The Journal of Experimental Biology 209, 945-955
Published by The Company of Biologists 2006
doi:10.1242/jeb.02102

Temperature resistance studies on the deep-sea vent shrimp *Mirocaris fortunata*

Bruce Shillito[1,*], Nadine Le Bris[2], Stéphane Hourdez[3], Juliette Ravaux[1], Delphine Cottin[1], Jean-Claude Caprais[2], Didier Jollivet[3] and Françoise Gaill[1]

[1]*UMR CNRS 7138 'Systématique, Adaptation et Evolution', Université Pierre et Marie Curie, 7 Quai St-Bernard, Batiment A, 75252 Paris Cedex 05, France,* [2]*Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Centre de Brest, DRO-EP, BP70, 29280 Plouzané, France and* [3]*Station Marine de Roscoff, UPR CNRS 9042, BP74, 29682 Roscoff Cedex, France*

*Author for correspondence (e-mail: Bruce.Shillito@snv.jussieu.fr)

Accepted 17 January 2006

Summary:

The shrimp *Mirocaris fortunata* is a hydrothermal vent species that is found at most vent-sites along the Mid-Atlantic Ridge. This endemic species is found across a hydrothermal gradient, with thermal conditions ranging from 2–9°C in ambient seawater to fairly warm values of about 25°C. We performed *in vivo* experiments on *M. fortunata* specimens originating from different sites and depths (850 m to 2300 m), both at atmospheric pressure and in pressurized aquaria, to characterise the upper thermal limits of this species. Atmospheric pressure results show that thermal physiology should be studied at each population's native pressure. At *in situ* pressure, shrimps from Menez Gwen (850 m depth) and Lucky Strike (1700 m depth) do not survive temperatures of 39°C, and the 'loss of equilibrium' response suggests that their critical thermal maximum (Ct_{max}), is about 36±1°C for both sites. This value is similar to those found for another vent shrimp, *Rimicaris exoculata*, which is thought to be a more temperature-resistant organism, so temperature resistance does not appear to be a crucial factor for explaining differences in distribution of shrimp species in a given vent site. Finally, the data for both vent shrimps are also comparable to those of other non-vent tropical caridean species.

Key words: hydrothermal vent, thermal stress, *Mirocaris fortunata*, Crustacea, IPOCAMP™.

Introduction

More than 25 years after their discovery (Lonsdale, 1977), there is still on-going discussion regarding the temperature limits that may be tolerated by deep-sea hydrothermal vent organisms (Fisher, 1998; Chevaldonné et al., 2000; Van Dover, 2004). Firstly, this is an environment where hot fluids issue substrates at temperatures ranging from less than 10°C to more than 350°C, which does indeed suggest that its endemic fauna may be highly thermophilic. Furthermore, on the basis of *in situ* observations and measurements, some vent metazoans, namely alvinellid polychaetes, have been reported to thrive in sustained temperatures of 60°C or more (Chevaldonné et al., 1992; Cary et al., 1999; Di Meo-Savoie et al., 2004), whereas biochemical data on the same species indicated much lower maximum habitat temperatures, below 50°C (Gaill et al., 1995) or below 35–40°C (Dahloff and Somero, 1991). Recently, other alvinellid species were reported to survive a 2 h exposure at 45°C in pressurised aquaria (Lee, 2004). On the other hand, *in vivo* experiments at native pressure demonstrated that at least some vent organisms, which occupy microhabitats such as those where the above-cited (Chevaldonné et al., 1992; Cary et al., 1999) thermophilic alvinellids live, could nevertheless do so without apparent temperature resistance capacities greater than 40°C (Shillito et al., 2001). Many of these apparent discrepancies result from difficulties in probing temperature accurately *in situ*, in the immediate surroundings of a given vent organism (Le Bris et al., 2005). Furthermore, direct tolerance tests *in vivo* on deep-sea creatures are difficult to achieve without using fairly heavy pressure-equipment; experimental aspects of deep-sea vent biology have been reviewed recently (Van Dover and Lutz, 2004).

Nevertheless, in addition to the obvious interest in finding exceptionally thermophilic metazoans, studying the thermal biology of vent creatures also allows testing of the influence of temperature on the distribution of species across a hydrothermal fluid gradient. At hydrothermal vent sites of the Juan de Fuca ridge, a correlation between lethal temperatures of different species and their distribution along the temperature gradient has been observed (Lee, 2004), although other studies on the same assemblages strongly suggest the potential importance of other factors, such as oxygen or food availability (Sarrazin et al., 1999).

All the above-cited species are found at Pacific Ocean

hydrothermal vents (East Pacific Rise, or Juan de Fuca Ridge). The Mid-Atlantic Ridge (MAR) vents also host highly specialized endemic fauna. Caridean shrimps dominate the vagile (mobile) megafauna at most MAR hydrothermal vent sites (Desbruyères et al., 2001). One of them, *Rimicaris exoculata* (Williams and Rona, 1986) is particularly abundant, forming dense swarms (>3000 individuals m^{-2}) around the chimneys expelling superheated sulfide-loaded fluid (Segonzac, 1993; Polz et al., 1998; Gebruk et al., 2000). Temperatures nearing 40°C have been reported within swarms of shrimps, and up to 70°C only a few centimeters from the swarms on the chimney-wall (Gebruck et al., 1993; Segonzac et al., 1993). Recently, we performed *in vivo* experiments in pressurized aquaria to determine the upper thermal limit (critical thermal maximum Ct_{max}) of *R. exoculata*. We demonstrated that the shrimp does not tolerate sustained exposure to temperatures in the 33–37°C range (proposed Ct_{max}), and suggested that their optimal temperature would probably lie below 25°C (Ravaux et al., 2003).

In the present study, we investigated the temperature resistance of another MAR caridean vent shrimp, *Mirocaris fortunata* (Martin and Christiansen, 1995; Komai and Segonzac, 2003), which is found from depths of 850 m to more than 3000 m, and which co-occurs with *R. exoculata* at several sites on the MAR. Although closely related to *R. exoculata*, *M. fortunata* nevertheless has a distinct lifestyle: it is most common within mussel beds, where it probably scavenges upon diverse sources (Gebruk et al., 2000). It is found across the vent gradient, from water of almost ambient temperature, to the point where *R. exoculata* swarms predominate (Desbruyères et al., 2001). The objectives of our work were multiple: (1) to evaluate first the possibility of *in vivo* experimentation with a deep-sea shrimp such as *M. fortunata*; (2) to test *in vivo* the temperature resistance of another vent organism, as only seven species have so far been studied that way (Mickel and Childress, 1982; Shillito et al., 2001; Lee, 2004), and only one of these was at the MAR (Ravaux et al., 2003); (3) to investigate whether or not temperature resistance varies with site of origin, particularly sites at different depths; (4) to see if the different distributions of two vent shrimps within a vent site (*M. fortunata*, across the vent gradient, and *R. exoculata*, swarming near the hot fluid sources) could be related to their temperature resistance.

Materials and methods

Specimen collection

Mirocaris fortunata Martin and Christiansen 1995 specimens were collected during two cruises: '*MARVEL*' (R/V *Atalante*, Nautile submersible, August 1997, for respirometry experiments at atmospheric pressure), and '*ATOS*' (R/V *Atalante*, ROV Victor6000 submersible, June 2001, for all other experiments presented in this work), along the Mid-Atlantic Ridge, at the Menez Gwen (37°50.6′N, 850 m depth), Lucky Strike (37°17.6′N, 1700 m depth) and Rainbow (36°14.0′N, 2300 m) vent sites. Animals were sampled with a suction device operated using the submersible's hydraulic arm, and stored inside insulated Perspex cylinders, until transferred to the ship. Upon recovery, the temperature of the water inside the cylinders ranged from 10 to 20°C. Most shrimps survived the trauma of collection, and the individuals that swam apparently normally were sorted for *in vivo* experiments. Further examination took place under binocular magnifying lenses, in order to select only *M. fortunata* specimens (2–4 cm length), which may be easily confused with small-sized *Chorocaris chacei*, another vent endemic shrimp species (Williams and Rona, 1986; Martin and Hessler, 1990). Our selection criterion was the presence (*M. fortunata*) or absence (*C. chacei*) of a sharp post-orbital prominence on the cephalothorax.

