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# Hydromineral Regulation in the Hydrothermal Vent Crab *Bythograea thermydron*

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**Abstract.** This study investigates the salinity tolerance and the pattern of osmotic and ionic regulation of *Bythograea thermydron* Williams, 1980, a brachyuran crab endemic to the deep-sea hydrothermal vent habitat. Salinities of 33‰–35‰ were measured in the seawater surrounding the captured specimens. *B. thermydron* is a marine stenohaline osmoconformer, which tolerates salinities ranging between about 31‰ and 42‰. The time of osmotic adaptation after a sudden decrease in external salinity is about 15–24 h, which is relatively short for a brachyuran crab. In the range of tolerable salinities, it exhibits an iso-osmotic regulation, which is not affected by changes in hydrostatic pressure, and an iso-ionic regulation for Na<sup>+</sup> and Cl<sup>-</sup>. The hemolymph Ca<sup>2+</sup> concentration is slightly hyper-regulated, K<sup>+</sup> concentration is slightly hyper-hypo-regulated, and Mg<sup>2+</sup> concentration is strongly hypo-regulated. These findings probably reflect a high permeability of the teguments to water and ions. In addition to limited information about salinity around hydrothermal vents, these results lead to the hypothesis that *B. thermydron* lives in a habitat of stable seawater salinity. The osmoconformity of this species is briefly discussed in relation to its potential phylogeny.

## Introduction

Hydrothermal vents, first discovered in 1977 on the Galapagos Ridge, are unique deep-sea habitats. They are char-

acterized by variable and extreme conditions of some physicochemical parameters, in particular by high temperature, high sulfide and metal content, high level of carbon dioxide, low level of oxygen and low pH (Truchot and Lallier, 1998; Sarradin *et al.*, 1998, 1999). To live in this environment, biological communities associated with the vents have developed behavioral, physiological, morphological, and reproductive adaptations such as symbiosis (Fisher, 1990), physiological and biochemical systems for sulfide detoxification (Powell and Somero, 1986; Cosson and Vivier, 1997; Geret *et al.*, 1998; Truchet *et al.*, 1998), behavioral and molecular responses to high temperature (Dahlhoff *et al.*, 1991; Dixon *et al.*, 1992; Segonzac *et al.*, 1993; Desbruyères *et al.*, 1998; Fisher, 1998), and specialized sensory organs to locate hot chimneys (Jinks *et al.*, 1998).

Among this vent fauna live endemic brachyuran decapod crustaceans (superfamily: *Bythograeoidea* Williams, 1980; family: *Bythograeidae* Williams, 1980; genera: *Bythograea* Williams, 1980; *Cyanagraea* de Saint Laurent, 1984; *Segonzacia* Guinot, 1989; *Austinograea* Hessler and Martin, 1989) (Tudge *et al.*, 1998). They have been found in all the known hydrothermal vents—*Bythograea* and *Cyanagraea* in the East Pacific, *Austinograea* in the West Pacific, *Segonzacia* in the mid-Atlantic (Tunnicliffe *et al.*, 1998). Information published on these brachyurans includes studies on their biogeography and evolution (Hessler and Wilson, 1983; Newman, 1985; Tunnicliffe, 1988), reproductive biology and larval development (Van Dover *et al.*, 1984, 1985; Epifanio *et al.*, 1999), and ecology and distribution (Van Dover, 1995; Guinot and Segonzac, 1997). Probably

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due to the difficulty of getting live specimens, physiological studies are scarcer and have addressed aspects of respiration (Lallier *et al.*, 1998), sulfide detoxification (Vetter *et al.*, 1987), and temperature or pressure effects on the mitochondria, heart rate, or oxygen consumption rate (Mickel and Childress, 1982a,b; Dahlhoff *et al.*, 1991) of these crabs.

