

Lake Baikal amphipods under climate change: thermal constraints and ecological consequences

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Abstract. Lake Baikal, the world's most voluminous freshwater lake, has experienced unprecedented warming during the last decades. A uniquely diverse amphipod fauna inhabits the littoral zone and can serve as a model system to identify the role of thermal tolerance under climate change. This study aimed to identify sublethal thermal constraints in two of the most abundant endemic Baikal amphipods, *Eulimnogammarus verrucosus* and *Eulimnogammarus cyaneus*, and *Gammarus lacustris*, a ubiquitous gammarid of the Holarctic. As the latter is only found in some shallow isolated bays of the lake, we further addressed the question whether rising temperatures could promote the widespread invasion of this non-endemic species into the littoral zone. Animals were exposed to gradual temperature increases (4 week, 0.8 °C/d; 24 h, 1 °C/h) starting from the reported annual mean temperature of the Baikal littoral (6 °C). Within the framework of oxygen- and capacity-limited thermal tolerance (OCLTT), we used a nonlinear regression approach to determine the points at which the changing temperature-dependence of relevant physiological processes indicates the onset of limitation. Limitations in ventilation representing the first limits of thermal tolerance (pejus (= "getting worse") temperatures (T_p)) were recorded at 10.6 (95% confidence interval; 9.5, 11.7), 19.1 (17.9, 20.2), and 21.1 (19.8, 22.4) °C in *E. verrucosus*, *E. cyaneus*, and *G. lacustris*, respectively. Field observations revealed that *E. verrucosus* retreated from the upper littoral to deeper and cooler waters once its T_p was surpassed, identifying T_p as the ecological thermal boundary. Constraints in oxygen consumption at higher than critical temperatures (T_c) led to an exponential increase in mortality in all species. Exposure to short-term warming resulted in higher threshold values, consistent with a time dependence of thermal tolerance. In conclusion, species-specific limits to oxygen supply capacity are likely key in the onset of constraining (beyond pejus) and then life-threatening (beyond critical) conditions. Ecological consequences of these limits are mediated through behavioral plasticity in *E. verrucosus*. However, similar upper thermal limits in *E. cyaneus* (endemic, Baikal) and *G. lacustris* (ubiquitous, Holarctic) indicate that the potential invader *G. lacustris* would not necessarily benefit from rising temperatures. Secondary effects of increasing temperatures remain to be investigated.

Key words: Amphipoda; breakpoint temperature; *Eulimnogammarus cyaneus*; *Eulimnogammarus verrucosus*; *Gammarus lacustris*; global warming; ion regulation; Lake Baikal; metabolic rate; thermal tolerance; ventilation.

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INTRODUCTION

Lakes are sentinel ecosystems of climate change (Adrian et al. 2009). Lake Baikal, the world's largest freshwater body, has a volume of 23,615 km³ (De Batist et al. 2006) and comprises 20% of global unfrozen surface freshwater resources. Since 1946 its mean surface water temperature increased by 1.21 °C (annual mean) and by 1.6 °C during summer (Hampton et al. 2008). Further, the ice-free season was prolonged by 18 d between 1869 and 2000 and the ice thickness in winter decreased by 12 cm in the southern basin between 1949 and 2000 (Shimaraev et al. 2002). By 2100 the surface water of Lake Baikal is projected to warm by more than 4.5 °C compared with present temperature (Moore et al. 2009).

Temperature is arguably the overarching abiotic factor for ectothermic organisms. Climate change will thus affect most aquatic ectotherms and challenge their physiological and ecological plasticity. This study focuses on the ecophysiological effects of rising temperature on amphipods, which comprise up to 90% of benthic biomass and species in Lake Baikal (Rusinek et al. 2012) and are key benthic decomposers in the complex nutrient cycling system of the lake. Lake Baikal is famous for its rich endemic amphipod fauna and is inhabited by more than 45% of the world's known gammaroidean species, all belonging to the suborder Gammaridea Latreille, 1802 (Takhetev et al. 2015). It is thus an ideal ecosystem to study ecosystem changes elicited through direct impacts of climate on the interaction of closely related species. Baikal endemics experienced stable abiotic conditions for millions of years with moderately fluctuating temperatures as contributing streams, currents, and winds are driving continuous mixing. Temperature fluctuates between 2.2 ° and 6.5 °C at 100 m depth (Kozhov 1963) and an annual mean temperature of 6 °C was measured in the littoral (Falkner et al. 1991, Weiss et al. 1991, Yoshioka et al. 2002). Hence, Baikal amphipods might be more vulnerable to a quickly changing environment than ubiquitous species like *Gammarus lacustris* Sars, 1863, a widespread amphipod of the Holarctic, including Siberia (Karaman and Pinkster 1977). The latter is a candidate species to widely invade Lake Baikal as it inhabits various shallow water bodies in proximity and even isolated bays of Lake Baikal

characterized by a comparatively high content of benthic organic matter, high temperatures in summer and low wave impact. At present, it is not found in the typical Baikal littoral benthos (Kozhova and Izmet'eva 1998, Timoshkin 2001). It is unknown whether climate change will affect the distribution patterns of ubiquitous and endemic species; the biotic and abiotic factors constituting the "immiscibility barrier" separating the endemic Baikal and the ubiquitous Palearctic faunas remain to be identified (Mazepova 1990, Timoshkin 2001).

Here we compare *G. lacustris* with two Baikal amphipods, *Eulimnogammarus verrucosus* (Gerstfeldt, 1858) and *Eulimnogammarus cyaneus* (Dybowsky, 1874), which are two of the most abundant species of the littoral and thus highly important components of the unique benthic ecosystem. They inhabit rocky habitats at depths of 0–15 m and 0–1.5 m, respectively. As a sedentary species of the upper littoral, *E. cyaneus* is exposed to higher temperature fluctuations than *E. verrucosus*, which migrates to deeper waters in summer (Weinberg and Kamaltynov 1998). Previous short-term studies of preference temperatures (Timofeyev et al. 2001, Timofeyev and Kirichenko 2004, Timofeyev and Shatilina 2007), heat shock proteins (Shatilina et al. 2011, Bedulina et al. 2013, Protopopova et al. 2014), enzyme activities, and products of metabolism (Axenov-Gribanov et al. 2012) indicated that *E. verrucosus* is more sensitive to thermal stress than *E. cyaneus* and *G. lacustris*, but ecophysiological thermal limits and performances at the whole animal level have not been investigated.

