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Specific dynamic action affects the hydrostatic pressure tolerance of the shallow-water spider crab *Maja brachydactyla*

Sven Thatje · Nathan Robinson

Abstract The bathymetric distribution of marine benthic invertebrates is likely governed by a combination of ecological and physiological factors. The present study investigates oxygen consumption and heartbeat rate in response to attempted feeding at 1, 100 and 150 atm in the shallow-water spider crab, *Maja brachydactyla*, from temperate European waters. No significant difference was evident between the resting heartbeat rate of specimens at 1 or 100 atm, which were 56 and 65 bpm, respectively (Mann–Whitney, $U=5382.0$; $n=95$, 98; $p=0.079$). However, at 150 atm the resting heartbeat rate was significantly higher than that observed for 100 atm at 108 bpm (Mann–Whitney, $U=149.0$; $n=45$, 98; $p<0.001$). At 150 atm, feeding was never observed and coupled with the elevated resting heartbeat rate; it is suggested by 150 atm continued survival is unfeasible. At 1 and 100 atm, feeding instigated a distinct increase in heartbeat rate, which remained elevated for over 30 h. This increase peaked within 1 h at 1 atm. At 100 atm, this required 4 h and postprandial oxygen consumption was significantly higher than at 1 atm (Kruskal–Wallis, $H=85.036$; $df=2$; $p<0.001$). Elevated hydrostatic pressure is hypothesized to extend the duration and the total metabolic energy devoted to specific dynamic action. The metabolic requirements of feeding under hyperbaric conditions may even reach such a critical demand that feeding is entirely inhibited.

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Introduction

Hydrostatic pressure in the marine environment is characterized by a linear increase of one atmosphere for every 10 m of depth, reaching up to 1,100 atm in the deepest ocean trench. Organisms that live beyond continental shelf depth require physiological adaptations to hyperbaric conditions, which are often absent from shallow-water species (Somero 1992). For the approximately 80% of the global biosphere which occurs below a depth of 500 m, the effects of hyperbaric conditions may consequently be an influential factor in all biochemical and structural evolution (Jaenicke 1983; Jannasch and Taylor 1984; Somero 1990).

Selectivity towards pressure tolerant mechanisms in deep-living species may be such a necessity that a substitution in efficiency does not even convey a competitive loss. Specifically, in the enzyme lactate dehydrogenase, essential for anaerobic respiration, there is an apparent trade-off between adaptation to pressure and catalytic efficiency (Murray and Siebenaller 1987). However, without adaptation the effects of elevated hydrostatic pressures may impair enzymatic function entirely (Mozhaev et al. 1996). At the organismal level, these effects culminate in an obvious impairment in motor functioning and results in immobilisation as conditions exacerbate (MacDonald and Teal 1975; Jain 1994).

Tolerance to ambient environmental factors provides a fundamental limit to the habitable conditions in which any organism can survive. However, even minor forms of stress are capable of impacting upon the aerobic scope and optimum performance of an organism, as demonstrated

for temperature stress (Frederich and Pörtner 2000). The mechanisms by which pressure and temperature act on chemical structures are different, since pressure effects result only from volume change, whereas temperature affects both energy and volume. The overall effect of increasing pressure or decreasing temperature on molecular structures may produce a similar outcome (Balny et al. 2002; Sébert et al. 2004). It has also been demonstrated that exposure to higher than native pressures may trigger an increase in the expression of heat shock proteins (Ravaux et al. 2003; Cottin et al. 2010), which are known to be involved in general stress situations (Feder and Hoffman 1999). It may thus be possible that the effects of temperature stress on aerobic scope may be equivalent to those for pressure (Balny et al. 1997). Specifically, as external conditions vary further from the optimal range for an organism, an increased homeostatic effort is required to maintain internal conditions within suitable boundaries. The increase in effort corresponds to an increase in mitochondrial activity, which is accompanied by an appropriate elevation in respiratory and circulatory functioning (Frederich and Pörtner 2000). This will continue until the maximum aerobic scope of the organism impedes further intensification of respiratory activity. Within this pejus tolerance range, growth in mitochondrial demand to counter stress increase is not equivalently matched by an increase in respiratory capacity (Pörtner 2001). When the mitochondrial oxygen demand, to normalise homeostatic processes, finally exceeds the respiratory capacity of the organism, there is a transition from aerobic to anaerobic mitochondrial metabolism (Stillman and Somero 2000). The point at which this mismatch occurs is termed the 'critical tolerance' and beyond this, the organism experiences an indefinitely sustainable level of respiration (Pörtner 2001). As a result, the Arrhenius break point in respiratory capacity may be used as an indicator of critical tolerance (Somero 2005).

Strongly linked to ventilation and both ambient oxygen concentrations and oxygen demand, cardiac activity in Crustacea has been used as a proxy for respiratory capacity (Frederich and Pörtner 2000). An Arrhenius response has been observed beyond 100 atm for the shallow-water European spider crab, *Maja brachydactyla*, suggesting this is the upper physiological limit of the species (Robinson et al. 2009). A similar response has also been observed for the vent-associated deep-sea species, *Bythograea thermydron* (Airriess and Childress 1994). However, in some species of crustaceans, the Arrhenius break points for mitochondrial respiration (Dahlhoff et al. 1991; Weinstein and Somero 1998) and enzymatic activity (O'Brien et al. 1991) may be considerably higher than those for observed cardiac activity (Stillman and Somero 2000). Functional failure in the cardiac muscle, prior to oxygen

limitation, may therefore form a 'weak link' in an organisms' physiological tolerance (Peck 1998). To accurately assess the true critical tolerance of an organism to a particular stress, both mechanistic and metabolic limits to performance must be measured.

Colonisation and speciation into the deep-sea is suggested to have primarily transpired through isothermal water masses, as found in the warmer waters of the Mesozoic and early Cenozoic (Young et al. 1997). Under such conditions, when migrating to depth does not incur strong transitions in temperature, pressure may instead become the dominant factor in restricting such movements. A tolerance to pressure may therefore define those species that are able to make such evolutionary transitions (Menzies et al. 1973). The capacity for evolutionarily rapid colonisation of deep-sea environments through such processes may even justify the close phylogenetic links between certain deep-sea species and their shallow-water counterparts (Distel et al. 2000; Hall and Thatje 2009).

Evidence that the warming of surface waters is reducing the physiological constraints that previously restricted emergence into shallow Antarctic waters could also relate to deep-sea environments (Thatje et al. 2005a; Aronson et al. 2007). If ambient temperatures rise beyond a species' optimal range, as long as the increase in hydrostatic pressure does not incur greater energetic cost, migration to depth may provide a suitable action. Rising temperatures may even facilitate colonisation of deeper waters considering the antagonistic effects of pressure and temperature on molecular processes (Balny et al. 2002). In conclusion, if considered in conjunction with phylogenetic evidence, knowledge of species' pressure tolerances may not only help to explain the distribution of extant aquatic species with respect to depth but also determine the potential of such species to respond to environmental change (Hall and Thatje 2009; Thatje et al. 2010).

