 °C than control crabs (Figs 6, 7).

Although leg membranes are not found in the majority of species of *Petrolisthes*, 16 of 79 species examined possessed such structures. Whether the leg membrane structures play a respiratory role in other species of *Petrolisthes*, including subtidal as well as intertidal species, is not known. Although we do not know the vertical distribution for many of the other species of *Petrolisthes* possessing the leg membrane, at least one species, *P. rathbunae*, is mainly subtidal (Haig, 1960). This suggests that totally aquatic species with very high metabolic rates might also utilize this structure for aquatic respiration, although this conjecture remains to be investigated.

The genus *Petrolisthes* is composed of species with widely differing morphological features (claw size, setae distribution, exoskeleton texture), and leg membranes were found on species with morphological features both similar to and very different from *P. cinctipes*. In addition, in two species (*P. tiburonensis* and *P. gracilis*), the leg membrane was only present on the second and third walking legs (middle and most posterior); the first pair had a complete exoskeleton. In one species (*P. hians*), the leg membrane was found only on the second leg. Without a phylogenetic tree for the genus, and a knowledge of the distribution and physiology of other species possessing the leg membrane, it is impossible to infer the function of the leg membrane and its adaptive significance in species other than *P. cinctipes*. An examination of the function of the leg membrane in additional species, together with the development of a phylogeny for the genus, is necessary to elucidate the evolutionary history of the leg membrane as an adaptive feature.

Leg membranes have evolved independently in at least two infraorders of decapod crustaceans: the family Ocypodidae (infraorder Brachyura) and the family Porcellanidae (infraorder Anomura). Maitland (1986) reports that the gas windows are typical in Scopimerinae, of the family Ocypodidae. The leg membrane of *Scopimera inflata* occurs on the dorsal side of the merus (Maitland, 1986), but the membrane in *Petrolisthes* spp. are all on the ventral side of the merus. This suggests that the leg membranes have evolved independently in the two groups of crabs.

In summary, temperature stress and aerial exposure are two predominant abiotic factors that influence distribution patterns in the intertidal zone. We have shown that *Petrolisthes cinctipes*, a mid to high intertidal inhabitant, has a wider thermal tolerance range than its congener *P. eriomerus*, which lives in the low intertidal and subtidal zones, and that *P. cinctipes* has additional respiratory structures which allow it to maintain an aerobic metabolic state while emersed. One very important finding was that *P. cinctipes* may encounter temperatures near or at their physiological tolerance limits. Increases in sea surface temperature, or in air temperature, that

are associated with global warming could cause lethal temperatures in the current microhabitat of *P. cinctipes*, the long-term effects of which might result in changes in the distribution and abundance of the species. Owing to the gregarious nature of settlement in *Petrolisthes* spp. (Jensen, 1989), a reduction in population size during one season could lessen recruitment and settlement in successive seasons and thus affect population size over a long time span. In addition to the potential importance of this species as an indicator of the effects of global warming on intertidal organisms, the different tolerances to temperature in *P. cinctipes* and *P. eriomerus* present a model system for the study of temperature adaptation in crustaceans. The genus is highly speciose, and species are found over a wide range of regions from temperate to tropical, in the intertidal and subtidal zones, some species having wide distributions and others narrow ones. These and additional congeneric species provide an excellent study system for addressing the roles that morphological, physiological and biochemical adaptations play in establishing and maintaining species distribution patterns and, in addition, provide an appropriate group of organisms to study temperature adaptation and the evolution of eurythermality.

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