Field observations of ecological consequences of climate change have been explained by the concept of oxygen- and capacity-limitation of thermal tolerance (OCLTT) (Pörtner 2010). Each species (and even life stage) has a limited thermal range of aerobic performance. Pejus (lat.) (= progressively deleterious) temperatures (T_p) characterize the first level of thermal limitations and the ecological thermal boundaries of a species. In the field, occurrence of temperatures beyond these thermal boundaries leads to decreasing abundance of a species (Pörtner and Knust 2007). Surpassing T_p implies decreased performance until reaching the critical temperature (T_c), which precludes long-term survival.

This study aimed to determine the points at which the changing temperature-dependence of relevant physiological processes indicates the onset of limitation in *E. verrucosus*, *E. cyaneus*, and *G. lacustris*. In light of the observations made, we discuss whether ecophysiological findings support an assessment of how increasing water temperatures will affect the three species and whether warming will be beneficial for *G. lacustris* in comparison with the Baikal endemics.

MATERIAL AND METHODS

Sampling site

Eulimnogammarus verrucosus (550–885 mg) and *E. cyaneus* (19–49 mg) were caught with a hand net (kick sampling) in the Baikal littoral in the vicinity of Bolshie Koty (51°9'137" N, 105°0'691" E) at depths of 0–1.2 m. The sampling site represents the beach zone, according to underwater landscape zoning (Kravtsova et al. 2004) characterized by algae-covered boulders (mainly *Ulothrix zonata*), half-immersed in sand, and intensive hydrodynamics. *G. lacustris* (26–145 mg) was sampled in the same way in "Lake 14" (51° 55' 14.39" N, 105° 4' 19.48" E). This well-aerated eutrophic pond is supplied by groundwater and connected to a backwater of Bolshie Koty river. It is inhabited by fauna common in freshwaters across the Holarctic. The ground consists of boulders and gravel, submerged in a silty sediment and covered by detritus and macrophytes. See Protopopova et al. (2014) for a map with sampling locations. The investigated amphipod species are depicted in the Supporting Information in Fig. S1.

Experimental setup and animal maintenance

Experiments were performed between June and October (2011–2013) in the Institute of Biology at Irkutsk State University and its field station in Bolshie Koty. Adult animals were selected with a similar or larger size than those of previous studies in which body length was used to classify adult animals (*E. verrucosus*: 30 mm, *E. cyaneus*: 14 mm and *G. lacustris*: 14 mm) (Timofeyev et al. 2001). Immediately after sampling, amphipods were transported to the laboratory in insulated boxes and sorted under temperature-controlled conditions. Two hundred individuals of *E. cyaneus*, 75 of

G. lacustris, or 25 of *E. verrucosus* were quickly placed into 2 L tanks (high-density polypropylene of food grade quality or glass) containing aerated 6 °C Baikal water and kept for at least 2 d with low illumination. About two-thirds of the tank bottoms were covered with pebbles from the littoral, precleaned with boiling Baikal water. Water was exchanged once every 1 to 3 d (increasing the exchange rate with rising temperatures). Commercial food for crustaceans (Tetra, Melle, Germany) was applied in 2011/2012 and animals were fed with a mix of amphipods, algae, water plants, and detritus collected in the Baikal littoral (frozen, air-dried at ≈ 30 °C and roughly mortared) in 2013. Food was supplied ad libitum and there was no visible difference in condition of animals fed with either food. Control animals were kept at a water temperature of 6 ± 0.8 °C, the reported annual mean temperature of the littoral. For *G. lacustris* an extra "Lake 14" control was set up containing water from "Lake 14" and animals were fed with alder, willow, and birch leaves collected in the pond. The cation composition (in μmol/L) was Na⁺: 210, K⁺: 10, Mg²⁺: 240, Ca²⁺: 300 in "Lake 14 and Na⁺: 220, K⁺: 40, Mg²⁺: 140, Ca²⁺: 450 in the Baikal littoral. In June 5.3–7.0 °C and 7.2–11 °C and in September 8.5–15 °C and 12.0–13.3 °C were monitored during sampling at depths of 0–1.2 m in Lake Baikal and "Lake 14," respectively. During long-term (4 week) experiments, water tanks were stored in an incubator (Sanyo MIR-254 (238 L), Osaka, Japan), and the water temperature was increased by 0.8 °C/d. In short-term (24 h) exposures, 2 L tanks were placed in water baths, increasing temperature by 1 °C/h by use of a thermostat (WiseCircu, Witeg GmbH, Wertheim, Germany). The experimental protocols were designed to mimic temperature fluctuations during a summer season and diurnal changes but went beyond natural habitat temperatures in order to clearly identify thermal limits. For each individual analysis, animals of a certain species were randomly taken out of 6–10 individual tanks.

Mortality

Dead animals were removed and counted daily to obtain the temperature-dependent cumulative mortality for each species excluding

cannibalized individuals. The Weibull model (5-parametric; sigmoidal) was fitted to the data in order to calculate lethal temperatures (LT) for certain percentages of the test group (LT_x%).

Routine metabolic rate

Routine metabolic rate (RMR), measured as oxygen consumption (MO₂) per gram of fresh weight (FW), was analyzed with optical sensors purchased from Presens, Regensburg, Germany: Microx TX3 and OXY4, Oxygen Micro-Optode (NTH-PS_t1-L5-TF-NS*35**x1,20-PC3,1-YOP), with airtight connections to flow-through respiration chambers. Sensors were calibrated to 0% oxygen in oversaturated sodium sulfite (AppliChem, Darmstadt, Germany) and to 100% air saturation in the running system. After calibration, the 100% value was recorded for several minutes, and animals were carefully placed in airtight Teflon® chambers closed with plexiglass lids. Tanks and plasticizer-free tubing (MHSL 2001, Ismatec, Wertheim, Germany) were submerged in thermostatic baths to ensure temperature stability (±0.2 °C). Flow rate was adjusted so that oxygen consumption was in steady state and the outflow drop in air saturation ranged between 6% and 23%. When the values were stable for about 20 min, the animals were removed from the chambers, quickly and gently dried with lint-free paper towel, weighed on a fine scale, immediately placed back into the incubation system, and allowed to recover from manipulation for at least 3 d. No differences in RMR were found between nontreated and previously treated animals. The Q₁₀ temperature coefficients were determined for each temperature increment to study irregular changes across temperatures.