The energetic requirements of feeding in particular are crucial when determining the overall metabolic capacity of an organism (Fig. 1a; McCue 2006). The postprandial increase in metabolism, termed specific dynamic action (SDA) reflects the energetic requirements of digestion; including the movement and handling of food, absorption and storage of nutrients, deamination of amino acids, synthesis of excretory products and elevated synthesis of proteins and lipids associated with growth (Jobling 1992). The cumulative impact of the various processes that form SDA may even exceed the metabolic cost of activity (Soofiani and Priede 1985). To facilitate these energetic requirements, feeding may elicit physiological responses as shown in the blue crab, *Callinectes sapidus*, which demonstrated an immediate increase in cardiac activity upon food detection (McGaw and Reiber 2000). Consequently, SDA may impact directly on the aerobic scope of
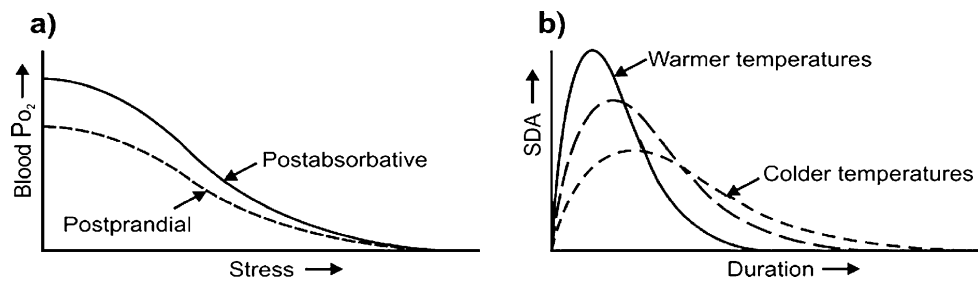
Fig. 1 The influence of SDA on aerobic scope. **a** The increased metabolic demand of postprandial processes reduces the aerobic scope (blood PO$_2$) to adapt to stress (modified from Peck 1998; McGaw 2005a). **b** The effect of digestion temperature on SDA in poikilotherms. Lower temperatures reduce the peak metabolic cost but extend the duration of the SDA response (modified from Wang et al. 2002)

an organism on a temporal basis by exerting additional demands on physiological systems (McGaw 2006). For the duration of the SDA, response critical tolerances may therefore be lowered and evidently feeding has been observed to increase mortality rate in osmoregulating marine Crustacea (Legeay and Massabuau 2000). The SDA scope can also be compared to the maximum metabolic scope of an organism, in order to estimate the residual energetic capacity during digestion (McCue 2006). If the metabolic scope is already nominal due to the impacts of additional stresses, the digestive processes that determine SDA could be impinged upon, even restricting successful feeding altogether (Peck et al. 2008).

Environmental variables, such as temperature, have little impact on the overall metabolic cost of SDA in poikilotherms (Robertson et al. 2002). However, the duration and momentary size of the SDA response may be affected (Fig. 1b; Whiteley et al. 2001). At low temperatures, the induced reduction in the rate of the SDA response may indeed prove beneficial if metabolic scope under these conditions is also diminished (Bennett et al. 1999). This may be crucial in the deep sea, where temperatures are generally low and stable, and species mostly have a reduced rate of metabolism, relative to corresponding shallow-water species (Childress et al. 1990). The SDA in Crustacea has been measured in response to temperature (Whiteley et al. 2001), but has never actually been quantified for any species under hyperbaric conditions. Thus, to date, whether the role of ambient pressure in defining the SDA response aids or hinders the hypothesized lower metabolic scope of deep-sea adapted species remains unknown.

This study aims to determine the physiological limits of the shallow-water crab *M. brachydactyla* to hydrostatic pressure. By also determining the effects of SDA in response to pressure, it is possible to assess whether the effects of feeding are of importance in defining the capacity of organisms to migrate to depth. Such knowledge is instrumental to all further experimentation that assesses the critical tolerances of aquatic invertebrates to hydrostatic pressure.

Materials and methods

Sampling and maintenance

Male specimens of *M. brachydactyla* were collected using baited trap at depths of 4–12 m from Southampton Water, United Kingdom, in November 2008. Only males with a CL between 75-90 mm were used. Specimens of this size were solely suitable due to the size of the carapace in relation to the heartbeat sensor (Robinson et al. 2009) and to accommodate specimens inside the pressure chamber used in the experimental procedure (IPOCAMP™; Shillito et al. 2001). As maturity occurs at CL over 96 mm, these specimens were not sexually mature (Corgos and Freire 2006).

Individuals were transferred to outdoor flow-through tanks. These tanks were subject to ambient temperature fluctuations, natural light cycles and were supplied with seawater at salinity of 32±1. After a minimum acclimation period of 1 week, specimens were cleaned and individually labelled with numbered tags. Labelled individuals were transferred to tanks in a constant temperature lab at 20±3°C and subjected to diurnal light:dark cycles of equal periodicity (12:12 h). Tanks were stocked with 1-μm filtered seawater, which was aerated continuously and completely replenished on a weekly basis. Specimens were allowed to acclimate under these conditions for a minimum of a week. All organisms were fed twice weekly with squid. Upon the termination of each successful experiment, the tested individual was returned to temperature controlled-conditions for an additional week, to observe for any subsequent deterioration in health. After feeding, specimens were maintained for an additional day to grant a suitable period for the previous meal be completely processed and excreted (Hill 1976). Recorded experiments therefore started 2 days after the previous feeding. Prior to experimentation, the IPOCAMP™ system was filled with 1-μm filtered seawater and the system was allowed to circulate for several hours (see Shillito et al. 2001). All experiments were conducted at 20°C, which slightly

exceeds the 8–17°C optimum temperature range for aerobic performance in this species (Frederich and Pörtner 2000).

Experimental pressure regimes and feeding under pressure

Experiments were used to test the effects of feeding at 1, 100 and 150 atm on *M. brachydactyla*. Specimens were fed squid equivalent of 1% of their wet mass (for description of feeding mechanism see Electronic Supplementary Material) on the second day of each treatment (Fig. 2). Under each pressure condition, three separate repeats were taken. For 1 atm experiments, the pressure was not adjusted over the length of the trail. For 100 and 150 atm experiments, in order to observe the basal heartbeat and respiration rate, pressure was always maintained at 1 atm on the first day until approximately 19:00. Pressure was then increased incrementally by 10 atm every 10 min until the appropriate pressure (100 or 150 atm) had been reached. If feeding was successful on the following day, pressure was maintained at the same level over the second day. On the third day, at approximately 20:00, pressure was reduced back to 1 atm at a similar rate to the pressure increase on the first day. Pressure was maintained at 1 atm until 08:00 on the fourth day when the experiment was ended (Fig. 2). Alternatively, if feeding was unsuccessful on the second day, pressure was decreased shortly after 12:00 on the day that the feeding mechanism was released.

Recording (cardiac) activity

Visual recording of the specimen was achieved using an endoscope and video recording over every light cycle during experimentation. As well as validating the onset of feeding, the timing and duration of any evident active behaviour was recorded. Cardiac activity was used as a measure of the circulatory capacity of the test organism.

The movements of the cardiac muscle were recorded using the photoplethysmographic technique developed by Depledge and Andersen (1990) and subsequently adapted for hyperbaric use (Robinson et al. 2009). The change in current measured by the sensor, reflecting the beating motions of the cardiac muscle, was converted into voltage and passed to the voltage amplifier (Electrode Amplifier; Vernier). The analogue signal is digitalised by the Vernier LabPro system (Vernier) and recorded using the Vernier LoggerPro software (Version 3.4.2; Vernier). The raw signal was recorded at a sampling rate of 1,000 min^{-1}. Cardiac activity was recorded continuously over the duration of the experiment; however, due the large amount of data collected, at 07:00 and 19:00 each day recording was stopped, saved and reset causing a 10–20 min gap in the heartbeat data.

