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Behavioural and respiratory response of the shallow-water hermit crab *Pagurus cuanensis* to hydrostatic pressure and temperature

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ABSTRACT

The evolutionary history of the Anomura has long been controversially discussed. One aspect that has received little attention is the dissimilarity in physiological tolerances of the related families Paguridae and Lithodidae to environmental conditions, and how this may have determined the divergence and radiation of the families into different distribution ranges, in particular with regard to the limited penetration of the deep sea by the Paguridae. This study investigates the physiological tolerances of the temperate shallow-water hermit crab, *Pagurus cuanensis*, to various temperature (5, 10, 15, 20 °C) and pressure regimes (1 to 100 atm) by measuring the standard metabolic rate (SMR) and behavioural changes. SMR was primarily affected by temperature, with a notably low rate at 5 °C throughout all pressures. Behaviour was affected by pressure, with an increase in pressure from 50 to 100 atmospheres (atm) resulting in reduced activity. It is suggested that this species can tolerate hydrostatic pressures greater than those found in its natural bathymetric range. It is discussed that a lack of physiological cold tolerance and ecological factors, such as the need to find gastropod shells for protection, are the principal restrictions maintaining *P. cuanensis* to a maximum depth of approximately 250 m. We hypothesize that temperate shallow-water invertebrates could indeed be able to penetrate greater depths as continental shelf waters warm up in the course of global climate change.

1. Introduction

The horizontal and vertical distribution ranges of marine organisms are controlled by a combination of biotic and abiotic factors (Carney et al., 1983). The two major abiotic factors that vary with depth are temperature and hydrostatic pressure. In relation to the depth distribution range of a species, the combined tolerance of temperature and hydrostatic pressure is potentially paramount in determining the upper and lower limits of the vertical region occupied by that species. The effect of temperature on the bathymetric distribution of marine organisms has been widely studied (e.g. Southward et al., 1995). However, hydrostatic pressure as a significant limiting factor has received relatively little attention in the past (Brauer et al., 1975; Jaenicke, 1983) and/or such studies have been constrained by the lack of available technology (Shillito et al., 2001; Robinson et al., 2009). Understanding the mechanisms by which these factors combined can drive the distribution limitations of species may advance comprehension of local biodiversity, as well as the potential for future radiation of species outside of their current range – an aspect particularly relevant in light of future climate change and the warming of surface waters (Tyler and Young, 1998).

The origin of deep-sea animals has been debated since the middle of the 19th Century (for review see Gage and Tyler, 1991). A relationship between the anomuran families Lithodidae (king crabs) and the Paguridae (hermit crabs) was first suggested by Milne Edwards (1836), in part due to the large, fleshy, asymmetrical abdomen of the king crabs (McLaughlin, 1983). It has been suggested that the large, primarily deep-sea king crabs (Feldmann, 1998) were descended from the small, primarily intertidal shell-dwelling hermit crabs through the process of 'carcinization'. The process of carcinization involves a long membranous abdomen becoming transformed into a reduced state, folded under the thorax and pressed against the sternum, negating the need for further protection of the abdomen from a gastropods shell or similar (McLaughlin and Lemaitre, 1997). Furthermore, this transition from hermit crabs to king crabs likely coincided with an increase in the abundance of the laminarid kelps (Saunders and Druehl, 1992; McGaw and Duff, 2008), which may have provided protection from predation and thus allowed the loss of the gastropod shell shelter. This hypothesis suggested the initial evolution of the soft-abdomen lithodids, followed by the fully carcinized subfamily Lithodinae (Zaklan, 2002; McGaw and Duff, 2008). Cunningham et al. (1992) assessed ribosomal RNA of both families and found close similarities between *Pagurus* and *Lithodes* species, placing the king crabs within the genus *Pagurus*, as well as estimating that the transition occurred between 13 and 25 million years ago. This is more than 50 million years later than the period from which fossils assigned to the genus *Pagurus* have been

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found (Cunningham et al., 1992, and references therein). On the other hand, there have also been noteworthy studies refuting the relationship between the king and hermit crabs. Martin and Abele (1986) first tentatively suggested that the close relationship proposed might not be correct (McLaughlin et al., 2007). In 1997, McLaughlin and Lemaitre strongly opposed the traditional view of lithodid crabs being descendant from hermit predecessors through carcinization based on their study on adult morphology. They even suggested an alternative hypothesis; that in fact the reverse had occurred, and that hermit crabs had evolved from the crab-like lithodid form (McLaughlin et al., 2004, 2007).

Species of the family Paguridae are distributed globally demonstrating a wide range of thermal tolerances from subpolar to tropical environments. A few exceptional species are also found terrestrially (Hazlett, 1981), while only few species are found in the deep sea (Selbie, 1921). The family Lithodidae is divided into two sub-families, the Hapalogastrinae which is constrained to the shallow North-Pacific and the Lithodinae, which is distributed globally but primarily restricted to the cold waters of the deep sea and shallow waters of high latitudes (Hall and Thatje, 2009). Differences in the distributions of the families may be related to the evolution of different tolerance levels to both temperature and hydrostatic pressure. The radiation of the Lithodinae into the deep sea from an intertidal ancestral state may concur with the pattern of onshore to offshore migration of species previously suggested (Jablonski et al., 1983).

This study will for the first time elucidate whether physiological limits have impaired a widespread invasion of the deep sea by the Paguridae. The study of the factors that govern the distribution ranges of both Lithodidae and Paguridae provides a case for physiological tolerances contributing to the constraint and radiation of other taxa into and through deep-sea environments.

2. Materials and methods

2.1. Sampling and maintenance

Individuals of the hermit crab *Pagurus cuanensis* were collected from Southampton Water, England, by dredging between August and October, 2008. Animals were maintained in approximately 10 l tanks containing filtered seawater (29 psu) and aeration; water was partially changed twice a week. The desired temperature, approximately 15 ± 1 °C (average water temperature in Southampton Water during September), was maintained by keeping the tanks in Cooled Incubators. To avoid cannibalism, the animals were kept separate by placing each into a small glass dish, which were then covered with a plastic grid. Feed consisted of shrimp food pellets, one pellet each administered every Monday and Friday. Hermit crabs were marked for identification using small numbered bee tags glued to their shells. In order to acclimatize the experimental animals and provide time to recover from collection and handling stress they were kept under these conditions for a minimum of a week prior to use. No mortality was recorded as a result of the sampling and maintenance conditions. When the three further experimental temperatures (5, 10 and 20 °C) were required, the temperature of the incubators was altered by a maximum of 1.5 °C per day and animals were allowed to acclimatize for a minimum of 5 days once the desired temperature was reached. No animal was used for an experiment more than once.