To our knowledge, no information is available on the hydromineral metabolism of the hydrothermal vent animals and particularly of the brachyuran crustaceans. Salinity is one of the main environmental factors exerting a selection pressure on aquatic organisms, and the successful establishment of a species in a given habitat depends on the ability of the organisms to adapt to, among other factors, the typical level and variations in salinity (Charmantier, 1998). This major adaptive process is achieved through different behavioral or physiological mechanisms. Osmoregulation is one of the most important of these mechanisms in some animal groups, including crustaceans. It has been explored in the adults of numerous crustacean species (reviews in Mantel and Farmer, 1983; Péqueux, 1995).

The present study has been conducted with one species of bythograeid crab from hydrothermal vents, *Bythograea thermydron* Williams, 1980. This crab is the most frequently observed [density about 20 individuals per m<sup>2</sup> (Guinot and Segonzac, 1997)] and captured species among brachyuran crustaceans on the East Pacific sites (Guinot, 1989). It is found predominantly in the warm water (>20°C) surrounding mussels and vestimentiferans on which it feeds, and also at the periphery of the vent areas where temperature is about 2°C (Grassle, 1986, cited by Epifanio *et al.*, 1999). These habitats, influenced by the spatially and temporally variable input of hydrothermal fluid, are greatly variable over short time and distance. Information on their salinity does not exist or is unpublished. It is thus unclear whether the salinity of the water surrounding the vents is as stable as the deep-sea water environment or is variable under the influence of the hydrothermal fluid. Physiological studies have indicated that adults of *B. thermydron* are tolerant of wide variations in temperature, dissolved oxygen, and hydrogen sulfide (Mickel and Childress, 1982a,b; Vetter *et al.*, 1987; Airries and Childress, 1994), but their ability to tolerate salinity variation and to osmoregulate is not known. The objectives of the present study were thus to evaluate the salinity tolerance and the pattern of osmoregulation of *B. thermydron*. The salinity of the natural habitat of the crab was also measured. As the hemolymph osmolality of crustaceans is mostly established by inorganic ions (essentially Na<sup>+</sup> and Cl<sup>-</sup>) (Péqueux, 1995), the ionic regulation of this crab was also studied.

## Materials and Methods

### Animals

Adults of *Bythograea thermydron* were collected by the submarine *Nautille*, using resin watertight containers (about

1 × 0.5 × 0.5 m), on the East Pacific Rise (EPR) on the 13°N and 9°N sites [12°46–50'N, 103°57'W and 9°50'N, 104°17'W (Tunnicliffe *et al.*, 1998)], at a depth of about 2500 m, during the HOPE 99 mission in May 1999. Only a small number of crabs were available, which resulted in 3 to 10 individuals for each experimental condition. As this species seems incapable of long-term survival outside the high-pressure environment of the deep sea (Mickel and Childress, 1982a; Airries and Childress, 1994), most of the crabs were transferred into aquaria with running aerated Pacific surface seawater as soon as they reached the ship *Atalante*, and they were used in the following hours for experiments conducted on board, at atmospheric pressure, at a water temperature of 13°C. Some of them were also exposed to high pressure (see below). Crab cephalothoracic widths were 6–8 cm. Their molt stages (Drach, 1939) were not checked, but soft (post-molt) crabs were not used in experiments.

### Ambient salinity

Water samples from the depth of the *Riftia pachyptila* ring on the 13°N and 9°N EPR sites were collected in 750-ml titanium syringes manipulated by the *Nautille*. The water osmolality in mosm/kg was measured on an automatic micro-osmometer (Wescor Varro 5520). The corresponding values of salinity in parts per thousand were calculated by interpolation of data according to Weast (1969).

