The metabolic costs of osmoregulation in a euryhaline fish, hogchoker (*Trinectes maculates*)

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Abstract

Fish that live in euryhaline environments in which the salinity varies substantially, such as estuaries, are required to regulate water and ions in their body through osmoregulation in order to combat passive diffusion across their cell membranes. This process involves the active transport of ions across cell membranes and requires energy, but the actual metabolic cost remains unclear. Estimates from previous studies range widely from 1.5% to 27% of the total energy budget of the fish. The purpose of this study was to attempt to determine the actual energetic costs of osmoregulation in a euryhaline fish, hogchoker (*Trinectes maculates*). Eighty-two fish were acclimated to either hypo-, iso-, or hyperosmotic conditions (0, 10, 30 ppt respectively) and their metabolic rates measured through static respirometry. There was a no significant difference in metabolic costs in any treatment; however, the activity of the active transport enzymes Na^+/K^+-ATPase and citrate synthase were both significantly elevated in the hyperosmotic treatment. The results suggest that while increased environmental salinity does present a challenge to the fish, the energetic costs of the physiological response are quite low at the whole organism level.

Introduction

The presence and movement of ions within a body play a critical role in how cells function in both terrestrial and marine habitats, affecting the overall performance of the organism. Varying levels of salts within the body, including Na^+, K^+, Cl^-, and Ca^{2+}, affect hydration, blood pH, and function and ability of muscles and nerves (Laurent and Perry, 1990). Due to the polar properties of water, ions are highly soluble. The majority of aquatic organisms have limited ability to control this passive movement of ions and are isosmotic, living in equilibrium with their respective environments. Vertebrates such as fish are able to regulate the amount of solutes, including ions, that are in their bodies. Freshwater fishes tend to lose ions and gain water because the potential gradient has a higher concentration of ions within the body than in the external environment. Saltwater fishes are the opposite, losing water and gaining ions because the salts in the external environment are at a much higher concentration than found inside the body. Most organisms are stenohaline – restricted to a narrow range of salinity – and the direction of ion movement varies depending on whether it is a freshwater or seawater species. However, some fishes are euryhaline and are capable of thriving across a range of salinity, which requires them to have the capability to maintain homeostasis at both lower and higher levels of ions.

When ion differences such as these occur, ions enter and exit the body through diffusion across two types of gradients: concentration and electrical. In concentration gradients ions diffuse passively from areas of high concentration to areas of low concentration until equilibrium is reached. In electrical gradients, ions actively move between areas of positive and negative electrical charge until the charge is neutral. Organisms seeking to regulate internal ion content must therefore have mechanisms to counteract these passive movements and maintain homeostasis.

In terrestrial environments, much of the burden of osmoregulation is carried by the kidney. Water is retained and excess ions are removed from the blood stream via diffusion across gradients, to be transported out of the body in a concentrated urine. In fishes, however, the gills are the main osmoregulatory organ for monovalent ions and the kidneys and intestines handle movements of divalent ions and water (McCormick et al. 1989).

Oxygen dissolves poorly in water, and because the primary function for which gills evolved was gas exchange for respiration, the gill structures are exceedingly thin in order to allow the oxygen to diffuse rapidly into the blood. This tremendous efficiency at gas exchange poses a problem when ion gradients are present between the organism and the environment, since ions and water are also able to rapidly diffuse across the membranes (McCormick *et al.*, 1989). The sites of osmoregulatory action in the gills are ionocytes – cells which facilitate the movement of ions using a number of transporter and channel proteins such as Na^+/K^+-ATPase (NKA) to create concentration and electrical gradients (McCormick *et al.*, 1989; Laurent and Perry 1990; Wood and Marshall 1994). Much of this process, including the role of NKA, involves active transport, using energy in the form of adenosine triphosphate (ATP) to pump ions against gradients. ATP is the basic unit of molecular energy. It is primarily created when glucose is broken down to pyruvate and then enters the complex series of aerobic enzyme reactions known as the citric acid cycle. One of the key enzymes in that process is citrate synthase; the presence of elevated levels of this enzyme can be used as an indicator of the presence of oxidative metabolism (McCormick *et al.*, 1989).

