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A cellular and metabolic assessment of the thermal stress responses in the endemic gastropod *Benedictia limnaeoides ongurensis* from Lake Baikal

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ABSTRACT

Our objective was to determine if the Lake Baikal endemic gastropod *Benedictia limnaeoides ongurensis*, which inhabits in stable cold waters expresses a thermal stress response. We hypothesized that the evolution of this species in the stable cold waters of Lake Baikal resulted in a reduction of its thermal stress-response mechanisms at the biochemical and cellular levels. Contrary to our hypothesis, our results show that exposure to a thermal challenge activates the cellular and biochemical mechanisms of thermal resistance, such as heat shock proteins and antioxidative enzymes, and alters energetic metabolism in *B. limnaeoides ongurensis*. Thermal stress caused the elevation of heat shock protein 70 and the products of anaerobic glycolysis together with the depletion of glucose and phosphagens in the studied species. Thus, a temperature increase activates the complex biochemical system of stress response and alters the energetic metabolism in this endemic Baikal gastropod. It is concluded that the deepwater Lake Baikal endemic gastropod *B. limnaeoides ongurensis* retains the ability to activate well-developed biochemical stress-response mechanisms when exposed to a thermal challenge.

1. Introduction

Temperature is one of the major environmental factors that influence all aquatic organisms. The temperature limits within which a species can successfully live are different, and for some species the limits can be very narrow. This range is primarily supported by the metabolic scope and the activity of stress-response mechanisms (Somero, 2002; Pörtner and Knust, 2007; Pörtner, 2010).

Different cellular mechanisms are involved in the maintenance of physiological homeostasis within the temperature range of a species. The most important of them is heat shock proteins, the antioxidative system and the mechanisms of alterations of general energetic metabolism (Viarengo et al., 1995; Blokhina et al., 2003; Chown and Storey, 2006; Timofeyev et al., 2008, 2009; Arad et al., 2010; Bedulina et al., 2010a; Pöhlmann et al., 2011; Mizrahi et al., 2012; Qian et al., 2012). These mechanisms allow an organism to overcome the negative consequences of thermal stress, including the accumulation of free radicals, protein degradation and energy depletion (Pörtner et al., 1999; Hofmann, 2005; Afonso et al., 2008; Tomanek, 2010).

In previous studies, it was shown that those species that evolved under thermally constant conditions have a decreased ability to cope with stress due to the reduction of basic cellular stress-response mechanisms. Such a reduction can be found in several Antarctic species (fishes, sea stars, gammarids, ciliates) and some thermosensitive hydrozoa that lost the heat-inducible expression of heat shock proteins (HSP) (Bosch et al., 1988; Brennecke et al., 1998; Hofmann et al., 2000, 2005; La Terza et al., 2007; Clark et al., 2008). Also, in Lake Baikal, deep-water amphipods have a reduced ability to activate antioxidant enzymes upon oxidative stress (Timofeyev et al., 2006). Therefore, the question of whether the reduction of a stress response is a common result of the evolution of animals in a thermally constant environment should be considered.

The endemic fauna of Lake Baikal is a promising object for such study. Lake Baikal in southeastern Siberia is the oldest and deepest freshwater body on earth, containing approximately 20% of the earth's liquid fresh water (Kozhova and Izmest'eva, 1998). The lake has an estimated age of 25–30 million years, and environmental conditions of the open water and deep-water zones have remained similar to their current states for the last 2 million years (Kozhova and Izmest'eva, 1998). The ecosystem of Lake Baikal is unique. To date, 2595 animal species have been described from Lake Baikal, with 80% of them being endemic. Gastropods (Gastropoda, Mollusca) are one of the largest groups of macroinvertebrates, represented by 148 species and 24 subspecies (Kozhova and Izmest'eva, 1998; Timoshkin et al., 2001).

Lake Baikal has specific abiotic characteristics: high oxygen content throughout the entire water column, stable, low water temperatures with long seasonal ice coverage and oligotrophic conditions.

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Environmental conditions below 30–50 m are characterized by very narrow fluctuations in temperature (3.5 to 6 °C at depths of 30–100 m and a constant temperature of 3.5 °C at depths below 100 m), constant oxygen concentrations and hydrochemical composition, and super-oligotrophic conditions (Kozhova and Izmest'eva, 1998). A rich fauna of endemic animals inhabits these depths, including dozens of gastropod species. The fauna of endemic gastropods in Baikal is suggested to be younger than 2–4 million years (Sherbakov, 2008). The evolution of deepwater gastropods in Lake Baikal is linked with the period of the formation of deepwater zones.

The adaptive evolution of gastropods along a gradient of environmental change could be related to the reduction of thermal fluctuation in their environment. We hypothesize that evolution in the stable thermal conditions of the Baikal sublittoral and deepwater zones could cause the reduction of the primary biochemical and cellular stress-response mechanisms in endemic gastropods inhabiting such zones. Our objective was to expose the animals inhabiting these thermally stable conditions to a thermal challenge to estimate whether they are able to express a thermal stress response.

2. Materials and methods

2.1. Animals

The endemic gastropod *Benedictia limnaeoides ongurensis* (Kozhov, 1936) was chosen as the object for this study. This species is omnivorous and typically inhabits sand–silt substrates with detritus across a wide depth range from 5 to 120 m (Sitnikova et al., 2004). This species can be acclimated to laboratory conditions and live there without loss of activity and feeding rate. So, high vertical and horizontal distribution, and dissemination of this species in Baikal allow them to be a promising object for laboratory exposure experiments. We hypothesized that evolution of this species in the stable cold waters of Lake Baikal resulted in a reduction of its thermal stress-response mechanisms at the biochemical and cellular levels.

According to our experimental data, the temperature causing 50% mortality in 24 h ($LT_{50}^{24\ h}$) was 25 °C for this species. Therefore, *B. limnaeoides ongurensis* appears to be a thermosensitive species.