### Preparation of media

Dilute media were prepared by adding fresh water to Pacific surface seawater (1002 ± 2 mosm/kg; approximately 34.6‰), and high-salinity media were prepared by adding ocean salts (Wimex, Germany) to seawater. Salinities were expressed as osmolality (in mosm/kg) and salt concentration (in parts per thousand). The osmolality of the media was measured with a Wescor Varro 5520 micro-osmometer. Media with the following osmolalities and corresponding salinities were prepared: 740 mosm/kg (25.4‰), 800 (27.5), 900 (31.0), 1002 (34.6), 1100 (38.2), 1200 (41.9), 1300 (45.7). Experiments were conducted at 13°C in 40-l aerated aquaria that were kept in the dark except at the time of sampling, when light was briefly necessary.

### Salinity tolerance

The objective of the experiment was to estimate the survival time of the crabs at different salinities. The crabs were transferred directly from seawater to the experimental media. Observations were made and dead individuals were removed 1, 2, 3, 5, 6, 12, 15, and 24 h after the beginning of the tests. The absence of body movement after repeated touches with a probe was considered as a proof of death.

### Hydromineral regulation

**Acclimation time.** To estimate the time necessary for hemolymph osmolality stabilization following a decrease in salinity, the crabs were first transferred from seawater (1002 mosm/kg), into a 740-mosm/kg medium. Hemolymph samples were taken from surviving animals after 0, 2.45, 5, and 15 h in the dilute medium. As 75% of the animals were dead at 15 h and 100% shortly afterward, a second experiment was conducted in an 800-mosm/kg medium. Survival was 60% at 12 h and 17% at 24 h. Hemolymph samples were taken from the surviving crabs after 0, 1, 2, 3, 6, 12, 24, and 48 h.

**Osmotic regulation.** The hemolymph osmolality of some crabs was measured as soon as they were brought on board. The crabs were then transferred to the different media, and their hemolymph osmolality was remeasured after a period of osmotic stabilization in each medium; the length of this period was determined from the results on adaptation time. A similar experiment was conducted under high pressure, at 15°C. The crabs were immersed in an 800-mosm/kg medium, in individual 400-ml containers set in a 19-l pressurized tank called "Incubateur Pressurisé pour l'Observation en Culture d'Animaux Marins Profonds" (IPOCAMP) (Shillito, unpub.). The crabs were subjected for 13 h to a pressure of 260 bars, which approximates the pressure at the site of capture. The hemolymph was then sampled and its osmolality was measured.

For sampling, the crabs were rinsed with deionized water and dried with absorbent paper. Hemolymph was sampled with a hypodermic needle mounted on a syringe and inserted at the basis of a posterior pereopod. The osmolality

of a 10- $\mu$ l sample of hemolymph was immediately measured on the Wescor Varro 5520 micro-osmometer.

**Ionic regulation.** Hemolymph from the same samples was quickly diluted to 25% in deionized water, stored in Eppendorf tubes, and kept at  $-80^{\circ}\text{C}$ . After transport to the Montpellier laboratory in liquid nitrogen, the hemolymph and media samples were dissolved in deionized water to the appropriate volume, and their ionic contents were determined using an amperometric Aminco-Cotlove chloridimeter for the titration of  $\text{Cl}^{-}$ , an Eppendorf flame photometer for  $\text{Na}^{+}$ ,  $\text{K}^{+}$ ,  $\text{Ca}^{2+}$ , and a Varian AA-1275 atomic absorption photometer for  $\text{Mg}^{2+}$ .

### Statistical analysis

Statistical comparisons of experimental data were performed by one-way analysis of variance (ANOVA) (Sokal and Rohlf, 1981) by using the software StatView 4.02 (Abacus Concept, Inc.).

