The Antagonistic Effect of Raised Salinity on the Aerobic Performance of a Rocky Intertidal Gastropod *Nassarius deshayesianus* (Issel, 1866) Exposed to Raised Water Temperature

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Abstract

Rocky intertidal organisms are facing pronounced fluctuations in environmental conditions even at small spatial and temporal scales. This heterogeneous habitat is a proper model system to investigate effects of physical parameters and their interactions on physiological performances of marine organisms. In the intertidal zone (especially in tidal pools), the temperature and salinity usually increase in concert during the daytime low tides. In this study, we used a rocky intertidal scavenger gastropod, *Nassarius deshayesianus*, as a model to test if high salinity can affect the metabolic performance of rocky intertidal mobile ectotherms at high temperatures. In August 2009, specimens were exposed to normal and elevated temperatures and salinities for 50 minutes and their specific metabolic rates were evaluated (N=12). At the high temperature treatment, metabolic rates were significantly lower when the salinity was high. Considering the concept of oxygen- and capacity- limited thermal tolerance, we will discuss that this antagonistic effect of increased salinity on the aerobic performance at the high temperature may lower thermal tolerance of intertidal ectotherms. Also *N. deshayesianus* showed high mortality after a few days of exposure to 36°C water temperature. This signifies that they may currently live close to the maximum limit of thermal tolerance in the hottest sea of the world, the Persian Gulf.

Keywords: Global warming, Iran, Persian Gulf, Scavenger gastropod, Specific oxygen consumption.

1. Introduction

It is now evident that global systems have been warming for the last decades at an unprecedented rate (IPCC, 2007) which altered the biogeographical distribution of many species in the oceans (Wethey and Woodin, 2008). While the warming is predicted to continue (IPCC, 2007), for the proper estimation of changes in biogeographical distribution of marine ectotherms in the future, it is crucial to know about their physiological responses to elevated temperatures (Somero, 2010). One of the methods to assess the current thermal tolerance and the future thermal sensitivity of marine organisms, is the determination of maximum critical temperature of death (CT_max) of target species by subjecting them to the gradual elevation of temperature (Brown et al., 2011). Nevertheless in many marine habitats, other physical and chemical parameters can interact to influence the physiological tolerance of a marine organism (Metzger et al., 2007; Walter et al., 2009). Hence, CT_max-based experiments may under- or over-estimate the current physiological tolerance of a target species. This can
subsequently result in an inaccurate prediction of the future condition of marine organisms faced with global warming.

In the rocky intertidal habitat, environmental parameters (i.e., temperature, desiccation, wave exposure, and salinity) not only differ between zones (between high and low intertidal), but also differ between microhabitats (scale of cm to m) and among sites along a coastline (scale of m to km) (Helmuth et al., 1999; Helmuth and Hofmann, 2001). This heterogeneous nature has made the intertidal habitat a proper model system to investigate the effect of different environmental parameters and their interactions on physiological performance of marine organisms (Helmuth, 2009 and 2010). In the intertidal zone (especially in tidal pools), the temperature and salinity usually increase together during the daytime low tides. In this study, we used a rocky intertidal scavenger gastropod, Nassarius deshayesianus, as a model to test if high salinities can affect the metabolic performance of rocky intertidal mobile ectotherms at high temperatures. For this, in August 2009, specimens were exposed to normal and elevated temperatures and salinities for 50 minutes and their specific metabolic rates were evaluated (N=12). Our study took place in Qeshm Island, the Persian Gulf. The Persian Gulf is the most extreme environment in which even subtidal organisms are faced with high temperatures (up to 36°C) and high salinities (more than 38 psu) in some places (Sheppard et al., 2010). Nevertheless, in Qeshm Island, the near shore water temperature reached to about 35 °C in summer 2009 (pers. obs.). In this study, we observed the antagonistic effect of raised salinity on the aerobic performance of N. deshayesianus at high temperature. Considering the concept of oxygen- and capacity-limited thermal tolerance, we will discuss that the increased salinity may lower thermal tolerance of intertidal ectotherms faced with high summer time temperatures. In another experiment, N. deshayesianus showed high mortality after a few days of exposure to 36°C water temperature. This result showed that N. deshayesianus may currently live close to the maximum limit of thermal tolerance in the hottest sea of the world, the Persian Gulf.

