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Acid-Base Regulation in Three Marine Teleosts: The Oyster Toadfish (*Opsanus tau*), the Winter Flounder (*Pseudopleuronectes americanus*), and the Long-Horned Sculpin (*Myoxocephalus octodecimspinosus*)

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ABSTRACT

In this study, three species of marine fish were exposed to a variety of dilute salinities in order to determine what effects exposure to diluted seawater may have on acid-base and ion balance. The oyster toadfish (*Opsanus tau*) and the winter flounder (*Pseudopleuronectes americanus*) are considered to be able to live in a broad range of salinities, euryhaline (Evans, 1979). The long-horned sculpin, *Myoxocephalus octodecimspinosus*, is considered to be a stenohaline fish, not able to withstand drastic changes in ambient salinity (Claiborne and Evans, 1988).

Initially, toadfish exposed to 20 mM diluted seawater took up ΔH^+ from the surrounding environment. However, after seven additional days in this salinity, net transfers returned to control levels. This indicates that toadfish are able to regulate the loss of ΔNH_4^+ and ΔHCO_3^- to the environment. Preliminary blood data show that these fish can also regulate [Cl^-] loss during exposure to low salinities. In 5 mM diluted seawater, ΔH^+ uptake increased approximately three times that observed in the 20 mM group. After an additional four days in 5 mM, net transfers were still significantly below control excretion rates and mortality was noted.

Flounder exposed to 20 mM diluted seawater did not show any significant change in excretion rates of the fish that were maintained in a tank of 20 mM diluted seawater an additional week, only one fish survived the entire week. Sculpin were able to withstand exposure to 20 mM diluted seawater for periods of 24 hours. During this 24-hour period fish exhibited a rapid loss of ΔHCO_3^-, but no change in

plasma pH. Sculpin survived 100 mM diluted seawater very well. Following 11 days of exposure to 100 mM seawater, excretion rates were not significantly different from seawater control values. When placed in 20 mM diluted seawater following a long 100 mM adaptation, fish did begin to lose ΔHCO_3^-, but this rate was significantly lower ($p \leq 0.05$) than the rate for fish that were transferred directly from seawater to 20 mM diluted seawater. Adjusting osmolarity and injections of epinephrine did not assist the sculpin in 20 mM diluted seawater. The kidneys are able to make up for 35% of the total ΔH^+ lost from the fish.

The ability of the toadfish and flounder to adjust acid-base losses in dilute salinities may be the key to their survival. It is interesting that the sculpin is able to maintain a constant pH during the acidosis that occurs in dilute salinities. Our indirect evidence suggests that bone demineralization is a source of the observed acid-base alterations during 20 mM exposure.

INTRODUCTION

Acid-base regulation in teleost fish or any other animal, involves the chemical reactions and the physiological mechanisms affecting the concentration of hydrogen ions, (H^+) in the various fluid compartments of the body (see review by Albers, 1970). The [H^+] depends primarily on the amount of the various acids present in the body fluids. The most important of these is carbonic acid (H_2CO_3) which is derived from CO_2, one of the major end products of metabolism. The predominant form of CO_2 in the blood is HCO_3^- at normal pH. The most important pH buffering system in vertebrates is the carbon dioxide - bicarbonate buffering system (Figure 1). This system is regulated by respiratory exchanges and the kidney.

Maintenance of a constant pH in the body fluids, to ensure homeostasis, is one of the most important tasks of regulatory systems in any animal (Heisler, 1984). While this has been studied extensively, the exact cellular mechanisms are not completely clear. Most enzyme systems catalyzing metabolic reactions have pH optima, thus changes in pH are expected to result in reduced metabolic performance (Heisler, 1984). During normal steady state conditions there is continuous production of surplus H^+ or OH^- ions due to $O_2\backslash CO_2$ exchange in the cells. These are eliminated from the body fluids by the excretory organs of the animals at the same rate as they are produced, such that pH in the body compartments is kept within narrow limits. During temperature changes and stress conditions, however, the capacity of the

excretory organs is usually not large enough to prevent transient acid-base disturbances. The concentration of CO_2 and the chemical composition of the body fluids are the principal factors governing acid-base balance. In teleost fish, acid-base regulation as well as ventilation, occur across the gills (Albers, 1970).

