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# Temperature shock tolerance and heat shock proteins in Arctic freshwater ostracod *Candona rectangulata* – preliminary results

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**Abstract**: *Candona rectangulata* is an ostracod species common in cold (<15°C ) shallow freshwater Arctic water bodies. This species is useful in palaeolimnological studies because only few known autecological data can be applied in reconstructions of palaeoclimate. Particular attention was paid to the temperature, which is the basic factor determining the geographic range of a species. In this study a wide tolerance of *C. rectangulata* to the temperature was demonstrated for the first time. Its high tolerance to the temperature changes seems to be based on induction of set of proteins belonging to the family of heat shock proteins. Using PAGE-SDS electrophoresis variation in the protein profile of non-model organism undergoing stress in the field (South Spitsbergen, near *Stanisław Siedlecki* Polish Polar Station) and in laboratory cultures was presented. These results could explain the eurythermic range of *C. rectangulata* and its good adaptation to the environmental conditions which normally do not exist in Arctic freshwater ponds.

Key words: Arctic, Spitsbergen, Ostracoda, temperature, adaptation.

## Introduction

*Candona rectangulata* Alm, 1914 was first described from Greenland. It is a species common in freshwater Arctic reservoirs, recorded from Novaya Zemlya (Alm 1914; Bronstein 1947), Franz Josef Land (Bronstein 1947), Spitsbergen (Olofsson 1918; Sywula *et al.* 1994; Wisniewska 1996; Wojtasik 2008), Canadian Arctic and Alaska (Delorme and Zoltai 1984; Forester and Brouwers 1985; Forester *et al.* 1987; Hevel *et al.*1990; Reid and Reed 1993). The species was described as completing its life cycle during a single season, the short Arctic summer; the eggs, laid in autumn, were regarded as overwintering (Olofsson 1918;

Bronstein 1947). Field observations showed the *C. rectangulata* life cycle, under conditions of Arctic freshwater reservoirs, to take several years to complete, due to the ostracod's ability to hibernate (Wiśniewska 1996). Analysis of hydrological conditions in water bodies has shown the tolerance of *C. rectangulata* to increasing salinity (Delorme 2001). According to Forester *et al.* (1987), *C. rectangulata* is sensitive to the water temperature exceeding 15°C. The species has been used in palaeolimnological research as an indicator of climatic changes (Meher *et al.* 1998; Smith 2000; Curry and Yansa 2004; Bunbury and Gajewski 2009).

The maximum temperature observed in the moraine and tundra reservoirs in the vicinity of *Stanisław Siedlecki* Polish Polar Station (Hornsund, South Spitsbergen) during the summer season was 14.5°C and 11.5°C, respectively. Hydrological surveys in the freshwater reservoirs studied showed their very good oxygenation and pH ranging from 6.54 to 10.39. Water conductivity was found to vary, depending on the reservoir and season (28.9–1980 µS/cm). The salinity of some shallow coastal water bodies was low (0.1–0.7 psu) or quite high (8.7–11.4 psu), the latter effect being produced by marine aerosols (Nowiński and Wiśniewska-Wojtasik 2006).

The organisms acclimate and survive over wide temperature ranges. The most universal stress answer in organisms is the induction of heat shock proteins (Hsp) expression. Hsps are an evolutionarily conserved family of proteins found in almost all taxonomic groups. These proteins were expressed at low levels under physiological conditions but show dramatically increased expression in cells experiencing different kinds of environmental stresses (heat shock, infection, inflammation, exposure of the cell to toxins or ultraviolet light, starvation, hypoxia, oxidative stress or water deprivation). At normal, physiological conditions Hsps are responsible for protein folding, assembly, secretion, degradation and regulation. During stressful situations Hsps protect endogenous proteins, preventing their irreversible aggregation and assist in protein refolding (Feder and Hofmann 1999; Hartl and Hayer-Hartl 2002; Liberek *et al.* 2008; Schumann 2009).