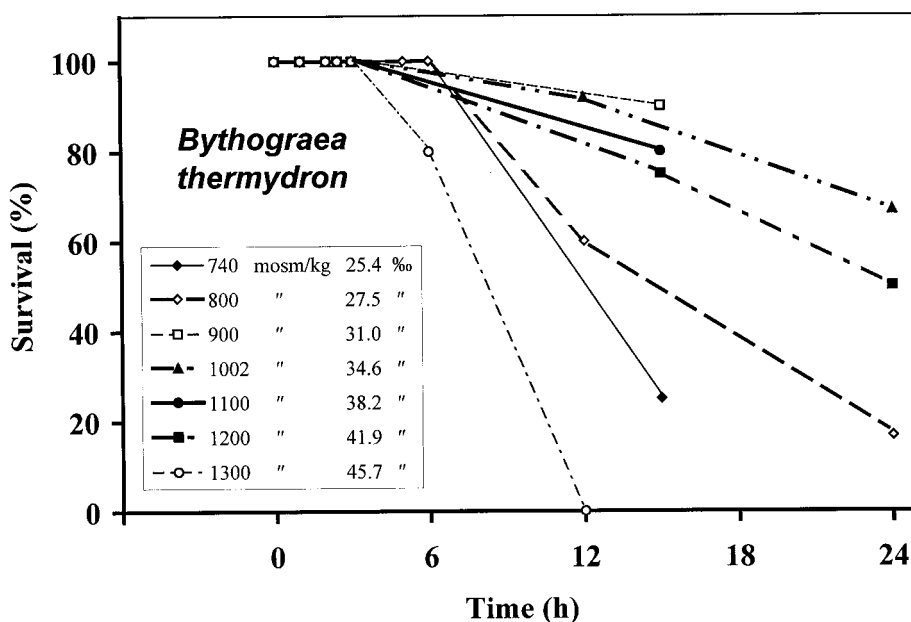
## Results

### Ambient salinity

The salinity measured from bottom seawater samples was 996–1007 mosm/kg at the 13°N EPR site, and 950 mosm/kg at the 9°N EPR site.

### Salinity tolerance

The survival rates of adults of *Bythograea thomydron* in Figure 1 were different according to salinity and decreased



**Figure 1.** *Bythograea thomydron*. Survival rate (in %) at different salinities according to the time of exposure. Number of crabs per condition at the start of the experiment: 3 to 10.

with the time of exposure (Fig. 1). They decreased sharply to less than 25% within 15–24 h at the highest (1300 mosm/kg) and lowest (740, 800 mosm/kg) salinities. Survival was higher in seawater (1002 mosm/kg) and in salinities ranging from 900 to 1200 mosm/kg.

#### Hydromineral regulation

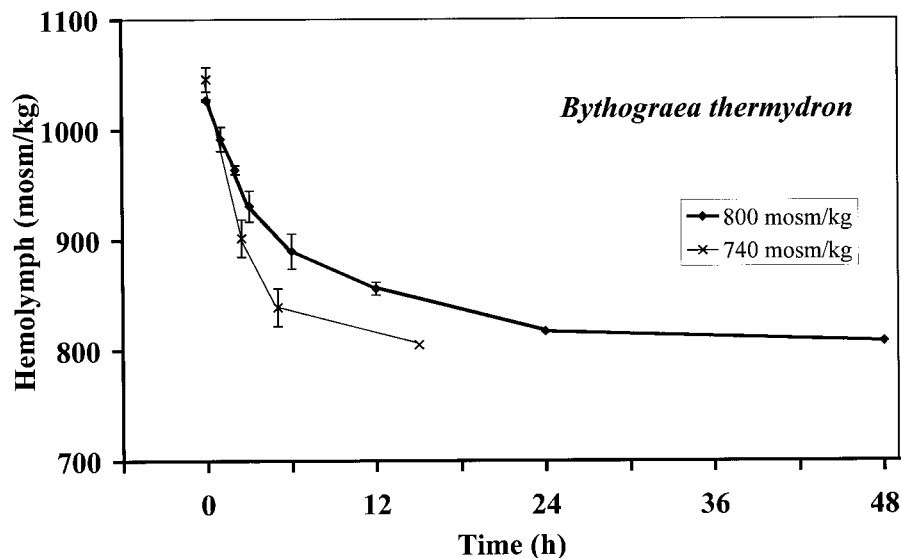
**Acclimation time.** The time of adaptation after a sudden change in salinity was evaluated at two low salinities (Fig. 2). In both media, the hemolymph osmolality decreased sharply within 12 h. After 15 h in the 740-mosm/kg medium, hemolymph osmolality had decreased to 805 mosm/kg—that is, to about 65 mosm/kg above the medium osmolality. As all crabs had died before 24 h, it was not possible to determine whether hemolymph osmolality had entirely stabilized at 15 h. After a transfer to the 800-mosm/kg medium, the hemolymph osmolality stabilized within 24 h. Its mean values were respectively 817 and 808 mosm/kg (no significant difference) after 24 h and 48 h in this medium. In subsequent experiments, the time of exposure to different media was based on these results and was kept in general at 15–24 h.