Ammonotelic teleosts use ammonia as the metabolic end product of protein catabolism (Forster and Goldstein, 1969; reviewed by Kormanik and Cameron, 1981). As intracellular NH_3 is released to the extracellular space it is immediately protonated to NH_4^+ (due to its high pk' value of approximately 9.6, (Cameron and Heisler, 1983), thereby again disrupting the acid-base balance of the fish. These animals must compensate for these changes by excreting H^+, NH_4^+, and HCO_3^- from the plasma into the surrounding media (Evans, 1980).

Teleosts face both ion and osmoregulatory problems associated with the environment in which they live. Marine teleosts have body fluids which are hypo-osmotic to seawater, thus these animals are subject to a net gain of ions (predominantly Na^+ and Cl^-) and a net osmotic loss of water. Freshwater teleosts face the opposite condition: a net ionic loss and an osmotic gain of water.

Homer Smith (1930) was the first to examine the mechanisms which enable marine and freshwater teleosts to offset their respective osmotic challenges. Smith (1930) demonstrated that marine teleosts drink seawater, yet they have body fluids hypo-osmotic to seawater. Marine teleosts must drink seawater to replace the water they lose to the hypertonic environment, therefore, they must excrete small volumes

of urine in order to conserve water. Monovalent ions (Na^+, Cl^-) are excreted via the gills and divalent ions ($SO_4^=$, Mg^{++}) are excreted renally via urine.

Freshwater teleosts are capable of using their gills to extract monovalent ions from the surrounding environment (Evans, 1979). These ions are needed to maintain their body fluid concentration. Excess water is excreted via the kidneys. Krogh (1937) suggested that the uptake of Na^+ in freshwater teleosts is probably in exchange for blood [NH_4^+], and the uptake of [Cl^-] is probably in exchange for blood [HCO_3^-]. Krogh (1937) proposed that Na^+/NH_4^+ exchanges were taking place because he found that Na^+ uptake was usually accompanied by a definite increase in ammonia excretion across the gills. Both Smith (1930) and Krogh (1937) conducted experiments using specialized chambers that separated the fishes' gills from the urinary papillae and anus. These experiments demonstrated that freshwater fishes excrete ammonia from the head end (gills).

Direct evidence for branchial Na^+/NH_4^+ and Cl^-/HCO_3^- exchanges was found through the use of radioisotopic tracers (Maetz and Garcia-Romeu, 1964). In this study, the injection of the carbonic anhydrase inhibitor, acetazolamide, was found to inhibit the influx of [Cl^-]. Maetz (See review by Evans, 1986) previously demonstrated that this inhibitor also prevented Na^+ uptake in goldfish. Maetz and Garcia-Romeu (1964) proposed a model for Na^+ and Cl^- uptake by the freshwater fish branchial epithelium cell (Figure 2).

The first evidence that Na^+/NH_4^+ exchange might not be the only mechanism for the removal of Na^+ from dilute freshwater came from de Vooys (1968), who

demonstrated that ammonia efflux from the carp (*Cyprinus carpio*) continued even after Na^+ was completely removed from the external environment. Kerstetter et al. (1970) found that Na^+ uptake by *Salmo gairdneri* was linked with acid rather than ammonia efflux (which could be inhibited by the injection of acetazolamide). This led to the proposal that Na^+/H^+, rather than Na^+/NH_4^+ was the dominant mode of Na^+ extraction from the environment of freshwater fishes. This proposal was supported by a later study which showed that amiloride inhibited Na^+ uptake along with acid efflux and ammonia efflux (Kirschner et al., 1973).

Evans (1986) proposed that cellular H^+ (produced by the carbonic anhydrase catalyed hydration of CO_2) could be exchanged for Na^+ at the apical surface of the branchial cell. Maetz (1973) had previously found that Na^+ uptake could be linked with ammonia efflux only if H^+ was considered to play a role. This supported the proposition that both Na^+/NH_4^+ and Na^+/H^+ are the modes of Na^+ uptake by freshwater fishes. Kerstetter and Kirschner (1972) found that Cl^- uptake was stimulated by the injection of $NaHCO_3^-$ or NH_4HCO_3. This uptake of Cl^-, however, could not be inhibited by acetazolamide. Epstein et al. (1973) found that Cl^- uptake, in two freshwater species, was inhibited by the addition of thiocyanate (SCN^-) to the external medium. Later De Renzis (1975) demonstrated that SCN^- inhibited Cl^- uptake and ΔHCO_3^- efflux in goldfish. It also appeared that the exchange was reversed in such a direction that Cl^- was lost from the fish and HCO_3^- was taken up by the fish. Claiborne and Heisler (1984) have also demonstrated that Cl^-/HCO_3^- may also be driven in the opposite direction from that normally attributed to freshwater

adapted carp. These animals appear to compensate for internal acidification via uptake of HCO_3^- in exchange for the loss of serosal Cl^-. It is evident in freshwater fish that acid-base balance takes precedence over ion regulation (Kormanik and Evans, 1979; and Toews et al., 1983). It is not certain what percentage of HCO_3^- excretion is through the Cl^-/HCO_3^- mechanism (Evans, 1986).