Oxygen-consumption measurements at 10°C, at different pressures, for shrimps collected at 1700 m depth (Lucky Strike)

At atmospheric pressure (MARVEL cruise)

Twelve shrimps were individually placed in polyethylene containers filled with surface seawater (16.5 ml volume), at atmospheric pressure. Another seawater container containing no shrimps was used as a control. Oxygen levels were determined after 1 h, using the Winkler method (s.d., 2%; 95% C.I. for N=1, ±4%) (Aminot and Chaussepied, 1983). The shrimps were further weighed fresh (to 0.1 mg precision), then dried at 60°C (for 48 h, then until constant mass was reached) and weighed again (to 0.1 mg precision), in order to obtain a fresh/dry mass correlation. The volume of the shrimp was ignored in our calculations of oxygen consumption (a 3 cm shrimp represents <1 ml volume).

At in situ pressure (ATOS cruise)

Ten shrimps were placed in polyethylene containers (210 ml vol.) filled with seawater, at *in situ* pressure (17 MPa), using the pressurized incubator IPOCAMP™ (see below). Another container without shrimps was also pressurized for use as a control. Oxygen levels were determined after 7 h, using a Clark-type micro-electrode (Unisense, Aarhus, Denmark) with an estimated precision of ±3%. These measurements were calibrated with air-equilibrated surface seawater (100%O_2) and surface seawater de-oxygenated by addition of sodium sulfite (0%O_2). The 100%O_2 solution was standardised using the Winkler method. The shrimps were dried at 80°C on board (48 h), and then later further dried at the laboratory at 80°C (to constant mass) and weighed (to 0.1 mg precision).

In both experiments, the O_2 uptake rates were checked against the control to preclude possible uptake from bacteria in the seawater. No measurable oxygen consumption was registered in the controls. For all 22 individuals, care was taken to check that the final oxygen concentration in the containers did not drop below 50% of the initial concentration. Final oxygen content was thus most likely above the oxygen level

below which shrimp O_2 consumption may decline rapidly (Prosser, 1973).

Survival at different temperatures, at atmospheric pressure, for shrimps collected at different depths

Groups of shrimps from the Menez Gwen (850 m depth) and Rainbow (2300 m depth) sites were maintained in 50-liter tanks at atmospheric pressure, first for 24 h at 5°C, later at different temperatures: 10°C (±1.5°C maximum deviation, m.d.), 16°C (±2°C m.d.) and 21°C (±1.5°C m.d.) (respectively 73, 68, 67 individuals for the Rainbow sampling, and 48 individuals at each temperature for Menez Gwen sampling). An additional experiment was carried out at 25°C (±1.5°C m.d.) for Menez Gwen samples (16 individuals). Constant oxygenation of the water was maintained by air bubbling and water circulation pumps. Oxygen and nitrite levels were periodically checked. Mortality was checked visually and by mechanical stimulation at different times throughout the experiments, dead shrimps being removed from the tanks. The experiments were interrupted after survival had reached less than 50%, or as a result of practical ship-time constraints.

Determination of Ct_{max} at in situ pressure, for shrimps collected at different depths

Pressurized incubator IPOCAMP™

The stainless steel pressure chamber (PV) has a volume of ca. 19 l, as previously described (Shillito et al., 2001). The general design of the pressure circuit was inspired by flow-through pressure systems utilized by Childress (Quetin and Childress, 1980), with flow rates that may exceed 20 l h^{-1} at 32 MPa maximum working pressure. Pressure oscillations due to pump strokes (100 r.p.m.) are <0.1 MPa, at working pressure. The temperature of the flowing seawater (filtered at 0.4 μm) is measured constantly, under pressure, in the inlet and outlet lines (±1°C). A more accurate temperature measurement (±0.1°C) is achieved inside the pressure vessel, through two Pt-100 probes positioned immediately 'upstream' and 'downstream' of the experimental cages (described below). Temperature regulation is powered by a regulation unit (Huber CC 240, Offenburg, Germany), which circulates ethylene glycol through steel jackets that surround the PV, and around the seawater inlet line. Finally, IPOCAMP™ allows video observations of the re-pressurized organisms through three separate view-ports, each offering a vertical descending view of the experimental cages. Each cage is a PVC cylinder of diameter 5 cm, topped by an inclined translucent lid, resulting in a height of 5–7 cm (for a schematic representation, see Ravaux et al., 2003). An endoscope (Fort, Dourdan, France) combined to a CCD camera (JVC, TK-C1380) is inserted in a given vertical view-port. The resulting view of the inside of the pressure vessel is then displayed on a TV monitor (JVC), and recorded (Sony SVO-9500 MDP videotape recorder).

Experiments for behavioural responses at in situ pressure

Specimens were placed in cages inside the pressure vessel at an initial seawater temperature of 10°C. Re-pressurization at 8.5 or 17 MPa (pressures occurring at the Menez Gwen and Lucky Strike sites, respectively) was achieved in less than 2 min. In all experiments, less than 2 h intervened between the time that the samples began decompression (ascent of the submersible) and the moment they were re-pressurized.

Four experiments were performed, using a total of 85 shrimps: two reference experiments, one of which involved two shrimp species, *M. fortunata* and *C. chacei*, and two heating experiments. The aim of these experiments was to evaluate the survival and behaviour of *M. fortunata*, either at constant 10°C temperature or throughout a lethal heat shock.

(1) Preliminary reference experiment, Lucky Strike samples. *M. fortunata* (N=14) and *C. chacei* (another vent shrimp species) (N=11) were placed in three cages of different sizes, and maintained during a 24 h period, at 10°C. For this preliminary experiment, only two shrimps remained motionless at the end of the experiment, which was a minimum 86% survival rate for *M. fortunata*. This experiment also allowed us to optimize experimental conditions (flow rates, size of cage, number of individuals per cage, video observation conditions, etc.).

(2) Reference experiment, Lucky Strike samples. 20 shrimps were maintained during a period of 20 h 45 min, at 10°C. This experiment was performed along with the respirometry experiment, involving a decompression/recompression event 7 h after the start, in order to allow retrieval of samples.

(3) Lethal heat shock, Lucky Strike samples. 20 shrimps, after 5 h at 10°C, were exposed to increasing temperatures, as previously described for the vent shrimp *Rimicaris exoculata* (Ravaux et al., 2003), until the temperature reached 40°C, followed by cooling to 10°C. The total time of experiment was 22 h. Maximum heating/cooling rates were 0.53°C min^{-1} and –0.47°C min^{-1}, respectively.

(4) Lethal heat shock, Menez Gwen samples. 20 shrimps were heat-exposed as described above. The total time of experiment was 20 h. Maximum heating/cooling rates were 0.52°C min^{-1} and –0.45°C min^{-1}, respectively.

Video analysis of behavioural responses at in situ pressure

For the four experiments described above, survival of the re-pressurized shrimps was determined in the final minutes of the experiments by identifying each individual and recording its movements. Survival could also be confirmed at atmospheric pressure after the experiments.

For video recording during the experiments, the endoscope was moved successively from the first to the third cage (3 min for each cage) at least once every hour at 10°C, and then was continuously rotated during the heat shock (each cage was then observed for 3 min before moving to the next one). The resulting behavioural data for 20 shrimps were pooled from the last 30 s in the first cage, the middle 30 s in the second cage, and the first 30 s in the third cage. Within each period of observation, the shrimps were individually classified into one of three exclusive categories (see also Table 1): C1 (motionless), C2 (moving) and C3 (active walking or swimming). A fourth behavioural character, C4 (loss of equilibrium) was also quantified, but was

Table 1. *Behavioural categories C1–C4, during periods of maintenance of M. fortunata shrimps from Lucky Strike or Menez Gwen at in situ pressure (17 MPa or 8.5 MPa) and 10°C*

	Behaviour*			
Number of individuals	C1	C2	C3	C4
Mean ± s.d.	4.3±2.2	10.3±2.7	5.4±2.8	0.5±0.9
Max.	8	16	12	4
Min.	1	5	1	0

Values are for 20 individuals, 27 observations.

The maintenance periods are from exp. 5, and the first 5 h of exps 6 and 7 (Figs 4, 5).

*Behavioural categories were defined as follows:

C1, 'Motionless'; no movement detected at normal tape-reading speed; this category was also designated when an individual's movement seemed to be the result of neighbouring shrimp 'pushing', with no apparent reaction of the individual.

C2, 'Moving'; any kind of detectable movement, at normal tape-reading speed, except that of category 3 (below): pereiopod or pleopod movements, scaphognathite beating, antennal lateral sweeping on dorsal side, cleaning of the mouth parts by rubbing them along each other.

C3, 'Active walking or swimming'; when the shrimps moved along a distance exceeding their own length in less than 30 s.