**Osmotic regulation.** Upon the arrival of the crabs on board the ship following their transfer from the bottom, their hemolymph osmolality was  $1025 \pm 4$  mosm/kg ( $n = 18$ ) and  $984 \pm 12$  mosm/kg ( $n = 29$ ) at the 13°N EPR and 9°N EPR sites respectively. The ability of the crabs to osmoregulate was then evaluated in the range of tolerable salinities between 900 mosm/kg and 1200 mosm/kg. The crabs osmoconformed in the whole range of tested salinities (Fig.

3A). The hemolymph osmotic concentration was close to that of the medium, different from it by only 9 to 22 mosm/kg, 15 mosm/kg on average.

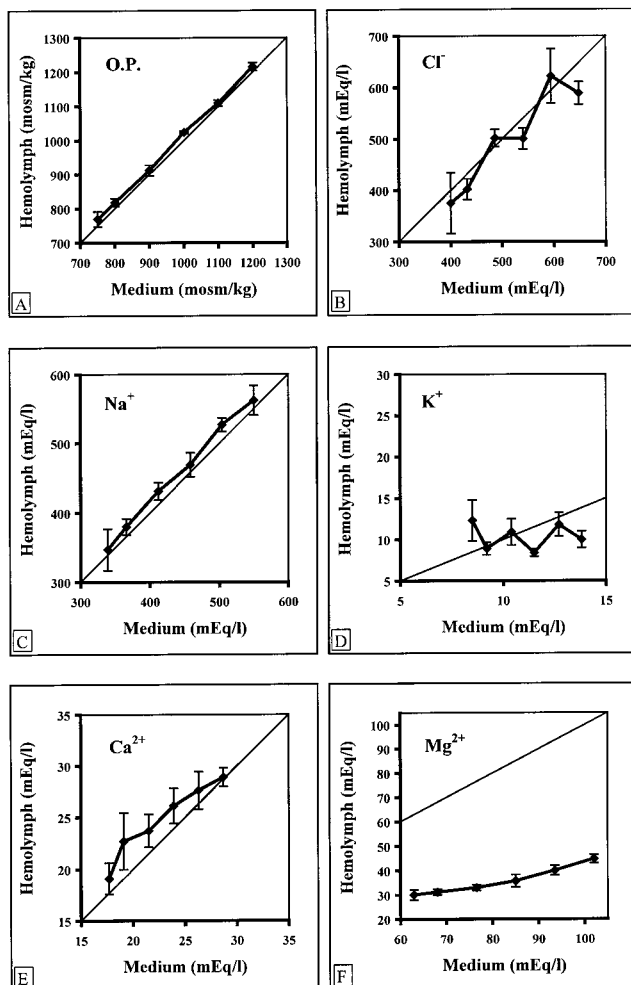
The hemolymph osmolality was also measured in crabs maintained in the 800-mosm/kg medium, under a pressure of 260 bars. The mean value of hemolymph osmolality following this treatment for 13 h was  $860 \pm 9$  mosm/kg ( $n = 3$ ), not significantly different from the value of  $856 \pm 6$  mosm/kg ( $n = 3$ ) in control crabs kept in the same medium for 13 h under atmospheric pressure.

