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

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Introduction

The presence and movement of ions within a body plays a critical role in how cells function in both terrestrial and marine habitats, affecting the overall performance of the organism (Laurent and Perry 1990). The majority of aquatic organisms have limited ability to control this passive movement of ions and are isosmotic, living in equilibrium with their environment. Vertebrates such as fish are able to regulate the amount of solutes,

Figure 1: juvenile hogchoker

including ions, that are in their bodies. 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.

The structure and large surface area of fish gills, which evolved to maximize diffusion of respiratory gasses, also permits the movement of metabolic waste products, ions, and water, and the gills function as the primary osmoregulatory organ for monovalent ions (McCormick et al. 1989). The site of osmoregulatory action in the gills are chloride cells which facilitate the movement of ions using a number of transporter and channel proteins such as Na^+/K^+-ATPase to create concentration and electrical gradients (McCormick et al. 1989; Laurent and Perry 1990; Wood and Marshall 1994). Much of this process involves active transport, using energy in the form of ATP to pump ions against gradients.

It has long been argued that osmoregulation must be metabolically demanding since it requires cellular energy reserves, and 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); however, direct measurement of these costs has been extremely difficult achieve and the results to date have been confounding (as high as 27% in some studies, e.g. Rao 1968).

The goals of this study were to expose a euryhaline flatfish, hogchoker (*Trinectes maculates*) (Fig. 1) to different salinity levels in order to determine differences in:
- resting metabolic rate,
- activity of key enzymes involved in osmoregulation (Na^+/K^+-ATPase) and oxidative metabolism (citrate synthase, a component in the Citric Acid Cycle) in the gills.

Methods

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, chilled tanks. Fish were gradually acclimated to target salinities of 0, 10, and 30 ppt, reflecting hyposmotic, isomotic, and hyperosmotic conditions, respectively, and allowed to acclimate for two weeks. Trials took place at the same time each day, and began by placing individual fish into custom constructed respirometer chambers (~250 ml) where they rested for two hours (Fig. 3). Chambers were supplied with recirculating water supply from their respective tank, along with a thin layer of sterilized sand on the bottom to promote quiescence. At the beginning of the trial, a 5-ml water sample was removed from each respirometer and the dissolved oxygen (DO) measured using a micro flow through oxygen probe (DO-166FT, Lazar Labs, Fig. 2), 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 two hours (Fig. 4).
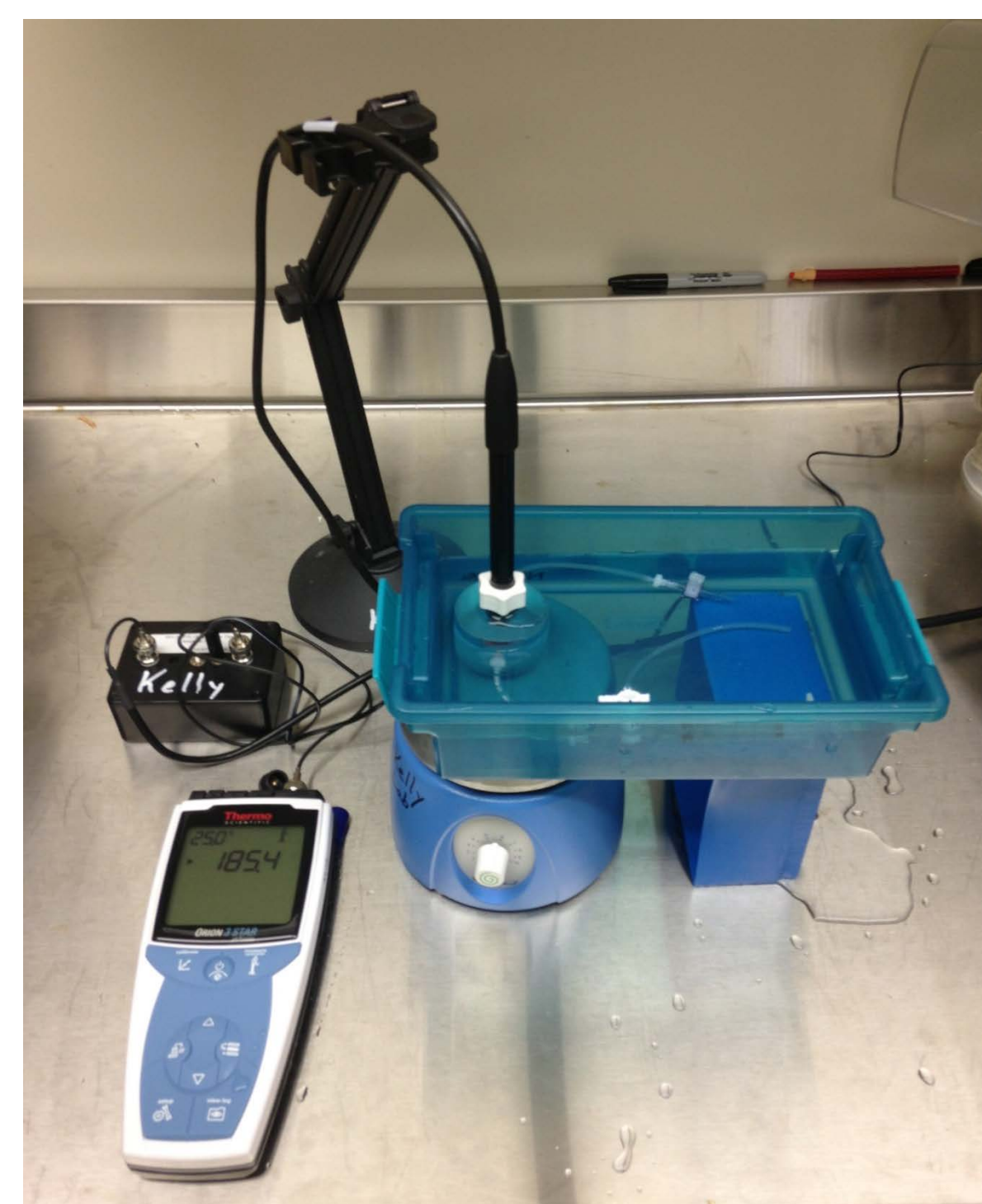