To reduce handling stress, animal parameters (Carapace Length = CL, weight, sex) were obtained following each experiment. In order to release the hermit crab from its shell, the shell was gently crushed in a vice, which makes the animal emerge from its shell and so could be slowly pulled away with forceps; a method that has been used previously and does not appear to cause injury or any mortality (Briffa and Elwood, 2005). Crabs were then thoroughly dried with blotting tissue and weighed to the nearest 1/100 g to determine the wet mass. Animals of both sexes were used.

2.2. Respiration rates

Hermit crabs were deprived of food for 4 days prior to the experiment to minimize errors in oxygen consumption due to bacteria in faeces. Prior to each experiment two plastic (1 l) containers, one containing filtered (30 µm) seawater and the other containing fresh tap water, along with six small pressure vessels were placed inside an incubator until the desired temperature for each test (5, 10, 15, 20 °C) was reached. The seawater was aerated for 30 min. The aeration system was removed 30 min before the start of each experiment to ensure that the water was fully oxygen saturated yet no longer contained air bubbles. An individual hermit crab was placed inside a 50 ml transparent plastic vial which was then filled with the oxygen saturated, filtered seawater and closed underwater to ensure that no air bubbles were trapped inside. The vial was placed inside a small pressure vessel that had been incubated at the experimental temperature (for details see Mestre et al., 2009), which was filled with the fresh tap water previously incubated. When required, the pressure vessel was pressurized to the desired level (20, 50, 100 atm) using a hydraulic (Maximator, Germany) hand pump. The increase in pressure was continuous and acute, taking 10 s or less. After 1 h the pressure vessel was removed from the incubator and the pressure released instantaneously. The plastic vial was removed and inverted gently three times to ensure the oxygen content of the seawater was homogenously mixed. The lid was then removed carefully to ensure no water was spilt. Using the microoptode and temperature sensor the oxygen percentage concentration and temperature were measured and recorded.

2.3. Standard metabolic rate

Oxygen was measured using a temperature controlled oxygen meter and microoptode (Microx TX 3, Presens, Germany). The microoptode was calibrated daily prior to use at the experimental temperature with 0% oxygen saturation as a seawater solution oversaturated with sodium sulphite (Na_2SO_3) and 100% oxygen saturation as fully aerated seawater that had been left to settle for 30 min. Oxygen measurements were temperature compensated using a temperature probe (PT 1000) positioned in close proximity (within mm) to the microoptode.

In order to measure the concentration of oxygen in the 100% oxygen saturated seawater prior to experimental use, the temperature and salinity of the seawater were measured. The temperature was measured using a temperature probe and the salinity was measured using a Hanna Instruments HI 9828 multiparameter water quality meter. The concentration of oxygen in 100% saturated seawater (µmol O_2 l^{-1}) was calculated using the equation from Benson and Krause (1984) (Eq. (1)):

$$\begin{aligned}\ln C^*_0 = {} & -135.90205 + 1.575701 \times 10^5 / T - 6.642308 \times 10^7 / T^2 \\ & + 1.243800 \times 10^{10} / T^3 - 8.621949 \times 10^{11} / T^4 \\ & - S(0.017674 - 10.754 / T + 2140.7 / T^2)\end{aligned} \quad (1)$$

where C^*_O is the concentration of oxygen in 100% saturated filtered seawater (µmol O_2 l^{-1}), T is the temperature in Kelvin and S is the salinity. In this experiment the conditions were at atmospheric pressure and within the $0\ °C < T < 40\ °C$ and $0 < S < 40$ boundaries set by Benson and Krause (1984). To determine anomalies caused by minor differences in seawater and in the calibration of the microoptode, the end point percentage oxygen saturation of the control vial containing only seawater was subtracted from 100%.

The percentage oxygen saturation in each sample at the end of each experiment (% O_2 *end*) was calculated by subtracting this difference from the end point % oxygen saturation value measured in each vial using the microprobe.

The ratio of the percentage oxygen saturation in the sample at the end of each experiment (% O_2 *end*) to the 100% oxygen saturation (C^*_O) was used to determine the oxygen concentration (cO_2) in each sample (μmol O_2 h^{-1}) (Eq. (2)):

$$(cO_2) = (C^*_O.\%O_2 end) / 100 \quad (2)$$

Since each vial was a closed system, the oxygen consumption rate (rO_2) of each individual hermit (μmol O_2 h^{-1}) could then be calculated using the following (Eq. (3)):

$$(rO_2) = (cO_2 start - cO_2 end) \times V / t \quad (3)$$

where cO_2 *start* is the oxygen concentration prior to closure of the vials, cO_2 *end* is the oxygen concentration when the vial is reopened at the end of the experiment (both in μmol O_2 h^{-1}), *V* is the volume of water in each vial in liters and *t* is the time each vial is closed in hours.

The volume of water inside each vial was measured by pouring the water into a 100 ml container on zeroed scales and recording the weight (1 ml = 1 g), whilst the hermit crab was held inside the vial. The vial was inverted twice and the hermit tapped to ensure that water was not trapped inside the shell. Five replicates were conducted for each pressure at each temperature. For each test condition a control was run. In order to eliminate any bias due to bacterial oxygen demand or calibration, any difference in oxygen concentration of the control vial from 100% was subtracted from the values of the experimental vials.

2.4. Behavioural measurements

Behavioural measurements were taken using the IPOCAMP™ pressure system (Shillito et al., 2001). The IPOCAMP™ was set running for 1 h prior to the start of each test to ensure that the saltwater inside was maintained at the desired test temperature (5, 10, 15, 20 °C). A hermit crab was selected at random and its shell secured to a clear plastic Petri dish using dental wax, ensuring that the opening of the shell faced upwards so that the hermit crab could be viewed from above at all times unless it was completely withdrawn inside the shell. This was then placed inside the IPOCAMP™ upon a tripod platform, which raised the position of the hermit crab inside the pressure chamber, allowing it to be viewed more clearly using en endoscope (see Ravaux et al., 2003). The IPOCAMP™ was then set running at atmospheric pressure for 30 min to allow the hermit crab to acclimatize and recover from handling stress. The video recorder was then set to record and the pressure altered at 20 min intervals from 1 atm–20 atm–50 atm–100 atm–50 atm–20 atm–1 atm, inclusive of the 20 min at atmospheric pressure at the start and end of each experiment. Repeats were conducted ($N = 5$) for each of the experimental temperatures (5, 10, 15, 20 °C). For the purpose of this study only the increase in pressure was analysed for comparison with SMR.