In 1964, Maetz and Garcia-Romeu stated that in marine fish Na^+ and Cl^- ions must be excreted along with ammonium and bicarbonate. Recent evidence clearly demonstrates that both marine elasmobranchs and teleosts possess at least Na^+/NH_4^+ and Na^+/H^+ exchange systems (Evans, 1986).

An early study of the efflux of Na^+ using the euryhaline (ability to survive in a broad range of salinities) molly (*Poecillia laetipinna*) demonstrated that Na^+ influx was saturable whether the fish was acclimated to freshwater or seawater (Evans, 1973). This Na^+ influx in seawater acclimated fish was inhibited by the addition of acid, ammonia, amiloride or K^+ to the external medium (Evans, 1975; 1986). Concurrently Payan and Maetz (1973) showed that Na^+ influx in the marine elasmobranch (*Scyliorhinus stellaris*) was stimulated by injection of either ammonia or HCl, but inhibited by the injection of acetazolamide. They suggested that the elasmobranch had retained the branchial ion exchange mechanisms of their freshwater ancestors and that these mechanisms were of importance in the maintenance of acid-base balance of body fluids.

This idea was supported by Evans (1975) who suggested that euryhalinity was therefore probably not limited by the lack of exchange systems for NaCl balance in

freshwater, but by the interactions between relatively inefficient uptake mechanisms and relatively high ionic permeabilities. Bently et al. (1976) showed that the rate of Na^+ influx into *S. canicula* slowed by acidifying the external seawater. The rate of titratable acid, (but not ammonia) efflux from the little skate (*Raja erinacea*) was inhibited by the addition of either Na^+ and K^+-free artificial seawater or with amiloride (Evans et al., 1979). In four species of marine teleosts Na^+ influx was inhibited by the addition of ammonia to the external medium. In addition, ammonia efflux was inhibited by 20 to 60% in three of the four species during exposure to Na^+ and K^+-free seawater (Evans, 1986). Injection of ammonia into the toadfish (*Opsanus beta*) stimulated Na^+ influx whereas addition of 200 mM ammonia to the outside Na^+ and K^+-free seawater stimulated Na^+ efflux from the animal (Evans, 1979 and 1986). It is also known that the ammonia efflux from a hypercapnic dogfish shark (*Squalus acanthias*) was inhibited 40% in Na^+-free artificial seawater, while the H^+ efflux from this species as well as the hypercapnic toadfish was completely inhibited in Na^+-free seawater (Evans, 1982). Substantial ammonia efflux from marine species (similar to freshwater species) is apparently not sensitive to external Na^+ (Evans, 1977).

Substantial evidence indicates that both Na^+/NH_4^+ and Na^+/H^+ are used for the excretion of excess acid in both freshwater and marine fish species (Evans, 1979; 1986). Zadunaisky (1984) showed that Cl^- extrusion by marine teleosts does not occur via Cl^-/HCO_3^-, and the removal of mucosal HCO_3^- does not alter the extrusion of Cl^- by the isolated opercular epithelium. However, Evans and Kormanik (1979)

found that Cl^- efflux from the toadfish, *Opsanus beta*, was related to the concentration of external ΔHCO_3^-. Silva et al. (1977) found evidence that chloride ions may be actively transported from the blood across the gills by a Na^+ dependent Chloride carrier (Figure 2).

DeRenzis and Maetz (1973) found that Na^+/H^+, Na^+/NH_4^+, and Cl^-/HCO_3^- could be involved in acid-base regulation since blood pH, rather than blood Na^+ and Cl^-, was altered by maintaining goldfish in either choline chloride, or sodium sulfate solutions (in an attempt to deplete either blood Na^+ or Cl^- respectively; DeRenzis and Maetz, 1973). Blood pH increased significantly in sodium sulfate solutions and decreased, (although not significantly) in either deionized water or Choline chloride solutions. They proposed that pH changes are secondary to cessation of Cl^-/HCO_3^- in sodium sulfate solutions and cessation of Na^+/H^+ and Na^+/NH_4^+ in choline chloride solutions: "Under the present experimental conditions it would seem that the stimulus pH-shift takes precedence over the stimulus internal Na^+ and Cl^- concentration drop in the feedback modulating the relative intensities of the branchial absorptions of sodium and chloride and thus regulating their internal concentrations" (DeRenzis and Maetz, 1973).