Figure 2: Set up and instrument for reading dissolved oxygen

Methods (cont.)

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, the fish were rapidly euthanized in an overdose of anesthetic (100 mg·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 Na^+/K^+-ATPase and citrate synthase enzyme activity. 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 α = 0.05.

Figure 3: example of the experimental respirometer chambers.

Figure 4: system of ten chambers held below water surface within treatment tank.

Results

Eighty-two juvenile hogchokers were initially used in this study; 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 with the respirometers used in this study. All data analyses were conducted on the remaining 63 fish (Table 1).

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

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

There was no mortality in any of the treatments. Metabolic rate (Fig. 5) and tissue water content (Fig. 6) 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. 7 A and B); however, 0 ppt and 10 ppt were not significantly different from each other.
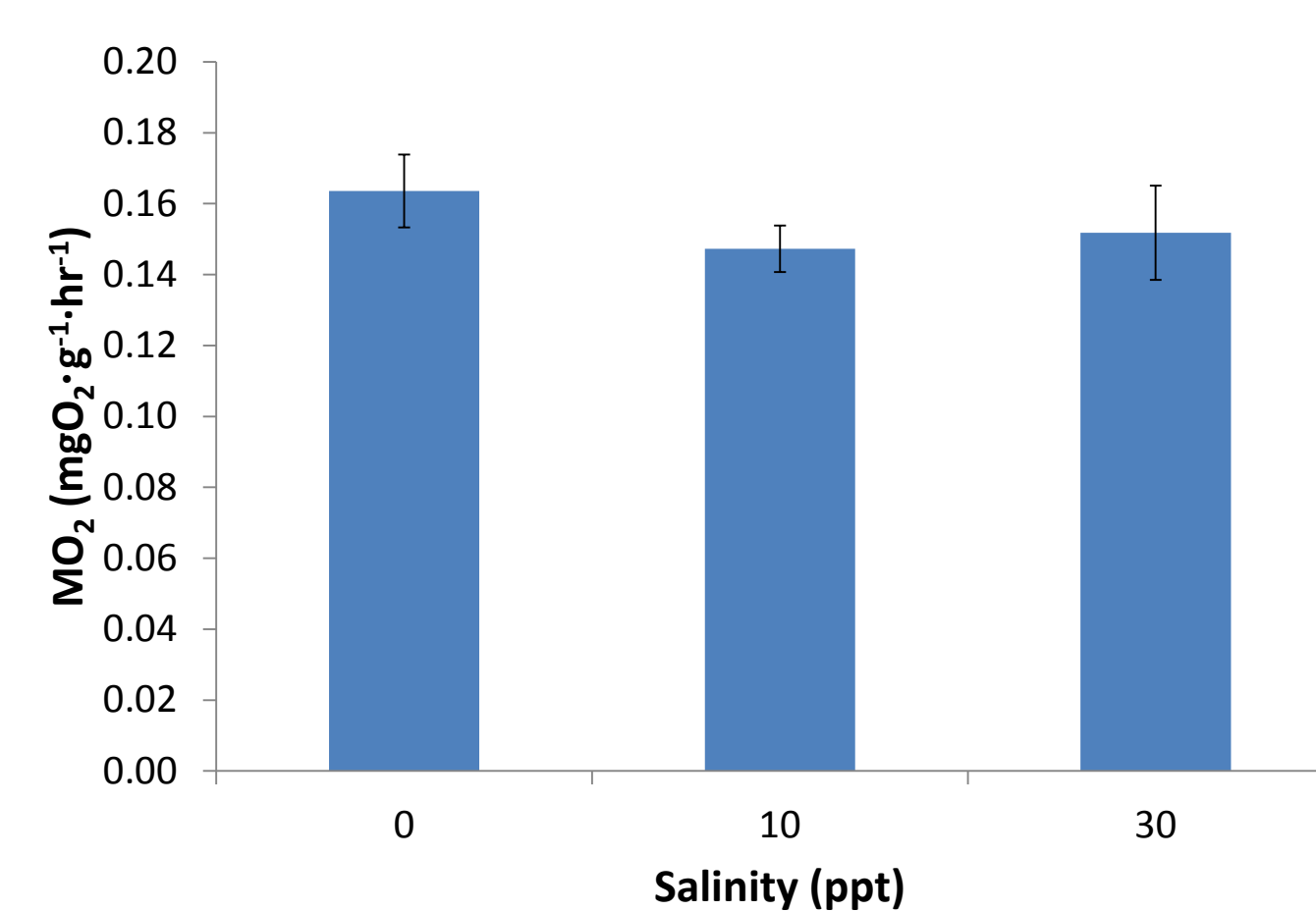