2.5. Video analysis

The videos were analysed using the software package Ulead. Behaviour was not recorded for the initial 5 min after an increase or decrease of pressure to allow the hermit crab to recover from any initial shock caused by the changing pressure. Behaviours were divided into two categories – states and events, depending on the relative length of time over which each behavioural incident occurred (Baeza and Fernández, 2002). Behaviours classed as states consisted of an action or position, which occurred over a period greater than 10 s (e.g. remaining withdrawn within the shell). Behaviour classed as events consisted of single specific actions or movements, which were completed in less than 10 s (e.g. wiping of the eye stalks with the third maxillipeds). States were quantified as proportion of time spent in each behavioural state (% of each 15 min period). Events were quantified as frequency of occurrences per unit time (15 min). The states and events recorded were as follows:

States

- 'Intermediate' (Fig. 1A) — time spent in a 'relaxed' position, with the chela, front two pairs of legs, eyestalks and front of the carapace clearly visible, but the soft abdomen held inside the shell not visible. Very little movement of the body was observed.
- 'Escaping' (Fig. 1B) — time spent extended further out of the shell than the 'Intermediate' position, with the soft abdomen visible and the hermit crab actively exploring where the shell was fixed with the dental wax and attempting to free itself. Movement of the body was almost continuous.
- 'Withdrawn' (Fig. 1C) — time spent with both the soft abdomen and the carapace hidden within the shell, either with legs withdrawn into the shell yet still visible, or totally withdrawn out of sight. No visible movement.
- 'Emerging' (Fig. 1D) — time spent moving between 'withdrawn' and 'intermediate' states as the hermit crab emerged from its protected position within the shell. This movement was often non continuous with many pauses; however, from the initial emerging motion to reaching the 'intermediate' state was counted.

States were recorded as total time spent in each state during each 15 min period under each pressure.

Events

Events primarily consisted of wiping of appendages with the third maxillipeds. The frequency of wipes of each type of appendage was counted for each 15 min period (excluding the initial 5 min after a pressure change) for each pressure. The individual events were defined as follows:

- Wiping of eye stalk/s — Number of times the third maxillipeds were used to wipe one or both eyestalks, placed behind the eye stalk/s and moved along from the base towards the end.
- Wiping of scaphognatites and antennule/s — Number of times the third maxillipeds were used to wipe the scaphognatites and one or both of the antennules, placed behind the scaphognatites and antennules and wiped with a downward motion.
- Wiping of antennae — Number of times the third maxillipeds were used to wipe one antennae i.e. placed behind the antennae and wiped along the length from the base towards the end.

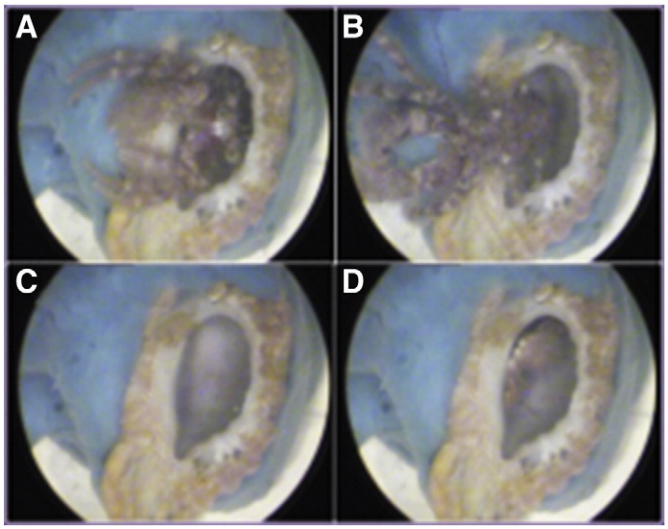

Fig. 1. The four behavioural states of *Pagurus cuanensis* in IPOCAMP™; (A) Intermediate, (B) Escaping, (C) Withdrawn, and (D) Emerging.

- Wiping of pereiopods — Number of times the third maxillipeds were used to wipe one of the pereiopods, placed partway down and wiped towards the end.
- Withdrawal — Number of times the hermit crab retreated further into the shell than the 'intermediate' position, often indicated by a sudden movement.

For events such as third maxilliped wiping of eye stalks, scaphognatites and antennules, each wiping motion was counted, regardless of whether one or both of each pair were wiped at a time. However, if more than one type of appendage was wiping simultaneously, this was counted as one event for each type of appendage. Scaphognatites were always wiped in the same motion as the antennules and therefore were grouped together.

2.6. Statistical analyses

Data transformation was necessary as the variances were heterogeneous (Underwood, 1981). The oxygen and behavioural events were found to have increasing variance with mean and therefore were square root transformed. However, as a large proportion of the behavioural events data were zero the transformation was completed using the square root of $n+1$. The behavioural states were proportions and therefore were arc-sine square root transformed. The data were analysed using Two-way Repeated Measures ANOVA. The post-hoc, multiple comparisons Holm–Sidak test (Sokal and Rohlf, 1995) was then used to determine which treatments produced the differences. As the data failed the normality test, 0.01 confidence limits were applied as opposed to the typical 0.05 to ensure statistically significant differences were not reported in error (Underwood, 1981).

3. Results

3.1. Effects of temperature on SMR when subjected to hydrostatic pressure

The standard metabolic rate (SMR) scaled with temperature (Fig. 2). However, the effect of temperature on the SMR was dependant on the pressure condition. At atmospheric pressure an increase in temperature resulted in an overall increase of the SMR. The smallest mean SMR was at 5 °C at $1.63 \pm 0.47\,\mu mol\,O_2\,h^{-1}\,g^{-1}$, the largest mean SMR was at 20 °C at $35.74 \pm 16.31\,\mu mol\,O_2\,h^{-1}\,g^{-1}$. In addition, the standard deviation increased with temperature. The two intermediate temperatures, 10 and 15 °C, however resulted in similar SMRs and standard deviations (15.51 ± 6.59 and $14.41 \pm 6.74\,\mu mol\,O_2\,h^{-1}\,g^{-1}$, respectively). At 20 atm the effect of temperature on SMR was less clear. For the three lower temperatures, SMR increased with temperature; however the SMR at 20 °C decreased to $12.66 \pm 8.74\,\mu mol\,O_2\,h^{-1}\,g^{-1}$, lower than the SMR of 10 and 15 °C, indicating the surpass of a physiological threshold. At 50 atm, the SMR also increased with temperature, but to a less distinctive degree than at 1 atm. In contrast, at 100 atm the three higher temperatures (10, 15 and 20 °C) were relatively similar, while the SMR at 5 °C the SMR remained low at $1.39 \pm 0.52\,\mu mol\,O_2\,h^{-1}\,g^{-1}$.