Up to 50 animals were collected in August near Cape Khorin-Irgi in the Ol'khonskie Vorota strait (Middle Baikal, N 53°03.442′, E 106°53.550′) using a benthic dragnet at depths of 30–40 m. Snail diameters ranged from 1.5 to 1.7 cm (n = 12), whereas masses ranged from 1.8 to 2.5 g (n = 25). Immediately following sampling, the gastropods were placed into a container with 6 °C water and with constant aeration, and then they were transferred to the laboratory. The gastropods were pre-acclimated in Lake Baikal water at constant conditions of 6 °C (which correspond to the highest temperature at 30–40 m in depth) and with intense aeration for one week. During the acclimation period, the gastropods were fed *ad libitum* with Tetramin food (Tetra, Germany).

2.2. Experimental design

To estimate snail thermotolerance at the biochemical level, the animals were exposed to a gradual temperature increase of 1 °C per hour, beginning at the temperature of pre-acclimation (6 °C) and continuing until the time point when 100% mortality occurred (modified method described in Sokolova and Pörtner (2003)). Thus, in 0.5 h temperature increased to 6.5 °C, in 1 h − 7 °C, etc. 6 individuals were placed into well-aerated glass aquaria containing 2 L of Baikal water. After every 5° of temperature elevation, 1 specimen was collected from each of 5 aquaria and shock-frozen in liquid nitrogen. Control samples were collected from an aquaria with a temperature of 6 °C before experiments were initiated.

2.3. Biochemical methods

Four critical factors of cellular thermal tolerance were tested under the experimental conditions: level of lipid peroxidation, synthesis of the heat shock protein HSP70, activity of antioxidative enzymes, and the state of energetic metabolism.

2.3.1. Lipid peroxidation level

To determine lipid peroxidation level, the concentration of Schiff bases was measured (Barata et al., 2005; Pietrzak et al., 2010). For extraction of Schiff bases, we used a mixture of heptane and isopropanol (ratio 1:1), followed by the addition of water, intensive stirring and use of a thermostation (30 min, 25 °C). This method led to the separation of homogenate from the heptane and isopropanol fraction. The supernatant of isopropanol fraction was obtained by centrifugation (16000 *g*, 2 min) and by the addition of 97% ethanol (ratio 1:3). Spectrophotometric analysis of samples was performed at λ = 400 nm in quartz cuvettes.

2.3.2. HSP70 level

The concentration of HSP70 (70 kDa) was determined using SDS-PAGE electrophoresis followed by Western blotting (Bedulina et al., 2013). Each specimen was separately disrupted under liquid nitrogen with a mortar and pestle, and the total proteins were extracted in 0.1 M Tris–HCl buffer (pH = 7.6) with the addition of 1% protease inhibitor cocktail (Amresco, no. Am. N-221 − 1.0) and 1% 1 M PMSF (no. Am O754—5.0). Samples were then centrifuged for 15 min at 7000 *g*, and the supernatant was dissolved in 0.5 M Tris–HCl loading buffer (pH = 6.8). SDS-PAGE electrophoresis in 10% polyacrylamide gel was followed by Western blotting to PVDF membranes. Ponceau Red (Amresco, no. Am-0860 − 0.05) staining was used as a loading control. The transfer membranes were then blocked overnight in 2.5% nonfat dry milk in TTBS (pH 7.5) and 0.02% sodium azide. For HSP70 identification was used as positive control (HSP70 from bovine brain, Sigma, Chemical Co. H9776). The blocked membranes were washed twice in TTBS for 10 min and incubated overnight in Bovine monoclonal Anti-HSP70 (monoclonal anti-heat shock protein 70, produced in mouse, Sigma Chemical Co., no. H5147) (Katsikatrou et al., 2011; Mizrahi et al., 2011), followed by two washes for 10 min in TTBS and then the incubation in secondary anti-mouse antibodies (Stressgen, no. SAB-101). As an internal control, the β-actin (45 kDa) level was estimated. The membranes were incubated for 24 h in the chicken anti-actin antibodies (polyclonal anti-actin antibody produced in rabbit, Sigma Chemical Co., no. A2668), and after two 10 minute-TTBS washes, they were incubated in secondary anti-rabbit antibodies (Sigma, Chemical Co. no. A9919) (Bedulina et al., 2013). The protein-antibody complexes were detected using BCIP-nBT. Densitometrical analyses were performed using ImageJ v.1.41 (Wayne Rasband).

2.3.3. Antioxidative enzymes activity

The activities of antioxidative enzyme peroxidase (EC 1.11.1.1) and catalase (EC 1.11.1.6) were estimated using the methods of Drotar et al. (1985) and Aebi (1984) modified according to Timofeyev et al. (2006) and Bedulina et al. (2010b), at λ = 340 nm for peroxidase and λ = 240 nm for catalase. The activity of lactate dehydrogenase (EC 1.1.1.27) was determined using the express-kit "LDH-vital" (Vital-Diagnostics Spb, Russia) at λ = 340 nm.

2.3.4. Metabolites

The level of glucose, glycogen, L-arginine, phospho L-arginine, L-alanine and acetate was measured using the NADH/NADPH-dependent enzymatic methods at λ = 340 nm according to Grieshaber et al. (1994), Bergmeyer (1985) and Sokolova et al. (2012). Deposited energetic metabolites (glycogen, phospho L-arginine) were hydrolyzed using the methods of Morris et al. (2005), Ivanina et al. (2010) and Sokolova et al. (2012). The level of lactate was determined using the

express-kit "Lactate-vital" (Vital-Diagnostics Spb, Russia). Spectrophotometry was performed with a Cary 50-spectrophotometer (Varian, USA).

2.4. Statistics

All experiments were performed with at least 5 biological replicates, and biochemical measurements were performed in triplicate for each sample. Data were analyzed statistically using one-way analysis of variance (ANOVA, general linear model) and the statistical package, Statistica 5.0 (StatSoft). Correlation analysis was used to quantify the relationship of measured parameters to temperature and between each other. Differences were considered to be significant at $p < 0.05$ (alpha 0.05). The results were compared with those from the control groups.