Since this study, many investigations have explored the role of branchial ion transfer mechanisms in the responses to a wide variety of conditions which induce acidosis, including: temperature changes (Rahn and Baumgardner, 1972; Heisler, 1984; Cameron, 1976; Cameron and Kormanik, 1982; Claiborne & Evans, 1992), hypercapnia (Cameron, 1976; Toews et al., 1983; Claiborne and Heisler, 1984; 1986;

Evans, 1982); infused or injected acid or base loads (Cameron 1980; Cameron and Kormanik, 1982; Evans, 1982; Claiborne and Evans, 1988), exercise (Turner et al., 1983; Holeton and Heisler,1983; Holeton et al., 1983); acidification of the external medium (see reviews by Fromm, 1980; Wood and McDonald, 1982; McDonald, 1983; Hobe et al., 1984); and hyperoxia (Hobe et al., 1984; Wood et al. 1984). Limitations in maintaining acid-base balance due to changes in ionic concentrations have only been studied in freshwater teleosts (Iwamma and Heisler, 1991). The need to regulate internal pH may increase ion fluxes which lead to osmotic and ionic stresses. However, if the ability of a fish to make necessary ionic exchanges is lacking, it may inhibit the necessary acid-base adjustments. It has been shown, in *Opsanus beta* (the gulf toadfish) that during external hypercapnia this species excretes H^+ (or uptakes HCO_3^-) at a rate six times faster than that of the freshwater carp (*Cyprinus carpio*; Evans, 1986). Freshwater trout (*Salmo gairdneri*) compensate for hypercapnia in approximately 22 hours. However, trout exposed to an environment with very low ion concentrations require 3 days for compensation to take place (Eddy et al., 1977; Janssen and Randall, 1975; Heisler, 1982). Iwamma and Heisler (1991) have found that trout adapted to higher salinities (300 mM NaCl) are able to compensate hypercapnic acidosis to a much greater extent than fish adapted to freshwater (3 mM NaCl). Several studies have also examined the effects of a rapid freshwater to seawater transition on acid-base balance and ion-regulation (Miline and Randall, 1976; Bath and Eddy, 1979; Perry and Heming, 1981). Perry and Heming observed that following the excursion to seawater, the plasma pH in the trout was

increased significantly due to an elevation of plasma [HCO_3^-]. This may have been due to a HCO_3^-/Cl^- exchange. Ion availability enhances the rate at which a fish may be able to compensate hypercapnic acidosis (Iwamma and Heisler, 1991).

The question of the role of the kidney-bladder complex in acid-base balance has often been ignored (Wood and Caldwell, 1978; Heisler, 1980,1982). In higher vertebrates, the kidney is responsible for the regulation of blood pH during stress situations such as chronic hypercapnia (Cameron and Wood, 1978). All that is known about any possible role of the teleost kidney is that the pH of urine is usually below that of blood (Hickman and Trump, 1969). Hodler et al. (1955) found that long-horned sculpin (*Myoxocephalus octodecimspinosus*) have an average urine pH of 6.25 versus an average blood pH of approximately 7.8. Hickman and Trump (1969) reported that in the southern flounder (*Platichthys fleus*), pH can range from 5.68 to 8.24, with the lowest pH values corresponding to the highest values of phosphate excretion. They also proposed that phosphate is being excreted in the form of H_2PO_4. Many recent experiments have reported a significant capacity for renal acid or base excretion in freshwater fish (Cameron and Wood, 1978; Wood and Caldwell, 1978; Kobayashi and Wood, 1980; Cameron, 1980; Wheatly et al., 1984). Three recent investigations compared the contribution of the kidney to that of the gills during environmental stresses, and concluded that the renal response is clearly significant in the freshwater fish studied (McDonald and Wood, 1981; Cameron and Kormanik, 1982). Studies of marine teleosts kidney output are extremely difficult to conduct due to the very low urine flow rates of these fish. The role of the kidney in ion

regulation and water balance has been studied more extensively. It has been shown in marine teleosts that less than 1% of the unidirectional efflux of Na^+ is lost renally (Evans, 1979). The major site of sodium efflux in marine teleost is the gills. In freshwater teleosts up to 27% of Na^+ loss occurs renally. The urinary bladder is responsible for modifying urethral urine before it is excreted, intermittently, out of the body (Cameron and Wood, 1978). Lahlou and Sawyer (1969) demonstrated that in *Opsanus tau*, the oyster toadfish, urethral urine contains higher concentrations of Na^+ and Cl^- than bladder urine. The bladder plays a very important role in conserving water in marine teleosts and in excreting excess water in freshwater fish (Evans, 1979).