It has long been argued that osmoregulation must be metabolically demanding since it requires cellular energy reserves. Some authors have argued that this cost can affect the growth and fitness of teleost fish (e.g. Beouf and Payan, 2001; Wood and Marshall, 1994). Theoretical calculations place the cost of ion transport at 0.5-1% of resting metabolic rate (McCormick *et al.*, 1989). Previous authors have attempted to experimentally measure the metabolic costs of

osmoregulation in fishes; however, direct measurement of these costs has been extremely difficult to achieve. The results to date have been confounding, as high as 27% in some studies (e.g. Rao, 1968).

The observed values of the metabolic cost of osmoregulation likely differ greatly from theoretical calculations because of methodological difficulties. Different methods have been used in this pursuit: some authors have measured the growth rate of fishes in various salinities (Peterson-Curtis 1997), some have measured dissolved oxygen consumption (Febry and Lutz 1987; McCormick et al. 1989; Peterson-Curtis 1997; Boeuf and Payan 2001), and some have measured the activities of chemicals and hormones within the body (McCormick 1989). One issue with the study of growth rate is that it is not solely determined by the metabolic costs; other factors include food intake and utilization or hormonal control. Measurement of oxygen consumption is the standard method for determining metabolic cost; however, osmoregulation is still likely a small cost compared to movement, digestion, and growth.

The goals of this study were to use a euryhaline flatfish, hogchoker (*Trinectes maculates*) (Fig. 1) in order to

Figure 1: juvenile hogchoker

experimentally measure the actual costs of osmoregulation. The hogchoker is a small flatfish native to New England. As adults, the species lives in estuaries and coastal regions of Long Island Sound, but it migrates into freshwater to spawn and juvenile fishes rear in that habitat before moving into saline conditions as they develop. Hogchoker were chosen as a preferred study species for this study because they are small, widely euryhaline, and sedentary. It was expected that the metabolic costs of osmoregulation would be elevated in hypo- and hyperosmotic conditions compared to fish acclimated to isosmotic water because the costs of ion and water transport would be higher in those conditions to compensate for diffusion. Gill NKA and citrate synthase levels were expected to be highest in hyperosmotic conditions because of the increased activity of NKA in seawater ionocytes and the increased demand for metabolic energy through aerobic cellular respiration.

Methods and Materials

Juvenile hogchokers were caught by seine net in low salinity water (~1 parts per thousand (ppt)) in the Quinnipiac River estuary in June 2013 and transported to the University of New Haven. Approximately thirty fish each were kept in three 150-L recirculating, filtered, chilled tanks. Fish were gradually acclimated to target salinities of 0, 10, and 30 ppt over a two week period, reflecting hyposmotic, isosmotic, and hyperosmotic conditions, respectively. Food was withheld from the fish in a specific treatment for 24 hours before the trial occurred. Trials took place at the same time each day to minimize the influence of any diel changes in metabolism. Trials began by placing individual fish in custom constructed glass respirometer chambers (~250 ml) where they rested for two hours (Fig. 2). Chambers contained a thin layer of sterilized sand on the

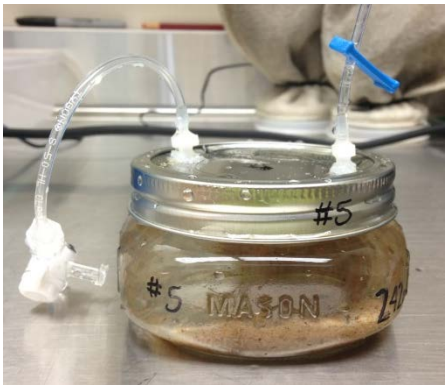

Figure 2: example of the experimental respirometer chambers.

bottom to promote quiescence; fish were provided with recirculating water supply from their respective acclimation tanks. At the beginning of the trial, a 5-ml water sample was removed from each respirometer and the dissolved oxygen (DO) was measured using a micro flow-through oxygen probe (DO-166FT, Lazar Labs), after which the chambers were sealed from the surrounding tank. In each trial, ten individuals from the same salinity treatment ran simultaneously for a duration of three hours.