Resting ventilation

Ventilation (pleopod beat rate) was analyzed in periods of resting defined as the time spent in relaxed posture without locomotor or intense grooming activity. Animals were transferred to Teflon® chambers with perforated depressions to allow unhindered water circulation in temperature-controlled 2 L tanks containing aerated Baikal water. As the animals did not show any reaction to red light, a 625 nm LED light source was used for illumination during 3 min video recording sequences. In the 4-week

study in June/July 2013, *E. verrucosus* was video-recorded in the flow-through systems during simultaneous measurements of RMR. As pleopods of *E. cyaneus* and *G. lacustris* are small and transparent, simultaneous recordings of ventilation rate and RMR were impossible for these species. After recordings individuals were treated in the same way as after measuring RMR. Video-taped ventilation rates were analyzed manually in slow motion (0.5× or 0.25×).

If irregular changes were noted in RMR or ventilation, movies recorded for studying ventilation rates were examined by eye for any changes in movement activity by determining the ratio of time spent in motion in comparison to resting periods.

Ion regulation

Extracellular concentrations of inorganic cations (Na⁺, K⁺, Mg²⁺, and Ca²⁺) were determined chromatographically using a Dionex ICS 1500/2000 equipped with an Ion Pac CS16 column operated at 40 °C. Methane sulfonic acid (30 mM) was used as an eluent at a flow rate of 0.36 mL/min. Hemolymph was extracted manually using capillaries with ultrafine tips. Animals were dried with paper tissue and capillaries were introduced dorsally preventing damage to the guts. Samples (0.5–2.5 µL) were diluted with 50 µL of H₂O and stored at –20 °C prior to analysis. For peak identification and quantification (based on peak areas) three-point calibrations of the Dionex Six Cation II Standard were used. All products for ion analyses were obtained from Dionex GmbH, Idstein, Germany.

Tissue characteristics

To study percentages of water and inorganic content, frozen animals were thawed, weighed, dried for 24 h at 40 °C in a drying cabinet and reweighed. We checked whether all water had evaporated after another 3 h in the drying cabinet. Eventually, animals were incinerated in a muffle furnace at 560 °C and the ash-weights were determined.

Abundance

Abundance was measured in the field on a relative scale by calculating the personal catching success per unit time at different ambient temperatures (5.3 to 15.0 °C). Animals were

sampled at ten sampling occasions in the period from the beginning of June to the beginning of September 2013. Only adult animals of the respective weight classes described in “Materials and methods: Sampling site” were taken into account. The amphipods were sampled at depths of 0–1.2 m.

Data analysis and statistics

Data were tested for normal distribution and homoscedasticity with the Ryan-Joiner and Levene’s test, respectively, using Minitab 17 (Minitab Inc, State College, Pennsylvania, USA). They are presented as means \pm standard error of the mean (SE) unless otherwise indicated. Standard statistical tests were applied to reveal significant differences ($P < 0.05$) and are mentioned in the results. If data showed no normal distribution, respective nonparametric tests were applied (e.g., ANOVA on ranks). The “Dynamic Fit Wizard” in SigmaPlot 13 (Systat Software Inc, San Jose, California, USA) was used to find the best-fit model (those with lowest Akaike’s information criterion) for the data sets of cumulative mortality, ventilation, and RMR. Candidate models were preselected by shape. Weibull and lognormal models were fitted to all data sets of mortality, ventilation and to RMR data sets of *E. verrucosus* and *G. lacustris* (6–22 °C). We aimed to find the upper breakpoint temperature (BPT) defined as the point where temperature stopped being the driver of ventilation (= $BPT_{Vent.}$) or RMR (= $BPT_{Ox.}$), i.e., when the slope of the curve started to flatten out and approach zero. The upper BPT was defined according to Marshall et al. (2013), i.e., as the point where the model’s slope equals 0.065 (nonlinear regression approach). The 95% confidence intervals (CI) are given in parentheses for the modeled BPT values of RMR and ventilation. In case none of the selected parametric models fitted the data sets, BPTs were estimated to correspond to the maximum values of the exponentially increasing rates that were followed by significant declines. These maximum values were similar to those derived by the nonlinear regression approach in this study (for comparison see Fig. 1). Only measurements at which survival was $> 75\%$ were taken into account to distinguish physiological phenomena that may appear right before death (e.g., a sudden

increase in heart rate) from physiological responses to rising temperatures. However, we emphasize that these values are only rough estimates. All model equations are available in the Supporting Information in Table S1.

RESULTS

Mortality

LT50 values of 21.1, 28.4, and 28.3 °C were derived from Weibull models fitted to the data sets of *E. verrucosus*, *E. cyaneus*, and *G. lacustris* (Fig. 1a–c; 4-week study, June/July 2013). In comparison to June/July, the LT50 values determined in September/October were similar in *E. verrucosus* (21.5 °C) and slightly higher in *E. cyaneus* and *G. lacustris* (30.4 and 30.3 °C) (Table 1).

Routine metabolic rate

At control temperature (6 °C), oxygen consumption ($\mu\text{mol g (FW)}^{-1} \text{h}^{-1}$) was lowest in *E. verrucosus* (3.3 ± 0.2) (Fig. 1a) and similar in *E. cyaneus* and *G. lacustris* (5.3 ± 0.4 and 5.7 ± 0.3 , respectively) (Figs. 1b and c; 4-week study, June/July 2013). RMR increased up to 15.0 (14.0, 16.1) °C in *E. verrucosus* determined as $BPT_{Ox.}$. At 20.4 °C RMRs decreased with increasing weights of *E. verrucosus* specimens (Pearson correlation, $P = 0.0029$) and beyond 20.4 °C only relatively small animals were still alive (ANOM, $P < 0.05$). *E. cyaneus* and *G. lacustris* increased RMRs exponentially up to 25.2 ° and 23.6 °C, respectively. As parametric models (e.g., Weibull or lognormal) failed to fit these data sets, $BPT_{Ox.}$ -values for *E. cyaneus* and *G. lacustris* could only be estimated and were considered to approximately correspond to the measured maximum values of the exponential increases, which were followed by significant declines in respiration rate (Figs. 1b and c).