3. Results

The concentration of Schiff bases in the control *B. limnaeoides ongurensis* was 17 ± 2.3 nMol/g wet mass. The temperature increase resulted in an elevated level of Schiff bases (40% above control value) beginning at 20 °C (Fig. 1A).

The constitutive concentration of HSP70 with a molecular mass of 71 kDa was detected in the control animals. The concentration of HSP70 increased significantly to 109% at 10 °C, followed by a decrease to the control value at 20 °C; however, at 15 °C, it was still significantly higher than the control value. The elevation of the temperature to 25 °C led to a decrease in the HSP70 level (Fig. 1B,C).

The antioxidative enzyme activity changed during the exposure. The elevation of the temperature to 10 °C resulted in a significant increase of peroxidase activity, 146% compared with the control level (0.010 ± 0.001 nKat/mg protein). The activity remained elevated in the range of 64–146% throughout the exposure (Fig. 2A). The control level of the catalase activity was 560.6 ± 24.6 nKat/mg protein. The gradual temperature increase resulted in a short increase in catalase activity at 18% above the control value at 10 °C, followed by a decrease in activity to the control levels (Fig. 2B).

The basal concentration of lactate in the control animals was 114.33 ± 17.1 nMol/g wet mass, and the lactate dehydrogenase activity was 92.1 ± 12.7 nKat/mg protein. During the exposure, the concentration of lactate increased significantly at 10 °C and 15 °C by approximately 50% above the control level, followed by a decrease to the control level at 20 °C and a significant decrease to 40% at 25 °C (Fig. 3A). In contrast, the lactate dehydrogenase activity rose by 50% of the control value at 20 °C and remained elevated at 25 °C (Fig. 3B).

Exposure to the gradual temperature elevation led to a significant decrease in glycogen and free glucose. The basal concentration of glucose in tissues was 0.1 ± 0.02 μMol/g wet mass and the concentration of glycogen was 8.2 ± 0.15 μMol/g wet mass. The concentration of free glucose decreased at 10 °C of exposure and remained decreased by 30–65% below the control value until the end of the exposure (Fig. 4A). The glycogen level began to decrease at 15 °C and continued to decrease by 35–40% until the end of the exposure (Fig. 4B).

The concentration of deposited phospho-L-arginine in the control group of animals was 60.9 ± 2.07 μMol/g wet mass with a concentration of L-arginine of 0.3 ± 0.01 μMol/g wet mass. Although the concentration of L-arginine did not change during the exposure (Fig. 5A), the phospho-L-arginine concentration decreased significantly by 10–15% at temperatures beginning from 15 °C. A temperature of 25 °C resulted in the return of the phospho-L-arginine concentration to the control value (Fig. 5B).

The concentrations of acetate and L-alanine rose significantly at 15 °C. The concentration of acetate increased by 19% above the basal level (2.6 ± 0.21 μMol/g wet mass) and then decreased with further temperature elevation (Fig. 6A). The concentration of another marker of anaerobiosis, L-alanine, gradually increased by 15% above the control value (0.3 ± 0.01 μMol/g wet mass) beginning at 15 °C until the end of the exposure (Fig. 6B).

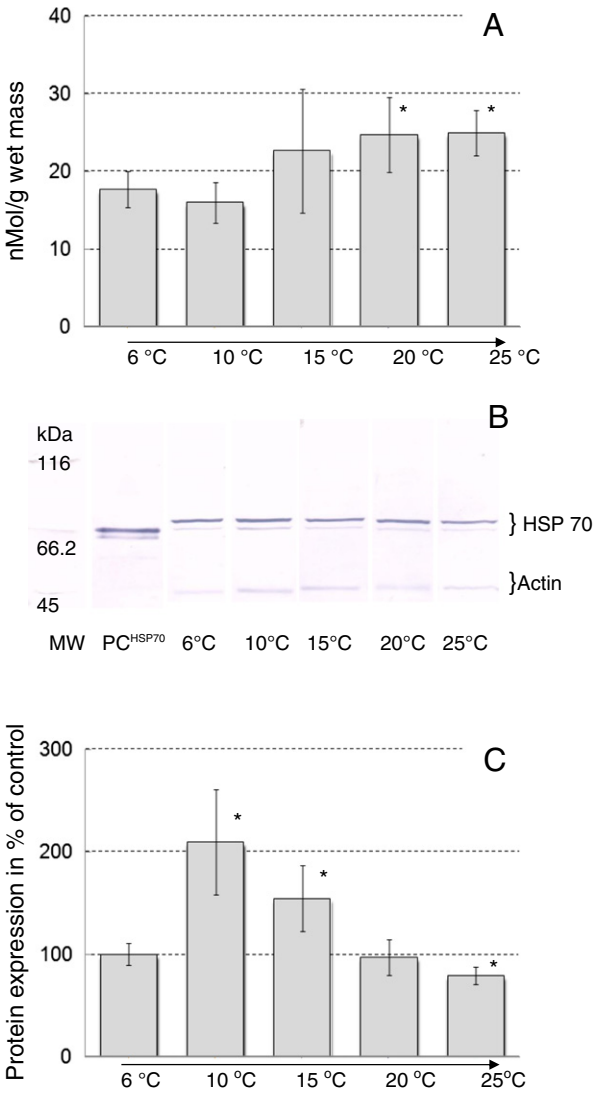

Fig. 1. Schiff bases levels (A) and HSP70 (B — Western Blotting and; C — levels) in Lake Baikal endemic gastropod *B. limnaeoides ongurensis* under expose to gradual thermal challenge from 6 °C (control) to 25 °C. *Indicates significant difference ($p < 0.05$) from the control (6 °C).

4. Discussion

Endemic gastropods inhabiting sublittoral and deepwater zones have not been tested previously for their stress-response. This is also the first report regarding the thermal stress-response of Baikal gastropods.