To convert cardiac activity into heartbeat rate, computer analysis was required to identify the characteristic slope of a single heartbeat and this was achieved using Chart (Version 5.5.6; ADinstruments). As the strength and form of the signal depends on the exact placement of the sensor above the cardiac muscle, this varied between specimens. The parameters used to define individual heartbeats from the cardiac signal were therefore redefined when analysing each trial (see Robinson et al. 2009).

The heartbeat rate was then calculated for discrete 5 min samples over the entire length of the experiment. Samples were divided into separate categories, defined by different experimental and behavioural conditions. These categories were:

- Resting during acclimation—all measurements taken at 1 atm on the first day of experimentation. Results from when the crab was in motion are excluded. Refers to acclimation to conditions inside the IPOCAMP™ and not experimental pressures.
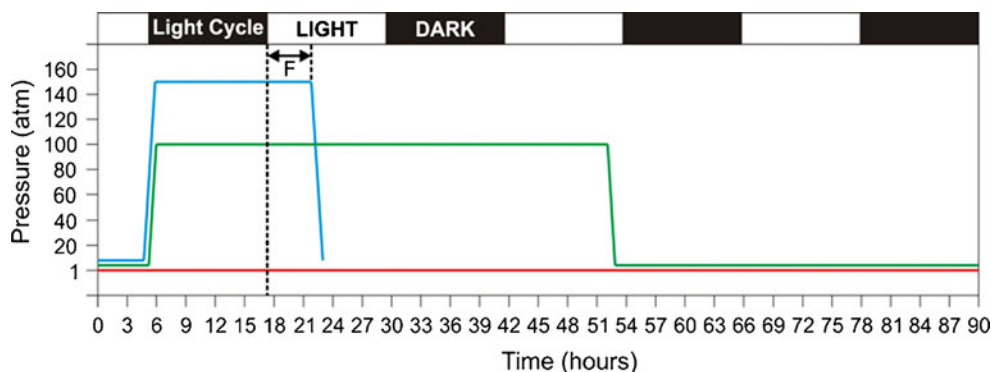

Fig. 2 *Maja brachydactyla*. Schematic overview of the pressure regimes used to compare the effects of 1 (*red/bottom line*), 100 (*green/middle line*) and 150 atm (*blue/top line*) on the feeding response. As feeding does not occur at 150 atm, the experiment runs over a shorter time frame. For clarity, the lines do not overlap, however, before and after pressurisation, all experiments were maintained at a uniform 1 atm. The *first dotted vertical line* represents the timing of food release. Pressure is reduced if feeding does not occur before the *second dotted line*. Incremental steps in pressure change are indicated as a *straight line* due to scale (cf "Materials and methods")

- Postprandial—starting at the 5-min sample that followed the onset of feeding, to exclude the direct locomotive impact of feeding. The postprandial increase in metabolism reflects the energetic requirements of digestion. The postprandial period is defined by elevated heartbeat level, before returning to levels equivalent of the acclimation period (resting during acclimation).
- Resting at experimental pressure—all measurements taken on the third day of experimentation for those where feeding was successful. Pressure is now at experimental level (1, 100 or 150 atm). When feeding is not successful, measurements are taken prior to depressurisation on the second day. Results from when the crab was in motion are excluded.
- Locomotion at experimental pressure—all measurements for when the crab was observed moving on the third day of experimentation. When feeding is not successful, measurements are taken prior to depressurisation on the second day.
- Resting after depressurisation—all measurements at 1 atm taken on the fourth day of experimentation for those where feeding was successful. For 1-atm experiments, no such depressurisation occurs. Results from when the crab was in motion are excluded.

Only measurements during the light portion of the day were used to infer the average heartbeat rate at all the mentioned conditions. The data for each three specimens from each pressure experiment were grouped before means were calculated to determine the average heartbeat rate during each condition. This value is termed the 'collective mean heartbeat rate'.

Oxygen consumption

The depletion of oxygen from the water of the IPO-CAMP™ by the specimens was measured using high precision micro-optode sensors (Fibre-optic oxygen meter Microx TX 3; PreSens; Germany). Oxygen saturation of the water was recorded as it passed out of the IPOCAMP™ into a small reservoir, which drained back into the head tank, measured oxygen depletion in respect to the residence time of the water inside the IPOCAMP™ during flow-through (see Electronic Supplementary Material). On the first and fourth day of sampling a minimum of three separate oxygen measurements were taken. On the second and third day, oxygen measurements began at 07:30, to coincide the beginning of the light cycle, and were repeated every 1.5 h. On both days, this continued for the entire light cycle plus a single measurement during the dark cycle. A two-point calibration at 20°C was undertaken daily using fully aerated seawater (100% O_2sat) and seawater over-

saturated with sodium sulphite (0% O_2sat) as standards. Using a constant value of salinity (S) at 32 and temperature (T) at 293°K, the maximum oxygen solubility was calculated using equation as given by Benson and Krause (1984):

$$
\begin{aligned}
\ln CO_{100\%} = & -135.90205 + 1.575701 \cdot 10^5/T \\
& - 6.642308 \cdot 10^7/T^2 + 1.243800 \cdot 10^{10}/T^3 \\
& - 8.621949 \cdot 10^{11}/T^4 \\
& - S\left(0.017674 - 10.754/T + 2.1407/T^2\right)
\end{aligned}
$$

Where CO is the concentration of oxygen in μmol/l at 100% saturation. Knowing the oxygen saturation of the sample ($DO\%_{sample}$) the actual concentration of oxygen in the sample (CO_{sample}) can then be calculated relative to 100% saturation using the equation:

$$
CO_{sample} = \left(CO \cdot DO\%_{sample}\right)/100
$$

Considering the residence time of water inside the IPOCAMP™ to be 1 h (for validation of method see Electronic Supplementary Material) and using the measured wet mass (g), this oxygen consumption can thus be converted into micromoles oxygen per gram hour. After conversion, the oxygen consumption rates from the first day of experimentation were averaged in order to indicate the basal oxygen consumption at 1 atm. Oxygen measurements on the subsequent 2 days were considered temporally in order to demonstrate the effects of SDA on oxygen consumption. Oxygen consumption measurements from the fourth day of experimentation were averaged.

Results

Feeding success at experimental pressure

At 1 atm, specimens readily fed in all three experiments. At this pressure, the mean time for feeding to be observed after the release of the feeding capsule was 8.6 min (SD±9.07; Fig. 3). At 100 atm, feeding was instead only successful in three of four attempted experiments and when feeding did occur it took longer to instigate (32.67±24.67 min, Fig. 3). However, this difference was not statistically significant (T test: $t=-1.582$, $df=4$; $p=0.189$). At 150 atm, no feeding attempt was observed.