In the present work we have analysed the protein expression patterns from field population and from different laboratory cultures of *C. rectangulata*. The acclimated laboratory cultures of *C. rectangulata* were additionally exposed to heat shock conditions. Similarities or dissimilarities by relative comparison of the protein patterns from SDS-PAGE gels were screened.

## Material and methods

Ostracods were collected from the shallow lake near *Stanisław Siedlecki* Polish Polar Station (77°N, 15°33'E) in Hornsund (Fig. 1), South Spitsbergen in September 2008. One part of living individuals of *C. rectangulata* collected from freshwater reservoir was prepared for an experiment after transfer from Spitsbergen. The sam-

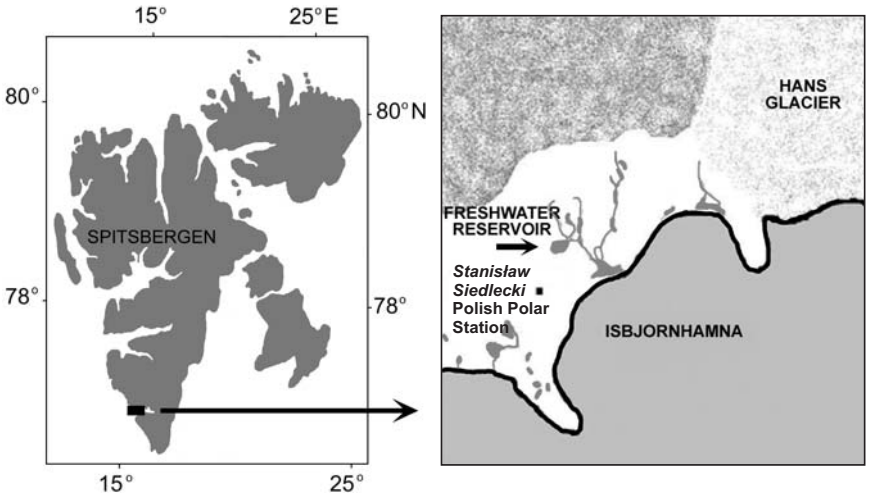


Fig. 1. Research area and location of the reservoir inhabited by *C. rectangulata*.

ple consisting of sediment, algae and ostracods was kept at temperature ranging from 4° to 10°C during transport, because it is a range of typical summer temperature of natural reservoirs in Spitsbergen (Nowiński and Wiśniewska-Wojtasik 2006). The other part of ostracods was cultured in the laboratory in the range of temperatures from 2°C up to 30°C; they were not additionally fed. In the culture there was only sediment with algae and detritus from natural reservoir from Spitsbergen. The culture water was not aerated; water losses were compensated by adding tap or distilled water. Cultures were maintained for one year under laboratory conditions (temperature 20–25°C). The culture and natural environment conditions are summarised in Table 1.

Table 1

Condition in the laboratory and in the natural conditions

| Condition | Parameters | | | | |
|---|---|---|---|---|---|
| | temperature [°C] | conductivity [µS/cm] | salinity [psu] | pH | sediment |
| laboratory culture | 20–22 | 187–250 | 0.0 | 8.0–9.0 | sandy-muddy with algae from spitsbergenian freshwater pond |
| natural environment | <14 | 187–263 | 0.0 | 7.9–9.0 | sandy-muddy with algae |

Twenty individuals of the ostracods were tested with rapid temperature change (from 2°C to 25°C) and temperature shock. Before the temperature shock for 10 minutes in 42°C, individuals were kept in the temperature 20°C for 5 hours. Ostracods, after 10 minutes of temperature shock in 42°C, were moved back to the temperature 20°C. During the experiment two visible activity of the individuals

was observed: the ostracods were moving or feeding, or they stayed inactive with closed shells.