C4, 'Loss of equilibrium' (non-exclusive); when a given shrimp rested on the bottom either in an 'upside-down' or 'sideways' position for more than 2 s.

not exclusive, therefore it could co-occur with any of the main three categories.

For each observation of 20 individuals during heating periods (successive sequences in the three cages), we determined the corresponding temperature error. We ignored any error of the probes themselves (±0.1°C). For a given point, the minimum possible temperature was considered to be the lowest recorded by the two probes (at the top and bottom of the cage), at the time of the first of the three sequences. In the same way, the maximum temperature was considered to be the highest recorded by the two probes, at the time of the last of the three sequences. One point is actually of 4 min duration (i.e. from the last 30 s of the first sequence, through the middle 3 min of the second, to the first 30 s of the third sequence). The resulting errors range from ±0.5°C (when reaching 40°C) to ±2.7°C (within the 15–25°C range). Such errors are rounded off to the upper half-unit throughout the text and in figures (when shown).

Results

Oxygen-level measurements at 10°C of shrimps collected at 1700 m depth

At atmospheric pressure

For shrimps originating from the Lucky Strike site, oxygen uptake rates followed a relation of the type $\dot{M}_{O_2}=0.699M_b^{0.941}$, r=0.877, N=12, P<0.005), where $\dot{M}_{O_2}$ is rate of oxygen uptake (μg O_2 h^{-1}) and M_b is body mass (mg), with dry mass (DW) ranging from 44 to 152 mg (Fig. 1). Mass-specific rates ranged from 0.338 to 0.791 mg O_2 h^{-1} g^{-1} DW (mean ± s.d.= 0.548±0.115, N=12). Expressed in terms of fresh mass (FW), and in units convenient for further comparison, these mass-specific rates ranged from 0.072 to 0.140 μl O_2 h^{-1} mg^{-1} FW (mean ± s.d.=0.099±0.019, N=12). In order to compare these data with those of the other respirometry experiments (at *in situ* pressure), only shrimps in the mass range 40–120 mg DW (N=9, see Fig. 1) were selected. In that case the mass-specific rates still ranged from 0.072 to 0.140 μl O_2 h^{-1} mg^{-1} FW, but the mean ± s.d. was 0.101±0.022 (N=9). The FW–DW relationship was: FW=3.838DW+3.099, where FW and DW are in mg (N=12, r=0.980, P<0.005).

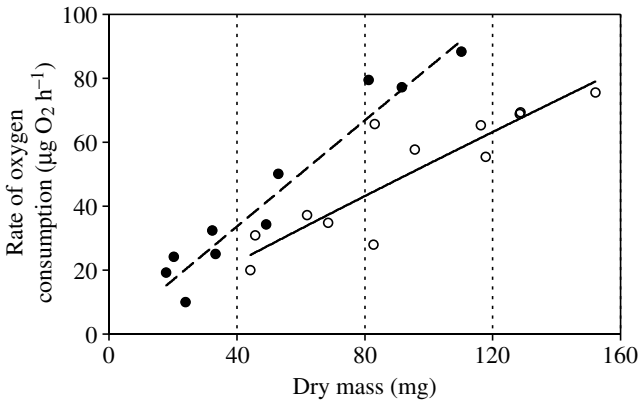

Fig. 1. Rate of oxygen consumption (μg O_2 h^{-1}) as a function of dry mass (mg), of *Mirocaris fortunata* individuals originating from the Lucky Strike vent site (1700 m depth), at 10°C. Open circles, solid line: atmospheric pressure experiment (exp. 0, 12 individuals). Closed circles, broken line: *in situ* pressure experiment (exp. 4, 17 MPa, 10 individuals). For correlation coefficients and P values, see text.

At in situ pressure

For shrimps originating from the Lucky Strike site, oxygen uptake rates followed a relation of the type $\dot{M}_{O_2}=0.870M_b^{0.991}$, r=0.907, N=10, P<0.005, with DW ranging from 18 to 110 mg (Fig. 1). Mass-specific rates ranged from 0.416 to 1.191 mg O_2 h^{-1} g^{-1} DW (mean ± s.d.=0.870±0.220). Using the FW–DW relationship established for the experiment at atmospheric pressure (only dry masses were available here), the mass-specific rates ranged from 0.074 to 0.211 μl O_2 h^{-1} mg^{-1} FW (mean ± s.d.=0.157±0.039, N=10). For comparative purposes, shrimps in the 40–120 mg (DW) range displayed rates from 0.126 to 0.179 μl O_2 h^{-1} mg^{-1} FW (mean ± s.d.=0.155±0.021, N=5). Inspection at atmospheric pressure just after decompression and oxygen level measurements revealed that all 10 shrimps were still alive.

Survival at atmospheric pressure and at different temperatures of shrimps collected at different depths

Shrimps originating from the deepest site, Rainbow (2300 m depth), and maintained at atmospheric pressure, reached 50% mortality after 14 h at 21°C, 20 h at 16°C and approximately

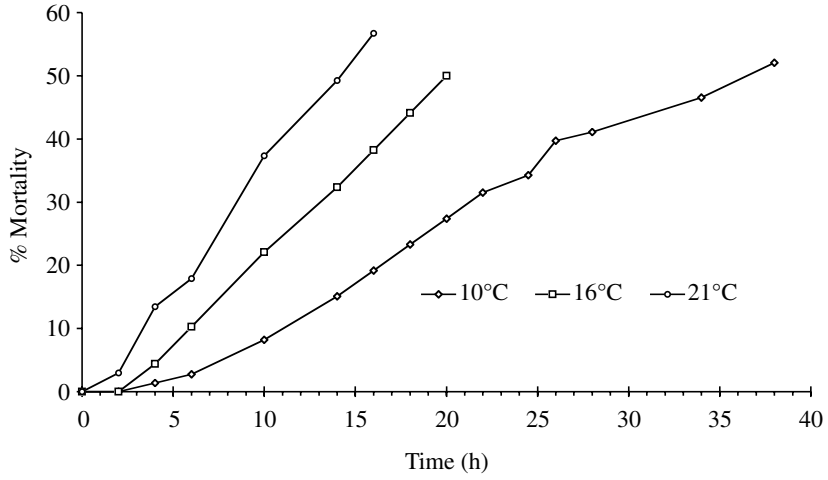

Fig. 2. Mortality (% of the initial pool) of *Mirocaris fortunata* individuals originating from the Rainbow site (2300 m depth) as a function of time, during maintenance at atmospheric pressure, at three different temperatures: 10°C, 16°C and 21°C (73, 68, 67 individuals, respectively).

36 h at 10°C (Fig. 2). In contrast, shrimps from the shallowest site, Menez Gwen (850 m depth), had still not been reached 50% mortality levels after 9 days: at atmospheric pressure, at 10, 16 and 21°C, mortalities were about 30–35% (Fig. 3), with no apparent pattern according to temperature. After the first 4 days, mortalities were still <10%, so another experiment was then initiated at a higher test temperature, 25°C. Due to ship-time constraints, this last experiment did not exceed 6 days. At that time, mortality was slightly less than 20%, in contrast to 10–15% at the other test temperatures (Fig. 3).

Determination of Ct_{max} at in situ pressure, for shrimps collected at different depths

Survival and behaviour at 10°C

At 10°C and 17 MPa pressure, survival was still 100% after 10 h, with all individuals moving during a given 3 min video sequence. This was 3 h after decompression and recompression in order to retrieve individuals selected for respirometry (see Materials and methods). In the last 15 min of the experiment, a video survey of the cages showed 70% survival (judged by movement). Inspection at atmospheric pressure just after decompression revealed that 13 shrimps (65%) were still alive and very reactive, while the others (35%) did not respond to mechanical stimulation, therefore appearing as dead, or at best moribund. Table 1 shows the distribution of behavioural categories amongst 20 shrimps at 10°C, averaged over 27 observations (also including the first 5 h of heat shock experiments, before temperature increase).

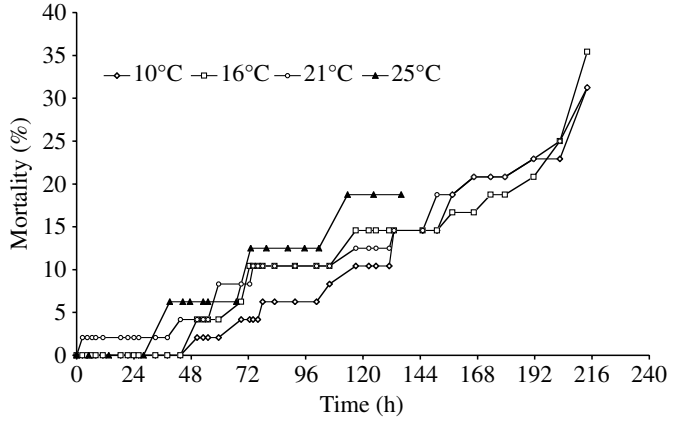

Fig. 3. Mortality (% of initial pool) of *Mirocaris fortunata* individuals originating from the Menez Gwen site (850 m depth) as a function of time, during maintenance at atmospheric pressure, at four different temperatures: 10°C, 16°C, 21°C and 25°C (48 individuals at each of the first three temperatures; 16 individuals for the 25°C experiment).