Heartbeat rate

Observations at 1 atm The collective mean heartbeat rate during acclimation was 80.82 bpm (±15.45 SD; Fig. 3). By the onset of the light cycle on the second day and
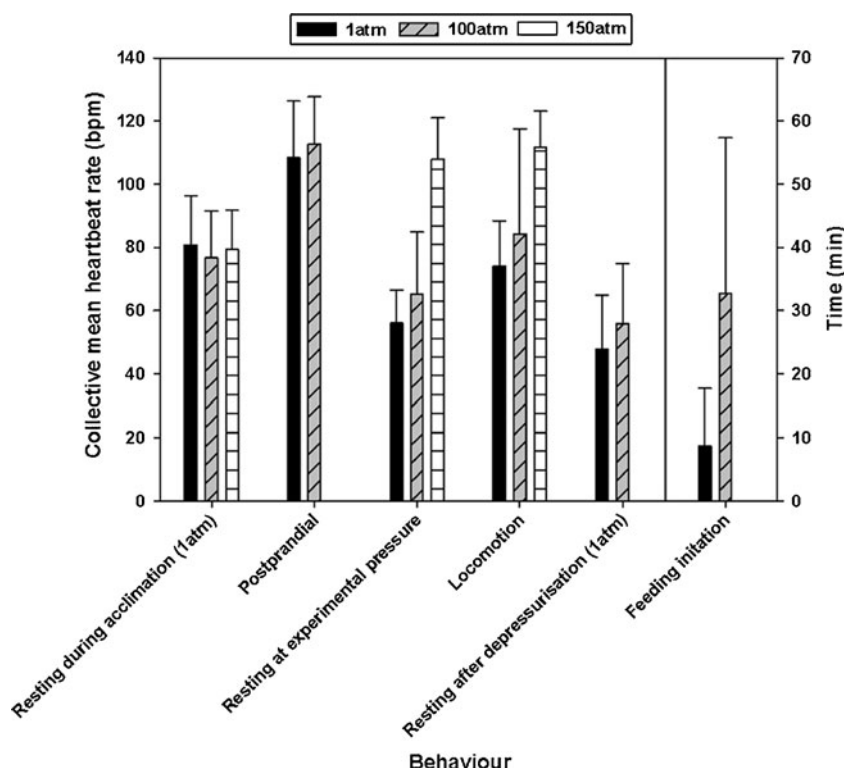
simultaneous with the occurrence of feeding, a distinct peak
in the heartbeat rate was evident in both successfully
recorded specimens. Reaching 108.43 bpm (SD±17.90),
the postprandial increase of 27.61 bpm from acclimation
was statistically significant (Mann–Whitney, $U=144.0$; $n=$
12, 67; $p<0.001$). In both cases, the heartbeat rate remained
elevated for approximately 1 h, before returning to levels
during acclimation (Fig. 4). This decline, lasting for over
60 h, actually continues far beyond acclimation levels and
was matched by a reduction in standard deviation. As a
result, the collective mean heartbeat rate during rest on the
day following feeding was only 56.07 bpm (SD±10.43),
significantly smaller than that observed postprandial
(Mann–Whitney, $U=1.0$; $n=12$, 95; $p<0.001$) or during
acclimation (Mann–Whitney, $U=652.0$; $n=67$, 95; $p<$
0.001). However, intermittently associated with periods of
movement, small peaks in the heartbeat rate do occur
throughout the third day of experimentation. During these
bouts of movement, the collective mean heartbeat rate was
74.02 bpm (SD±14.47), which was significantly higher
than that observed the same day during resting (Mann–
Whitney, $U=410$; $n=25$, 95; $p<0.001$). While the heartbeat
rate during movement does remain significantly lower than
the postprandial heartbeat rate (Mann–Whitney, $U=41$; $n=$
12, 25; $p<0.001$), there was no significant difference
between the heartbeat rate during movement or acclimation
(Mann–Whitney, $U=1039$; $n=25$, 67; $p=0.025$).

The postprandial decline in heartbeat rate continues until
the beginning of the dark cycle on the fourth day of

experimentation and as a consequence the collective mean
heartbeat rate during rest that day was the lowest recorded
from all experiments at any pressure (Fig. 4). At 47.68 bpm
(SD±17.27), it was even significantly different from the
heartbeat rate during rest on the previous day (Mann–
Whitney, $U=4716$; $n=75$, 95; $p<0.001$).

Observations at 100 atm Starting at 1 atm, the collective
mean heartbeat rate during acclimation was 76.66 bpm (SD
±14.98; Fig. 3). When pressure was increased to 100 atm,
two of the three specimens demonstrated a steady and steep
increase in heartbeat rate, which peaked at either 142.67 or
133.33 bpm depending on the specimen (Fig. 5). These
peaks occurred after approximately 4 h, after which a