Protein extracts were obtained from *C. rectangulata* specimens subjected to different temperatures. The ostracods were collected, washed twice with 1 ml 10 mM Tris/HCl pH 8.0 and homogenized in 200 µl of Tris buffer at 4°C. Sample protein concentration was determined according to Bradford (1976), with bovine serum albumin as a standard. Samples containing equal amounts of proteins were dissolved in Laemmli (1970) lysis buffer, separated by 0,1% SDS–12% PAGE and stained with silver method or transferred to a nitrocellulose membrane according to Sambrook *et al.* (1989). Molecular Weight Markers from Sigma were used (range 14 to 66 kDa). For Hsp70 immunodetection polyclonal rabbit antiserum against *Escherichia coli* Hsp70 – DnaK (diluted to 1:500), anti-rabbit peroxidase conjugates (Sigma; diluted to 1:3000) and substrates: 4-chloro-1-naphtol and $H_2O_2$ (Sigma) were used. As *C. rectangulata* is a rather small ostracod (about 1 mm long shell for adults), in one experiment there were used some 100 individuals. Protein expression patterns were compared in ostracods from field and laboratory conditions.

## Results and discussion

Laboratory cultures allowed to obtain information on the temperature tolerance of *C. rectangulata*. We compared the cell-associated protein profiles of *C. rectangulata* from Arctic natural environment condition and long term cultures

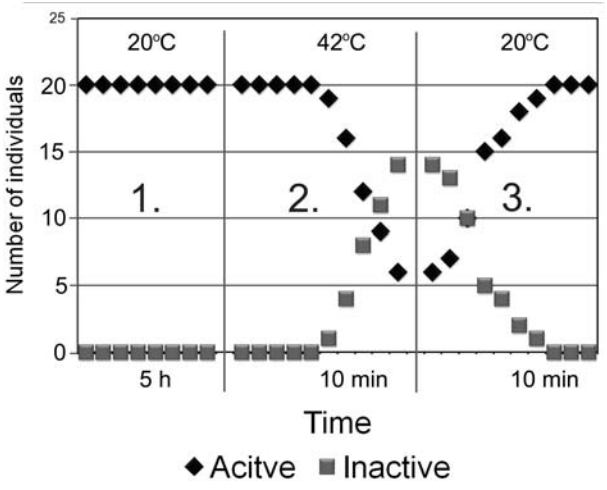


Fig. 2. Reaction of *C. rectangulata* for temperature increase: **1**. before the temperature shock the individuals were kept in temperature 20°C for 5 hours; **2**. temperature shock in 42°C, 10 minutes; **3**. individuals were moved back to temperature 20°C; black square – number of active (moving) specimens, grey squares – number of inactive (closed shells) specimens.

The ostracods were found to be tolerant to short period (up to a few hours a day) of the temperature above 30°C. Rapid temperature changes over a wide range (from 2°C to 25°C) occurring several times a day were not lethal and did not inhibit the activity of the ostracods exposed. Temperature shock (10 min., 42°C) was not lethal but individuals remarkably changed their activity (moving) to inactivity (closed shells); the result of the experiment is shown in Fig. 2.

Field and laboratory cultured animals share the majority of proteins; however, striking differences in several abundant proteins in steady-state profiles occur (Fig. 3). Two proteins are unique to Arctic animals: one approximately 90 kDa and second about 40 kDa. Levels of four proteins of molecular mass of 18 kDa, 30 kDa, 70 kDa and 100 kDa approximately are greatly enhanced in laboratory-acclimated individuals. These marked differences in protein profiles must be a reflection of laboratory acclimation. Identifying the specific products present in field and laboratory animals will provide an insight into the understanding of this acclimation/thermal tolerance mechanism.