3.2. Effect of pressure on SMR at different temperatures

The effect of pressure on SMR was dependent upon the experimental temperature, with each temperature showing different relationships of SMR with pressure (Fig. 2). At 5 °C the SMR was the lowest at all pressures, however an increase in pressure caused a steady increase of SMR from 1 atm to 50 atm (1.63 ± 0.466 to $4.82 \pm 2.07\,\mu mol\,O_2\,h^{-1}\,g^{-1}$). The SMR then decreased at 100 atm to $1.39 \pm 0.52\,\mu mol\,O_2\,h^{-1}\,g^{-1}$, a similar value to that at 1 atm. In contrast, at 10 °C the SMR displayed the inverse of this pattern, decreasing slightly from 1 atm to 20 atm (15.51 ± 6.59 to $12.73 \pm 3.86\,\mu mol\,O_2\,h^{-1}\,g^{-1}$), and more sharply to $6.60 \pm 3.04\,\mu mol\,O_2\,h^{-1}\,g^{-1}$ at 50 atm. The SMR then increased at 100 atm to $14.86 \pm 4.54\,\mu mol\,O_2\,h^{-1}\,g^{-1}$. At 15 °C the SMR increased from 1 to 20 atm from 14.41 ± 6.74 to $18.55 \pm 6.08\,\mu mol\,O_2\,h^{-1}\,g^{-1}$. The SMR then decreased at 50 atm $10.76 \pm 2.25\,\mu mol\,O_2\,h^{-1}\,g^{-1}$, and increased again to $12.65 \pm 3.36\,\mu mol\,O_2\,h^{-1}\,g^{-1}$ at 100 atm. At 20 °C, the SMR at 1 atm was much higher than the other temperatures, at $35.74 \pm 16.31\,\mu mol\,O_2\,h^{-1}\,g^{-1}$. This value decreased by 23.08 to $12.66 \pm 8.74\,\mu mol\,O_2\,h^{-1}\,g^{-1}$ at 20 atm, increased slightly at 50 atm to $15.42 \pm 8.09\,\mu mol\,O_2\,h^{-1}\,g^{-1}$ and remained fairly constant to 100 atm.

A statistical interaction was found between the effects of temperature and pressure on SMR ($F = 5.052$, $p < 0.001$). Temperature also had a significant effect on SMR ($F = 47.471$, $p < 0.001$). A Holm–Sidak post-hoc multiple comparison test found that these differences were between the 5 °C and the higher three temperatures, as well as between 10 and 20 °C.

3.3. Effects of temperature and pressure on behavioural events

The effects of temperature and pressure on the rate of wiping of the eyestalks and with the third maxillipeds (Fig. 3) were non-significant. However, the lowest rate of wiping was consistently found at 5 °C, which decreased with pressure, and the highest rate was at

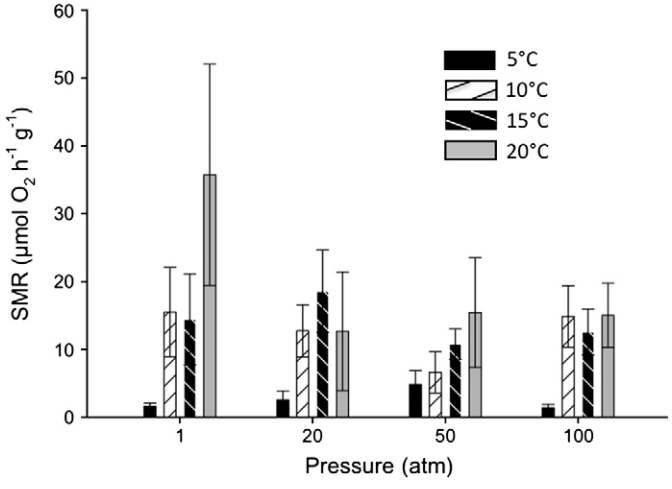

Fig. 2. *Pagurus cuanensis*. Standard metabolic rate ($\mu mol\,O_2\,h^{-1}\,g^{-1}$) maintained under temperature and pressure conditions for 1 h; ($N = 5$ in each treatment).

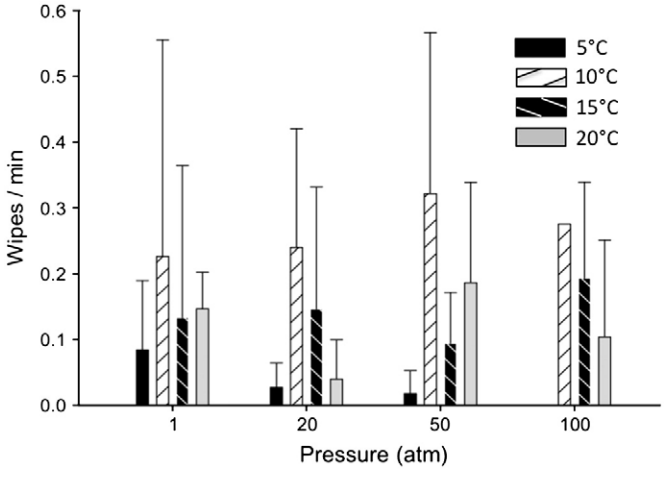

Fig. 3. *Pagurus cuanensis*. Frequency of wiping of the eyestalks with the third maxillipeds under different temperature and pressure conditions; ($N = 5$ in each treatment).