Fig. 4 Response to feeding in heartbeat rate (*bottom*), oxygen ▶
consumption (*top*) and behaviour (*middle*) in three *Maja brachydactyla* specimens (**a–c**) for 1 atm. Food was released at the beginning of
the light cycle on the second day. Heartbeat rate is indicated by *grey
lines* representing continual 5-min samples. *Black circles* are the
averages of these 5-min samples over a period of 30 min. Oxygen
consumption measurements during the first and fourth day were
averaged from a minimum of three discrete measurements. For the
second and third day, each column represents single measurements
taken at 1.5 h intervals. Behaviour was only assessed during the light
period of the day. On the behaviour bar, periods of movement are
indicated by *grey L* squares, *grey F* squares indicate feeding and *D*
indicates postprandial digestion. White sections represent rest,
indicated by a lack of movement. The uncharacteristically uniform
single in **c** is attributed to a faulty sensor, and data were not considered
in the analysis
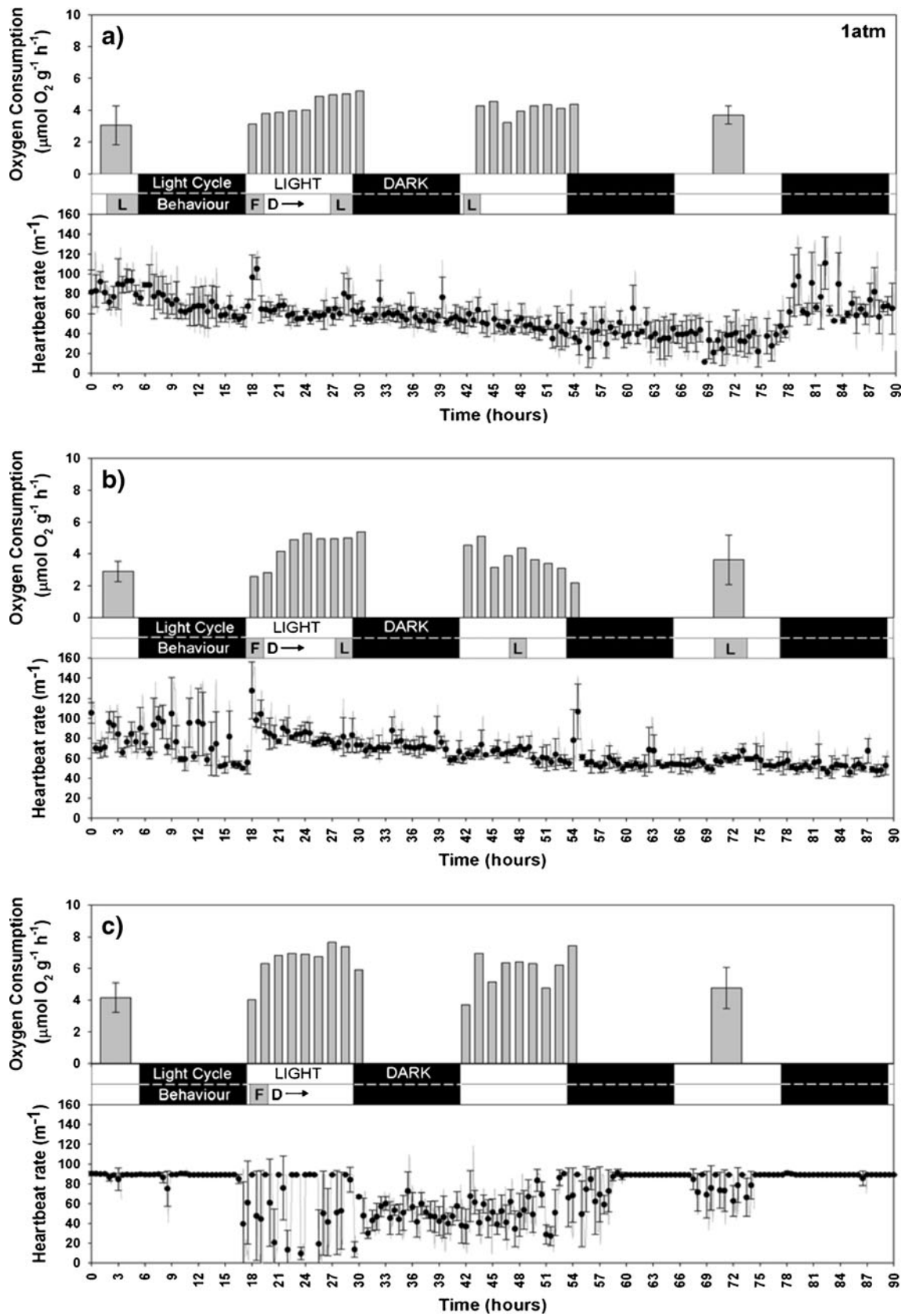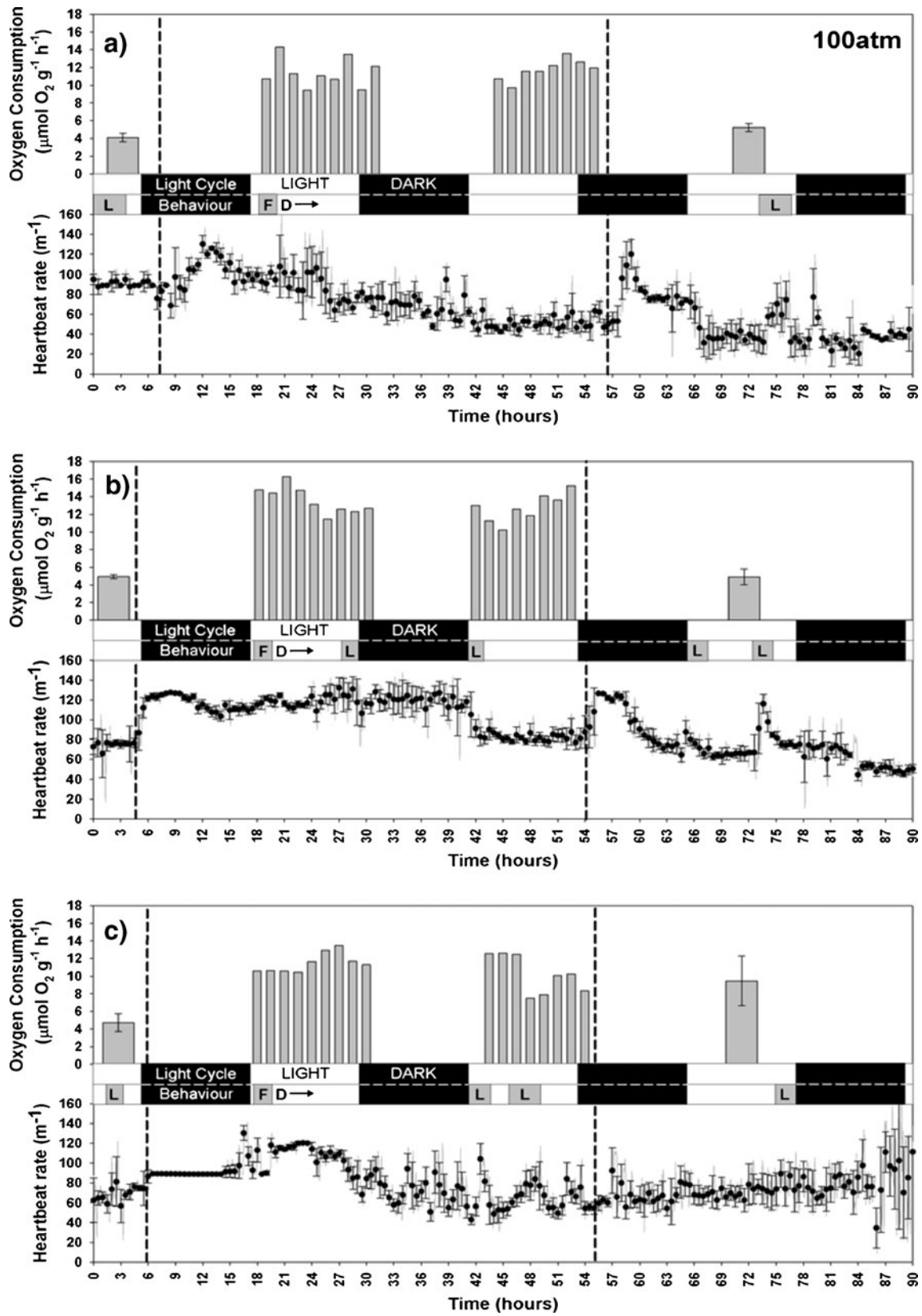
◀ **Fig. 5** Response to feeding in heartbeat rate (*bottom*), oxygen consumption (*top*) and behaviour (*middle*) in three *Maja brachydactyla* specimens (**a**–**c**) for 100 atm. The *left-most black dotted line* indicates when pressure was raised to 100 atm, the *right-most black dotted line* indicates when returned to 1 atm. Food was released at the beginning of the light cycle on the second day. Heartbeat rate is indicated by *grey lines* representing continual 5-min samples. *Black circles* are the averages of these 5 min samples over a period of 30 min. Oxygen consumption measurements during the first and fourth day were averaged from a minimum of three discrete measurements. For the second and third day, each column represents single measurements taken at 1.5 h intervals. Behaviour was only assessed during the light period of the day. On the behaviour bar, periods of movement are indicated by *grey L* squares, *grey F* squares indicate feeding and *D* indicates postprandial digestion. White sections represent rest, indicated by a lack of movement. The uncharacteristically uniform single in **c** (hours 6–14) is attributed to a faulty sensor, and data were not considered in the analysis

decline was observed. In the single remaining specimen, the true cardiac response was hidden by a minor sensor malfunction, which extended over a period of 9 h (Fig. 5c).

At 100 atm, feeding once again prompted an increase in heartbeat rate. This increase was observed 30 min after feeding was instigated and peaked closer to 6 h later. This response was accompanied by a marked reduction in the standard deviation as the peak was approached. The collective postprandial heartbeat rate was still significantly higher, at 112.56 bpm (SD±15.08), than that observed during acclimation (Mann–Whitney, U=197.0, n=12, 105; p<0.001). Over the subsequent 30 h, a general reduction in the heartbeat rate is apparent although individual patterns vary prominently (Fig. 5). The average decrease in heartbeat rate over this time is enough to lower the collective mean on the third day during periods of rest to 65.14 bpm (SD±19.73), which is significantly lower than the postprandial (Mann–Whitney, U=127.0; n=12, 98; p<0.001) or acclimation heartbeat rate (Mann–Whitney, U=7261; n=98, 105; p<0.001). This period of rest is interspersed with bouts of movements, for which the collective mean heartbeat rate increases to 84.19 bpm (SD±33.36). This is significantly higher than the resting heartbeat rate that day (Mann–Whitney, U=1747; n=53, 98; p<0.001) but lower than the postprandial rate (Mann–Whitney, U=592.0; n=12, 53; p<0.001). Furthermore, there is no significant difference between the heartbeat rates when moving or during acclimation (Mann–Whitney, U=282.5; n=53, 105; p=0.877).