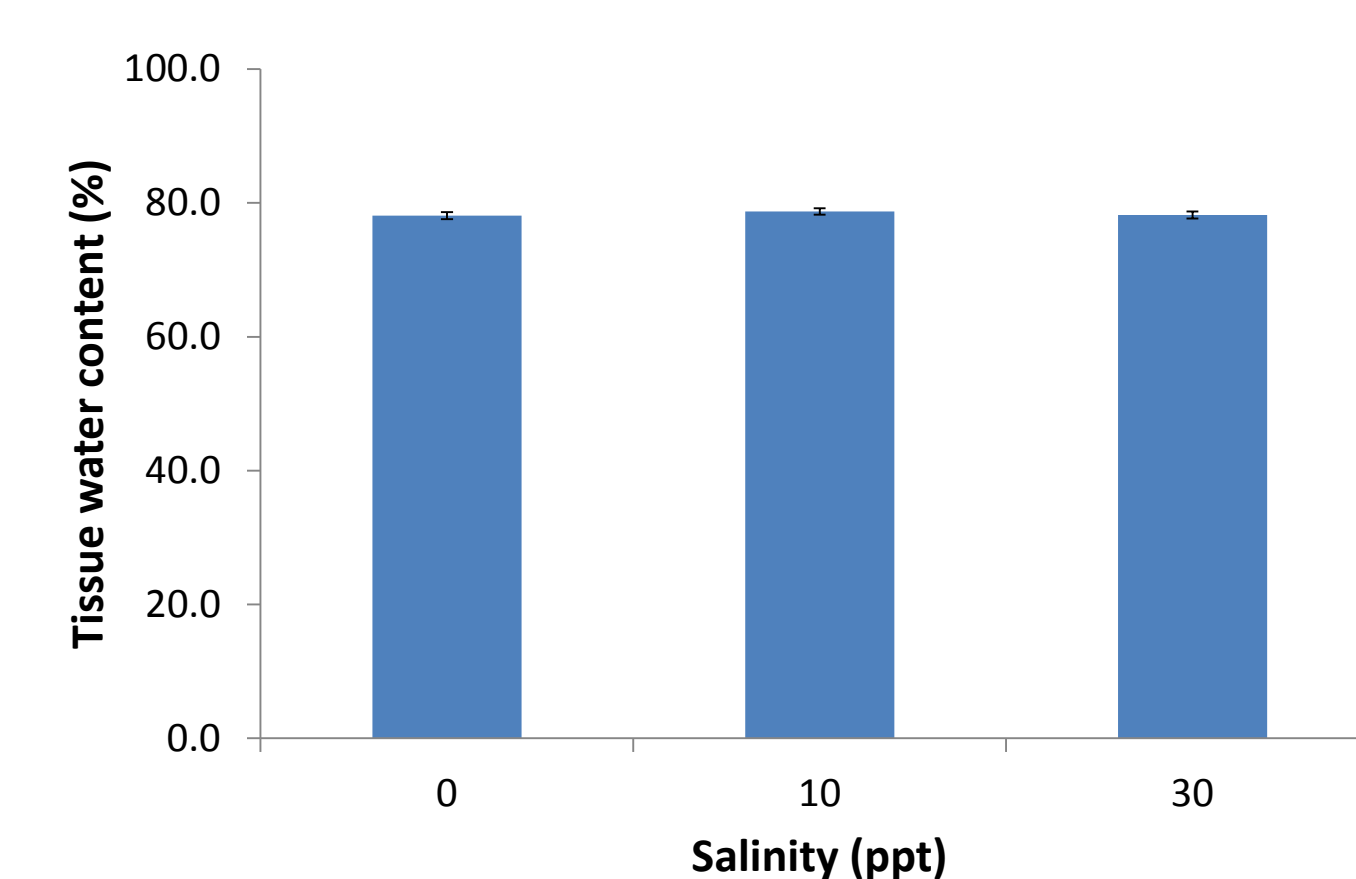Figure 5: Mean (±SEM) metabolic rate.
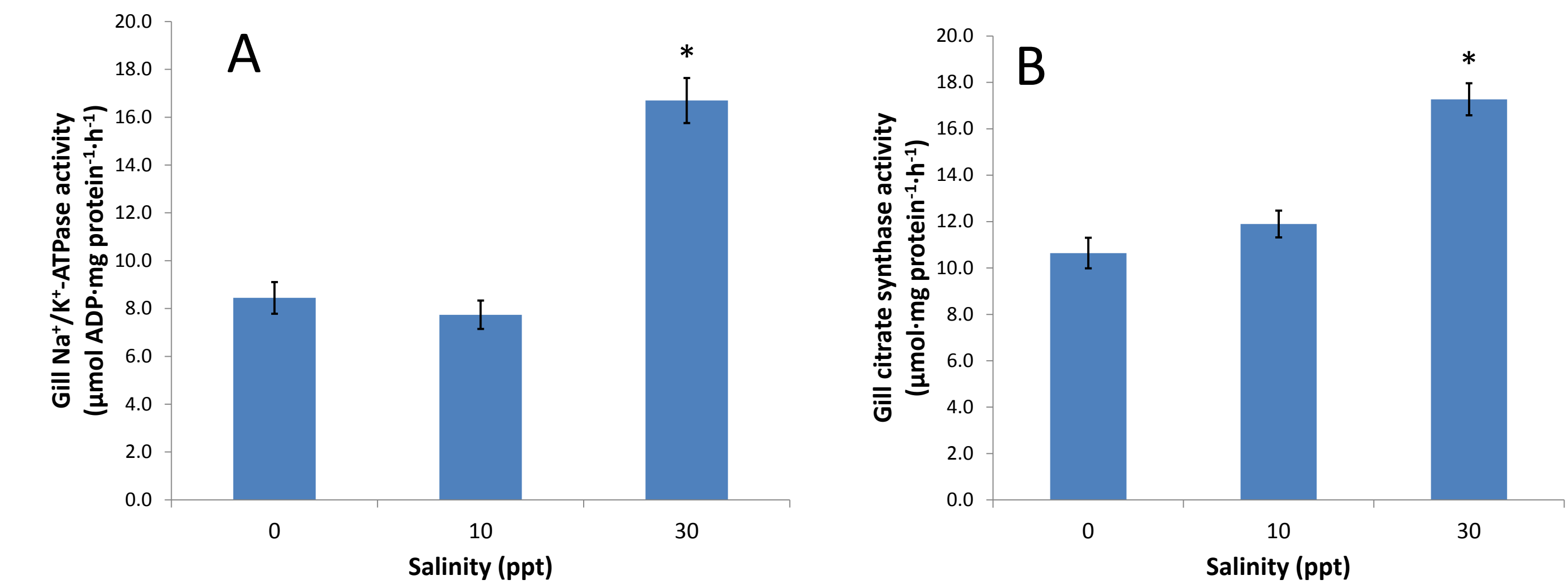

Figure 6: Mean (±SEM) tissue water content.

Figure 7: 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 fishes; 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, 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 hogchoker'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

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