Numerous previous studies have shown that temperature increases provoke the activation of some cellular and biochemical stress-response mechanisms such as HSP70 and antioxidative system due to the accumulation of reactive oxygen species and the degradation of cellular proteins (Hofmann, 2005; Chown and Storey, 2006; Afonso et al., 2008; Arad et al., 2010; Tomanek, 2010; Pöhlmann et al., 2011; Mizrahi et al., 2012). In addition, temperature increase leads to the depletion of the free- and deposited energetic metabolites and to the switch from aerobic to anaerobic metabolism (Grieshaber et al., 1994).

As our study shows, the temperature increase led to the accumulation of Schiff bases as a marker of lipid peroxidation, an increase in HSP70 content, and increased concentrations of lactate, acetate and L-alanine. Significant activation of antioxidative enzymes and the

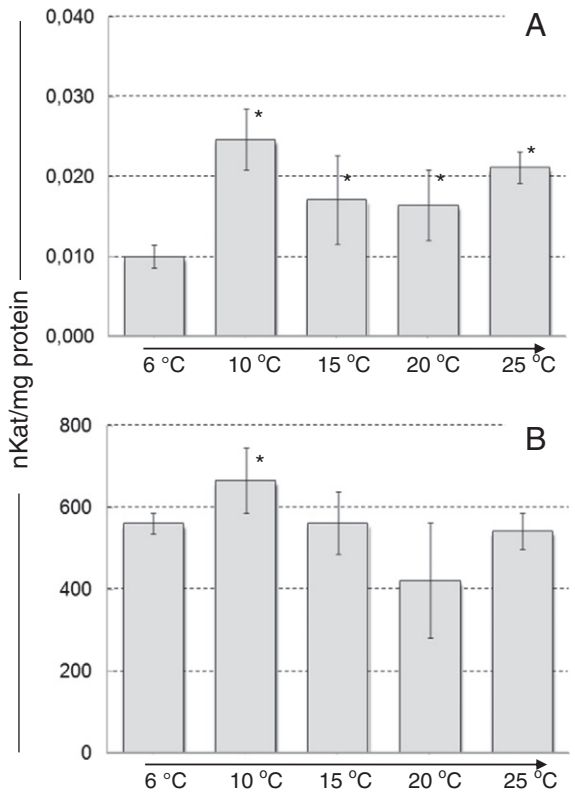

Fig. 2. Peroxidase (A) and Catalase (B) activities in Lake Baikal endemic gastropod *B. limnaeoides ongurensis* under expose to gradual thermal challenge from 6 °C (control) to 25 °C. *Indicates significant difference ($p < 0.05$) from the control (6 °C).

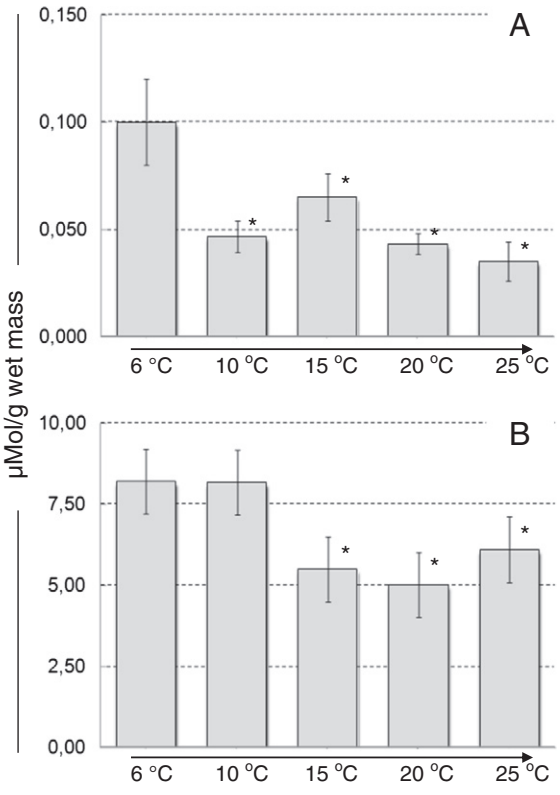

Fig. 4. Glucose (A) and Glycogen (B) levels in Lake Baikal endemic gastropod *B. limnaeoides ongurensis* under expose to gradual thermal challenge from 6 °C (control) to 25 °C. *Indicates significant difference ($p < 0.05$) from the control (6 °C).

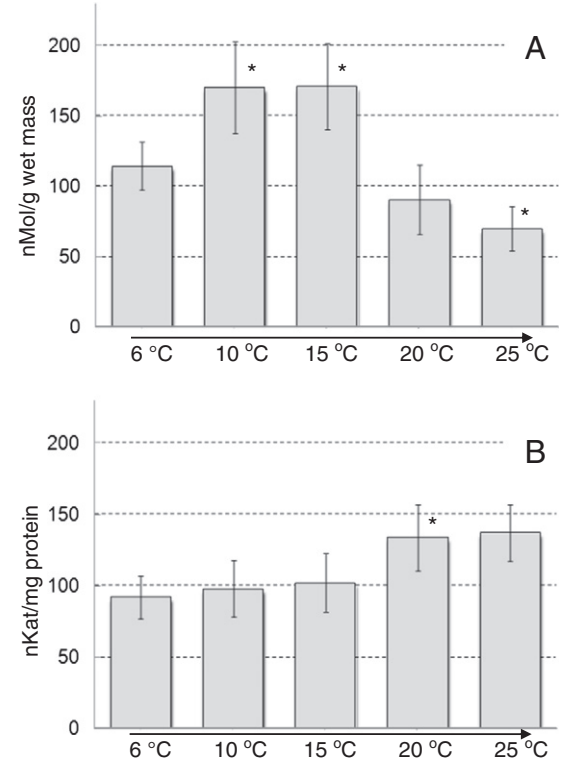

Fig. 3. Lactate content (A) and lactate dehydrogenase activities (B) in Lake Baikal endemic gastropod *B. limnaeoides ongurensis* under expose to gradual thermal challenge from 6 °C (control) to 25 °C. *Indicates significant difference ($p < 0.05$) from the control (6 °C).