At the end of the trial period, a final DO reading was taken from each sealed chamber. The change in DO reflects the amount of oxygen used by the fish for aerobic respiration and is a metric of metabolic rate (MO_2). After the trial, the fish were returned to a holding tank of the same salinity to recover from handling. Once a round of one trial from each salinity was completed, those fish were rapidly euthanized in an overdose of anesthetic (100 $mg \cdot L^{-1}$ MS222 tricaine methanosulfonate, Western Chemical). For each, length and wet weight were recorded and the gill basket dissected and stored in SEI buffer at -80°C for later determination of NKA and citrate synthase enzyme activity. NKA and citrate synthase activity was determined with a kinetic assay as described in McCormick (1993) and McCormick et al. (1989), respectively. Head, viscera and fins were removed and the trunks were dried for twenty four hours at 60 °C, after which a dry weight was obtained. Results were analyzed by one way ANOVA and Tukey-Kramer post hoc test, with significance accepted at $\alpha = 0.05$.

Results

Trials were conducted on a total of 82 juvenile hogchokers; however, 19 fish under 0.5 g were later omitted

from analysis because it was difficult to precisely measure MO_2 in such small fish in the respirometers used in this study. All statistical analyses were conducted on the remaining 63 fish (Table 1). There was no mortality in any of the treatments. Metabolic rate (Fig. 3) and tissue water content (Fig. 4) were not significantly different among any treatment. There was a significant difference between the activity of both Na^+/K^+-ATPase and citrate synthase in the 30 ppt treatment compared to the other treatments (Fig. 5 A and B); however, 0 ppt and 10 ppt were not significantly different from each other.

Treatment	Number of fish	Mean mass (g)	Mean Total Length (cm)
0 ppt	22	1.28 ± 0.10	4.5 ± 0.11
10 ppt	18	1.28 ± 0.11	4.5 ± 0.12
30 ppt	23	1.25 ± 0.12	4.4 ± 0.14

Table 1: Experimental treatment groups used in this study (mean ± SEM).

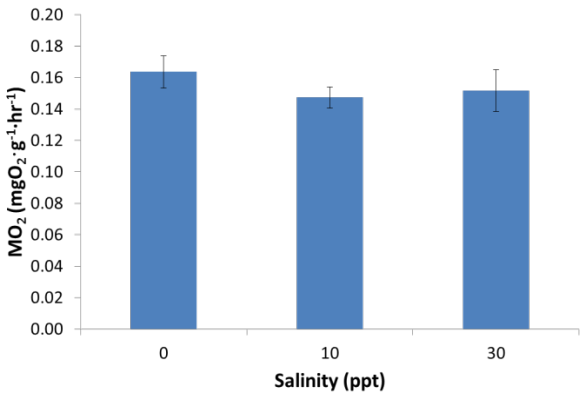

Figure 3: Mean (±SEM) metabolic rate.

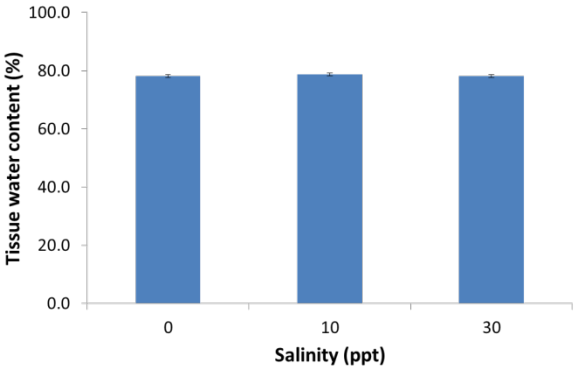

Figure 4: Mean (±SEM) tissue water content.