In two of the three experiments at 100 atm, a distinct peak in heartbeat rate was observed within 4 h after decompression (Fig. 5a, b). This change only lasted between 5 and 15 h and by the onset of the light cycle on the following day, the collective mean heartbeat rate had already returned to 56 bpm (SD±18.99), which is not significantly different to the observed heartbeat rate under pressure (Mann–Whitney, U=2614.5; n=98, 120; p=0.03).

Only a single specimen did not demonstrate an obvious peak in the heartbeat rate after decompression (Fig. 5c).

Observations at 150 atm The collective mean heartbeat rate during acclimation was 79.45 bpm (SD±12.28; Fig. 3). When pressure was raised to 150 atm a unanimous increase in heartbeat rate was observed, which peaked within 1 h of pressurisation. Over time, a decline from this peak heartbeat rate was only observed in two specimens (Fig. 6a, c). Nonetheless, the heartbeat remained relatively constant after these initial fluctuations, exhibiting minimal variation and approximating at 130 bpm in two specimens (Fig. 6a, b) and 95 bpm in the third specimen (Fig. 6c). Consequently, the collective mean heartbeat rate for rest at 150 atm, at 107.92 bpm (SD±13.18), is far greater than that observed during acclimation (Mann–Whitney, U=256.5; n=15, 11; p<0.001). While pressurised conditions were maintained, the nominal variation in the heartbeat rate exhibited for the duration of the experiment meant that the minor increase in heartbeat rate to 111.53 bpm (SD±11.57) observed during period of movement was not significant (Mann–Whitney, U=360.5; n=32, 45; p=0.030). As feeding did not occur at 150 atm, all three trials were terminated early (Fig. 6).

Comparison between pressure regimes For all experimental procedures at 1, 100 and 150 atm, the collective mean heartbeat rate during acclimation ranged from 76.66 to 80.82 bpm, and there was no statistically significant difference between each pressure regime (Mann–Whitney, 1:100 atm U=3184.0; n=67, 105; p=0.296; 1:150 atm U=3920.0; n=67, 111; p=0.543; 100:150 atm U=6629.0; n=105, 111; p=0.081). The only statistically significant difference between heartbeat rate at 1 and 100 atm experiments was observed during resting after decompression. In this instance, heartbeat rate was lower during 1 atm experiments (Mann–Whitney, U=5574.0; n=75, 120; p<0.005).

Oxygen consumption

Response to feeding at 1 atm The mean oxygen consumptions for each specimen during the light cycle of the first day were similar, only ranging between 2.89–4.15 μmol O_2 g^{-1} h^{-1} with standard deviations from 0.63–1.21 (Fig. 4). Following feeding however, a general increase in oxygen consumption was evident. Only in a single specimen was this increase not consistent for the duration of the second day and began to decline after 8 h (Fig. 4c). In each instance, the final measurement that day, 10.5 h later, is almost double the initial measurement of the day. At this level, oxygen consumption is now beyond the range of the standard deviation for collective mean oxygen consumption during
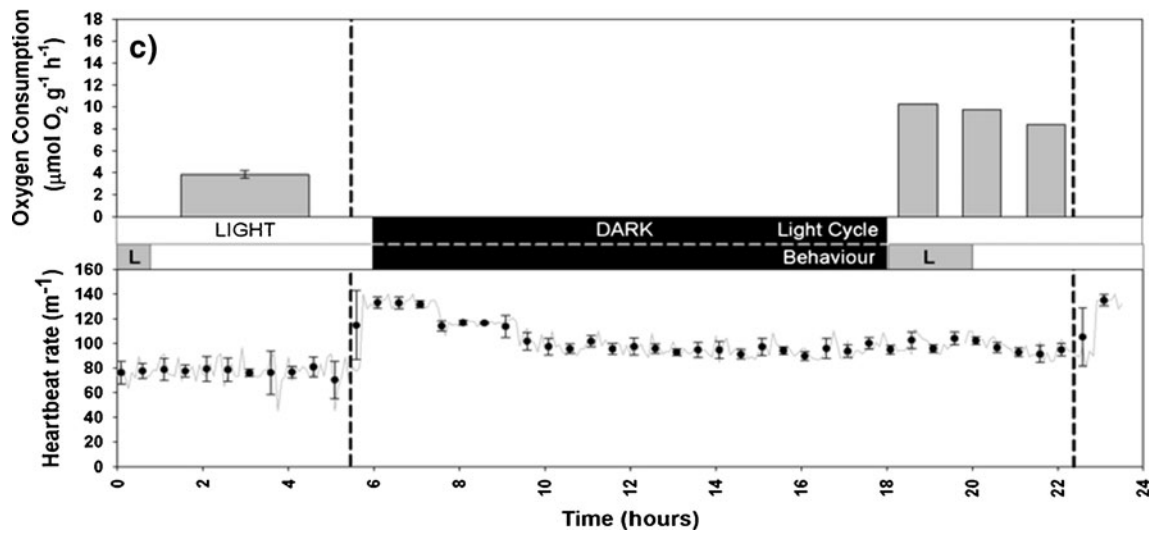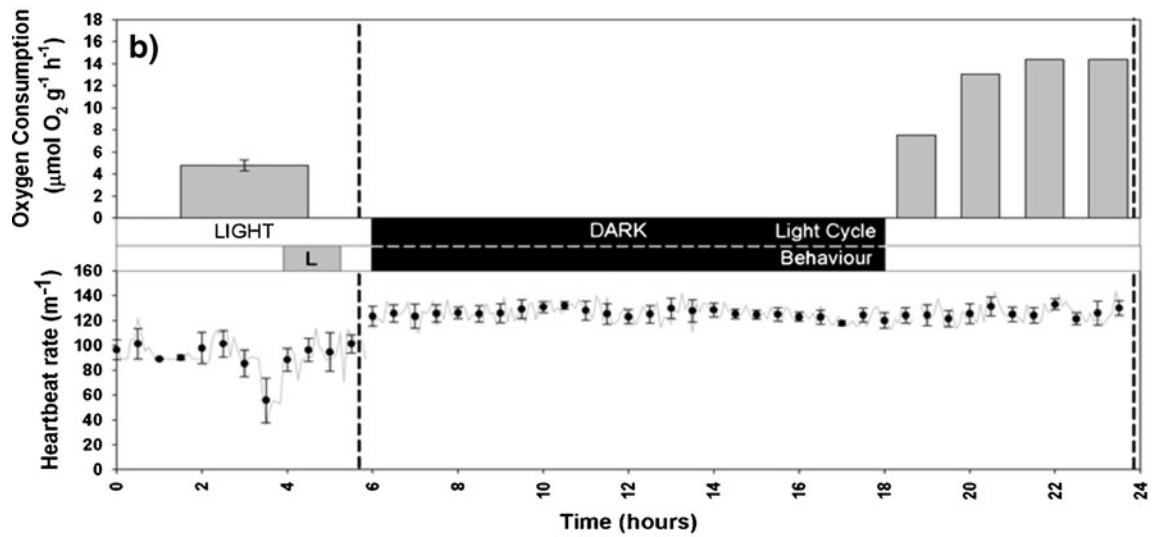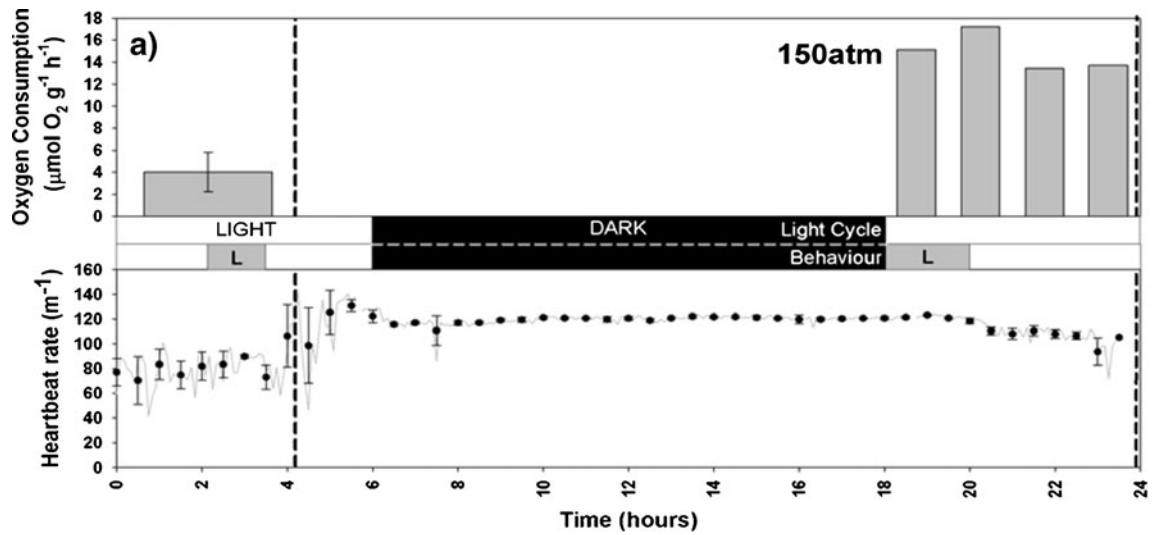
◀ **Fig. 6** Response to feeding in heartbeat rate (*bottom*), oxygen consumption (*top*) and behaviour (*middle*) in three *Maja brachydactyla* specimens (**a**–**c**) for 150 atm. Food was released at the beginning of the light cycle on the second day. Heartbeat rate is indicated by *grey lines* representing continual 5-min samples. *Black circles* are the averages of these 5-min samples over a period of 30 min. Oxygen consumption measurements during the first and fourth day were averaged from a minimum of three discrete measurements. For the second and third day, each column represents single measurements taken at 1.5 h intervals. Behaviour was only assessed during the light period of the day. On the behaviour bar, periods of movement are indicated by *grey L squares*, *grey F* squares indicate feeding and *D* indicates postprandial digestion. *White sections* represent rest, indicated by a lack of movement. Feeding did not occur at 150 atm and thus all experiments were terminated after a period of 24 h