Lethal heat shocks

Both experiments involved a temperature rise reaching almost 40°C (Figs 4, 5), through which none of the shrimps survived. From the time temperature had reached 40°C, and until the experiments were stopped more than 13 h later, all the shrimps were in the same position, lying on their side or back on the bottom of the cages, in a *post mortem* curved body-shape position. The temperature was 39°C (±1°C and ±0.5°C for Menez Gwen and Lucky Strike experiments, respectively) when the shrimps were last observed moving, in both experiments. At this temperature, the number of 'motionless' individuals (17 and 14 for MG and LS experiments, respectively) was well above the maximum observed in reference experiments (8; see Table 1). In order to simplify the presentation of data, the 'motionless' category is not directly represented in Figs 4 and 5, but may be inferred from the representation of 'total movement' (total movement= C2+C3=20–C1).

For the heat shock involving Lucky Strike individuals (Fig. 4), the 'motionless' population reached a peak shortly after the beginning of heating (13°C; 8 individuals), and further decreased until there was only one 'motionless' individual left when the temperature reached 29±1°C. Both numbers correspond to the boundaries of the range observed during 10°C maintenance periods (1–8 individuals; Table 1). From 29±1°C to 39±0.5°C, the number of motionless individuals sharply increased to 17 individuals (when shrimps were last seen moving), a value that was clearly out of the range observed at 10°C (Table 1). Regarding 'active moving', a peak in movement activity (8 individuals) seemed to occur when the temperature reached 29±1°C, and was maintained until 36.5±1°C, although behaviour category numbers remained within the ranges observed at 10°C (i.e. below 12 individuals 'actively moving'; see Table 1). Between 36.5±1°C and 39±0.5°C, 'active movement' disappeared.

For the heat shock involving Menez Gwen individuals (Fig. 5), the 'motionless' population reached a peak (13 individuals) shortly after the beginning of heating (13°C

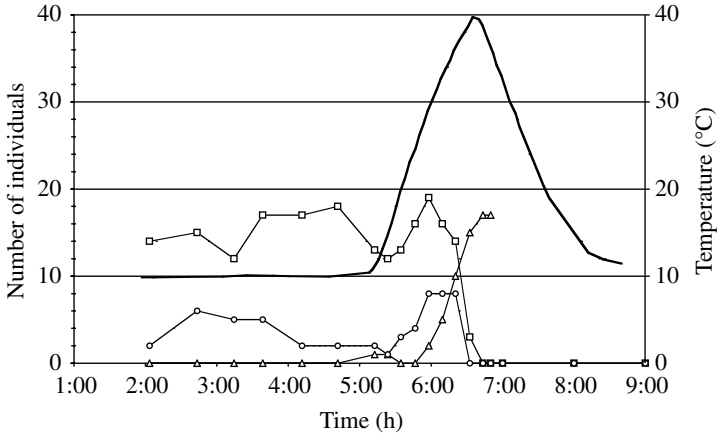

Fig. 4. Distribution of behavioural categories throughout a lethal heat shock applied to Lucky Strike *M. fortunata* shrimps (20 individuals), at *in situ* pressure (17 MPa, exp. 6). Squares, total movement, i.e. C2+C3=20–C1; circles, moving more than body length, i.e. C3; triangles, loss of equilibrium, i.e. C4; solid bold line, temperature (°C). For an explanation of the behavioural categories, see Table 1.

temperature). This peak clearly exceeds the maximum population (8 individuals; Table 1) observed during 10°C maintenance periods. The number of 'motionless' shrimps further decreased until all individuals were moving, when the temperature reached 30±1.5°C. Regarding 'active moving', a peak in movement activity occurred at about 30±1.5°C, with a maximum (9 individuals) at 33±1°C. At 36.5±1°C, 'active movement' decreased (5 individuals), and had disappeared when the temperature reached 39±1°C.

During heating, although active behaviour qualitatively appeared to increase for both experiments, it was difficult to quantify any type of spasmodic behaviour that would suggest loss of locomotory coordination, such as flicking of abdomen with no resulting displacement. However, beyond 30°C, shrimps were often seen lying on their side or back (C4 category, Fig. 6), at the bottom of the cage, although still alive, as indicated by movements after such a 'pause'. This position was rarely observed at 10°C (see Table 1): after a resting period along the vertical walls of the cage, the shrimps swam towards, or passively landed, on the cage bottom, but were nearly always in a natural 'upright' position upon reaching the bottom. Occasionally, a shrimp would contact the bottom in a 'sideways' position, but would instantaneously right itself up upon contact. In the case of heat exposure experiments, the number of shrimps losing their balance exceeded 50% (10 individuals) upon reaching 36.5°C. Comparison with data in Table 1 shows that this response is clearly linked to the temperature increase. Finally, violent backward displacements, due to single rapid movement of the abdomen, were occasionally observed for some individuals between 30°C and 36°C. Such movements had also been observed in reference experiments, but only shortly after pressurization, or during decompression events.

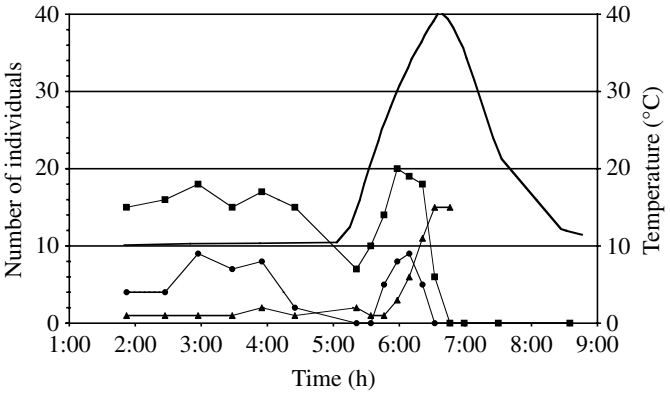

Fig. 5. Distribution of behavioural categories throughout a lethal heat shock applied to Menez Gwen *M. fortunata* shrimps (20 individuals), at *in situ* pressure (8.5 MPa, exp. 7). Squares, total movement, i.e. C2+C3=20–C1; circles, moving more than body length, i.e. C3; triangles, loss of equilibrium, i.e. C4; solid bold line: temperature (°C). For an explanation of the behavioural categories, see Table 1.

Discussion

The following discussion focuses first on the conditions of maintenance (pressure and temperature) of the deep-sea shrimp *Mirocaris fortunata* on board an oceanographic ship. Then the results of our heat-exposure experiments are discussed in terms of the thermal biology of this hydrothermal vent creature.

Conditions of maintenance

Choice of 10°C as a reference temperature

Before discussing the thermal biology of *M. fortunata*, it is important to consider the choice of a 10°C reference temperature, which is thought to be within the preferred thermal range of the studied species. *M. fortunata* is believed to scavenge from diverse sources, and is fairly widely distributed throughout the hydrothermal vent habitat; although it is often observed within mussel (*Bathymodiolus azoricus*) beds, it has been observed at the periphery of vent communities, but also forming aggregations next to hydrothermal flange pools at the base of active chimneys. Desbruyères and collaborators (Desbruyères et al., 2001) reported temperature measurements among microhabitats where *M. fortunata* was present: at Rainbow, discrete measurements (N=5), indicated temperatures of 11.2±4°C. At Lucky Strike, the same type of probing yielded values of 6.8±1.3°C (N=11), 9.5±3.4°C (N=6) and 13.9±0.5°C (N=6). Furthermore, 5-day time series using autonomous probes recorded values of 8.2±1.7°C, 11.4±2.6°C and 14.4±3.0°C, with a maximum temperature of 24.6°C, and a minimum of about 4°C. Finally, although analogous data are lacking from the Menez Gwen site, it can be noted that the temperature of ambient water at this site (i.e. water not influenced by hydrothermal warming) is about 9°C. All these data suggest that 10°C is a temperature which is adequate for long-term survival of *M. fortunata*.