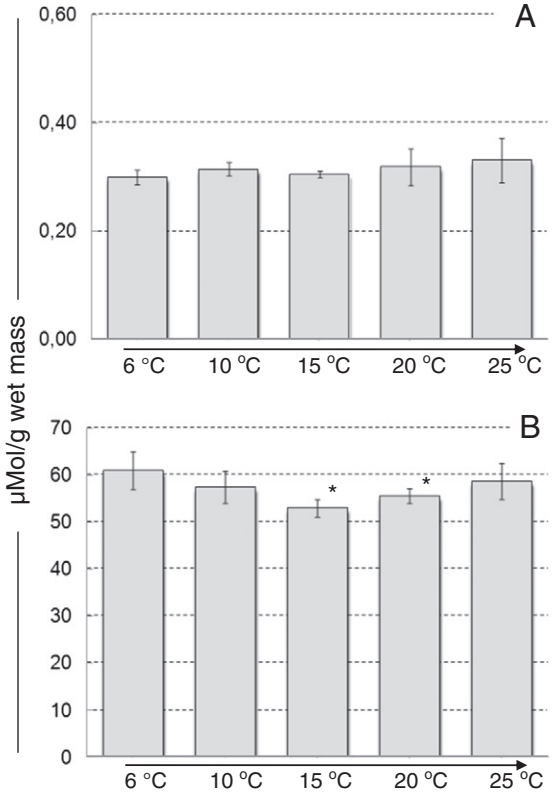

Fig. 5. L-arginine (A) and Phospho-L-arginine (B) levels in Lake Baikal endemic gastropod *B. limnaeoides ongurensis* under expose to gradual thermal challenge from 6 °C (control) to 25 °C. *Indicates significant difference ($p < 0.05$) from the control (6 °C).

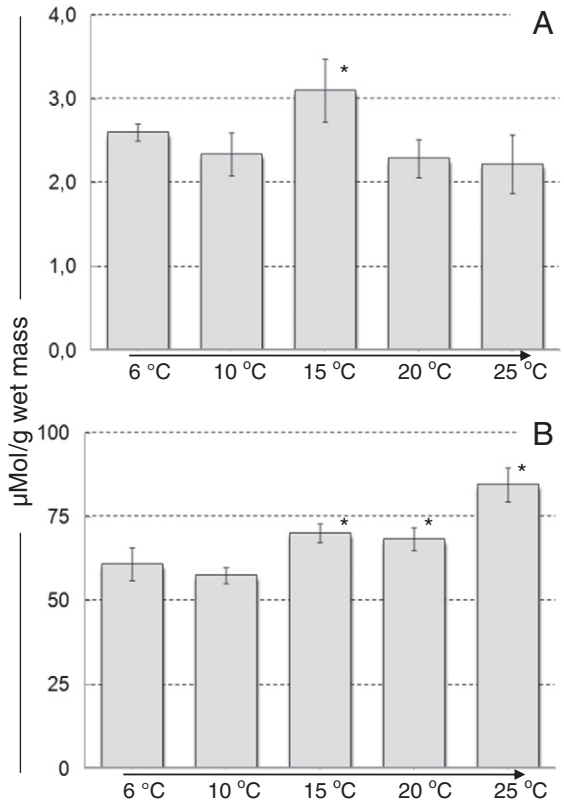

Fig. 6. Acetate (A) and L-alanine (B) levels in Lake Baikal endemic gastropod *B. limnaeoides ongurensis* under expose to gradual thermal challenge from 6 °C (control) to 25 °C. *Indicates significant difference ($p < 0.05$) from the control (6 °C).

depletion of energetic metabolites (glucose, glycogen and phosphagens) were also observed in *B. limnaeoides ongurensis*.

As mentioned above, the induction of HSP70 is a well-known key mechanism of thermal resistance. These proteins play an important role in protein homeostasis and folding, both in normal physiological conditions and under a variety of proteotoxic stresses, acting as molecular chaperones and preventing protein degradation (Hofmann, 2005; Tomanek, 2010; Shatilina et al., 2011; Bedulina et al., 2013). An increase in the HSP70 concentration in *B. limnaeoides ongurensis* begins to occur at 10 °C and seems to act as a protection against protein denaturation. Protein denaturation should result from direct temperature influence, and acidosis should develop due to lactate accumulation. In addition, the increase in lactate concentration could be indirect evidence of destructive processes in the mitochondrial membranes, where the electron transport chain is localized. Because the damaged membranes are not able to create a H+-gradient, pyruvate converts into lactate to release the hydrogen carriers, such as FAD+ and NAD+, which are necessary for glycolysis (Mathews et al., 2000; Larade and Storey, 2002; Philp et al., 2005). The final stage of exposure at high temperatures causes a simultaneous decrease in lactate concentration and an increase in lactate dehydrogenase activity which indicates the utilization of acetate to obtain energy under conditions of general metabolic depression.

This metabolic depression, with a predominance of oxidizing, catabolic and proteolytic processes, is a well-known reaction to thermal stress (Grieshaber et al., 1994). Our results show that the elevation of temperature to 15 °C leads to several metabolic switches and the depletion of the available phosphagen energy. As a consequence, gastropods use the deposited phosphagens, such as phospho-L-arginine. Energy released from the deposited phosphagens allows the functioning of cellular metabolic pathways, for example the synthesis of new HSP70. Decreases of the glucose and glycogen concentrations indicate the intensification of metabolism and the growing energy consumption under thermal stress. The progressive physiological and energetic depression corresponds to the elevation of the lipid peroxidation rate, the level of L-alanine and acetate, and the decrease in HSP70 and lactate during the last stage of exposure. These changes could be due to switching from aerobic to less efficient anaerobic metabolism.