the first day. On the third day relative to the first day, oxygen consumption is still elevated. However, in comparison to the peak oxygen consumption observed on the second day it was reduced. In addition, no uniform pattern is observed between repeats and only a single crab demonstrated a general decrease in oxygen consumption. Mean oxygen consumption between specimens was contained by the fourth day, within the standard deviation of measurements from the first day. No statistical significance is observed between oxygen measurements on these days (*t* test, $t=-0.985$, $df=16$; $p=0.339$). Throughout, no distinct correlation between oxygen consumption measurements and any observation of movement was apparent (Fig. 4).

Response to feeding at 100 atm The mean oxygen consumptions for each specimen during the light cycle of the first day were similar, only ranging between 4.09–4.94 μmol O_2 g^{-1} h^{-1}, with standard deviations from 0.23–1.03 (Fig. 5). After pressurisation to 100 atm, the first oxygen consumption measurement on the second day of the experiment had now amplified by 262.10% to a collective mean of 12.02 μmol O_2 g^{-1} h^{-1}. Although during the light cycle of this day these values fluctuated by up to 5.80 μmol $O_2 g^{-1}$ h^{-1}, no measurement decreased to those observed on the first day at 1 atm. Still at 100 atm on the third day, oxygen consumption measurements remained equivalent to those of the second day. The same as on day two, there was no uniform pattern between specimens with one specimen indicating a decreasing trend (Fig. 5b) while another specimen indicated an increasing trend (Fig. 5c). After decompression on the fourth day, although mean oxygen consumption fell back to an average of 5.21 and 4.90 μmol $O_2 g^{-1}$ h^{-1} for two specimens (Fig. 5a, b), it remained high in a single specimen at 9.46 μmol $O_2 g^{-1}$ h^{-1} (Fig. 5c). Due to this third specimen, there was no statistically significant difference in oxygen consumption between the first and fourth day of experimentation for the specimens collectively (*t* test, $t=-2.184$, $df=16$; $p=0.054$).

Response to feeding at 150 atm The mean oxygen consumption of each specimen during the light cycle of the first

day was similar, ranging between 3.89 and 4.72 μmol O_2 g^{-1} h^{-1} (SD±0.35–1.71; Fig. 6). After pressurisation, the lowest oxygen measurement for any specimen was 7.49 μmol $O_2 g^{-1}$ h^{-1}. No other distinct temporal patterns in oxygen consumption prior to pressurisation were observed.

Comparison between pressure regimes Between all trials during acclimation at 1 atm on the first day of experimentation, no statistical difference in oxygen consumption between each experimental pressure was observed (one-way ANOVA: $F_{2,\ 26}=1.117$; $p=0.334$). Grouping the oxygen consumption measurements for separate experimental pressures (second and third day) demonstrates an evident statistical difference between regimes (Kruskal–Wallis: $H=85.036$, $df=2$; $p<0.001$). Furthermore, comparing 100 and 150 atm directly indicates that this significant difference is not between these two regimes (Mann–Whitney, $U=229.0$; $n=11$, 51; $p=0.347$).

Discussion

Functional limitations in the cardiac muscle

During both rest and activity, heartbeat rate of *M. brachydactyla* was significantly higher at 150 atm than at either 1 or 100 atm (Fig. 3). Two of the specimens used to determine this collective mean heartbeat rate consistently demonstrated heartbeat rates which approached on 130 bpm (Fig. 6a, b). The third specimen exhibited a heartbeat rate closer to 95 bpm, but still showed a similarly unvarying pattern in output (Fig. 6c). Evidently, the cardiac muscle in the later individual was not performing at the maximum rate possible under optimum conditions for this species. However, the lack of variation infers the cardiac muscle was performing at the maximum rate allowable under these conditions, specifically pressures of 150 atm. The heartbeat rate may have therefore passed an Arrhenius break point and was subsequently limited in exertion by the capacity for sufficient oxygen to perfuse into the cardiac muscle (Lannig et al. 2004). Specifically, if *M. brachydactyla* is unable to acclimate to pressures of 150 atm, the detrimental effects of hyperbaric conditions on biological membranes may instigate functional failure in membrane dynamics (Heinemann et al. 1987; Theron and Sébert 2003). The increase in heartbeat rate may therefore be a homeostatic response, to correspond to the increasing energetic demands of routine processes (Avent 1975). Indicatively, by 150 atm, all specimens demonstrated disjointed movements, which are often observed just prior to the complete immobilisation associated with increasing hydrostatic pressures (MacDonald 1972).

Oxygen limitation to performance

Grouped oxygen consumption measurements for *M. bra-chydactyla* were statistically higher at 100 atm than at 1 atm, whilst a direct comparison of 100 atm and 150 atm did not show statistically significant difference. Oxygen consumption has previously been linked to an increase in hydrostatic pressure beyond optimum tolerance ranges, and such changes are associated with an acclimation response and return to basal levels after time (Mickel and Childress 1982). In the present case, elevated oxygen consumption may instead be accredited to the effects of SDA at 100 atm. The lack of statistically significant difference between the oxygen consumption rates at 100 or 150 atm may suggest that digestion at 100 atm is eliciting an equivalent metabolic response to rest at 150 atm. Although no statistically significant increase in heartbeat rate is facilitating this increase in oxygen consumption, a minor elevation in the heartbeat rate for each of the three categorised behaviours (postprandial; resting at experimental pressure; locomotion) at 100 atm relative to 1 atm is apparent (Fig. 3). This may coincide with observations by Frederich and Pörtner (2000) that an elevation in haemolymph circulation often precedes an increase in heartbeat rate.