In all species, the highest thermal sensitivity of RMR, deduced from the largest Q_{10} -values, occurred close to the upper $BPT_{Vent.}$. This leap in RMR was most pronounced in *G. lacustris* between 22.0 ° and 23.6 °C coinciding with a significant increase in movement activity from $52 \pm 20\%$ to $93 \pm 13\%$ (t test, $P = 0.002$). For the other species no such sudden elevations of movement activity were statistically detectable. Passing the $BPT_{Ox.}$

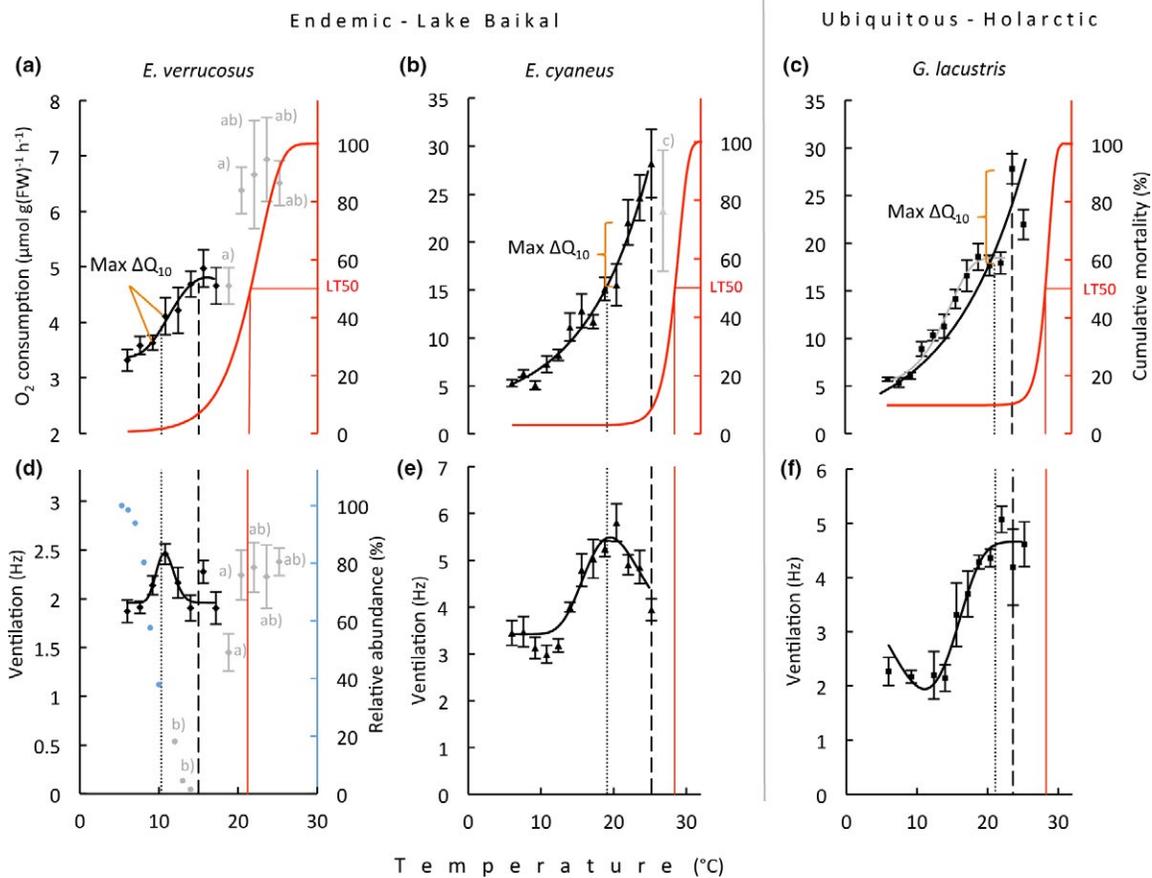


Fig. 1. Routine oxygen consumption (black symbols, a–c), cumulative mortality (red lines, a–c) and resting ventilation (black symbols, d–f) of *E. verrucosus* (left panels), *E. cyaneus* (central panels), and *G. lacustris* (right panels) exposed to gradual temperature increase (0.8 °C/d, 6–30 °C) (experimental period: June/July 2013). Model fits are represented by solid lines and model equations are summarized in the Supporting Information in Table S1. Field abundance (0–1.2 m depth) at different ambient temperatures is only depicted for *E. verrucosus* (blue dots, d). On each panel, vertical lines indicate the upper BPT_{Vent.} (black dotted lines) and the upper BPT_{Ox.} (black dashed lines) and the LT50 values (red solid lines), respectively. Gray symbols mark data subsets in which a) > 25% of the investigated population had died, b) negative deviations from the overall mean animal weight were stated (ANOM, $P < 0.05$) or c) only three replicates could be analyzed and were excluded from further analyses. Data are displayed as means \pm SE ($n = 5–18$).

coincided with the onset of exponentially increasing mortality in all species.

Resting ventilation

The results of the 4-week study (June/July 2013) are depicted in Fig. 1d–f. A BPT_{Vent.} of 10.6 (9.5, 11.7) °C was determined for *E. verrucosus* (Fig. 1d). Ventilation in *E. cyaneus* (Fig. 1e) and *G. lacustris* (Fig. 1f) was insensitive to temperatures up to 12.4 ° and 14.0 °, respectively. Beyond those temperatures it

increased exponentially, reaching an upper BPT_{Vent.} of 19.1 (17.9, 20.2) °C in *E. cyaneus* and 21.1 (19.8, 22.4) °C in *G. lacustris*. Individuals of *G. lacustris* incubated in “Lake 14”-water showed a BPT_{Vent.} of 21.8 (19.2, 24.4) °C (data not depicted).

Table 1 compiles BPT_{Vent.} values from experiments differing with regard to duration and season. The BPT_{Vent.} values of the two Baikal species from a 24-h study slightly exceeded those determined in 4-week investigations from the same

Table 1. LT50 and BPT_{Vent.} (95% CI) of *E. verrucosus*, *E. cyaneus*, and *G. lacustris*. Values are displayed for long-term (4 week; 0.8 °C/d) studies conducted in different seasons (start of experiment in June or September) and for long- and short-term (24 h; 1 °C/h) studies performed in the same season (September), (n.a. = not analyzed).