**Ionic regulation.** The results concerning hemolymph ion concentrations in the different media are given in Figure 3B–F. In seawater,  $\text{Na}^+$  and  $\text{Cl}^-$  were the main osmoeffectors in hemolymph since they accounted for about 95% of the total hemolymph osmolality, and this trend was retained in all media. The hemolymph  $\text{Cl}^-$  concentration followed that of the medium in the whole range of tolerable salinities. It tended to be slightly hypo-regulated in most media (Fig. 3B).  $\text{Na}^+$  regulation was iso-ionic; hemolymph  $\text{Na}^+$  concentration constantly remained slightly above that of the medium, by 8 to 23 mEq  $\text{Na}^+/\text{l}^{-1}$  (Fig. 3C).  $\text{K}^+$  was slightly hypo-regulated (by approximately 2.5 mEq  $\text{K}^+/\text{l}^{-1}$ ) in the media in which concentrations were above 10.5 mEq  $\text{K}^+/\text{l}^{-1}$  (900 mosm/kg), and it was slightly hyper-regulated (by approximately 3.5 mEq  $\text{K}^+/\text{l}^{-1}$ ) in the lowest salinity (800 mosm/kg, 9.3 mEq  $\text{K}^+/\text{l}^{-1}$ ) (Fig. 3D). Hemolymph  $\text{Ca}^{2+}$  concentration was slightly hyper-regulated (by 1.2 to 3.6 mEq  $\text{Ca}^{2+}/\text{l}^{-1}$ ) at most tested salinities (Fig. 3E). Hemolymph  $\text{Mg}^{2+}$  concentration was strongly hypo-regulated (by about 33 to 57 mEq  $\text{Mg}^{2+}/\text{l}^{-1}$ ) over the entire range of salinities (Fig. 3F).



**Figure 2.** *Bythograea thermydron*. Change in hemolymph osmolality according to the time after rapid transfer from Pacific surface seawater ( $1002 \pm 2$  mosm/kg) to dilute media at 740 mosm/kg and 800 mosm/kg. Error bars: mean  $\pm$  SD;  $n$ : 4 to 6 individuals.





**Figure 3.** *Bythograea therymydron*. Variations in hemolymph osmolality (A: O.P.) (osmotic pressure in mosm/kg) and ionic concentrations (B to F) (in  $\text{mEq} \cdot \text{l}^{-1}$ ) after hemolymph osmolality stabilization (about 15–24 h), in relation to the osmolality or ionic concentration of the medium. Time of exposure to the different media was 24 h (A) or 15–24 h (B to F). Error bars: mean  $\pm$  SD;  $n$ : 3 to 12 individuals; isoconcentration lines are drawn.

## Discussion

### Salinity tolerance

The limited number of available animals and lack of time and space on board the ship prevented long-term tolerance experiments. Specimens of *Bythograea therymydron* survived for 24 h in a narrow range of salinities ranging from about 31‰ to 42‰. These crabs, unable to withstand a great extent of salinity fluctuations, are thus stenohaline animals. They share this feature with other species of decapods whose habitat is most often restricted to seawater, for example, the Majidae, the Cancridae, and the Calappidae (review in Mantel and Farmer, 1983; Péqueux, 1995).

### Acclimation time

In *B. therymydron*, the time required to reach an osmotic steady-state after a sudden decrease in external salinity was

about 15 to 24 h. This is short for a brachyuran crab, similar to the 15 h required for osmotic equilibration in osmoconformers such as the Majidae *Maja* sp. and *Hyas* sp. transferred to 75‰ seawater (Prosser and Brown, 1965). Osmotic adaptation requires longer times in strongly osmoregulating species, such as 48 h in osmoregulating crabs (Charmantier, 1998) and up to 96 h in crayfish (Susanto and Charmantier, 2000). The short acclimation time found in *B. therymydron* probably indicates a relatively high exchange of water and ions between the organism and the external medium and a high permeability of the body surface in this species; it also reflects the weak salinity stress applied.