Most recent studies concerning the ability of the kidney to play a role in acid-base physiology were carried out by Maren et al., (1992). This group was able to show that the long-horned sculpin can regulate urinary pH in the range of 6.1-7.8. This study (Maren et al., 1992) also showed that in response to an injection of $NaHCO_3$, the renal response is insignificant in an attempt to correct for alkalosis. However, when this species was injected with an acid load, urinary pH decreases and urinary H^+ increases 2.5-fold. The kidneys are able to compensate for 28 % of the excess acid given (Maren et al., 1992). When Maren et al.,(1992) injected fish with methazolamide to induce carbonic anhydrase inhibition , there was no alteration in urine composition. This agreed with earlier findings that carbonic anhydrase was found to be localized only in hematopoietic tissue (Maren and Wiley, 1965).

Carbonic anhydrase was not found in the nephrons of the longhorned sculpin (Maren et al., 1992).

In summary, fish must be able to make a number of osmoregulatory adjustments in order to survive a transition from seawater to freshwater (Walton and Claiborne, 1987). Many euryhaline species are capable of adapting to dilute salinities (Evans,1984). To ensure survival in a dilute environment, transbranchial Na^+/NH_4^+, Na^+/H^+, and Cl^-/HCO_3^- exchanges thought to be used by these animals, must be modified. These exchanges affect both salt and acid-base movements, thus the need to alter ion transfers may also disturb mechanisms for acid-base regulation (Walton and Claiborne, 1987).

In the present study, acid-base and ion-regulation during exposure to low salinities were investigated in three marine teleosts: *Opsanus tau*, the oyster toadfish; *Pseudopleuronectes americanus*, the winter flounder; and *Myoxocephalus octodecimspinosus*, the long-horned sculpin. The first two species are thought to be euryhaline. Traditionally the sculpin has been considered to be capable of surviving only in a very narrow range of salinities and thus is stenohaline. By exposing these fish to lower salinities, such as those encountered by a fish living in an estuary, the fish will be undergoing a low salinity stress. The purpose of this study was to investigate the linkage of acid-base and ion regulation during periods of low salinity exposure. I also investigated the limiting factors prohibiting the sculpin from surviving in low salinities.

METHODS AND MATERIALS

Maintenance and Preparation of Animals

Long-horned sculpin and winter flounder were supplied by commercial fishermen working at Mount Desert Island, Salsbury Cove, Maine. Oyster toadfish were supplied by commercial fishermen in Savannah, Georgia and Marineland, Florida.

Toadfish were transferred to Georgia Southern College and kept in aerated, filtered seawater (20°C) made up with artificial sea salts (Instant Ocean). Toadfish were fed fresh rock shrimp 1 to 2 times weekly. Sculpin and flounder were held in large Fiberglas tanks supplied with running seawater pumped from Frenchman's Bay. Before use, fish were held for 2 to 6 days without feeding.

In order to measure blood acid-base and ion changes without unnecessary stress to the animal (see discussion by Cameron, 1984), sculpin were chronically cannulated for blood collection by methods used by Claiborne and Evans (1988). Each fish was anesthetized (Ms-222, 1:10,000) and placed on a moist tray. Aerated seawater was periodically placed over the gills during surgery. The cut tip of a 23 gauge needle, connected to a short length (20-30 cm) of heparinized, Ringer-filled tubing (PE-50) was inserted into the afferent artery of the third branchial arch and secured in place with a suture.

