Normoxic and anoxic energy metabolism of the southern oyster drill
*Thais haemastoma* during salinity acclimation

A direct calorimetric study

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Abstract

The energetic cost associated with salinity acclimation was determined in the marine gastropod *Thais haemastoma* by direct calorimetry under normoxic and anoxic conditions. Snails were collected from Caminada Pass near Grand Isle, Louisiana (Longitude 90°2'W; Latitude 29°2'N) in September 1987. Metabolic heat flux of snails acclimated to and measured at 10 or 30% S was similar at 15.06 or 16.39 J g⁻¹ dry wt h⁻¹, respectively, (corresponding to 0.76 or 0.83 ml O₂ g⁻¹ dry flesh wt h⁻¹) under normoxic conditions, and 2.39 or 2.53 J g⁻¹ dry wt h⁻¹ under anoxic conditions. Inter-individual variability was high, obscuring the effect of salinity gradient on heat flux. When standardized to the pre-transfer control level of each individual under anoxic conditions, a significant increase (55%) of energy expenditure was observed for snails transferred to hyperosmotic conditions. In contrast, heat flux varied insignificantly in individuals in the anoxic 30 to 10% S transfer. After transfer of individuals from 10 to 30% S under normoxic conditions, heat flux was depressed initially to 38% of the control rate, but recovered after 14 h to a higher metabolic rate (56%) than the pre-transfer control rate. After transfer of individuals from 30 to 10% S under normoxic conditions, heat flux was depressed to 28% of the control rate, followed by a 20 h period of recovery to the control rate. The energy cost of intracellular hyposmotic regulation was less than hyperosmotic regulation under anoxic conditions. The retraction of the foot of *T. haemastoma* after normoxic salinity transfers did not generally correlate with the time course of metabolic heat flux.

Introduction

No single hypothesis can account for the effects of salinity on the metabolic energy demand of marine invertebrates (Kinne 1971). Although anisosmotic processes are passive in osmoconformers, isosmotic intracellular osmoregulation is an active process. In osmoconforming molluscs, the oxygen-consumption rate of *Modiolus demissus demissus* (Baginski and Pierce 1975), *Rangia cuneata* (Henry et al. 1980), and *Mytilus edulis* (Widdows 1985) decreases upon transfer of individuals to hyperosmotic conditions. The oxygen-consumption rate also shows a decrease during transfer of individuals to hyposmotic conditions in *Crassostrea virginica*, *Mercenaria mercenaria*, *Modiolus demissus* (van Winkle 1968), *R. cuneata* (Henry and Mangum 1980), and *Mytilus edulis* (van Winkle 1968, Bayne 1973, Widdows 1985). In addition, the oxygen consumption of *Modiolus demissus*, *Mytilus edulis* and *Thais haemastoma* declines as the salinity fluctuates in either direction from the acclimation salinity and increases as the ambient salinity returns to the acclimation salinity (Findley et al. 1978, Shumway and Youngson 1979, Stickle and Sabourin 1979). An energetic interpretation of these changes of oxygen consumption is complex, since osmoregulatory energy demand and oxygen availability are superimposed in bivalves and snails due to their shell closing and retraction behavior (Bayne 1973).

In previous studies, metabolic rates were measured respiratorically as oxygen consumption. By this method, anaerobic metabolism involved in intracellular osmoregulation (Baginski and Pierce 1975) is not detected. The use of perfusion microcalorimetry allows the determination of total metabolic heat flux, including both aerobic and anaerobic sources of heat (Gnaiger 1983 a). Applying direct calorimetry in this study, we determined (1) the metabolic costs of isosmotic, intracellular hyperosmotic and hyposmotic regulation of *Thais haemastoma* during salinity transfers of individuals under normoxic and anoxic conditions, and (2) the retraction behavior of *T. haemastoma* during salinity transfers.

Materials and methods

Snails

*Thais haemastoma*, ranging in length from 15 to 25 mm, were collected from pilings and bulkheads in the vicinity of Caminada Pass near Grand Isle, Louisiana (Longitude 90°2'W; Latitude 29°2'N) in September 1987. The ambient
water temperature and salinity was 28°C ± 1°C and 26%, respectively. The snails were held in 38-liter aquaria (25°C ± 0.5°C) containing artificial seawater of the same salinity as in the field. The snails were acclimated by stepwise changes of 2% S per day to the target salinity and maintained for two weeks before the experiments began. Prior to the experiments, they were provided access to oysters (Crassostrea virginica) ad libitum. Thereafter, the snails were starved for three days before they were used in the experiments.