Parameter	Endemic – Lake Baikal						Ubiquitous – Holarctic		
	<i>E. verrucosus</i>			<i>E. cyaneus</i>			<i>G. lacustris</i>		
Season	June	September	September	June	September	September	June	September	September
Duration	4 week	4 week	24 h	4 week	4 week	24 h	4 week	4 week	24 h
LT50 (°C)	21.1	21.5	29–29.5†	28.4	30.4	n.a.	28.3	30.3	n.a.
BPT _{Vent.} (95% CI) (°C)	10.6 (9.5, 11.7)	9.6 (8.7, 10.5)	(11.4)‡	19.1 (17.9, 20.2)	20.8 (20.0, 21.6)	21.3 (20.5, 22.1)	21.1 (19.8, 22.4)	21.8 (20.5, 23.1)	≥ 27

† Axenov-Gribanov et al. (2012).

‡ Value could be derived from the model but with low confidentiality.

experimental season (September/October). They were determined as 11.4 °C (24 h) and 9.6 (8.7, 10.5) °C (4 week) in *E. verrucosus* and 21.3 (20.5, 22.1) °C (24 h) and 20.8 (20.0, 21.6) °C (4 week) in *E. cyaneus*. We were unable to determine the BPT_{Vent.} for short-term-incubated *G. lacustris* as they tended to increase ventilation rates until reaching lethal temperatures (BPT_{Vent.} ≥ 27 °C). In the 4-week study performed in September/October, *G. lacustris* showed a BPT_{Vent.} of 21.8 (20.5, 23.1) °C.

Ion regulation

Extracellular ion concentrations are displayed in Fig. 2 (4-week study, June/July 2013). Potassium concentrations tended to be higher at 25.2 °C in all species and were positively correlated with temperature (6–25.2 °C) in *E. cyaneus* and *G. lacustris* (Pearson correlation, $P < 0.001$) (Fig. 2h–i). Sodium and calcium concentrations showed no clear trends in the investigated species. In the hemolymph of *E. verrucosus* and *G. lacustris* [Mg²⁺] was reduced by 16–31% and 28–45%, respectively, at temperatures above 14.0 °C and negatively correlated with temperature (Pearson correlation, $P < 0.05$). *E. cyaneus* showed large interindividual variation and no clear temperature-dependent trend in [Mg²⁺] (Fig. 2b). Only *G. lacustris* showed progressively decreasing [Mg²⁺] over time at 6 °C (control). Likewise, in temperature treatment and control incubations with water from “Lake 14” [Mg²⁺] also decreased in *G. lacustris* (Pearson correlation, $P = 0.033$) suggesting no effect from water from different sources on extracellular ion concentrations. No differences

were seen between the effects of short- and long-term gradual temperature increases on ion levels except for [K⁺] that in contrast to long-term experiments were not correlated with temperature (6–24 °C) in short-term studies. Data for controls (6 °C), 24-h studies and data recorded in different seasons in *E. verrucosus* are available in Supporting Information (Tables S2 & S3; Fig. S2). They are consistent with the patterns of regulation described here.

Tissue characteristics

The percentage of ash-weight based on the animal’s wet weight was not significantly different between *E. verrucosus* (7.8 ± 0.4%) and *G. lacustris* (6.7 ± 0.3%), but higher in *E. cyaneus* (9.4 ± 0.3%) (ANOVA; $P < 0.001$; Holm-Sidak (post hoc test)); *E. verrucosus* vs. *E. cyaneus*, $P = 0.015$ and *E. cyaneus* vs. *G. lacustris*, $P < 0.001$). The water content of *G. lacustris* tissue was higher than found for the Baikal amphipods (ANOVA; $P < 0.001$; Holm-Sidak (post hoc test)); *E. cyaneus* vs. *G. lacustris*, $P < 0.001$ and *E. verrucosus* vs. *G. lacustris*, $P = 0.014$) (Table 2).

Abundance

Temperature and field abundance of *E. verrucosus* (550–885 mg) were negatively correlated (Spearman rank correlation, $P < 0.001$; Fig. 1d). The highest relative sampling success was recorded in June at water temperatures of 5–6 °C. At 14 °C, only a few small adults and juveniles of *E. verrucosus* were found. At temperatures ≥ 12 °C individuals showed lower weights than the overall mean (ANOM, $P < 0.05$). For