Due to difficulty in cannulating toadfish, blood samples were drawn from the caudal artery. The fish was quickly removed from the water with a net, it could be placed on its back in a v-shaped tray. An 18 gauge needle, connected to a 1 cc syringe, was inserted into the tail one inch below the anus. By keeping the needle on the midline of the body one can feel the tip of the needle slightly touch the spine. The needle can then be backed out of the fish slightly and, by drawing back on the syringe, blood can be removed from the caudal artery/vein. The fish were then returned to experimental chambers connected to running seawater for a recovery period. Fish involved in longterm dilution experiments were kept in the appropriate solution during anesthesia and recovery periods. Urinary catheters were placed in the urinary papillae of the long-horned sculpin as described by Wood and Randall (1973). One end of a short length of Ringer-filled tubing (25-30 cm) was perforated and inserted into the urinary papillae. A suture was then made around the papillae in order to keep the catheter in place. Another suture was made to attach the catheter to the body of the fish approximately 2.5 cm from the urinary papillae.

Following surgery fish were placed in darkened Plexiglas boxes (volume = 2.0-2.5 L) and allowed to recover for 12 to 48 hours. Fishes (toadfish and flounder) that did not undergo surgery were placed in boxes 12 to 24 hours prior to the start of an experiment. This enabled the fish to adjust to the experimental chamber and water flushing procedures. Depending upon the experiment, cannulae or catheters were passed out of the experimental chambers in order for blood or urine collection to take place without disturbing the fish. Urine collection tubes were treated with 10 uL of

streptomycin solution (.250 gm/L) in order to prevent bacterial growth over long collection periods (Calla, 1977). During recovery and adjustment periods, experimental chambers were supplied with running seawater or were flushed periodically with artificial seawater. During experimental periods or low salinity control periods, chambers were aerated with airstones connected to electric aquarium pumps. Prior to the experiment, running seawater was disconnected, and boxes were closed off so changes in the water surrounding the fish could be measured for each experimental period.

Analytical Techniques

Each blood sample (sculpin or toadfish) was analyzed to determine the pH (I.L. model 213 or radiometer electrode with an Orion E Ionanalyzer EA 920 pH meter) and total CO_2 (Capni-Con II; Cameron Instrument Inc.). Total osmolarity was measured with a Wescor osmometer. Plasma and urine [Na^+] were measured by IL flame photometer. Plasma and urine [Cl^-] were measured with a Haake-Buchler Digital chloridometer. Plasma [PO_4^{3-}] was measured by assay (Sigma). [Ca^{++}] in plasma and water samples were measured with a Perkin-Elmer atomic absorption spectrophotometer. Ion measurements will be reported in mM/kg or mM/L for each sample taken. Urine ion concentrations will be expressed in mM/kg/hr.

Plasma Pco_2 and [HCO_3^-] were calculated by the following arrangements of the Henderson-Hasselbalch equation:

$$Pco_2 = Tco_2/(10^{pH-pK'}+1)*Sco_2$$

and

$$[HCO_3^-] = Tco_2 - (Pco_2 * Sco_2)$$

where values for Sco_2 and pK' are computed according to the methods of Boutilier et al. (1984).

Urine samples were analyzed for pH and titratable acidity immediately after collection as described by Wood and Caldwell (1978) and Kobayashi and Wood (1980). Urine samples (0.150-1.00 mL) were titrated with 0.1 N NaOH to a pH of 7.8 (as modified from Cameron and Kormanik 1982). Urine samples were also analyzed for $[NH_4^+]$ (Solorzano, 1969). Total renal acid output was calculated by the equation:

$$((\text{titratable acidity})*\text{flowrate (mL/kg/hr)}+((\Delta NH_4^+)*\text{flowrate})$$

Water samples (20 mL) were taken periodically and analyzed for ΔHCO_3^- (Δ indicates the net change between the fish and the surrounding environment) measured by titrating duplicate 8 mL samples to a pH to 3.7 with 0.1 N HCl with a syringe micrometer burette (model SB2, Micrometric Instrument Company) according to the methods of Cameron and Kormanik (1982). Phosphate buffers used to calibrate pH electrodes were made according to instructions described in Data for Biochemical Research. Phosphate buffers D and E were mixed in 450 mM NaCl when electrodes were used for titrating water samples. The same buffers were made with deionized water for electrodes used for urine and blood analyses. Measurements of $[NH_4^+]$ were also determined by the methods of Solorzano (1969).

Analysis of Data

Calculations of ΔHCO_3^+ and ΔNH_4^+ were obtained as the difference between the concentrations at the beginning and end of each experimental period. Changes in the external concentrations in mM/kg of these parameters can then be calculated for all time periods by multiplying the measured concentration of the specific ion (mM/mL) by the total volume of the water in the experimental chamber (total volume = volume of box - (volume of samples - weight of fish)). Initial control measurements were made while fish remained in seawater (400 to 480 mM; measured as $[Cl^-]$). Following a control period, fish were exposed to diluted seawater (5, 20 and 100 mM; measured as $[Cl^-]$). All measurements were made in duplicate and recorded as an average per fish. Results will be given as mean $\pm$ SE with n equaling the number of fish used for the experiment. Paired and un-paired Student's t-test were utilized where appropriate.