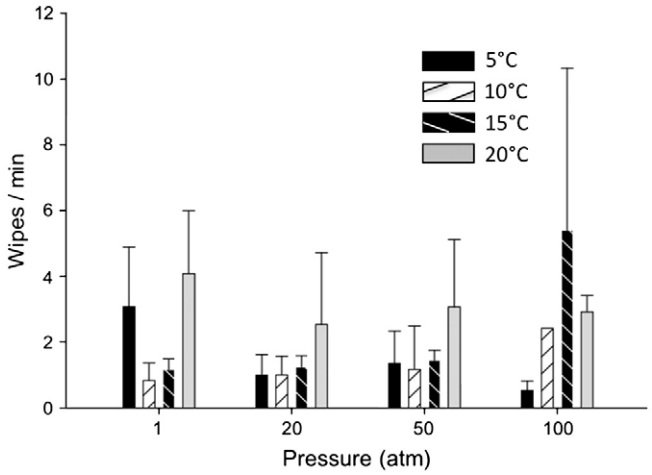

Fig. 4. *Pagurus cuanensis*. Frequency of wiping of the scaphognatites and antennules with the third maxillipeds under different temperature and pressure conditions; ($N = 5$ in each treatment).

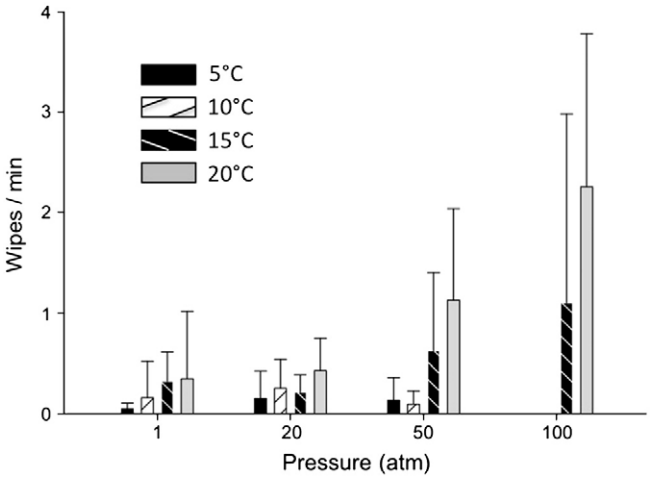

Fig. 6. *Pagurus cuanensis*. Frequency of wiping of the pereiopods with the third maxillipeds under different temperature and pressure conditions; ($N = 5$ in each treatment).

10 °C at all pressures. The effects of temperature and pressure (Fig. 4) showed a significant interaction on the rate of wiping of the scaphognatites and antennules ($F = 3.999$, $p < 0.001$). At 1 atm the frequency of wiping was low at 10 and 15 °C, at 0.83 ± 0.53 and 1.17 ± 0.32 wipes min^{-1}, respectively. The rate of wiping of the antennules was higher at 5 and 20 °C at 3.08 ± 1.81 and 4.08 ± 1.90 wipes min^{-1}, respectively.

At 20 atm the rate of wiping of the scaphognatites and antennules at 5 °C was reduced to a similar value to 10 and 15 °C, the rate of which remained stable. The rate at 20 °C also decreased by a lesser extent to 2.54 ± 2.18. The values at all temperatures remained relatively unchanged at 50 atm, although at each temperature the rate marginally increased by an average of 0.3 wipes min^{-1}. At 100 atm the rate of wiping of the scaphognatites and antennules at 5 °C decreased to its lowest value at 0.53 ± 0.28 wipes min^{-1}, at 10 and 15 °C the rates increased to the highest values for each temperature (2.41 ± 0.00 and 5.40 ± 4.92). However, at 20 °C the rate remained very similar to that at 50 atm. The standard deviations were largest at 20 °C except for at 100 atm where the distinctly large standard deviation was at 15 °C (4.92).

The rate of wiping of the antennae with the third maxillipeds (Fig. 5) was significantly affected by pressure ($F = 15.778$, $p < 0.001$). Furthermore, the frequency of antennae wiping at both 1 and 100 atm was statistically different to 20 and 50 atm ($p < 0.001$). The rate of wiping of the antennae reached a maximum at all temperatures at 20 and 50 atm, while the rate of wiping was lower and more affected by temperature at 1 and 100 atm. At 1 atm the rate of wiping showed an overall increase with temperature to a maximum at 20 °C of 0.200 ± 0.021 wipes min^{-1}. At 20 and 50 atm, the wiping at all temperatures increased to similar values of approximately 0.3 wipes min^{-1}. At 100 atm the pattern of rates of wiping for each temperature resembled that at 1 atm.

There was no significant difference with temperature or pressure found for the rate of wiping of the pereiopods with the third maxillipeds. However, the results show that at each pressure there was a general increase in rate of wiping with temperature (Fig. 6). At 5 and 10 °C the values remained low and decreased to zero at 100 atm, while the rates of wiping at 15 and 20 °C increased at 50 atm and increased again at 100 atm up to 1.100 ± 1.880 and 2.256 ± 1.524, respectively.

At the three lower pressures (1, 20, 50 atm) the mean number of withdrawals into the shell per minute remained low at less than 0.6 withdrawals min^{-1} at all temperatures (Fig. 7), with no withdrawals at 10 and 15 °C. At 100 atm the rate of withdrawals at 5 °C decreased to 0, while the rate for 10 and 20 °C increased to 1.40 ± 2.07

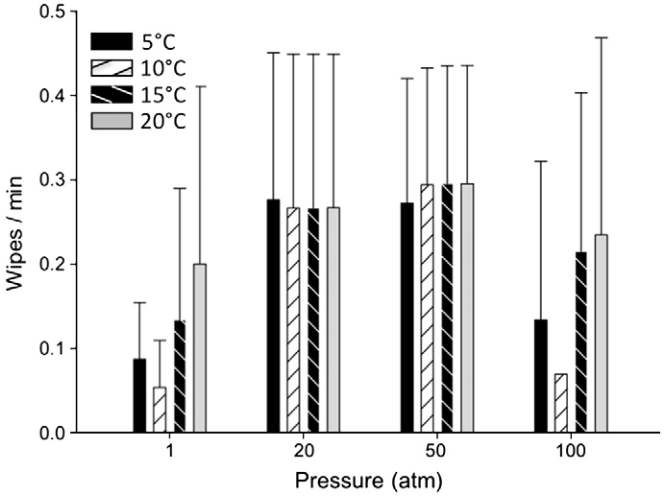

Fig. 5. *Pagurus cuanensis*. Frequency of wiping of the antennae with the third maxillipeds under different temperature and pressure conditions; ($N = 5$ in each treatment).