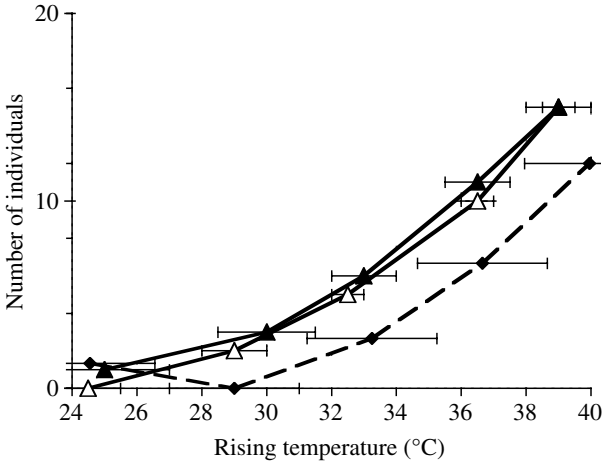

Fig. 6. Data for loss of equilibrium (i.e. C4; see Table 1) for *Mirocaris fortunata* (open triangles, filled triangles, from Figs 4 and 5, respectively) are compared to those for *Rimicaris exoculata* (solid diamonds, broken line), another vent shrimp studied previously (Ravaux et al., 2003). The latter data are the result of experiments using 15 individuals, which are here multiplied by 1.33 (20/15) to allow comparison with experiments (20 individuals).

Viability and experimental stress effects

The initial experiment in this work occurred during the *MARVEL* cruise, in 1997, where oxygen consumption of *M. fortunata* from the Lucky Strike vent field (1700 m depth) was measured at 10°C and at atmospheric pressure (Fig. 3). The results, extrapolated to 10 g shrimps, may be compared to exhaustive data obtained for other caridean shrimps. At 10°C, and for depths varying from 1250 m to surface water, oxygen consumption values ranged from 0.026 to 0.094 μl O_2 h^{-1} mg^{-1} FW for 12 species (Childress et al., 1990). The present results, 0.101 μl O_2 h^{-1} mg^{-1} FW, yielded 0.059 μl O_2 h^{-1} mg^{-1} FW when extrapolated to a 10 g shrimp, using equations provided by Childress and collaborators (1990). This suggested that *M. fortunata* metabolic rates, although measured at atmospheric pressure, were, if not 'normal', at least not reflecting that of moribund animals. Moreover, *M. fortunata* survived the possibly traumatic effects of recovery from depths as great as 1700 m for at least a few hours.

While oxygen uptake rates measured at *in situ* pressure (0.155 μl O_2 h^{-1} mg^{-1} FW) are also comparable to data reported in the literature (extrapolated to 0.089 μl O_2 h^{-1} mg^{-1} FW according to Childress et al., 1990), they are nevertheless clearly higher (50% higher) than those obtained at atmospheric pressure. Within the overlapping mass range (40–120 mg, see Fig. 1), mass-specific oxygen uptake rate means are significantly different (Student test: t=4.21, d.f.=12, P<0.01). This difference may be due to different volumes used for our two experiments, which may have restricted shrimp activity during the atmospheric pressure experiment (16 ml *vs* 210 ml). Different experimental durations (1 h *vs* 7 h) for our end-point measurements could also provide an explanation to these differences: it may be expected that hyperventilation due to stress was most important just after manipulating and conditioning the shrimps. In that case, one would expect uptake values for long durations (the 7 h *in situ* pressure experiment) to be lower than those for short experiments (1 h at atmospheric pressure), due to an averaging effect between the initial hyperventilating state and later stages. This was not the case, so this latter explanation cannot alone explain the observed tendency. Differences in oxygen uptake rates at *in situ* pressure, with respect to atmospheric pressure, were reported for the vent crab *Bythograea thermydron*. In that study, however, measurement at *in situ* pressure showed a decrease of metabolic rate, compared with measurements at atmospheric pressure (Mickel and Childress, 1982). Even though experimental biases (volume, duration) may be partly responsible for the observed differences in our study, it is likely that lower uptakes also reflect the initiation of deleterious effects due to exposure to atmospheric pressure. Future experiments should include testing these hypotheses.

During the ATOS cruise (2001), further experiments (Figs 2 and 3) investigated mortalities at atmospheric pressure, for shrimps originating from shallow and deep sites (Menez Gwen and Rainbow, 850 m and 2300 m depths, respectively). The mortality rates observed were strikingly higher for the shrimps recovered from Rainbow than for those recovered from Menez Gwen. Indeed, Rainbow samples all reached 50% mortality within 48 h, whereas mortality of the Menez Gwen samples did not exceed 5% during the same period. It is very unlikely that the differences between shrimp mortality patterns from different sites reflect differences in thermal adaptation of the two groups. Recalling that 10°C is very likely to be within the thermal preferendum of this species, the mortalities observed at this temperature in our experiments are obviously artefactual, i.e. linked to experimental stress.

Experimental stress involved strong mechanical stimuli through suction-capture, followed by decompression during the submersible ascent. Further manipulation when sorting species, and prolonged exposure to atmospheric pressure, also probably contribute to the observed mortalities. Clearly, *M. fortunata* individuals originating from deep areas are much more adversely affected by experimental stress than are their shallower congeners, pointing to pressure effects (decompression and exposure at atmospheric pressure) being responsible for the difference between the two groups. It also appears that temperature effects are more important for Rainbow samples, with about 15, 30 and almost 50% mortality at 10, 16, and 21°C, respectively after 14 h, with no such differences between Menez Gwen samples at any time (no more than 5% difference in mortality). The ecological meaning of such temperature effects for Rainbow samples is unclear, but at least the increased mortality underlines the necessity to keep temperatures low when attempting to preserve freshly recovered deep-sea fauna. By contrast, the lower Menez Gwen mortalities show that these individuals are in much better physiological condition than their Rainbow congeners. From there, given that 10°C can be considered within the preferred

thermal range of this species, and that there are no obvious mortality differences between the 10°C, 16°C and 21°C experiments, we suggest that sustained temperatures as high as 20°C are tolerated *in situ* by *M. fortunata* originating from the Menez Gwen site.

In the first reference experiment at *in situ* pressure (preliminary), less than 15% mortality was observed after 24 h at 10°C. In the second one, mortality was 30% after 20 h, but this higher value may be explained by the additional decompression/recompression event that occurred after 7 h, in order to retrieve samples used for the respirometry experiment. We did not obtain enough samples to attempt a survival experiment at 10°C and atmospheric pressure, so it is impossible to evaluate directly the benefits of maintaining these creatures at their native pressure. Nevertheless, the depth of Lucky Strike, 1700 m, is closer to the Rainbow depth (600 m shallower than the latter) than that of Menez Gwen (850 m deeper), and the obvious traumatic effects of exposure to atmospheric pressure in the Rainbow shrimps, added to the possible pressure effects in the respirometry experiment, led us to carry out heat-exposure experiments directly at *in situ* pressure. For comparison, Menez Gwen samples were also heat-exposed at their *in situ* pressure.

Thermal biology of Mirocaris fortunata

Ct_{max} determination

The critical thermal maximum (Ct_{max}) is defined as the temperature at which the animal is no longer capable of proper locomotion and starts to move in a jerky, uncoordinated way (Wehner et al., 1992; Gehring and Wehner, 1995; Cuculescu et al., 1998). According to this, signs of loss of locomotory coordination are indicators of the Ct_{max}, and the onset of spasms (OS) was suggested as the response that corresponded best to the definition of the Ct_{max} as being a 'thermal trap' (reviewed in Lutterschmidt and Hutchinson, 1997a; Lutterschmidt and Hutchinson, 1997b). The onset of spasms shortly preceeds heat coma and death. In previous similar work with another hydrothermal vent shrimp, *Rimicaris exoculata* (Ravaux et al., 2003), Ct_{max} was indicated by very characteristic spasmodic movements of the abdomen (OS), which first appeared in the 33–37°C (±2°C) temperature range, during the rapid decrease in activity that followed the peak of 'active movement'. Such behaviour continued until the temperature had reached 40°C, which was also the last point where signs of life were observed. However, behavioural responses to heating vary among taxa, so that the OS may not always be detectable, or quantifiable. Alternative responses may however be considered for estimating Ct_{max}. One is the 'loss of righting reflex' (LRR) response, upon checking an organism's ability to recover its normal 'upright' position, after probing by the experimenter (Cuculescu et al., 1998). In the case of shrimps, several studies proposed the loss of equilibrium (LOE) response as an end-point for Ct_{max} determination (Nelson and Hooper, 1982; Hernandez et al., 1996; Diaz et al., 1998; Diaz et al., 2002; Manush et al., 2004; Selvakumar and Geraldine, 2005), since this corresponds to a loss of balance of the shrimp, an equivalent of the LRR response but without active probing by the experimenter. Although the correspondence of these responses with the definition of Ct_{max} is currently debated, they are nevertheless reliable indicators of imminent heat coma and death. Finally, Lutterschmidt and Hutchinson (Lutterschmidt and Hutchinson, 1997a; Lutterschmidt and Hutchinson, 1997b) recommend inter-species comparison to be carried out using the same behavioural response, and similar heating rates (in the 0.5–1.5°C min^{-1} range). If the rate is too low (e.g. 1°C h^{-1}), heating rates may allow for acclimation effects (i.e. 'heat-hardening') to occur during the experiment. By contrast, high rates (exceeding 3°C min^{-1}) may not allow the body temperature to match the environmental temperature. With heating rates of 1°C min^{-1}, and in the case of animals weighing 150 g or less, it was demonstrated that the body temperature and the environmental temperature did not differ significantly (Lutterschmidt and Hutchinson, 1997a). Our study deals with fresh masses of less than 1 g, and heating rates of about 0.5°C min^{-1}, so it may be assumed that in our experiments the body temperature of our samples matched the experimental temperature.