Experimental Protocol

I. Toadfish series

I.A. Seawater control

Following a 12-hour control period, (n=10; 339 $\pm$ 46g), fish remained in normal seawater for 312 hours. Fluxes were measured during the initial 72 hours and the final 72 hours (n=4; 502 $\pm$ 24g). Following the initial 72 hours of measurement, toadfish were returned to buckets filled with seawater provided with an individual pump and airstone for a period of 168 hours. At the end of 168 hours, fish

were returned to experimental chambers. All water samples in this series were taken at 12-hour intervals.

I.B. 20 mM series

Following a 12-hour seawater control period, a group of fish (n=8; 268 ± 42g) was exposed to 20 mM diluted seawater for 312 hours. Fluxes were measured during the initial 72 and the final 72 hours (n=6; 316 ± 41g). Between the two 72-hour measurement periods fish were returned to buckets of 20 mM diluted seawater for 168 hours.

I.C. 5 mM series

Following a 12-hour seawater control period, a group of fish (n=6; 293 ± 41g) was exposed to 5mM diluted seawater for 228 hours. Fluxes were measured during the intiial 72 hours and the final 72 hours. Between the two 72-hour measurement periods fish were returned to buckets of 5 mM diltued seawater for 96 hours.

I.D. Plasma series

A fourth group of fish (n=5 409 ± 41g) were placed in seawater for a control period of 24 hours. Control blood samples were taken at hour 12 and 24. Fish were placed in 20 mM diluted seawater for 96 hours with blood samples taken every 24 hours for 96 hours. Following exposure to 20 mM diluted seawater, fish were returned to seawater for 48 hours with blood samples taken at 24-hour intervals. Blood was analyzed for pH, [Tco_2], and [Cl^-] according to the methods previously described.

II. Flounder series

The experimental group consisted of 4 fish (230 $\pm$ 42 g). Following a 10-hour control period, fish were placed in 20 mM diluted seawater. Water samples were taken every 12 hours for a total of 168 hours. Of the fish remaining in 20 mM seawater for an additional week, one survived. Water samples were then taken for this fish an additional 72 hours.

III. Sculpin series

III.A. Sculpin 20 mM series

III.A.1. Seawater/20 mM/seawater series

Ten sculpin, (213 $\pm$ 1g) were placed in seawater for a 10-hour control period. Fish were then placed in 20 mM diluted seawater for 24 hours and then returned to seawater for an additional 24 hours. Samples were taken at hour 4, 8, 12, 24, 26, 28, 34, 46, 58, and 70.

Concentration of plasma ions [PO_4^{3-}], [Ca^{++}], [Na^+], and [Cl^-], pH, Tco_2 and osmolarity were measured in separate groups of cannulated fish. Samples were taken at approximately the same times as water samples in the above experiment.

Sculpin, catheterized for urine collection (204 - 444g; n=9), were exposed to 24 hours of seawater and then placed in 20 mM diluted seawater for 24 hours. Fish were then returned to seawater for an additional 24 hours. Urine samples were collected every 12 to 24 hours and were analyzed for pH, [Na^+], [Cl^-], and [NH_4^+].

In an additional experiment, three fish (169 $\pm$ 7g) following a 10-hour control period, were placed in 20 mM diluted seawater for 48 hours and then returned to

seawater for an additional 24 hours. Only water fluxes were measured for these fish. Samples were taken at hour 8, 12, 24, 26, 46, 54, 58, 70, 82, and 94.

III.A.2. Intracellular pH series

Two fish were placed in 20 mM diluted seawater for 23 hours. Fish were then anesthetized and a 1 gram white muscle tissue sample was taken from the fish. This tissue sample was freeze clamped in liquid nitrogen. Intracellular pH of the tissue could then be determined by the methods described by Portner et. al.(1990). The intracellular pH of two additional fish (one control and one after fish was made to swim around large holding tank for ten minutes) was also tested using this technique described by Boutilier (1984).