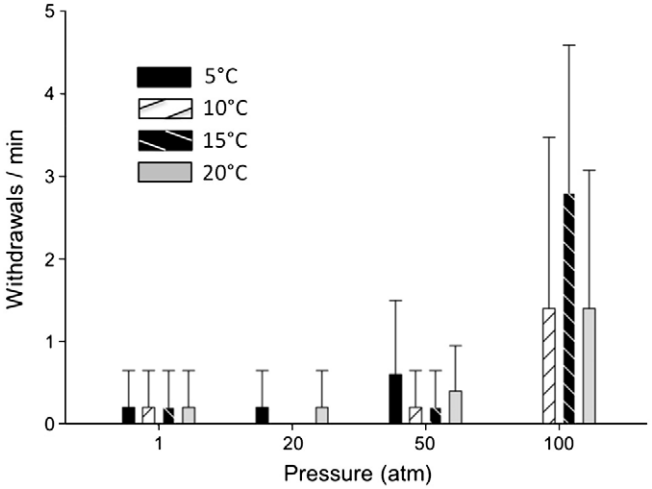

Fig. 7. *Pagurus cuanensis*. Frequency of withdrawals into the shell under different temperature and pressure conditions; ($N = 5$ in each treatment).

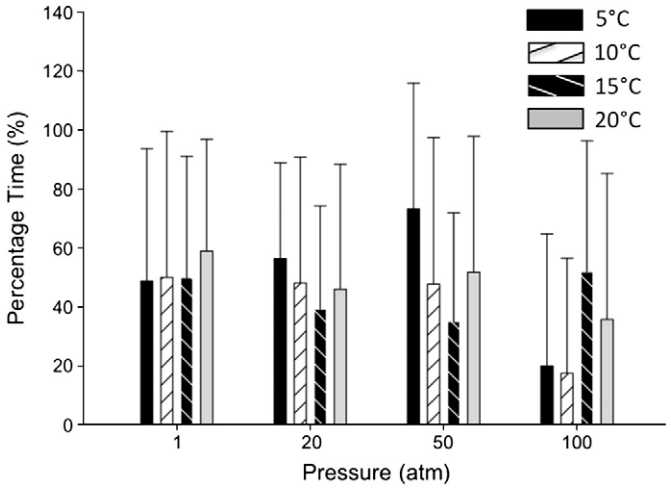

Fig. 8. *Pagurus cuanensis*. Percentage of time spent in the intermediate state under different temperature and pressure conditions; ($N=5$ in each treatment).

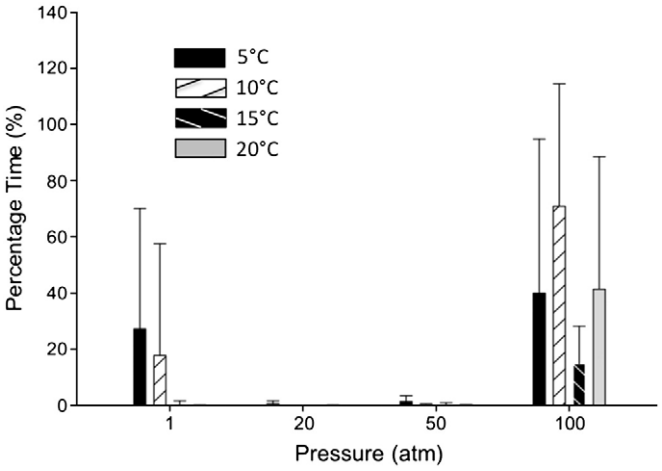

Fig. 10. *Pagurus cuanensis*. Percentage of time spent in the withdrawn state under different temperature and pressure conditions; ($N=5$ in each treatment).

and 1.40 ± 1.67 withdrawals min^{-1}, respectively. At 15 °C the rate also increased but to a rate twice that of 10 and 20 °C, to 2.80 ± 1.79. Care must be taken when analyzing this behaviour, as it indicates the number of withdrawals per minute, not the amount of time withdrawn. If a hermit was already withdrawn when data collection for a pressure began and remained withdrawn throughout, the number of withdrawals per minute is still zero (e.g. 5 °C at 100 atm). Therefore this behavioural event, withdrawing, should be compared with the behavioural state withdrawn (see Fig. 10). Statistical analysis found a significant effect of pressure ($F=13.108$, $p<0.001$) and also a significant interaction between the effects of temperature and pressure ($F=3.734$, $p=0.001$).

3.4. Effects of temperature and pressure on behavioural states

There was no significant effect of pressure or temperature on the percentage of time spent in the intermediate 'resting' position, which remained almost constant at each temperature and pressure (Fig. 8). The percentage of time spent in the escaping state (Fig. 9) was significantly affected by pressure ($F=7.080$, $p<0.001$), with the lowest average values for 10, 15 and 20 °C at 100 atm, and the lowest value for 5 °C at 50 atm (Fig. 9). *Post hoc* comparisons placed the significant differences between 100 atm and each of the lower three pressures. With the exception of 5 °C, where the lowest average percentage of time escaping was found at 50 atm ($2.71 \pm 6.06\%$), the lowest percentage of time spent escaping was at 100 atm. The highest percentage of time spent attempting to escape was found at 20 atm at all temperatures, with the highest average value of $51.22 \pm 27.41\%$ at 15 °C.

The percentage of time spent withdrawn was negligible at all temperatures at 20 and 50 atm and at 15 and 20 °C at 1 atm (Fig. 10). At 1 atm the animals spent an average of 27.18 and 17.78% of time escaping at 5 and 10 °C, respectively. The percentage of time spent withdrawn was highest at every temperature at 100 atm, with the highest mean value at 10 °C of $70.87 \pm 43.55\%$. There was a significant difference with pressure $F=12.533$, $p<0.001$), and the differences were found between 100 atm and each of the other pressures ($p<0.001$).

The percentage of time spent emerging was also significantly affected by pressure ($F=5.350$, $p=0.003$), with the highest values at 100 atm at 10, 15 and 20 °C (Fig. 11). However, the percentage of time spent emerging was highest at 5 °C at all pressures except for 100 atm where no emerging behaviour was displayed. At 100 atm the highest average percentage of time spent emerging was at 20 °C at $19.40 \pm 32.42\%$. Significant differences also lay between 100 atm and each other pressure ($p<0.004$). In a single individual at 5 °C, the crab removed itself entirely from its shell and then remained completely exposed for the duration, mid-way through the 20 min spent at 20 atm.