The Ct_{max} of M. fortunata

Fig. 6 first shows that movement activity increases up to a temperature of 29–30°C, suggesting that the Ct_{max} is above this limit. It is a general observation that an increase in activity preceeds the point when Ct_{max} is reached, e.g. sea urchins (Hernandez et al., 2004), or pseudoscorpions (Heurtault and Vannier, 1989) or shrimps (Rodriguez et al., 1996). This increased activity possibly reveals thermal discomfort for *M. fortunata* in the 25–30°C range, although neither of the activity responses (C2 and C3) fell out of the reference range observed at 10°C (Table 1). A future investigation of the heat-shock protein response at such sublethal temperatures would certainly help to indicate possible heat stress, as previously achieved in the case of *Rimicaris exoculata* (Ravaux et al., 2003). Secondly, it can be safely concluded that Ct_{max} is <39°C, when only a few shrimps are still moving. So, the Ct_{max} of *M. fortunata* is clearly within the 30–40°C range. Spasmodic movements were observed in the case of *M. fortunata*, but were difficult to characterize. Another behavioural response, the loss-of-equilibrium response (LOE), was identified and quantified. Fig. 6 shows that LOE increases above the reference level (Table 1) when the temperature reaches 30°C, and 50% of the experimental population shows signs of loss of equilibrium when the temperature is about 36°C. Beyond 36°C, movement activity rapidly decreases, with only a few shrimps still moving at 39°C. As for several other shrimp studies (see above), we propose that the temperature at which LOE occurs corresponds to the Ct_{max} of *M. fortunata*.

Both groups of *M. fortunata* seem to have very similar Ct_{max} values (Fig. 6). Ct_{max} is not constant within a given species, and may vary according to habitat temperature or experimental acclimation temperature: variations in Ct_{max} by as much as 4–10°C have been reported between summer-caught and

winter-caught crabs (Cuculescu et al., 1998), or fish acclimated at different temperatures (Rajaguru, 2002). This suggests a relationship between temperature resistance and tolerable or preferred habitat temperature (Tsuchida, 1995). Although the ambient water temperature at Menez Gwen is higher than at Lucky Strike (8.8°C *vs* 4.5°C) (Desbruyères et al., 2001), the previously cited temperature recordings among shrimps of Lucky Strike or Rainbow do not suggest significant differences in habitat temperature. Pressure is another possible factor that may cause differences in thermal biology of a given species. Pressure-dependant thermal characteristics have been reported for various biological systems, from isolated biomolecules to whole organisms (Summit et al., 1998; Kaneshiro and Clark, 1995; Holden and Baross, 1995; Kaneko et al., 2000). Temperature resistance properties of *M. fortunata* do not appear to be influenced by depth of occurrence, at least in the 800–1700 m depth range. Further similar studies on the deeper-occurring *M. fortunata* of Rainbow (2300 m depth) would certainly help to test the generalization of this observation to a greater bathymetric range.

Inter-species comparison

Unlike *Mirocaris fortunata*, which is rather broadly distributed across the vent-fluid influence gradient (see above), the closely related vent shrimp *R. exoculata* is believed to occur at the hot end of the hydrothermal biotope in order to provide essential elements to the abundant epibiosis that it hosts in its gill chamber, and on which it feeds (Rieley et al., 1999; Wirsen et al., 1993; Zbinden et al., 2004). As summarized previously (Ravaux et al., 2003), discrete temperature measurements as warm as 25°C to nearly 40°C have been reported within swarms of *R. exoculata* (Desbruyères et al., 2001; Van Dover et al., 1988; Gebruk et al., 1993). Moreover, Gebruk and collaborators (Gebruk et al., 2000) reported that up to 30% of collected specimens were damaged (scalded cuticle) by heat exposure. Although the highest temperatures reported there (>30°C) are unlikely to be within the preferendum of *R. exoculata* (Gebruk et al., 2000; Ravaux et al., 2003), these data nevertheless depict a species that could possibly be more temperature-resistant than *M. fortunata*. At first sight, our results do not support this view, with a Ct_{max} of about 36±1°C for the latter, in the same range as that found for *R. exoculata* (33±2°C to 37±2°C). However, as discussed previously, the Ct_{max} values that we propose for these two species were not determined according to the same behavioural responses (loss of equilibrium *vs* onset of spasms), although the heating rates were identical (Ravaux et al., 2003). Accordingly, and because exact quantification of spasmodic behaviour was impossible for *M. fortunata*, we re-examined our previous behavioural study of *R. exoculata*, and determined the LOE response (Ravaux et al., 2003). We found that the LOE response, as defined in the present study (50% of individuals showing signs of LOE), occurred at about 38.5±2°C for *R. exoculata*, based on interpolation of behavioural data points at 36.5°C and almost 40°C (Fig. 6). Comparing species that differed by more than 10°C in temperature resistance (Northeast Pacific vents), Lee (Lee, 2004) noted the importance of temperature resistance in limiting the distribution of organisms at the hottest end of the hydrothermal vent gradient. This does not seem so obvious for these two MAR vent shrimps, whose critical temperature may differ only slightly (36±1°C for *M. fortunata*, *vs* 38.5±2°C for *R. exoculata*). It is likely that several other factors account for their different distributions within a given vent site, such as nutritional modes, which are clearly different (Gebruk et al., 2000), and possibly tolerance to other environmental factors (oxygen, sulfide levels, etc.).

Temperature resistance values found for both vent shrimps may be compared to those of non-vent shrimp species. Several studies on the Ct_{max} (based on the LOE response, with heating rates in the range 0.3–1°C min^{-1}) of five tropical freshwater caridean shrimp species, each acclimated at different temperatures ranging from 20°C to 35°C (usually for 1 month), yielded values in the 34–43°C range (Nelson and Hooper, 1982; Hernandez et al., 1996; Diaz et al., 1998; Diaz et al., 2002; Manush et al., 2004; Selvakumar and Geraldine, 2005). These shrimps all live in water where the temperature is rarely cooler than 20°C, most of the time is about 28–30°C, and may reach 35°C. Another point of comparison is the temperate-climate shrimp *Palaemon serratus*, which naturally encounters temperatures in the 14–25°C range along the Mediterranean coast (Richard, 1978), and for which extreme temperature limits (the temperature at which immediate death occurs upon exposure to it, according to the author) were reported to be in the 31–37°C range, when acclimated at temperatures within natural range.

The critical thermal maximum is one of several upper thermal limits that may be measured for a given organism. The so-called median lethal temperatures (or 'LT_{50}', corresponding to 50% mortality) will lead to heat death, depending on exposure time. At the lower boundary of this range, it is possible to define the upper incipient lethal temperature, a temperature leading to 50% mortality over an indefinitely long exposure time (Lutterschmidt and Hutchinson, 1997b). This temperature marks the boundary between the 'tolerance' and 'resistance' zones, and may be considered as the temperature below which there is no significant mortality due to heat stress. Although it has been proposed that the incipient lethal temperature is ecologically more relevant than Ct_{max}, its determination is problematic when only a limited amount of samples is available, whereas Ct_{max} may be determined from one single experiment. Moreover, although there is still some debate about the possibility of accurately predicting incipient lethal temperature from Ct_{max}, various studies suggest that the former are several degrees lower than the latter: in a study of seven fish species, Rajaguru (Rajaguru, 2002) found that incipient lethal temperatures were 3–5°C lower than the corresponding Ct_{max}. Closer to *M. fortunata*, Nelson and Hooper (1982) showed that incipient lethal temperature is <33°C for the glass shrimp *Palaemonetes kadiakensis*, while the measured Ct_{max} was ca. 37°C at least. Lastly, the Ct_{max} of the vent annelid *Hesiolyra bergi* was in the 41–46°C range, but

this creature did not survive 4 h exposures at 39°C (Shillito et al., 2001). Overall, these data suggest that sustained exposure in the 30–35°C range is likely to induce significant mortality for *M. fortunata*.