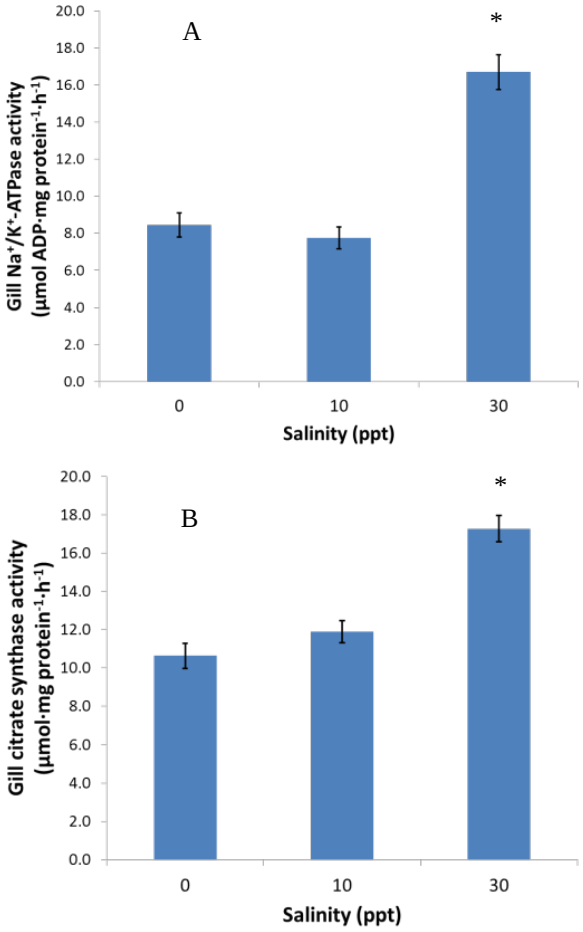

Figure 5: Mean (±SEM) activity of A) Na+/K+-ATPase and B) citrate synthase enzymes in gill tissue. * indicates treatments that were significantly different ($p<0.001$, one way ANOVA and Tukey's HSD post hoc tests).

Discussion

Fish use the energy extracted from consumed food for many tasks including movement, growth, reproduction, and digestion as well as the continuous basal costs of maintaining respiration and cellular function. Among many tasks included in that resting metabolic expense is the cost of maintaining osmoregulatory homeostasis. Since much of osmoregulation requires active transport, it has long been argued that this expense is significant and potentially limiting to fish. However, researchers have found it hard to measure these costs and estimates vary from as low as 0.5% to as high as 29% of the resting metabolic rate (McCormick et al. 1989). This study attempted to measure osmoregulatory costs by combining modern analytical methods with a sedentary, euryhaline subject species. An earlier study of MO_2 in hogchoker reported that juveniles exhibited significantly lower rates in 7 ppt than in 0 ppt, and intermediate rates at 15 ppt (Peterson-Curtis 1997). That author postulated that these costs explained a seasonal migration for yearling hogchokers from freshwater into less

energetically costly brackish water. The results of this study do not support that finding. While there was a slight trend to lower MO_2 from 0 ppt to 10 ppt, the magnitude was small and not significant. Additionally, there was no difference in tissue water content between salinities, indicating that the fish were well within the limits of their capacity to maintain homeostasis, and were not stressed, even at levels similar to full strength seawater. Interestingly, both Na^+/K^+-ATPase and citrate synthase enzyme activity were considerably higher in the 30 ppt treatment. This suggests that the fish were working harder at the cellular level to osmoregulate, reflected in both the amount of ATP-requiring movement of ions and the amount of mitochondrial activity. Since these large increases in energy-requiring activity in the gills do not translate into measurable increases in the metabolic rate of the animal, these results suggest that a very small percent of a fish's total energy budget goes to osmoregulation, supporting the theoretical arguments proposed by McCormick et al. (1989).

Conclusions

Understanding the costs of this critical component of metabolism furthers our understanding of osmoregulation in fishes in general and provides insight into the evolution of the often dramatic migratory movements observed in euryhaline and anadromous species of fish such as salmon. Additionally, an understanding of the energetic costs of osmoregulation could have applied value, permitting aquaculturists to grow fish more rapidly. However, at least in the present study, the actual costs of osmoregulation appear to be small and are likely not limiting to the animal.

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Acknowledgments

I appreciate the support of the University of New Haven's Summer Undergraduate Research Fellowship program. I would like to specifically thank my mentor, Dr. John Kelly, for the help and guidance through this research project. Thanks are also due to Patrick Vogt for providing tank preparation and lab assistance. Fish were captured with the help of Patrick Vogt, Lauren Kircher, Danielle Perry, and Sam Gurr. Assistance with enzyme assays was generously provided by Steve McCormick with help from Mike O'Dea and staff at the USGS Conte Anadromous Fish Research Center.

Biography

Jessica Norstog is currently a junior at the University of New Haven, majoring in Marine Biology and Environmental Science. She plans to continue her education to a doctorate degree, striving for a career in fish physiology research.