2. Material and Methods

2.1. Characteristics of Study Sites

This study was undertaken in rocky intertidal shores with gentle slopes located at Qeshm Island in the Persian Gulf. The intertidal organisms at study sites experience a semidiurnal tidal regime. Study sites were located at the southern coast of Qeshm Island (Qeshm Site: 26° 51’ 34” N, 056° 08’ 52.8” E and ‘Naz Site’: 26° 48’ 52.0” N, 056° 06’ 40.8” E). Naz Island or Naz Site is located approximately 6 Km away from ‘Qeshm site’ and is about 300 m away from the southern shore of Qeshm. Naz Site with a northward orientation is protected from direct wave action. Conversely, Qeshm site is exposed to moderate wave action and is oriented southward. Due to the gentle slope of the shore, waves do not exceed a height of about 50 cm (pers. obs). The study was conducted in August 2009 when near shore water temperatures (during mid day high tide) ranged from 31 to 35°C and the salinity was about 37 psu at southern shores of Qeshm Island (pers. obs). During the mid-day low tides, salinities up to 41 psu were measured at rock pools located at mid to high tide levels (pers. obs.). All measurements of temperatures and salinities were done by a portable multi-meter (WTW-Germany).

2.2. Study Organism

Nassarius deshayesianus is a gastropod (family: Nassariidae) which occurs at the mid to low rocky intertidal zones distributed along the shores of the Oman Sea and the Persian Gulf (Bosch et al., 1995). It is most active at low tides during the night (pers. obs).

2.3. Sampling and Acclimation

48 specimens were sampled for 50 min exposure to elevated temperature and salinity (first experiment)
all of a similar size, during 6 subsequent low tides, i.e. 8 individuals per site and night (i.e. totally 96 specimen). Specimens were immediately transported within 20 min to the laboratory where they were acclimated at 31 °C and salinity of 37 psu for 2.5 days in the aquarium tank filled up with the artificial sea water. Each gastropod was fed bivalves once the morning after. We changed the water in all aquaria once at the second day of acclimation. Six additional specimens were collected during low tide from two study sites and acclimated in order to compare the survival rates of the test organisms at highly elevated temperature (36 °C).

2.4. Experimental Set up and Oxygen Consumption Measurements

After acclimation periods, gastropods were randomly exposed for 50 min to elevated temperature and salinity (36°C and 40.5 ±0.1 psu). A separate water bath (± 0.25°C) was used for each temperature. The experimental units were 250 ml aerated aquaria, placed inside the baths but isolated from the surrounding water. To keep the salinity level inside the aquaria constant, the aeration inside aquaria were as low as possible (bubble by bubble) and aquaria were clogged by aluminum sheets to prevent the evaporation. In all phases of the experiment, artificial sea water, which was made from mixing distilled water with the natural sea salt was used. Using one oxygen meter, the oxygen consumption of each animal was measured in the last 30 min of thermal stress period in order to estimate the metabolic rate (Willmer et al., 2005). Since N. deshayesianus stays immobile when a prey stimulus is absent, the rate of metabolism was not confounded by variations in the activity patterns between individuals. 16 replicates were measured subsequently during the course of one day while this procedure was repeated at 6 subsequent days. For the metabolic rate measurement, the animals were transferred to 70 ml containers saturated with oxygen which served as respiration chamber with the same temperature as the respective water baths. Afterwards, we waited 15 min. for relaxation of the animal. In order to ensure that the gastropods respired normally, water movement in the chamber was maintained with a magnetic stirrer (Irwin et al., 2006). The oxygen concentration in the chambers was determined directly after animals were relaxed and after a 30 min period. To determine the specific oxygen consumption, the dry body weight of each animal was measured by freezing at -60°C followed by placing in 10% acetic acid to dissolve the shells. Next, the soft remaining body was dried by an electrical dryer at +65°C and weighing was carried out after the constant weight had achieved within about 5 h. Finally, the specific oxygen consumption (mg/mg/h) was determined by dividing the oxygen consumption of each gastropod in one hour by its dry body weight.