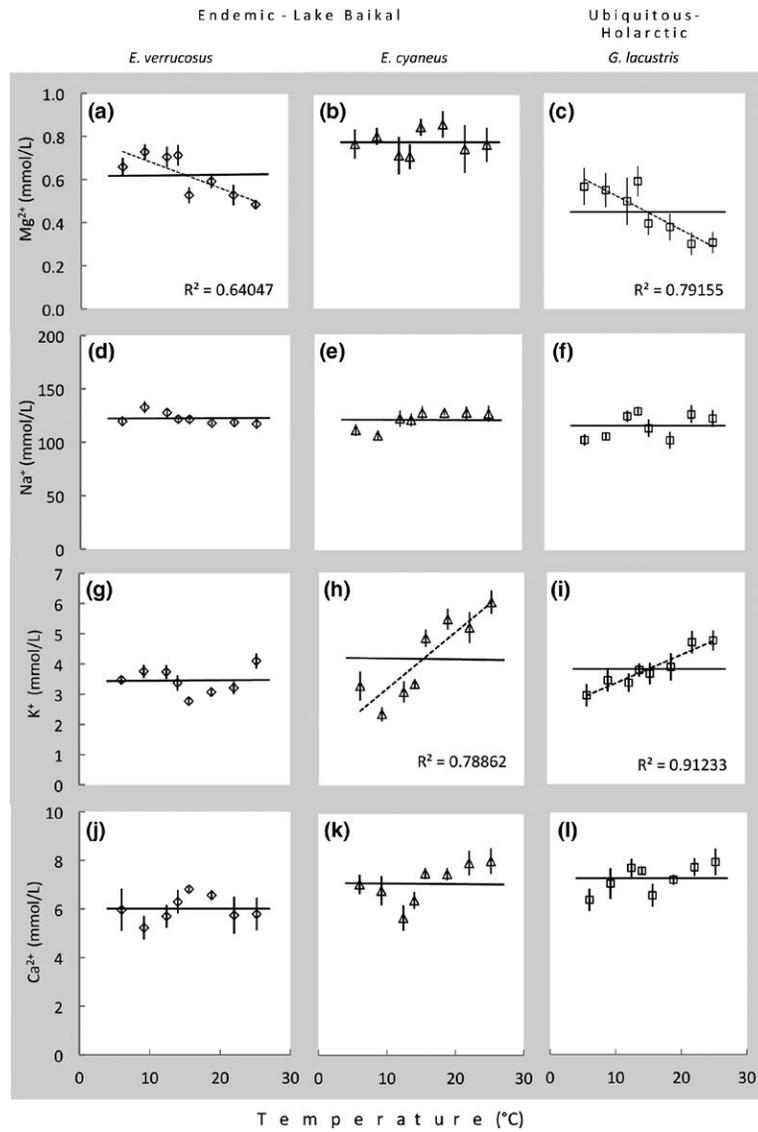


Fig. 2. Concentrations of Mg^{2+} , Na^+ , K^+ , Ca^{2+} in hemolymph of *E. verrucosus* (right panels), *E. cyaneus* (central panels), and *G. lacustris* (left panels) exposed to gradual temperature increase ($0.8\text{ }^{\circ}C/d$) (experimental period: June/July 2013). Horizontal black lines (a–l) represent mean ion concentrations and dashed lines are linear fits (h and i). Data are presented as means \pm SE, ($n = 5\text{--}14$).

Table 2. Wet weight, water content and inorganic content of *E. verrucosus*, *E. cyaneus*, and *G. lacustris*. Statistically significant differences (ANOVA, $P < 0.05$) are indicated by different superscripted letters in each column. Values represent means \pm SE, $n = 5\text{--}6$.

Species	Wet weight (mg)	Water content (%)	Inorganic content (percentage of wet weight)
<i>E. verrucosus</i>	$693.9^A \pm 94.4$	$73.6^A \pm 0.5$	$7.8^A \pm 0.4$
<i>E. cyaneus</i>	$41.8^B \pm 4.1$	$71.0^A \pm 1.2$	$9.4^B \pm 0.3$
<i>G. lacustris</i>	$65.6^C \pm 9.8$	$77.4^B \pm 0.8$	$6.7^A \pm 0.3$

E. cyaneus sampling success was independent of ambient temperatures (5.3–14 °C; $R^2 = 0.024$) and highly variable over time (data not displayed). Field abundance was not quantitatively evaluated for *G. lacustris* as the small pond only provided few sampling sites and some of them were not accessible due to the funnel-shape of this small waterbody. However, we observed that adult individuals were far less frequently found in regions close to the water's edge when water temperatures reached up to 22 °C in July and August.

DISCUSSION

Our discussion mainly focuses on the results for long-term studies (4 week; 0.8 °C/d) conducted in June/July (Figs 1 and 2). These are considered to be most ecologically relevant as animals were sampled at water temperatures close to control values (6 °C) and the rate of temperature change in the experiments is considered to be similar to field conditions. Thus, at 1–1.2 m depth water temperatures of around 6 °C were measured in the beginning of July, whereas by the end of the month up to 20 °C can be reached. Inter-experimental comparisons regarding duration and season (Table 1) are discussed in a separate section. Experiments performed in September/October addressed the question whether the animals had adapted to higher temperature during summer whereas short-term studies (24 h, 1 °C/h) reflect the responses to quick, e.g., diurnal, changes in the thermal regime.

Mortality, routine metabolic rate, and resting ventilation

The limit of oxygen supply capacity is likely key in the onset of constraining (beyond $BPT_{Vent.}$) and then life-threatening (beyond $BPT_{Ox.}$) conditions. Hypoxemic conditions (beyond $BPT_{Ox.}$) correlate with the start of exponentially increasing mortality in all species. The observed leaps in RMR reflect the highest thermal responsiveness and indicate a metabolic switch. As they occurred close to the first limit of thermal limitation represented by the $BPT_{Vent.}$, they might reflect a startling or escape response supported by the corresponding loss of field abundance in *E. verrucosus* and significant increase in movement activity in *G. lacustris*.

Metabolic rate “sets the pace of life” and changes are thus fundamental to any animal (Brown et al. 2004). Evidently, beyond the limitations in ventilatory capacity the situation was progressively turning deleterious for *E. verrucosus* as a slight increase in the mortality rate (lag phase) was visible. The upper limit of the thermal optimum of *E. verrucosus* found in this study matches previous findings of a threefold increase in heat shock protein levels (Hsp70) and a doubling of catalase activity at 11 °C in a 24-h study (1 °C/h) (Axenov-Gribanov et al. 2012) indicating that physiological and biochemical transition phases go hand in hand (Pörtner 2010).

In all species studied here, resting ventilation failed to continually follow the increasing oxygen consumption rate, indicating an extended pejus range, which in a study investigating spider crab *Maja squinado* was also paralleled by heart rate leveling off (Frederich and Pörtner 2000). Ventilation comprises a significant fraction of metabolic costs (Glass and Wood 2009) and can thus reach capacity limits before the aerobic metabolic capacity becomes exploited. In addition, heart rate, oxygen affinity, and oxygen transport capacity of hemocyanin are likely to play a crucial role for maintaining aerobic metabolism at elevated temperatures or hypoxic conditions (Booth et al. 1982, Wilkes and McMahon 1982, Giomi and Pörtner 2013). Factors involved in modulating hemocyanin oxygen transport include changes in pH and lactate levels (independent of pH), in $[Mg^{2+}]$ as well as in hormonal determinants (Mangum 1997, McMahon 2001). In the optimum range, hemocyanin might remain oxygen-saturated and might not be involved in tissue oxygen supply. Diffusion processes at low oxygen demand might play a large role for oxygen supply in highly oxygenated Baikal water, contributing to energy savings and low costs of oxygen supply in the cold. At low temperatures, RMR was even independent of ventilation in the similar-sized *E. cyaneus* and *G. lacustris* but not in the much larger *E. verrucosus*.

Eulimnogammarus verrucosus is considerably more thermally sensitive than *E. cyaneus* and *G. lacustris*, which may partly be due to its large body size, consistent with the allometry of thermal tolerance seen, e.g., in fishes (Pörtner et al. 2008). Warming exploits the capacity of the

system to increase metabolic rate, reaching levels similar to those after maximum exercise before it collapses. Aerobic metabolic scope depends on three parameters, namely oxygen uptake, distribution and cellular exploitation. Oxygen uptake is a function of gill surface, which was shown to scale to body mass by an allometric coefficient of 0.792–0.843 in different species of the genus *Gammarus* (Moore and Taylor 1984). Thus, small animals have a relatively enlarged gill surface area, which is beneficial if oxygen is limited, likely contributing to higher thermal tolerance.