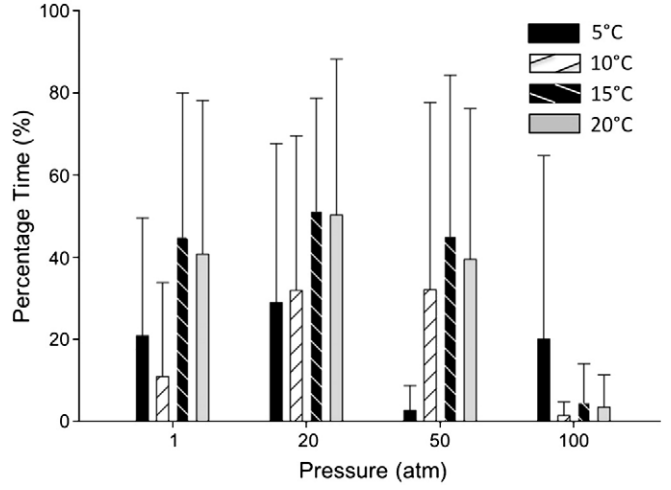

Fig. 9. *Pagurus cuanensis*. Percentage of time spent in the escaping state under different temperature and pressure conditions; ($N=5$ in each treatment).

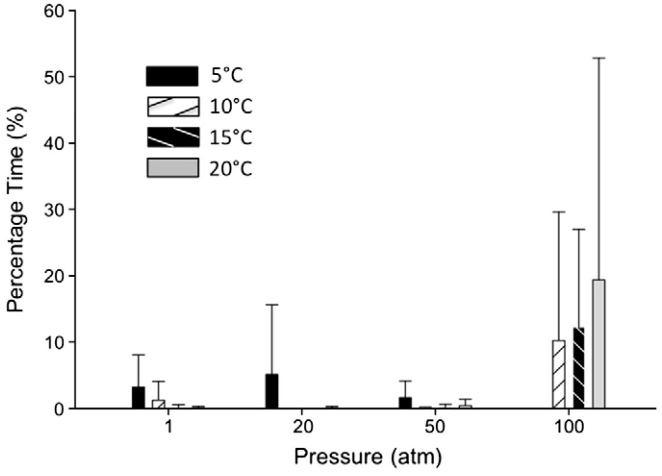

Fig. 11. *Pagurus cuanensis*. Percentage of time spent in the emerging state under different temperature and pressure conditions; ($N=5$ in each treatment).

4. Discussion

4.1. Influence of pressure and temperature on the SMR

The SMR in *P. cuanensis* is positively correlated with temperature when pressure was not a contributing factor, similar to previous research into crustacean metabolic rate (Stillman and Somero, 1996; Alekseeva and Zotin, 2001). The little overall effect of increased pressure on SMR at the lower three temperatures (5, 10 and 15 °C) suggests that temperature is the more dominant factor affecting oxygen consumption. However, at higher pressures the difference of SMR with temperature resulted in a distinctly smaller range. This is in contrast with the results of Childress (1977), who found that in the mid-water copepod *Gaussia princeps* oxygen consumption was more sensitive to temperature at higher pressures. Teal (1971) demonstrated that for some vertically migrating decapods, decreasing metabolism brought about by lower temperatures was offset by an increase in metabolism related to higher pressures, potentially as an adaption to maintain predatory behaviour at depth. It is possible that *P. cuanensis* undertakes a similar regulatory practice when subjected to hydrostatic pressure. Hermit crabs have previously demonstrated only a temporary increase in oxygen consumption when subjected to stressful conditions (i.e. salinity stress; Shumway, 1978). It is therefore possible that an increase in pressure may produce the same result of only a temporary metabolic increase that would not be measurable with the techniques used within this study. This would also suggest acclimation to pressure, at least in the short term and/or an attempt to maintain the normal metabolic rate, which implies an additional energetic cost. In addition, activity in hermit crabs results in greater oxygen consumption (Herreid and Full, 1986). Strenuous activity i.e. hermits attempting to free their shell from the dental wax, decreased significantly at 100 atm. This was not correlated with a decrease in SMR at the same pressure, possibly a result of the balance between decrease in SMR related to reduced activity and an increase in SMR related to pressure stress. The low SMR found at all pressures at the temperature indicative of the deep sea (5 °C) suggests that the hermit crabs would be capable of only limited activity at depth implying a potentially unviable lifestyle.

4.2. Influence of hydrostatic pressure and temperature on behaviour

The behaviour of organisms can represent a direct or indirect response to unfavourable conditions. Individuals of *P. cuanensis* demonstrated numerous behavioural changes when subjected to changes in hydrostatic pressure.

The high rate of wiping of the scaphognatites and antennules at both 5 and 20 °C at atmospheric pressure may be a consequence of temperature stress as the antennules bear sensory setae (Ingle, 1993). At 20 °C, an increased rate of wiping of these appendages occurred, which may imply stress or increased activity with higher temperatures. At 100 atm the mean rate of wiping of the scaphognatites and antennules increased with temperature up to 15 °C but at 20 °C the rate was restrained to that displayed at the previous two pressures, suggesting that the individuals reach a maximum wiping rate at this temperature when subjected to pressures 20 atm and above, as energy may be reserved for maintaining more basic functions.

The rate of wiping of the antennae with the third maxillipeds revealed two very distinct patterns. The antennae also bear sensory setae (Ingle, 1993) and so may indicate a response to stressful conditions. At the lowest and highest pressures the rate of wiping of the antennae followed remarkably similar relationships with temperature. The lowest rate of wiping at 10 °C suggests the optimal or least stressful temperature, while the rate of wiping then increased from 5 to 15 to 20 °C, possibly indicating increasing levels of both low and high of temperature stress. However, at 20 and 50 atm the rate of wiping was increased to an apparent maximum equal rate at all temperatures, implying pressure stress regardless of temperature. The lack of this display of maximum wiping rate at 100 atm may be a result of energy being directed elsewhere, as previously suggested. However, a further possibility is that high hydrostatic pressure impairs the function of the mechanoreceptor sensory setae on the antennae, resulting in the rate of wiping being related solely to the metabolic rate and temperature, as found at atmospheric pressure.

Hermit crabs can withdraw into gastropod shells for protection from environmental factors such as predation, desiccation, extremes of salinity and competition for shells from other individuals (Elwood, 1995; Pechenik et al., 2001); retraction of a hermit crab into its shell may be viewed as a defensive behaviour. The significant increase in the number of withdrawals into the shell per minute at 100 atm strongly suggests that the crabs can sense and become agitated by high-pressure conditions. This is supported by the increase in the amount of time spent emerging at 100 atm, a result of both the induced withdrawal response and a possible insecurity under the conditions causing rapid alterations in decisions. This reaction to high pressure however, was not displayed at 5 °C, where three individuals remained exposed and two remained withdrawn the entire time. This demonstrates that the effect of pressure was more distinct at lower temperatures, either causing the withdrawal response to be maintained for the duration or not at all. The cause of this discrepancy is unknown, but is possibly due to the low temperature combined with high pressure impairing decision-making abilities, or stimulating an altered behaviour.