Finally, the survival rate of animals exposed to 36 °C water temperature was evaluated by putting acclimated specimens in aquaria (similar to the previous experiment) and recording mortality rates in subsequent days.

2.5. Experimental Design and Statistical Analysis for Metabolic Rate Comparison

A completely randomized block design was employed (50 min exposure to raised temperature and salinity). "Temperature" (with 2 levels), "Salinity" (with 2 levels) and "Site" (with two levels) were used as main factors; and "Day" (with 6 levels) was used as the blocking factor. The specific oxygen consumption of test organisms was negatively correlated with the dry body weight at all treatment levels (r /g149 -0.6, N= 12, P<0.001). This is in agreement with earlier findings for other organisms which are regarded as Klieber law (Willmer et al., 2005) which means that bigger specimens had a lower specific oxygen consumption than smaller ones. Therefore, unavoidable size differences between sampled specimens in different samples could confound our result by increasing the residual (unexplained) variation. Since the correlation coefficient was more than 0.4, it was the
most appropriate to use the dry body weight as the covariate in the analysis of covariance (Sheskin, 2004). Analysis of covariance (ANCOVA) is a statistical test in which the effect of a continuous predictor or covariate will be accounted and then a large reduction in the error sums of square may be achieved (Field, 2005).

So, we analyzed our data using general linear models (GLM) dialog of Statistica 8 in which we assessed full factorial effect of the Temperature and Salinity and main effects of the Day and Site. We used the planned comparison analysis for pair wise comparison of samples.

Planned comparison uses the same model as the main analysis, so is the only test which is appropriate for the pair wise comparison of samples in ANCOVA (for more details on planned comparison in ANCOVA (Field, 2005). All of the statistical procedures were done via Statistica 8.

3. Results

3.1. Metabolic Rate Comparisons

Temperature and salinity had significant effects on respiration rates of Nassarius deshayesianus after 50 min exposure to raised temperature and salinity (Table 1). At elevated temperature (36°C), the specific respiration rate of specimens was lower when the salinity was high (40.5 psu) (Fig. 1).

The planned comparison analysis confirmed that, in elevated temperature, there was a significant difference between the specific metabolic rate of specimens exposed to high and normal salinity treatments (F=7.54; P<0.01).

3.2. Survival Rate Experiment

All the specimens survived at 31°C but at 36°C, 3 specimens died after 2 days and 3 specimens survived up to 3, 4 and 7 days (N=6). At 36°C test organisms stopped feeding and went to heat coma (a few hours before death). The gastropods in heat coma turned upside down with their feet extended and were non-responsive to mechanical stimuli.

Table 1. Analysis of covariance on temperature, salinity, site, day and dry body weight affecting respiration rates of Nassarius deshayesianus after 50 min exposure to raised temperature and salinity

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>1</td>
<td>0.000000</td>
<td>5.8013*</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.000002</td>
<td>59.8623*</td>
</tr>
<tr>
<td>Salinity*Temperature</td>
<td>1</td>
<td>0.000000</td>
<td>2.1997</td>
</tr>
<tr>
<td>Site</td>
<td>1</td>
<td>0.000000</td>
<td>1.7219</td>
</tr>
<tr>
<td>Day</td>
<td>5</td>
<td>0.000000</td>
<td>4.5485*</td>
</tr>
<tr>
<td>Dry body weight</td>
<td>1</td>
<td>0.000005</td>
<td>120.5549*</td>
</tr>
</tbody>
</table>

*= P<0.05

Fig. 1. Specific oxygen consumption of Nassarius deshayesianus exposed to 50 min normal and elevated temperatures and salinities (plotted after using the dry body weight as covariate and computing for covariate at their means). Diamonds denote for normal summer time temperature (31°C) and quadrates denote for elevated temperatures (36°C). Vertical bars denote 0.95 confidence intervals.