### Hydromineral regulation

*B. therymydron* osmoconformed over the narrow range of tolerable salinities. When salinity varied, the hemolymph osmolality tended to follow the external osmolality, with a slight positive difference of only about 15 mosm/kg. This is probably due to the colloid osmotic pressure of plasma proteins. *B. therymydron* is therefore an osmoconformer like the Majidae *Libinia emarginata*, *Pugettia producta* (Mantel and Farmer, 1983), *Maja* sp. (Potts and Parry, 1963), and *Chionoecetes* sp. (Mantel and Farmer, 1983; Hardy *et al.*, 1994); the Cancridae *Cancer antennarius* (Jones, 1941; Gross, 1964) and *C. pagurus* (Péqueux, 1995); and the Calappidae *Calappa hepatica* (Kamemoto and Kato, 1969). As already noted by different authors (reviewed in Mantel and Farmer, 1983; Péqueux, 1995), osmoconformity does not permit survival at salinities widely different from seawater, and these osmoconformers are marine stenohaline species. As in other crustaceans, the osmolality of the hemolymph of *B. therymydron* was mostly due to inorganic ions, essentially  $\text{Na}^+$  and  $\text{Cl}^-$  (Péqueux, 1995), which accounted for about 95% of the total hemolymph osmolality. The regulation of these ions was almost iso-ionic. In these crabs, hemolymph  $\text{Cl}^-$  and  $\text{Na}^+$  concentrations respectively represented about 93% and 102% of the same ion concentrations in the medium. There is thus a slight excess of  $\text{Na}^+$  and a slight deficit of  $\text{Cl}^-$  in the hemolymph compared to the medium, as is noted in other osmoconformers such as the Majidae *Libinia emarginata* (Gilles, 1970), *Maja* sp. (Potts and Parry, 1963), and *Chionoecetes opilio* (Hardy *et al.*, 1994); the Cancridae *Cancer antennarius* (Gross, 1964); and the Calappidae *Calappa hepatica* (Spencer *et al.*, 1979).

In *B. therymydron*, the hemolymph  $\text{Ca}^{2+}$  concentration was slightly hyper-regulated, as noted by Prosser (1973) in marine crustaceans.  $\text{K}^+$  concentration was slightly hyper-hypo-regulated. However, among crustaceans,  $\text{K}^+$  is often found in higher concentration in the hemolymph than in the medium (Mantel and Farmer, 1983).

In *B. therymydron*,  $\text{Mg}^{2+}$  concentration was strongly hypo-regulated. The concentration of this ion was about 44% of that found in the medium, a percentage included in the

standard range of hemolymph  $Mg^{2+}$  concentration for brachyurans, that is, between 20% and 80% of the medium concentration (Prosser, 1973). As in several species of crabs,  $Mg^{2+}$  might be excreted through the antennal glands (Morritt and Spicer, 1998). Other osmoconformers such as *Maja squinado* or *Hyas sp.*, which are relatively “unresponsive” (slow-moving) species, have higher hemolymph  $Mg^{2+}$  concentration (about 80% of that of seawater) (Robertson, 1960; Frederich *et al.*, 2000). *B. thermydron* exhibits a hemolymph  $Mg^{2+}$  concentration closer to that of more “active” crabs such as *Carcinus maenas* and *Pachygrapsus marmoratus*, in which the ion concentration is below 50% of that found in the medium (Robertson, 1960; Frederich *et al.*, 2000). This fact can be related to the active locomotor behavior of *B. thermydron* (Williams, 1980; Guinot, 1988; Guinot and Segonzac, 1997), which is evident in visual observations and video monitoring (Jean-Yves Toullec, pers. obs.) that show the crabs frequently moving on chimneys, in and out of the warm areas, and among the vestimentiferans or mussels on which they feed. In addition, these results show that the crabs had retained a strong ability to hypo-regulate  $Mg^{2+}$  in their hemolymph after their transfer to the surface and one or two days of exposure to different media. Thus, their osmoconformity and their  $Na^+$  and  $Cl^-$  iso-regulation most probably result from a specific pattern and not from damage to the integument or serious stress due to the pressure change associated with bringing the crabs to the surface.