The lack of additional heartbeat rate increase at 150 atm may suggest a functional limitation of the cardiac muscle, which may occur prior to oxygen limitation. This supports previous work suggesting that mechanical failure in the cardiac muscle is the preliminary factor that restricts tolerance to adverse environmental conditions (Somero 2005). If the resting metabolic rate at 150 atm requires the maximum functional capacity of the cardiac muscle, then it is possible to suggest no further increase in heartbeat rate can occur. Unable to increase further to meet increasing mitochondrial demand, anaerobic respiration may be necessitated. The capacity of the heart to perform is subsequently limited by the available oxygen supply, as well as the likely accumulation of anaerobic by-products (Pörtner 2001). As mitochondrial oxygen demand increases, the effects of oxygen limitation on the cardiac muscle are amplified (Somero 2005). The accumulation of chemical by-products formed during anaerobic respiration is correlated to the length of time that the specimen was under the conditions that caused the stress response (Herreid 1980). The duration for continued survival at this level of metabolism would be limited.

Effects of hydrostatic pressure on SDA

In the supralittoral isopod, *Ligia pallasii*, the immediate excitement of the cardiac muscle was suggested to occur in response to chemosynthetic stimulus of the olfactory organs upon release of the feeding mechanism. The initial increase

in heartbeat rate may also be attributed to the manipulation of food prior to ingestion and mechanical breakdown in the foregut (Carefoot 1990). During these processes, it has been observed in *Cancer magister* that blood flow is diverted to the musculature tissue, instigating an instantaneous increase in heartbeat rate (McGaw 2005a, b). In *M. brachydactyla*, at 1 atm the extent of this increase momentarily reaches 130 bpm, suggesting that the act of feeding is energetically demanding. While comparable trends are apparent in similar species, equivalently high cardiac responses to feeding appear uncommon (Whiteley et al. 2001). For *M. brachydactyla* it may be that the highest respiratory output is purposefully utilised in the process of feeding. As the extent of exertion is often correlated to the necessity of the action, then it may be advantageous for this species to respond to any available food source as quickly as possible.

Subsequent to active feeding, blood flow is diverted to the digestive organs and a peak in heartbeat rate is observed within 1 h under optimal conditions (McGaw 2005b). After this immediate increase, a shallow but consistent decline in heartbeat rate is apparent (McGaw 2006). This steady decline in heartbeat rate over the subsequent 30 h observed in the present study has also been observed in the blue crab *C. sapidus* (McGaw and Reiber 2000). In *C. sapidus*, the decrease in heartbeat rate was also matched by an increase in the stroke volume of the heart. Even though the heartbeat rate was decreasing, flow rate of haemolymph from the cardiac muscle remained consistently elevated for over 24 h (McGaw and Reiber 2000). For *M. brachydactyla*, an increased level of metabolism is also indicated by oxygen consumption rates, which remain elevated for a minimum of 12 h.

The length of the SDA response in crustaceans is correlated with meal size (McCue 2006). In this study, this was relatively small at 1% of the specimens wet mass, while comparative studies predominantly used a meal size of 3% wet body mass (Legeay and Massabuau 2000; McGaw 2006). It is therefore possible that the quantity of provided food plays an important factor in defining the physiological performance of ectotherms under hyperbaric pressure conditions. In addition to meal size, it remains unknown whether specimen size affects the SDA. However, specimen size should not have affected the present study because only specimens of comparable size had been used.

While the postprandial peak in heartbeat rate observed in *M. brachydactyla* occurred within 1 h at 1 atm, at 100 atm, this peak was observed after approximately 4 h. Furthermore, as the initial heartbeat rate prior to this increase was higher at 100 than 1 atm, yet the peak heartbeat rate was comparable, the overall increase in heartbeat rate at 100 atm was smaller. It has been previously observed in poikilotherms that the integrated SDA response incurs a relatively

fixed metabolic cost, which is independent of environmental factors such as temperature (Robertson et al. 2002; Wang et al. 2002). Instead, the duration and peak size of the SDA response may be accordingly affected (Whiteley et al. 2001). It is therefore possible that the energetic demands of digestive processes at higher pressures may prolong the SDA response. As such, the effects of elevated hydrostatic pressure on the duration of SDA may have a similar effect to low temperatures (Brockington and Clarke 2001). Analogous effects of low temperatures and high pressure on physiological processes have been previously observed (Airriess and Childress 1994; Balny et al. 2002). While in the present study no distinct postprandial pattern was observed in the oxygen consumption at 100 atm, it remained statistically higher than that observed at 1 atm. This suggests that under hyperbaric conditions, the metabolic cost of digestion may actually be amplified in effect as well as prolonged. Consistently high oxygen demand during digestion at 100 atm could reduce the aerobic scope to deal with other impacting factors. The effects of feeding and digestion may therefore be increasingly important in defining aerobic scope at elevated pressures (McCue 2006). The lack of successful feeding at 150 atm will ultimately affect continued survival. This supports the necessity for considering both physiological and ecological factors when defining the capacity for an organism to tolerate impacting environmental factors.

Capacity for deep-sea invasion

M. brachydactyla can evidently survive short-term exposure to the pressures that are apparent at depths up to 1,500 m (Fig. 6). Considering the successful feeding events demonstrated in the present work, continuous survival at depths of 1,000 m might be possible (Fig. 5). As such, it is unlikely that tolerance to hydrostatic pressure alone is restricting the movements of this species into deeper waters. Indeed, the range of any marine organism is rarely restricted by a single environmental or ecological factor (Airriess and Childress 1994; Somero 2005). Hydrostatic pressure may therefore work in conjunction with other environmental factors, especially temperature, to instigate a collaborative limit to the distribution of this species. In particular, low temperatures may often reduce tolerance to elevated pressures (Kaye and Baross 2004; Mestre et al. 2009). Thus, the additive effects of lower temperature and higher pressure may restrict the vertical movements of the species. As such, colonisation of deeper waters by this species may be increasingly plausible in an isothermal water column. Such conditions, which predominated during the Mesozoic and Cenozoic, have already been hypothesised to assist past shallow-water faunal exchange with the deep-sea (Young et al. 1997; Thatje et al. 2005b).

Movement to depth during such events could minimise the evolutionary adaptations required to colonise these environments. Once adapted to the deep sea, a tolerance to low temperature could evolve independently as the temperatures of the deep ocean cooled with time (Elderfield and Ganssen 2000).

Conclusion

Marine ectotherms are specifically adapted to particular hydrostatic pressures. The range of this tolerance is potentially attributed to the capacity of biological structures, at all levels of organisation, to withstand hydrostatic pressure and retain optimal functioning. The implications of hyperbaric conditions may therefore extend to physiological processes and SDA in particular may be affected. It is herein hypothesized that the energetic cost of SDA increases with hydrostatic pressure in both size and duration. The metabolic requirements of feeding under hyperbaric conditions may even reach such a critical demand that feeding is entirely inhibited. This emphasizes the importance of holistic studies that consider physiological limits in a variety of routine behaviours to more accurately define an organism's true capacity to withstand or adapt to hyperbaric conditions.

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