Correlation analyses demonstrated a close interconnection between aerobic and anaerobic metabolism and the main cellular and biochemical stress-resistance mechanisms (Table 1). The highest positive correlations were found between concentrations of HSP70 and lactate ($r = 0.67$), concentrations of glycogen and phospho-L-arginine ($r = 0.89$), and the activity of lactate dehydrogenase and the concentration of L-alanine ($r = 0.78$). The highest negative correlations were detected between concentrations of glucose and L-arginine ($r = -0.94$), glycogen and L-alanine ($r = -0.96$) and Schiff base concentrations ($r = -0.97$), lactate dehydrogenase activity and catalase activity ($r = -0.67$), and the concentrations of lactate ($r = -0.77$), glucose ($r = -0.75$) and glycogen ($r = -0.70$). The changes in the concentrations of phospho-L-arginine, L-alanine, Schiff bases and the activity of lactate dehydrogenase exhibited a positive correlation with temperature ($r = -0.87$, -0.89, -0.90, -0.95, respectively). Negative correlations with temperature were found for the concentrations of lactate ($r = -0.61$), glucose ($r = -0.79$) and glycogen ($r = -0.77$).

The beginning of the accumulation of HSP70 and lactate together with activation of the antioxidative enzymes occurred when the temperature reached 10–15 °C. The temperature increase to 15–20 °C resulted in the depletion of deposited energetic metabolites and the accumulation of anaerobic end products. At 25 °C, an increase in the lipid peroxidation rates with maximal accumulation of L-alanine was observed simultaneously with a decrease in the concentrations of HSP70 and lactate. This latter increase, together with the activation of lactate dehydrogenase, can be taken as the final stage of the stress response in *B. limnaeoides ongurensis*. This final stage is characterized by the exhaustion of the thermal resistance abilities followed by enhanced lipid structural distractions together with anaerobic depression. Such a sequence of biochemical and bioenergetic reactions corresponds to the results of previous ecophysiological studies and it is commonly reported for aquatic animals (Pörtner, 2012).

The temperature of activation of cellular and biochemical stress-response mechanisms can be a critical point for defining the thermal window and the thermotolerance for *B. limnaeoides ongurensis*. This temperature corresponds to the ecological parameters of the species as a sublittoral and deepwater stenothermal gastropod. However, the results of this study clearly show that the temperature exposure activates the cellular and biochemical mechanisms of thermal resistance, such as heat shock proteins and antioxidative enzymes, together with the alteration of energetic metabolism in *B. limnaeoides ongurensis*.

In contrast with studies of Antarctic fishes, hydrozoa and deepwater amphipods, which exhibited a reduced stress response (Feder and Hofmann, 1999), the Lake Baikal endemic gastropod *B. limnaeoides ongurensis* retains the ability to activate their well-developed biochemical stress-response mechanisms. A possible explanation could be the young evolutionary age of Lake Baikal endemic gastropod species, which are considered to be of Pleistocene origin and less than 2–4 million years old (Sherbakov, 2008). In comparison with Antarctic species such as *Trematomus* fishes, whose age is estimated at 15–25 million years (Eastman, 1993), these gastropods should be considered the very youngest group. Thus, they possibly have not had sufficient evolutionary time to develop narrow adaptation to deepwater conditions and to lose their stress response ability. Another possible reason of conserved stress response to thermal stress in studied Benedictiidae family could be relation with a shallow or littoral species. Thus, a Benedictiidae family has a morphological similarity to European genus *Lithoglyphus* and phylogenic narrow similarity to North American genus *Fluminicola* (family Lithoglyphidae), and Amnicolidae family (genus *Erchai* from central Asia) (Sitnikova et al., 2004).

Table 1
Correlation analyses of cellular and metabolic assessment in Lake Baikal endemic gastropod *B. limnaeoides ongurensis*.

	HSP70	Lactate	Glucose	Glycogen	L-arginine	Phospho-L-arginine	L-alanine	Acetate	Schiff bases	Lactate dehydrogenase	Peroxidase	Catalase	Temperature
HSP70		0.84	−0.06	0.49	−0.29	−0.21	−0.71	0.22	−0.73	−0.59	0.54	0.73	−0.53
Lactate	0.84		0.24	0.31	−0.62	−0.44	−0.64	0.71	−0.61	−0.77	0.22	0.66	−0.61
Glucose	−0.06	0.24		0.49	−0.88	0.39	−0.50	0.55	−0.51	−0.75	−0.82	0.14	−0.79
Glycogen	0.49	0.31	0.49		−0.39	0.71	−0.62	−0.07	−0.92	−0.70	−0.01	0.75	−0.77
L-arginine	−0.29	−0.62	−0.88	−0.39		0.00	0.71	−0.78	0.58	0.88	0.62	−0.24	0.87
Phospho-L-arginine	−0.21	−0.44	0.39	0.71	0.00		0.12	−0.50	−0.42	−0.15	−0.29	0.21	−0.32
L-alanine	−0.71	−0.64	0.50	−0.62	0.71	−0.12		−0.29	0.85	0.78	0.15	−0.40	0.89
Acetate	0.22	0.71	0.55	−0.07	−0.78	−0.50	−0.29		−0.17	−0.64	−0.35	0.20	−0.45
Schiff bases	−0.73	−0.61	−0.51	−0.92	0.58	−0.42	0.85	−0.17		0.86	−0.02	−0.77	0.90
Lactate dehydrogenase	−0.59	−0.77	−0.75	−0.70	0.88	−0.15	0.78	−0.64	0.86		0.26	−0.67	0.95
Peroxidase	0.54	0.22	−0.82	−0.01	0.62	−0.29	0.15	−0.35	−0.02	0.26		0.45	0.37
Catalase	0.73	0.66	0.14	0.75	−0,24	0.21	−0.40	0.20	−0.77	−0.67	0.45		−0.53
Temperature	−0.53	−0.61	−0.79	−0.77	0.87	−0.32	0.89	−0.45	0.90	0.95	0.37	−0.53	

However, the verity of these explanations needs support using molecular approaches of complex Baikal gastropods phylogenetic analysis and evaluation of energetic metabolism gene expression and changes in proteome. This should be the promising topic of future studies with Lake Baikal gastropods.