It has been stated as a unifying principle that climate warming benefits small organisms (Daufresne et al. 2009, Storch et al. 2014). Consistent with these studies and field observations in fish (Pörtner and Knust 2007), mortality occurred earliest in the largest individuals of *E. verrucosus* during the experimental temperature increase. Furthermore, at 20.4 °C RMR decreased with increasing animal weight. This indicates that smaller animals have a higher physiological capability to deal with elevated temperatures. However, other aspects also need to be considered when assessing the overall consequences of elevated temperatures for the species such as behavioral adaptations as discussed below. As smaller individuals have a smaller number of offspring the absence of large individuals will result in a declining population. Since food web structures and dynamics are mainly determined by body size (Woodward et al. 2005, 2010a,b, Barnes 2008, Yvon-Durocher et al. 2010), ecosystems would be severely affected by any temperature-mediated changes in organism sizes.

Ion regulation

The decrease in $[Mg^{2+}]$ seen in *E. verrucosus* and *G. lacustris* at > 14 °C but not in *E. cyaneus* might be linked to increased activity as low extracellular $[Mg^{2+}]$ in crustaceans go together with increased heart rate and support muscular activity. This might trigger behavioral changes, which in turn might influence field abundance. Moreover, a decrease in $[Mg^{2+}]$ can counteract a temperature-dependent decrease in oxygen affinity of hemocyanin and support enhanced oxygen demand.

Magnesium is a modulator for numerous enzyme systems (Morritt and Spicer 1993) and important for regulatory processes concerning

metabolism and growth. Decreasing intracellular $[Mg^{2+}]$ correlate with lowered protein synthesis rates (Terasaki and Rubin 1985). However, as our findings are not uniform across the species, we suggest that the adjustment and regulation of Mg^{2+} is species-specific and possibly reflects its involvement in the control of hemocyanin or locomotor activity level more than other aspects.

The facultative hibernator *G. lacustris* might depend on elevated temperatures in summer to increase activity levels, forage and fill up energy stores. Animals caught at 6–7 °C in June were mostly found inactive in leaf litter suggesting that these individuals were still in their hibernation retreats. When kept at 6 °C after removing them from wintering substrates, extracellular $[Mg^{2+}]$ progressively decreased. Moreover, individuals of *G. lacustris* that were caught in June showed a higher mortality than those sampled in September when they were kept at 6 °C for 4 weeks.

The positive correlation of extracellular $[K^+]$ and temperature in *E. cyaneus* and *G. lacustris*, which are much smaller than *E. verrucosus*, is possibly due to the fact that small specimens were prone to membrane damage when extracting hemolymph with a glass capillary as the effect was absent in *E. verrucosus*. We suggest that the stability of membranes decreased during long-term warming as this effect was only visible in the 4-week studies. There is, however, no close relationship between sublethal thermal limits and $[K^+]$.

In summary, ion regulation is a rather insensitive parameter when investigating sublethal thermal constraints in Baikal amphipods. This might be an indication of their excellent ion regulation capacities. Baikal amphipods need to efficiently take up essential ions as Baikal water is of extremely low ionic content ($Na^+ = 155$; $Ca^{2+} = 402$; $Mg^{2+} = 126$; $Cl^- = 12$; $SO_4^{2-} = 57 \mu\text{mol/kg}$; Zerbst-Boroffka et al. (2000)).

Abundance

Decreasing abundance of adult *E. verrucosus* at the field sampling sites paralleled rising water temperatures and can be explained through a decline in physiological performance and associated aerobic scope. Consistent with the allometry of thermal tolerance the largest animals reacted most sensitively. Similar to the field situation, *E. verrucosus* preferred temperatures of 5–6 °C in a previous behavioral laboratory study,

which showed progressive decreases in abundance with rising temperatures in the experimental temperature gradient (Timofeyev and Shatilina 2007). It has previously been hypothesized that the migration patterns of *E. verrucosus* are temperature-dependent (Weinberg and Kamaltynov 1998). The present data suggest that migration behavior is initiated when temperatures exceed pejus limits. Considering that ecological niches are densely occupied in the Baikal littoral by 60–70% of the highly diverse macroinvertebrate fauna (Takhteev et al. 2003) the seasonal and diurnal migration behavior of *E. verrucosus* indicates a high ecological competitiveness of this species. This dominance of *E. verrucosus* is possibly due to the large size of adult specimens and their low metabolic rate; the latter may become advantageous as food availability decreases further with increasing depth in the oligotrophic lake. *E. verrucosus* is clearly dependent on the ability to escape to deeper zones, as in the upper littoral its T_c of 15.0 (14.0, 16.1) °C can easily be surpassed in summer.

Eulimnogammarus cyaneus is found in the upper littoral throughout the year and experiences temperatures from close to the freezing point up to 20 °C. It rarely faces its preferred temperature of 11–12 °C determined in a behavioral study (Timofeyev and Kirichenko 2004). A broad thermal tolerance range has been suggested as the most important trait for a species successful under climate change (Tepolt and Somero 2014), yet eurythermal species living at the edge of their thermal window are likely to be affected by global warming (Somero 2010). *E. cyaneus* was probably able to occupy its current niche due to its wide thermal window but summer temperatures in the littoral can reach the pejus range (> 19.1 °C). If temperatures exceed 19.1 °C in summer for extended periods of time, *E. cyaneus* may suffer as it, due to its small size and lower competitiveness, might not be able to conquer deeper habitats as niches are densely occupied. At any site this will depend on the community responses given that ecological interactions in the Baikal benthos are profoundly complex. In the bay of Bolshie Koty, 93 species of amphipods were found at depths of 0–20 m (Kravtsova et al. 2003). While *E. verrucosus* is less thermally tolerant than *E. cyaneus*, its temperature-related migration indicates behavioral plasticity.

The abundance of *G. lacustris* was difficult to quantify but it was noticeable that only few small adult individuals and large numbers of juveniles were found close to the water's edge in August and September when up to 22 °C were reached in "Lake 14". These findings suggest that *G. lacustris* also escaped rising surface temperatures in summer to stay below T_p .