Although exceptionally high temperature tolerance has been reported in the case of Pacific vent annelid species (Cary et al., 1998; Lee, 2004), our study reveals an organism with a rather moderate temperature resistance (<40°C), as suggested for a few other EPR vent organisms (Dahloff and Somero, 1991; Shillito et al., 2001; Mickel and Childress, 1982). As proposed previously (Shillito et al., 2001; Ravaux et al., 2003), an active thermoregulatory behaviour would nevertheless permit short exposures to temperatures above the Ct_{max}, as in the case of *Seothyra* sp., a small (max. 300 mg FW) desert spider that continues to hunt at temperatures exceeding 65°C, well above its 49°C Ct_{max} (Lubin and Henschel, 1990). In the case of short exposure to lethal temperatures, the larger size of *R. exoculata* (ca. twice as large as *M. fortunata*) may be an advantage, despite having similar Ct_{max}. Significant differences may appear between body and environmental temperatures for temperature increases of 10°C min^{-1} or more (a situation possibly encountered by a shrimp moving in the proximity of a vent smoker) (Lutterschmidt and Hutchinson, 1997b). These differences would obviously be more important for larger size animals (meaning a lower body temperature at a given time), and therefore permit longer exposure to 'bursts' of higher environmental temperatures for the latter. In other words, upon exposure to brutal temperature increases, *M. fortunata*'s body temperature would meet the Ct_{max} first.

Finally, little is known about temperature resistance of non-vent caridean species in the deep sea. With the exception of areas such as the Mediterranean or Red Seas, temperatures of the deep sea rarely exceed 5°C, and by comparison with coldwater crustacea (Lahdes, 1995; Cuculescu et al., 1998) upper thermal limits (such as Ct_{max}) may be expected not to exceed 20°C. In that respect, vent shrimps could be regarded as thermophilic organisms. Further studies on related species living in colder habitats, such as the surrounding deep sea, or cold-seeps (see Shank et al., 1999, for vent shrimp phylogeny), will provide interesting insights into the adaptive significance of temperature resistance at hydrothermal vents.

Conclusions

(1) The tolerance of the deep-sea shrimp *Mirocaris fortunata* to the trauma of collection was sufficient to allow further *in vivo* experimentation on-board ship. However, deeper-originating samples (2300 m depth) were not appropriate for experimentation at atmospheric pressure. By contrast, shrimps originating from shallower zones (Menez Gwen, 850 m depth) survived for several days, with equivalent survival rates at 10, 15 and 20°C.

(2) Heat exposure experiments carried out at *in situ* pressure showed that *M. fortunata* displays signs of severe heat stress at about 36±1°C (the proposed Ct_{max}), and does not survive at temperatures above 39°C. A comparison with similar data obtained for other non-vent caridean shrimps reveals that the temperature resistance of this species is similar to those of temperate-climate and tropical-climate-adapted species.

(3) The heat exposure experiments were carried out using shrimps obtained from different sites and depths (Menez Gwen, 850 m and Lucky Strike, 1700 m), and there was no evidence that temperature resistance varied according to site and/or depth (36±1°C and 36.5±0.5°C for Menez Gwen and Lucky Strike shrimps, respectively).

(4) Comparison with another vent shrimp, *Rimicaris exoculata*, using the same behavioural criteria as in the present work, suggests that the latter may have a slightly higher temperature resistance (38.5±2°C). Whether this putative difference is consistent with differences in distribution across the hydrothermal vent gradient, as suggested for other vent species (Lee, 2004), remains to be seen.

We thank the captain and crew of N/O *Atalante,* along with the teams of the submersibles Nautile and ROV *Victor* (Ifremer), for their assistance throughout this work. We also wish to thank D. Desbruyères and P. M. Sarradin, chief scientists of the *MARVEL* and *ATOS* cruises, respectively, and also M. Zbinden and E. Thiébaut. This research was funded by the EC programs VENTOX (EVK3-1999-00056P), and AMORES. Experiments described in this paper complied with the current laws in France.

References

Aminot, A. and Chaussepied, M. (1983). *Manuel des Analyses Chimiques en Milieu Marin*. Brest: CNEXO (Centre National pour l'Exploitation des Océans).

Cary, S. C., Shank, T. and Stein, J. (1998). Worms bask in extreme temperatures. *Nature* **391**, 545-546.

Chevaldonné, P., Desbruyères, D. and Childress, J. J. (1992). Some like it hot... and some even hotter. *Nature* **359**, 593-594.

Chevaldonné, P., Fisher, C. R., Childress, J. J., Desbruyères, D., Jollivet, D., Zal, F. and Toulmond, A. (2000). Thermotolerance and the 'Pompeii worms'. *Mar. Ecol. Prog. Ser.* **208**, 293-295.

Childress, J. J., Cowles, D. L., Favuzzi, J. A. and Mickel, T. J. (1990). Metabolic rates of benthic deep-sea decapods crustaceans decline with increasing depth primarily due to the decline in temperature. *Deep-Sea Res.* **37**, 929-949.

Cuculescu, M., Hyde, D. and Bowler, K. (1998). Thermal tolerance of two species of marine crab, *Cancer pagurus* and *Carcinus maenas*. *J. Therm. Biol.* **23**, 107-110.

Dahlhoff, E. and Somero, G. (1991). Pressure and temperature adaptation of cytosolic malate dehydrogenases of shallow- and deep-living marine invertebrates: evidence for high body temperatures in hydrothermal vent animals. *J. Exp. Biol.* **159**, 473-487.

Desbruyères, D., Almeida, A., Biscoito, M., Comtet, T., Khripounoff, A., Le Bris, N., Sarradin, P.-M. and Segonzac, M. (2000). A review of the distribution of hydrothermal vent communities along the Northern Mid-Atlantic Ridge: Dispersal vs environmental controls. *Hydrobiologia* **440**, 201-216.

Desbruyères, D., Biscoito, M., Caprais, J.-C., Colaço, A., Comtet, T., Crassous, P., Fouquet, Y., Khripounoff, A., Le Bris, N., Olu, K. et al. (2001). Variations in deep-sea hydrothermal vent communities on the Mid-Atlantic Ridge near the Azores plateau. *Deep-Sea Res.* **48**, 1325-1346.

Di Méo-Savoie, C. A., Luther, G. W. and Cary, S. C. (2004). Physicochemical characterization of the microhabitat of the epibionts associated with *Alvinella pompejana*, a hydrothermal vent annelid. *Geochim. Cosmochim. Acta* **68**, 2055-2066.

Diaz, F., Sierra, E., Bückle, F. and Garrido, A. (1998). Critical thermal maxima and minima of *Macrobrachium rosenbergii* (Decapoda: palaemonidae). *J. Therm. Biol.* **23**, 381-385.

Diaz, F., Sierra, E., Re, A. D. and Rodriguez, L. (2002). Behavioural thermoregulation and critical thermal limits of *Macrobrachium acanthurus* (Wiegman). *J. Therm. Biol*. **27**, 423-428.

Fisher, C. R. (1998). Temperature and sulphide tolerance of hydrothermal vent fauna. *Cah. Biol. Mar*. **39**, 283-286.

Gaill, F., Mann, K., Wiedemann, H., Engel, J. and Timpl, R. (1995). Structural comparison of cuticle and interstitial collagens from annelids living in shallow sea-water and at deep-sea hydrothermal vents. *J. Mol. Biol.* **246**, 284-294.

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

Gebruk, A. V., Southward, E. C., Kennedy, H. and Southward, A. J. (2000). Food sources, behaviour, and distribution of hydrothermal vent shrimps at the Mid-Atlantic Ridge. *J. Mar. Biol. Assoc. UK* **80**, 485-499.

Gehring, W. J. and Wehner, R. (1995). Heat shock protein synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara desert. *Proc. Natl. Acad. Sci. USA* **92**, 2994-2998.