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References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
Afonso, L.O.B., Hosoya, S., Osborne, J., Gamperl, A.K., Johnson, S., 2008. Lack of glucose and hsp70 responses in haddock *Melanogrammus aeglefinus* (L.) subjected to handling and heat shock. J. Fish Biol. 72 (1), 157–167.
Arad, Z., Mizrahi, T., Goldenberg, S., Heller, J., 2010. Natural annual cycle of heat shock protein expression in land snails: desert versus Mediterranean species of *Sphincterochila*. J. Exp. Biol. 213 (20), 3487–3495.
Barata, C., Navarro, J., Varo, I., Riva, M., Arun, S., Porte, C., 2005. Changes in antioxidant enzyme activities, fatty acid composition and lipid peroxidation in *Daphnia magna* during the aging process. Comp. Biochem. Physiol. B 140, 81–90.
Bedulina, D.S., Timofeyev, M.A., Zimmer, M., Zwirnmann, E., Menzel, R., Steinberg, C.E.W., 2010a. Different natural organic matter isolates cause similar stress response patterns in the freshwater amphipod *Gammarus pulex*. Environ. Sci. Pollut. Res. 17, 261–269.
Bedulina, D.S., Zimmer, M., Timofeyev, M.A., 2010b. Sub-littoral and supra-littoral amphipods respond differently to acute thermal stress. Comp. Biochem. Physiol. B 155, 413–418.
Bedulina, D.S., Evgen'ev, M.B., Timofeyev, M.A., Protopopova, M.V., Garbuz, D.G., Pavlichenko, V.V., Luckenbach, T., Shatilina, Z.M., Axenov-Gribanov, D.V., Gurkov, A.V., Sokolova, I.M., Zatsepina, O.G., 2013. Expression patterns and organization of the hsp70 genes correlate with thermotolerance in two congener endemic amphipod species (*Eulimnogammarus cyaneus and E. verrucosus*) from Lake Baikal. Mol. Ecol. 22, 1416–1430.
Bergmeyer, H.U., 1985. Methods of enzymatic analysis. Vol. VI. Metabolites 1: Carbohydrates. Vol. VIII. Metabolites 3: Lipids, Amino Acids and Related Compounds. VCH Verlagsgesellschaft, Weinheim.
Blokhina, O., Violainen, E., Fagerstedt, K.V., 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bot. 91, 179–194.
Bosch, T.C., Krylow, S.M., Bode, H.R., Steele, R.E., 1988. Thermotolerance and synthesis of heat shock proteins: these responses are present in *Hydra attenuata* but absent in *Hydra oligactis*. Proc. Natl. Acad. Sci. U. S. A. 85 (21), 7927–7931.
Brennecke, T., Gellner, K., Bosch, T.C.G., 1998. The belt of a stress response in *Hydra oligactis* is due to reduced Asp70 mRNA stability. Eur. J. Biochem. 255 (3), 703–709.
Chown, S.L., Storey, K.B., 2006. Linking molecular physiology to ecological realities. Physiol. Biochem. Zool. 79 (2), 314–323.
Clark, M.S., Fraser, K.P.P., Peck, L.S., 2008. Lack of an HSP70 heat shock response in two Antarctic marine invertebrates. Polar Biol. 31 (9), 1059–1065.
Drotar, A., Phelps, P., Fall, R., 1985. Evidence for glutathione peroxidase activities in cultured plant cells. Plant Sci. 42, 35–40.
Eastman, J.T., 1993. Antarctic Fish Biology — Evolution in a Unique Environment. Academic Press, San Diego (322 pp.).
Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61 (1), 243–282.
Grieshaber, M.K., Hardewig, I., Kreutzer, U., Pörtner, H.O., 1994. Physiological and metabolic responces to hypoxia in invertebrates. Rev. Physiol. Biochem. Pharmacol. 125, 44–147.
Hofmann, G.E., 2005. Patterns of Hsp gene expression in ectothermic marine organisms on small to large biogeographic scales. Integr. Comp. Biol. 45 (2), 247–255.
Hofmann, G.E., Buckley, B.A., Airaksinen, S., Keen, J.E., Somero, G.N., 2000. Heat-shock protein expression is absent in the antarctic fish *Trematomus bernacchii* (family nototheniidae). J. Exp. Biol. 203 (15), 2331–2339.
Hofmann, G.E., Lund, S.G., Place, S.P., Whitmer, A.C., 2005. Some like it hot, some like it cold: the heat shock response is found in New Zealand but not Antarctic notothenioid fishes. J. Exp. Mar. Biol. Ecol. 316 (1), 79–89.
Ivanina, A.V., Sokolov, E.P., Sokolova, I.M., 2010. Effects of cadmium on anaerobic energy metabolism and mRNA expression during air exposure and recovery of an intertidal mollusk *Crassostrea virginica*. Aquat. Toxicol. 99, 330–342.
Katsikatrou, M., Anestis, A., Portner, H.O., Kampouris, T., Michaelidis, B., 2011. Field studies on the relation between the accumulation of heavy metals and metabolic and HSR in the bearded horse mussel *Modiolus barbatus*. Comp. Biochem. Physiol. C 153, 133–140.
Kozhov, M.M., 1936. Molluscs of Lake Baikal. Systematics, distribution, ecology, some data on the genesis and history. Proc. Limnol. Stn SB AS USS, Moscow-Leningrad, Publ. Acad. Sci., 8 (350 pp., in Russian).
Kozhova, O.M., Izmest'eva, L.R., 1998. Lake Baikal. Evolution and Biodiversity (447 pp.).
La Terza, A., Passini, V., Barchetta, S., Luporini, P., 2007. Adaptive evolution of the heat-shock response in the Antarctic psychrophilic ciliate, *Euplotes focardii*: hints from a comparative determination of the hsp70 gene structure. Antarct. Sci. 19 (2), 239–244.
Larade, K., Storey, K.B., 2002. A profile of metabolic responses to anoxia in marine invertebrates. In: Storey, K.B., Storey, J.M. (Eds.), Sensing, Signaling and Cell, Adaptation. Elsevier Science B.V., pp. 27–46.
Mathews, C.K., Van Holde, K.E., Ahern, K.G. (Eds.), 2000. Biochemistry, 3rd ed. Addison Wesley Longman, San Francisco (1186 pp.).
Mizrahi, T., Heller, J., Goldenberg, S., Arad, Z., 2011. Heat shock protein expression in relation to reproductive cycle in land snails: implications for survival. Comp. Biochem. Physiol. A 160, 149–155.
Mizrahi, T., Heller, J., Goldenberg, S., Arad, Z., 2012. Heat shock proteins and survival strategies in congeneric land snails (Sphincterochila) from different habitats. Cell Stress Chaperones 17 (5), 523–527.
Morris, S., van Aardt, W.J., Ahern, M.D., 2005. The effect of lead on the metabolic and energetic status of the Yabby, Cherax destructor, during environmental hypoxia. Aquat. Toxicol. 75, 16–31.
Philp, A., Macdonald, A.L., Watt, P.W., 2005. Lactate — a signal coordinating cell and systemic function. J. Exp. Biol. 208, 4561–4575.
Pietrzak, A., Michalak-Stoma, A., Chodorowska, G., Szepietowski, J.C., 2010. Lipid disturbances in psoriasis: an update. Review article. Mediat. Inflamm. http://dx.doi.org/10.1155/2010/535612.
Pöhlmann, K., Koenigstein, S., Alter, K., Abele, D., Held, C., 2011. Heat-shock response and antioxidant defense during air exposure in Patagonian shallow-water limpets from different climatic habitats. Cell Stress Chaperones 16 (6), 621–632.
Pörtner, H.O., 2010. Oxygen- And capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. 213 (6), 881–893.