3.3. Field Temperature and Salinity Measurements

In August 2009, near shore water temperatures (during midday high tides) reached to about 35°C and the salinity was about 37 psu at southern shores of Qeshm Island (pers. obs.). During the mid-day low tides, the salinity was up to 41 psu at mid to high tide rock pools (pers. obs.).
4. Discussion

A carnivorous scavenger gastropod, *Nassarius deshayesianus*, was resistant to laboratory condition and also usually stayed immobile when a prey stimulus was absent. Hence, rate of metabolisms was not confounded by variation in activity patterns of individuals. So, this species and perhaps other scavenger gastropods with similar behaviors could successfully be used as the model organism to evaluate effects of physical and chemical parameters on metabolic rates of rocky intertidal mobile ectotherms.

In this study, at one degree higher than maximum near shore water temperature (i.e. 36°C), the metabolic rate of specimens subjected to elevated salinity treatment (i.e. 40.5 psu) was significantly lower than that of specimens subjected to normal salinity (Fig. 1). At the elevated temperature (36 °C), the elevated energetic needs resulted in higher oxygen consumption (Fig. 1). But the reduced metabolic performance at high- temperature and salinity treatment was most likely due to the inhibitory response of the respiratory system to avoid losing osmotic balance in the new hyper-osmotic environment (Cheung and Lam, 1995). Hence the hyper-salinity induced reduction in metabolism could result in the lowered capacity of the animal to support the required oxygen of tissues at high temperature (i.e. decrease at aerobic performance). Regarding the concept of oxygen- and capacity-limited thermal tolerance (Portner, 2010), the aerobic scope (i.e. increasing aerobic performance from resting to maximum) matched the thermal window of water breathers.

On the other hand, when the temperature passes the optimal range, oxygen transport system can no longer support the energetic needs of a marine organism and these decrease physiological performances. This has been demonstrated for many different taxa of marine animals (Portner, 2010). Furthermore, the reduction in the aerobic scope and subsequent reduction in thermal tolerance have been caused by other climatic factors including acidification (Metzger et al., 2007; Walter et al., 2009), hypoxia and heavy metal pollution (Lannig et al., 2008). At the intertidal zone (especially tidal pools), the temperature and salinity usually increase in concert (especially at the mid-day low tides). Regarding the observed lessening effect of the raised salinity on the aerobic performance of *N. deshayesianus* at high water temperature, we suggest that the thermal tolerance of intertidal ectotherms may decrease by the antagonistic effect of the increased salinity at high water temperatures. In our study, gastropods were suddenly exposed to the elevated temperature and salinity.

This is different from naturally occurring changes that happen gradually in the field. Hence, future studies with more naturally simulated experiments are needed to verify our result. Nevertheless, in case of the presence of antagonistic effect of increasing salinity on aerobic performance of a marine water breather in the natural habitat, CT<sub>max</sub>-based experiment (using only thermal increase) may under- or over-estimate the current physiological tolerance of the target species. This can subsequently result in an inaccurate prediction of the future condition of a marine organism facing with global warming.

According to climate change synthesis report by intergovernmental panel for climate change (IPCC) in 2007, mean annual water temperature of the Persian Gulf has increased by 0.2 to 2 °C from 1970 to 2004. Also, recent models of global warming have predicted an increase of 1.8 to 4 °C at global average temperature (according to the different developmental scenarios) (IPCC, 2007).

The highest near shore water temperature (during mid-day high tides) in summer 2009 was about 35°C (pers. obs.). At 36°C most of our test organisms ceased feeding, went to heat coma (a few hours before death) and finally died after 2-3 days. According to the closeness of 36°C to the present naturally experienced temperature by *N. deshayesianus* in summers, this species may be damaged by the predicted future rise in
temperature due to the global warming. This study further emphasizes the importance of research regarding effects of global warming in the hottest sea of the world, the Persian Gulf.

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References