Exposure to high pressure did not affect the hemolymph osmolality of *B. thermydron* exposed to low salinity, when compared to crabs kept at atmospheric pressure. In these deep-sea hydrothermal crabs, osmoconformity thus appears to be unaffected by a change in hydrostatic pressure. This contrasts with the few tested epibenthic crabs in which osmotic and ionic regulation may vary in relation to pressure. For instance, short-term exposure (1–3 h) to pressure of 50–100 bars significantly affected the concentration of the inorganic ions ( $Na^+$ ,  $K^+$ ,  $Cl^-$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ) in hemolymph of *Carcinus maenas* (Péqueux and Gilles, 1984), but changed only the  $Ca^{2+}$  content of the hemolymph in *Eriocheir sinensis* (Sébert *et al.*, 1997).

#### Ecological implications

Because *B. thermydron* is a marine stenohaline osmoconformer, we may hypothesize that this species occupies a deep hydrothermal habitat where salinity is stable and close to that of seawater. This hypothesis has been verified in the present study through direct measurements of the ambient salinity. The salinity of the hydrothermal water directly measured on samples taken on the 9°N and 13°N EPR was approximately 32.7‰ to 34.3‰–34.7‰. These values are close to the salinity of standard Pacific seawater, 34.62‰ (Ivanoff, 1972). The osmoregulation pattern of *B. thermy-*

*dron* confirms that these crabs live in an environment where salinity is stable and close to 33‰–35‰. In addition, these values of salinity add to the knowledge of the ambient parameters of the deep-sea hydrothermal environment (Mid-Atlantic Ridge or East Pacific Rise) where  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Cl^-$ ,  $PO_4^{3-}$ ,  $NO_2^-$ ,  $NH_4^+$ ,  $NO_3^-$ , and  $NO_2^-$  concentrations have been measured (Truchot and Lallier, 1998; Sarradin *et al.*, 1998, 1999).

#### Phylogeny and osmoregulatory adaptation

The phylogenetic origin of the Bythograeidae is disputed (Delamare Deboutteville and Guinot, 1981; Guinot, 1988, 1990). According to Williams (1980), Tudge *et al.* (1998), and Sternberg *et al.* (1999), *Bythograea thermydron* exhibits some similarities to Potamoidea (Potamidae), Portunoidea (Portunidae), and Xanthoidea (Goneplacidae; Xanthidae; Trapeziidae). The marine stenohaline osmoconformer *B. thermydron* may thus have derived from families consisting mainly of crab species that are able to strongly osmoregulate (Jones, 1941; Shaw, 1959; Robertson, 1960; Ballard and Abbott, 1969; Kamemoto and Kato, 1969; Harris and Micallef, 1971; Taylor *et al.*, 1977; Birchard *et al.*, 1982; Blasco and Forward, 1988; review in Mantel and Farmer, 1983). During its evolution, *B. thermydron* would have lost its ancestor’s osmoregulatory ability, which had become superfluous in an environment where salinity is stable. A similar, if not identical, pattern of evolution has been reported in some freshwater caridean shrimps—for example, *Palaemonetes paludosus* (Dobkin and Manning, 1964) and *P. argentinus* (Charmantier and Anger, 1999). These species, which live in fresh water or in low-salinity habitats, have lost the useless function of hypo-regulation usually present in osmoregulatory caridean shrimps and have retained only the capacity to hyper-regulate.

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