Pörtner, H.O., 2012. Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. Mar. Ecol. MEPS 470, 273–290.

Pörtner, H.O., Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315, 95–97.

Pörtner, H.O., Peck, L., Zielinski, S., Conway, L.Z., 1999. Intracellular pH and energy metabolism in the highly stenothermal Antarctic bivalve *Limopsis marionensis* as a function of ambient temperature. Polar Biol. 22 (1), 17–30.

Qian, Z., Liu, X., Wang, L., Wang, X., Li, Y., Xiang, J., Wang, P., 2012. Gene expression profiles of four heat shock proteins in response to different acute stresses in shrimp *Litopenaeus vannamei*. Comp. Biochem. Physiol. C 156 (3), 211–220.

Shatilina, Z.M., Riss, H., Protopopova, M.V., Trippe, M., Meyer, E.I., Pavlichenko, V.V., Bedulina, D.S., Axenov-Gribanov, D.V., Timofeyev, M.A., 2011. The role of the heat shock proteins (HSP70 and sHSP) for the thermotolerance of freshwater amphipods from contrasting habitats. J. Therm. Biol. 36 (2), 142–149.

Sherbakov, D.Y., 2008. Molecular-genetic studies of evolutionary mechanisms on baicalian endemics. In: Rusinek, O.T., Fialkov, V.A. (Eds.), Evolution of Life in the Process of Abiotic Changes on the Earth. RAS, Novosibirsk, pp. 425–429.

Sitnikova, T.Y., Starobogatov, Y.I., Shirokaya, A.A., Shibanova, I.V., Korobkova, N.V., Adov, F.V., 2004. Chapter 1. Gastropoda. In: Tomoshkin, O.A., Sitnikova, T.Y., Rusinek, O.T., et al. (Eds.), Index of Animal Species Inhabiting Lake Baikal and its Catchment Area, Book 2. Nauka, Novosibirsk, pp. 937–1003.

Sokolova, I.M., Pörtner, H.O., 2003. Metabolic plasticity and critical temperatures for aerobic scope in a eurythermal marine invertebrate (*Littorina saxatilis*, Gastropoda: Littorinidae) from different latitudes. J. Exp. Biol. 206 (1), 195–207.

Sokolova, I.M., Frederich, M., Bagwea, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar. Environ. Res. 79, 1–15.

Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integr. Comp. Biol. 42 (4), 780–789.

Timofeyev, M., Shatilina, Z.M., Kolesnichenko, A., Kolesnichenko, V., Steinberg, C.E.W., 2006. Specific antioxidants reaction to oxidative stress promoted by natural organic matter (NOM) in two endemic amphipod species from Lake Baikal. Environ. Toxicol. 21, 104–110.

Timofeyev, M., Shatilina, Z.M., Bedulina, D.S., Protopopova, M.V., Pavlichenko, V., Grabelnich, O.I., Kolesnichenko, A.V., 2008. Evaluation of biochemical responses in Palearctic and Lake Baikal endemic amphipod species exposed to $CdCl_2$. Ecotoxicol. Environ. Saf. 70 (1), 99–105.

Timofeyev, M., Shatilina, Z.M., Protopopova, M.V., Bedulina, D.S., Pavlichenko, V.V., Kolesnichenko, A.V., Steinberg, C.E.W., 2009. Thermal stress defense in freshwater amphipods from contrasting habitats with emphasis on small heat shock proteins (sHSPs). J. Therm. Biol. 34, 281–285.

Timoshkin, et al., 2001. Index of Animal Species Inhabiting Lake Baikal and Its Catchment Area: In 2 volumes. — Novosibirsk: Nauka. Vol. I: Lake Baikal. Book 1 (832pp.).

Tomanek, L., 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. J. Exp. Biol. 213 (6), 971–979.

Viarengo, A., Canesi, L., Martinez, P., Peters, L., Livingstone, D., 1995. Prooxidant processes and antioxidant defense systems in the tissues of the Antarctic scallop (*Adamussium colbecki*) compared with the Mediterranean scallop (*Pecten jacobaeus*). Comp. Biochem. Physiol. B 111, 119–126.