Length of time spent in shell has been suggested to relate to motivation (e.g. hunger combined with a startle stimulus, Elwood et al., 1998). Billock and Dunbar (2009) found that when both deprived of a shell and starved, the hermit crab *Pagurus samuelis* was more motivated to find a shell than food, demonstrating the importance of the protective shell. Additionally, hermit crabs are known to withdraw into the shell when subjected to both low salinity and low oxygen conditions (Lancaster, 1988). In agreement with this, the distinct increase of the length of time spent within the shells at 100 atm suggests that the motivation to adopt this position as a defensive response to pressure is greater than the motivation to attempt to escape from the conditions. Many individuals remained withdrawn for the duration of the exposure at the highest pressure, and for a significant period following, which included depressurisation at a similar rate to the previous pressurisation. Reese (1969) argued that the shells of hermit crabs act as a microhabitat, which increases physiological tolerances to, or removes the individual from extreme conditions. However, while withdrawn the individual forsakes the opportunity to undertake activities such as feeding and mating. This suggests that while all animals tolerated the high-pressure conditions in the short term, the typical lifestyle would not be possible, rendering long-term survival at 1000 m depth unviable.

The use of behaviours to maintain homeostasis in decapod crustaceans has been explored (e.g. Huntingford et al., 1995 and references therein). For example, increased swimming activity has been observed in the larvae of the hermit crab *Discorsopagurus schmitti* as a result of increasing pressure (Gherardi, 1995). Shumway (1978) found that exposing hermit crabs to low salinities resulted in increased activity, most likely in an attempt to move away from the unfavourable conditions, and only withdrew into the shell when escape was impossible. This may explain the increase in the percentage of time spent attempting to escape from the dental wax which restrained movement of the shell at all temperatures from 1 to 20 atm, as the greater pressure could motivate the crab to increase energy spent endeavouring to maintain itself within its optimal depth distribution. In addition, the exposed position in which the experimental animals were fixed in order to maintain observation of behaviours would, in the natural environment, render them vulnerable to predation. This evidently explains the relatively high amount of time invested in attempting to free itself. However, increased activity results in higher

oxygen consumption and therefore energetic cost (Herreid and Full, 1986). It is likely that the decrease in the escaping behaviour at higher pressure may be due to energy being reserved for maintaining basic functions such as respiration, with freeing its protective shell no longer the priority. In nature, this would result in the individual no longer capable of, or primarily motivated to remove itself from the detrimental conditions.

Under high pressure and low temperature conditions, it was also noted in some individuals that movements became distinctly robotic, resulting in tremors. This behaviour has been noted previously for shallow-water species (Brauer, 1984; Wilcock et al., 1978), and is thought to be a symptom of high-pressure neurological syndrome (Somero, 1992). As pressure had different behavioural effects at varying pressures, it is suggested that there is a hierarchy in the stress responses of *P. cuanensis* when subjected to pressure.

4.3. Distribution and radiation of the Anomura

The evolutionary history of the Anomura has long been controversially discussed (McLaughlin and Lemaitre, 1997, and references therein). An interesting aspect to the separation of the families of the Lithodidae and Paguridae is the differences in the distribution, both horizontally and vertically. While there are large overlaps in the distribution of the families, each contains species, which inhabit environments inaccessible to the other. For example, there are species of hermit crab, which are terrestrial and they are commonly found in tropical regions (e.g. Hazlett, 1981). In contrast, there are no terrestrial king crabs, and there is a limit to the temperature range with a maximum of about 16 °C for successful larval development (Hall and Thatje, 2009). The Lithodidae are divided into two sub-families, Hapalogastrinae and Lithodinae. The Hapalogastrinae are constrained to the North Pacific and inhabit depths less than 200 m, yet the Lithodinae are distributed globally, with a maximum temperature threshold of 13 °C, resulting in a deeper distribution to greater than 3000 m, and intertidal species being found only at high latitudes (Hall and Thatje, 2009). This intolerance to high temperatures resulted in the radiation of the Lithodinae from the North Pacific through the deep sea to avoid the high temperatures (Zaklan, 2002). Therefore the spread of the Lithodinae occurred through a temperature bottleneck (Hall and Thatje, 2009). This is fundamentally similar to the pattern of increasing depth distribution of *P. cuanensis* with movement southwards in the North Atlantic (Forest, and Guinot, 1956; Ingle, 1993). While some species of hermit crab exist in the deep sea, the vast majority is found in intertidal or sub tidal habitats (Selbie, 1921; Wolff, 1961). Data from the present study demonstrate that the depth range of a hermit crab can be markedly smaller than its scope of physiological tolerance to pressure may suggest. A possible reason for the greater success of deep-sea existence of the Lithodinae is the adaptation of having a strongly calcified exoskeleton, negating the need for gastropod shell protection, a resource, which becomes limited with depth. This is further supported by the radiation into the deep sea by the fully armoured Lithodinae, but the restriction to depths not more than 200 m of the soft abdomened Hapalogastrinae (Zaklan, 2002; Hall and Thatje, 2009).

The SMR of *P. cuanensis* was substantially reduced at 5 °C, suggesting that intolerance to cold water may inhibit radiation into deeper habitats. In addition, deep-sea lithodids must have evolved to effectively tolerate high hydrostatic pressure. The behaviour of *P. cuanensis* at 100 atm, representative of 1000 m, indicated that hydrostatic pressure had detrimental effects on the individuals. The Lithodidae are suggested to be descendent from a shallow-water, cold-eurythermal population in the North-East Pacific (Hall and Thatje, 2009), agreeing with the hypotheses of near shore to offshore evolution (Jablonski et al., 1983). If the Lithodidae and Paguridae have a common ancestor, evolution of divergent traits and physiological tolerances are most likely to have led to dissimilarities in the distribution of these families. It is therefore hypothesized that differences in physiological tolerances to temperature and pressure combined and the limiting effect of the necessity of locating shells for protection, are among the main causes for the disparity in the geographical and bathymetric range of the families.

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