Inter-experimental comparison—exposure duration and season

Long-term experimental warming led to falling thermal thresholds, which was most striking when comparing the LT50 values of *E. verrucosus*. These findings are likely due to the time dependence of lethal limits (Peck et al. 2009). Exploiting the passive range of tolerance during longer-term protocols possibly results in lower LTs. Considering ventilation, the divergence of limits between long- and short-term warming was highest in *G. lacustris*. Hence, we conclude that it is the most tolerant to diurnal temperature increases among the studied species, consistent with its shallow water habitat characterized by restricted hydrodynamics.

In comparison to June/July, the $BPT_{Vent.}$ of *E. cyaneus* and *G. lacustris* were marginally higher in September/October possibly due to acclimatization to higher temperatures during summer. In support of this, their LT50 values also exceeded those determined in June/July, which is likely mirrored in slightly right-shifted T_c following thermal acclimation. In contrast, the $BPT_{Vent.}$ of *E. verrucosus* was slightly lower in late summer and the LT50 remained unchanged probably because this species escapes rising temperatures and might thus not acclimatize.

[Mg²⁺] decreased similarly in 24-h and 4-week experiments, indicating its early decrease and then stabilization. [K⁺] was not positively correlated with temperature in *E. cyaneus* and *G. lacustris* in 24-h, but in 4-week studies. Maintaining a high stability might be energetically costly and effects thus only discernible in the long-term studies.

Gammarus lacustris—a potential invader to Lake Baikal?

To date *G. lacustris* is outcompeted by Baikal endemics in most Baikal habitats; low food availability, lack of hibernation retreats, and/

or intensive hydrodynamics might limit its distribution. Further, we observed that at low temperature (6 °C) Baikal amphipods were active whereas *G. lacustris* individuals were more rigid (sampling time: June). Animals sampled in September were more active than those caught in June indicating seasonal differences in physiological status causing different reactions to cold temperature. However, the observations suggest that the *Eulimnogammarus* species are more cold-tolerant than *G. lacustris* and should benefit from this advantage during cold seasons. Our results also show that the upper thermal limits of *G. lacustris* and *E. cyaneus* are similar and from the perspective of tolerance to high temperature *G. lacustris* should not be more competitive at elevated temperatures, but the competitive advantage from cold adaptation of Baikal endemics may be diminished.

Low metabolic rates likely improve a species' fitness in the oligotrophic Lake Baikal. Even though mass-specific metabolic rates generally decrease with increasing body size, *E. cyaneus* (endemic, Baikal) has a lower mass-specific metabolic rate than the larger *G. lacustris*, (ubiquitous, Holarctic), which could be due to higher activity of the latter. Assuming that energy efficiency and savings are optimized in food-limited Baikal amphipods, it is likely that *G. lacustris* would be affected by low food availability. However, increasing temperatures will possibly lead to higher amounts of benthic organic matter, which could benefit *G. lacustris*.

Considering the intensive hydrodynamics in the Baikal littoral, we hypothesize that resistance to mechanical stress is another key parameter that prevents *G. lacustris* from invading the Baikal littoral. *G. lacustris* is generally found in lakes and slow-moving rivers (Matafonov 2007). A preliminary experiment revealed that in comparison to *E. verrucosus* and *E. cyaneus*, *G. lacustris* was less resistant to mechanical stress when gradually increasing pressure was applied to their exoskeleton by use of a glass capillary (diameter: 100 µm). Exoskeletons of *G. lacustris* were highly elastic and compressed completely in 45% of the tested animals. In contrast, Baikal species resisted equal pressure with little deformation. In addition, the inorganic content of whole animals was lowest in *G. lacustris*. This might indicate a lower resistance to mechanical

disturbance than in Baikal endemics. It remains to be shown whether the exoskeleton of *G. lacustris* can evolve to resist the enormous physical disturbances in the Baikal littoral. Furthermore, a soft exoskeleton increases predation risk as we observed predation pressure to be highest on freshly molted amphipods. The abundance of *G. lacustris* correlates negatively with that of fish but positively with the occurrence of submerged aquatic vegetation (Anteau et al. 2011). Bullhead fish prey on benthic amphipods in Lake Baikal and submerged vegetation is absent around Bolshie Koty. This may also prevent the settlement of *G. lacustris* along the rocky shores of Lake Baikal but this situation may change due to ongoing coastal eutrophication (Silow 2014, Timoshkin et al. 2014).

CONCLUSIONS

Our data show the complex interaction of physiological constraints and habitat characteristics in shaping ecological patterns. In all investigated amphipod species, oxygen supply constraints became limiting at high temperatures. Comparing the two endemic species inhabiting the littoral of Lake Baikal, *E. verrucosus* ($T_p = 10.6$ (9.5, 11.7) °C) is significantly less thermally tolerant than *E. cyaneus* ($T_p = 19.1$ (17.9, 20.2) °C). By determining the upper (pejus) threshold temperature of physiological performance we could explain the warming induced retreat and loss of abundance of *E. verrucosus* in its natural habitat.

At first sight *E. verrucosus* may be more adversely affected by climate change than *E. cyaneus*, but as the former is able to escape from unfavorable conditions it might be the latter more sedentary species which is more vulnerable to increasing water temperatures despite its higher thermal tolerance. Temperatures of 20 °C can be reached in the upper littoral of Bolshie Koty bay, slightly exceeding the T_p of *E. cyaneus*. Following recent climate models for the Baikal region, this temperature is likely to be reached or even surpassed more often in future summer seasons and could negatively impact the fitness of *E. cyaneus*. If it is unable to escape from the rising temperatures to colder, deeper waters this species might be affected, e.g., suffer from reduced body size and, consequently, lower numbers of offspring.

Gammarus lacustris ($T_p = 21.1$ (19.8, 22.4) °C) showed similarities in thermal tolerance to *E. cyaneus* and displayed no distinct characteristics of thermal physiology that would benefit *G. lacustris* at higher temperatures. A combination of various other ecophysiological traits might prevent *G. lacustris* from becoming established in Lake Baikal. The importance of the variables food limitation, degree of cold adaptation, and sturdiness of exoskeletons to biomechanical stress remain to be investigated in future studies as well as further secondary factors of global warming and eutrophication.

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DATA ACCESSIBILITY

Data presented here are available at: <http://dx.doi.org/10.1594/PANGAEA.845942>

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SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1308/supinfo>