Hernandez, M., Bückle, F. and Diaz, F. (1996). Critical thermal maximum of *Macrobrachium tenellum*. *J. Therm. Biol.* **21**, 139-143.

Hernandez, M., Bückle, F., Guisado, C., Baron, B. and Estavillo, N. (2004). Critical thermal maximum and osmotic pressure of the red sea urchin *Strongylocentrotus franciscanus* acclimated at different temperatures. *J. Therm. Biol.* **29**, 231-236.

Heurtault, J. and Vannier, G. (1990). Thermorésistance chez deux pseudoscorpions (Garypidae), l'un du désert de Namibie, l'autre de la région de Gênes (Italie). *Acta Zool. Fennica* **190**, 165-171.

Holden, J. F. and Baross, J. A. (1995). Enhanced thermotolerance by hydrostatic pressure in the deep-sea hyperthermophile *Pyrococcus* strain ES4. *FEMS Microbiol. Ecol*. **18**, 27-34.

Kaneko, H., Takami, H., Inoue, A. and Horikoshi, K. (2000). Effects of hydrostatic pressure and temperature on growth and lipid composition of the inner membrane of barotolerant *Pseudomonas* sp. BT1 isolated from the deep-sea. *Biosci. Biotechnol. Biochem*. **64**, 72-79.

Kaneshiro, S. and Clark, D. (1995). Pressure effects on the composition and thermal behavior of lipids from the deep-sea thermophile *Methanococcus jannaschii*. *J. Bacteriol*. **177**, 3668-3672.

Komai, T. and Segonzac, M. (2003). Review of the hydrothermal vent shrimp genus Mirocaris, redescription of *M. fortunata* and reassessment of the taxonomic status of the family Alvinocarididae (Crustacea: Decapoda: Caridea). *Cah. Biol. Mar*. **44**, 199-215.

Lahdes, E. (1995). Acute thermal tolerance of two Antartic copepods, *Calanoides acutus* and *Calanus propinquus*. *J. Therm. Biol*. **20**, 75-78.

Le Bris, N., Zbinden, M. and Gaill, F. (2005). Processes controlling the physico-chemical micro-environments associated with Pompeii worms. *Deep-Sea Res.* **52**, 1071-1083.

Lee, R. W. (2004). Thermal tolerances of deep-sea hydrothermal vent animals from the Northeast Pacific. *Biol. Bull*. **205**, 98-101.

Lonsdale, P. (1977). Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at oceanic spreading centers. *Deep-Sea Res*. **24**, 857-863.

Lubin, Y. D. and Henschel, J. R. (1990). Foraging at the thermal limit: burrowing spiders (*Seothyra*, Eresidae) in the Namib desert dunes. *Oecologia* **84**, 461-467.

Lutterschmidt, W. I. and Hutchinson, V. H. (1997a). The critical thermal maximum: data to support the onset of spasms as the definitive end point. *Can. J. Zool*. **75**, 1553-1560.

Lutterschmidt, W. I. and Hutchinson, V. H. (1997b). The critical thermal maximum: history and critique. *Can. J. Zool*. **75**, 1561-1574.

Manush, S. M., Pal, A. K., Chatterjee, N., Das, T. and Mukherjee, S. C. (2004). Thermal tolerance and oxygen consumption of *Macrobrachium rosenbergii* acclimated to three temperatures. *J. Therm. Biol*. **29**, 15-19.

Martin, J. W. and Hessler, R. R. (1990). *Chorocaris vandoverae*, a new genus and species of hydrothermal vent shrimp (Crustacea, Decapoda, Bresiliidae) from the western pacific. *Contrib. Sci*. **417**, 1-11.

Martin, J. W. and Christiansen, J. C. (1995). A new species of the shrimp genus Chorocaris Martin and Hessler 1990 from hydrothermal vents along the Mid-Atlantic Ridge. *Proc. Biol. Soc. Wash*. **108**, 220-227.

Mickel, T. J. and Childress, J. J. (1982). Effects of pressure and temperature on the EKG and heart rate of the hydrothermal vent crab *Bythograea thermydron* (Brachyura). *Biol. Bull*. **162**, 70-82.

Nelson, D. H. and Hooper, D. K. (1982). Thermal tolerance and preference of the freshwater shrimp *Palaemonetes kadiakensis*. *J. Therm. Biol*. **7**, 183-187.

Polz, M. F., Robinson, J. J., Cavanaugh, C. M. and Van Dover, C. L. (1998). Trophic ecology of massive shrimp aggregations at a Mid-Atlantic Ridge hydrothermal vent site. *Limnol. Oceanogr.* **43**, 1631-1638.

Prosser, C. L. (1973). Oxygen: Respiration and metabolism. In *Comparative Animal Physiology* (ed. C. L. Prosser), pp. 165-211. Philadelphia: W. B. Saunders Co.

Quetin, L. B. and Childress, J. J. (1980). Observations on the swimming activity of two bathypelagic mysid species maintained at high hydrostatic pressure. *Deep-Sea Res. A* **27**, 383-391.

Rajaguru, S. (2002). Critical thermal maximum of seven estuarine fishes. *J. Therm. Biol.* **27**, 125-128.

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

Richard, P. (1978). Tolerance aux temperatures extrêmes de *Palaemon serratus* (Pennant): influence de la taille et de l'acclimatation. *J. exp. Mar. Biol. Ecol.* **35**, 137-146.

Rieley, G., Van Dover, C. L., Hedrick, D. B. and Eglinton, G. (1999). Trophic ecology of *Rimicaris exoculata*: a combined lipid abundance/stable isotope approach. *Mar. Biol.* **133**, 495-499.

Sarrazin, J., Juniper, S. K., Massoth, G. and Legendre, P. (1999). Physical and chemical factors influencing species distributions on hydrothermal sulfide edifices of the Juan de Fuca Ridge, northeast Pacific. *Mar. Ecol. Prog. Ser*. **190**, 89-112.

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

Selvakumar, S. and Geraldine, P. (2005). Heat-shock protein induction in the freshwater prawn *Macrobrachium malcolmsonii*: Acclimation-influenced variations in the induction temperatures for hsp70. *Comp. Biochem. Physiol*. **140A**, 209-215.

Shank, T. M., Black, M. B., Halanych, K. M., Lutz, R. A. and Vrijenhoek, R. C. (1999). Miocene radiation of deep-sea hydrothermal vent shrimp (Caridea:bresiliidae): evidence from mitochondrial cytochrome oxidase subunit I. *Mol. Phylogenet. Evol.* **13**, 244-254.

Shillito, B., Jollivet, D., Sarradin, P.-M., Rodier, P., Lallier, F., Desbruyères, D. and Gaill, F. (2001). Temperature resistance of *Hesiolyra bergi*, a polychaetous annelid living on deep-sea vent smoker walls. *Mar. Ecol. Prog. Ser*. **216**, 141-149.

Summit, M., Scott, B., Nielson, K., Mathur, E. and Baross, J. (1998). Pressure enhances thermal stability of DNA polymerase from three thermophilic organisms. *Extremophiles* **2**, 339-345.

Tomanek, L. and Somero, G. N. (1999). Evolutionary and acclimation-induced variation in the heat-shock reponses of congeneric marine snails (genus Tegula) from different thermal habitats: implications for limits of thermotolerance and biogeography. *J. Exp. Biol.* **202**, 2925-2936.

Tsuchida, S. (1995). The relationship between upper temperature tolerance and final preferendum of japanese marine fish. *J. Therm. Biol*. **20**, 35-41.

Van Dover, C. L. and Lutz, R. A. (2004). Experimental ecology at deep-sea hydrothermal vents: a perspective. *J. Exp. Mar. Biol. Ecol*. **300**, 273-307.

Van Dover, C. L., Fry, B., Grassle, J. F., Humphris, S. and Rona, P. A. (1988). Feeding biology of the shrimp *Rimicaris exoculata* at hydrothermal vents on the Mid-Atlantic Ridge. *Mar. Biol*. **98**, 209-216.

Wehner, R., Marsh, A. C. and Wehner, S. (1992). Desert ants on a thermal tightrope. *Nature* **357**, 586-587.

Williams, A. B. and Rona, P. A. (1986). Two new Caridean shrimps (Bresiliidae) from a hydrothermal field on the Mid-Atlantic Ridge. *J. Crust. Biol*. **6**, 446-462.

Wirsen, C. O., Jannasch, H. W. and Molyneaux, S. J. (1993). Chemosynthetic microbial activity at Mid-Atlantic Ridge hydrothermal vent sites. *J. Geophys. Res.* **98**, 9693-9703.

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