Calorimetric experiments

In both low to high (10 to 30%) and high to low (30 to 10%) salinity transfers, the heat flux of individual snails was measured in either an open-flow (perfusion) or a static modular microcalorimetry system (2777 Thermal Activity Monitor, ThermoMetric) described by Suurkuusk and Wadsö (1982, see also Gnaiger 1983b). The flow through the 3.5 ml stainless steel perfusion chamber was 20 ml h⁻¹ of normoxic (> 150 torr) or anoxic seawater. The level of anoxia (< 0.5% air saturation) obtained in the perfusion calorimeter was previously checked with a Cyclobios Twin-Flow respirimeter (Gnaiger 1983c).

Heat flux of individual snails was recorded continuously during perfusion with normoxic seawater for 120 h (24 h: pretransfer salinity; 25 to 120 h: target salinity). Subsequently, the perfusion medium was switched to seawater equilibrated with nitrogen, and the anoxic heat flux was recorded in several experiments. In the static 25 ml stainless steel chambers, heat flux was recorded in anoxic seawater at the pre-transfer salinity for 24 h and thereafter at the target salinity for another 24 h. Ammonium production under anoxic conditions at both the pre-transfer and post-transfer salinity was low, ranging from 0.1 to 0.4 μM NH₄ produced per snail over a 24 h period. The NH₄ concentration at the end of the 24 h experiment would have been no higher than 17 μM in the static chambers, while Thais haemastoma has been shown to tolerate NH₄ loading up to 350 μM without any apparent adverse effect (Kapper et al. 1985). The salinity transfer without intrusion of oxygen was possible by flushing anoxic seawater through stainless steel capillaries which were completely sealed during heat-flux measurements (Gnaiger 1983b).

Hourly averages of heat flux were calculated from instantaneous rates read at intervals of 1 min from the chart-recorder traces, expressed in J g⁻¹ dry flesh wt h⁻¹ (1 J h⁻¹ = 0.278 mW). The mean pre-transfer steady-state flux (control period) was determined as the last 12 h interval at the pre-transfer salinity. The standardized heat flux was calculated as a percentage of this control. One-way ANOVA and Schefê’s multiple-comparison tests (SAS Institute, Inc. 1982) were used to test the significance of variation of the standardized mean heat-flux.

Behavioral observations

The retraction behavior of a separate group of snails was observed in two 38-liter aquaria for 96 h after the target salinity was reached. Separate groups of snails were used for heat flux and behavioral observations because both the perfusion and static calorimeter chambers were opaque and positioned in the thermopiles of the calorimeter, making behavioral observations impossible. The salinity changes were established by gradually siphoning and refilling the aquaria with seawater. Observations were made at 3 h intervals during the first 12 h and thereafter at 12 h intervals. In this study, retraction behavior is calculated as the percentage of snails with the operculum closed and the foot, siphon and tentacles withdrawn.

Results

The heat flux of Thais haemastoma stabilized within the first 12 h period of normoxia after introduction to the pre-transfer salinity. The average heat flux was observed in snails acclimated to and measured at 10 or 30% S (15.06 or 16.39 J g⁻¹ dry wt h⁻¹, respectively; Table 1). Under these conditions, the ratio of calorimetric and simultaneous respirometric measurements were in agreement with the theoretically expected oxycaloric equivalent of 440 to 470 kJ/mol O₂ (Gnaiger 1983d). This was particularly true when heat and oxygen flux were averaged over several hours (unpublished results). Thus, the average heat flux of 15.06 or 16.39 J g⁻¹ dry wt h⁻¹ corresponds to an oxygen flux of 0.76 or 0.83 ml g⁻¹ dry wt h⁻¹.

The heat flux among individuals varied considerably in all experiments (Figs. 1C, 2C, 3B, 4B). The interindividual variability of metabolic activity obscured to a large extent the effects of salinity on heat flux (Table 1). Significant salinity effects were readily apparent when the heat flux of an individual during treatments were expressed relative to the pre-transfer period (standardized heat flux expressed as a percentage), because snails with a relatively high metabolic heat flux during the pre-transfer control period had a relatively high flux throughout the experiment.

After transfer from normoxic 10 to 30% S in the perfusion calorimeter, the mean standardized heat flux decreased within the first 2 h to a minimum of 38% of the pre-transfer control value, and returned to the control level after 14 h (Fig. 1A). In the 30 to 10% S transfer, the post-transfer standardized heat flux was reduced to a minimum of 28% of the control, followed by a longer period of recovery to the pre-transfer level after 20 h (Fig. 2A).

After the recovery period, standardized heat flux remained significantly higher than the control flux up to 120 h after transfer of snails to hyperosmotic conditions (Fig. 1A). In contrast, the standardized heat flux leveled off at a value insignificantly different from the control after transfer of snails to hypoosmotic conditions (Fig. 2A).

Under anoxic conditions in the closed calorimeter chamber, heat flux of Thais haemastoma declined rapidly during the first 12 h and attained a nearly stable level by 24 h. The average anoxic heat flux was similar at 10 and 30% S, with values of 2.39 and 2.53 J g⁻¹ dry flesh wt h⁻¹, respectively (Table 1). In contrast to the response observed in the