Enhanced tolerance to environmental stresses provides expression of heat shock proteins (Clegg *et al.* 1998; Brown *et al.* 2004). We have tested Hsps induction profiles in laboratory cultured ostracods. Individuals were taken after long term cultures (6 months) and shocked in 42°C by 30 min. Control group was from culture with temperature range of 20–22°C. Protein profiles of cell lysates were generated by SDS–PAGE and silver staining (Fig. 4A). There was a remarkable increase in the level of approximately 90 kDa protein, which was also present in field population (Fig. 3). Hsp90 is classified to the family of heat shock proteins

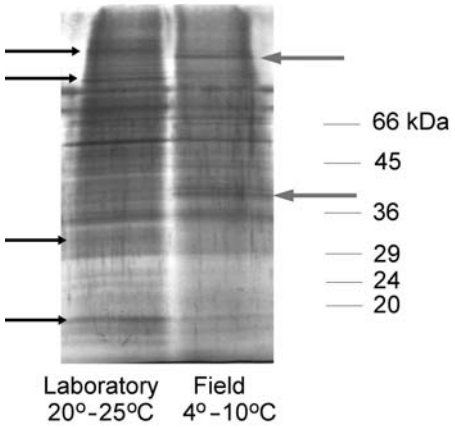


Fig. 3. SDS-PAGE of proteins from *C. rectangulata* from the field and from the laboratory cultures. The samples containing equal amounts of protein were resolved by 0.1% SDS–12% PAGE. The gel was stained with silver. The positions of unique proteins are shown by arrows. Black arrows show proteins overrepresented in the laboratory cultured animals; the proteins enriched in field ostracods were marked by grey arrows. Positions of molecular weight markers (Sigma M.W. 14.00–66.00 kDa) were indicated on the right.

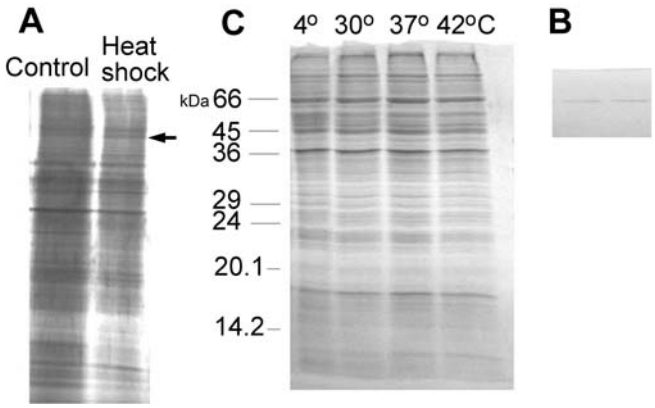


Fig. 4. **A**. Induction of heat shock proteins in *C. rectangulata* from laboratory cultures. Samples of cell extracts corresponding to the same amount of proteins were prepared and analysed by 0.1% SDS–12% PAGE. The gel was stained with silver. The position of the most abundant protein in heat stressed cells is indicated by arrow. **B**. Western blotting analysis of Hsp70/Hsc70 in the extracts of laboratory cultured animals exposed to heat shock (42°C, 30 min). Aliquots of cell extracts were subjected to 0.1% SDS–12% PAGE and Western blotting with anti-DnaK serum. **C**. Profile of proteins of *C. rectangulata* exposed to different temperatures. Whole cell extract (5µg) was separated by 0.1% SDS–12% PAGE and stained with Coomassie Brilliant Blue.

and is the most abundant chaperone in cells. It plays an essential role in the folding of a large number of proteins participating in cell cycle regulation and signal transduction (Pearl and Prodromo, 2001). Further experimental work is necessary to confirm identification of this protein.

Preliminary experiments by Western blotting analysis have been undertaken to examine endogenous levels of primary family of heat shock proteins – Hsp70. Typically, cell will synthesize constitutive and inducible isoforms of Hsp70. In *C. rectangulata* only one band for Hsp70 data was visible (Fig. 4B). Since no induced response was observed we supposed antibody we used was not specific to the inducible Hsp70 isoform. We compared also extracts obtained from cold shocked (1.5 h , 4°C) and mild heat- shocked (1.5h; 30°C or 37°C) cultures (Fig. 4C). The profiles of all protein spots were similar for all conditions.

## Conclusion

The present results indicate that *C. rectangulata*, an ostracod of Arctic distribution, is in fact rather resistant to the temperatures higher than 15°C. The increase of the temperature activates the expression of the heat shock proteins which enable these animals to accommodate to the thermal conditions other than those typical of their natural geographic range.

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