III.A.3. Seawater/low salinity epinephrine series

Two sculpin (210 g; mean weight) were injected intramuscularly with epinephrine (2×10^{-5} mM/kg) mixed in sculpin ringers after being exposed to 20 mM seawater for 20 hours. Water samples were taken at hour 4, 10, and 20. Blood samples were taken at hour 0, 20, 20.5, 21.5, and 24.5.

III.A.4. Low salinity/seawater osmolarity series

Two sculpin (260 and 210 g) were placed in water composed of 20 mM NaCl and enough mannitol (700mM) to increase the osmolarity to that of seawater (900-1000 mOsm) for seven hours. Water samples were taken at hour 2, 4, and 7 during exposure to the low salinity/high osmolarity. Blood samples were taken before the experimental period began and then at hour .5, 1.5, 4, and 7 while in low salinity/high osmolarity exposure. After hour 7, one fish was returned to seawater and a

blood sample was taken at hour 7.5. Blood samples were analyzed for pH, Tco_2, $[Na^+]$, $[Cl^-]$, $[PO_4^{3-}]$, and Total osmolarity.

III.B. Sculpin seawater/100 mM diluted seawater series

Following a 10-hour control period, sculpin (n=5; 242 $\pm$ 0.03g) were placed in 100 mM seawater for 46 hours. Water samples were taken at hour 4, 8, 12, 22, 28, 36, and 46. Blood samples were measured on a separate group of fish (n=8 177 $\pm$ 21g). Blood was analyzed for $[Na^+]$ and $[Cl^-]$ and total osmolarity at hour 0, 2.5, 10, 24, 48, 72, 96, and 120.

III.C. Sculpin 100 mM/20 mM diluted seawater series

III.C.1. Long-term 100 mM/20 mM diluted seawater series

Eight sculpin (234 $\pm$ 16g) were placed in large aerated plastic containers of 100 mM seawater (14 °C) for 240 horus. Every 12 hours water was carefully drained from the containers and replaced with freshly mixed 100 mM seawater. Water samples were taken at hour 8, 14, and 24 while fish were in 100 mM. Then fish (n=7) were placed in 20 mM diluted seawater for an additional 70 hours. Water samples were continued at hour 28, 36, 48, 54, 62, 72, 76, 84, and 94. Blood samples were taken on a different group of fish (n=8; 264.8 $\pm$ 16.2) taken at hour 2, 9, 24, 18, and 70 of the 20 mM diluted seawater exposure. Blood samples were analyzed for pH, Tco_2, $[Na^+]$, $[Cl^-]$, $[Ca^{++}]$ and $[PO_4^{3-}]$. One fish (320 grams) from this group was placed back into seawater following the 24 hours in 100 mM seawater. Water samples were taken for an additional 70 hours at hour 4, 12, 24, 30, 38, 48, 52, 60, and 70.

III.C.2. 96 hours 100 mM/20 mM diluted seawater series

A group of sculpin (n=4; 268 $\pm$ 1 g) were placed in 100 mM seawater for a period of 96 hours. During the last 18 hours of the 96-hour period, control water samples were taken. The fish were then placed in 20 mM diluted seawater for 48 hours. Water samples were taken at hour 8, 12, 24, 36, and 48. Two catheterized sculpin were placed in 100 mM for 96 hours for urine collection. Fish were anesthetized and allowed to recover in order for a control collection to be made the last 24 hours of the 96 hour 100 mM dilute salinity exposure. Fish were then placed in 20 mM seawater for 48 hours.

III.C.3. Low salinity/100 mM osmolarity series

Two sculpin (311.5 $\pm$ 30g) were exposed to 100 mM seawater for 11 days. These fish were then placed in 20 mM seawater with mannitol added to make the total osmolarity of the solution 220 mOsm for 20 hours. This is the approximate osmolarity of 100 mM seawater. Blood samples were taken at hour 0 (just prior to the change of salinity) and hour 1, 4, 8, and 21. Water samples were taken at hour 0, 2, 4, 8, and 20. Blood samples were analyzed for pH, $[Na^+]$, $[Cl^-]$ and Tco_2.

III.D. Sculpin acetazolamide series

Three fish (217 $\pm$ 2 g) were injected with acetazolamide, a carbonic anhydrase inhibitor, following a 24 hour sampling period while the fish were in seawater. Urine collections were made as often as possible in order to measure Tco_2, pH, H^+ ΔNH_4^+, and flow rate. Two of these fish were also cannulated so that blood samples could be taken.

RESULTS

I. Toadfish series

Whole body excretion rates ($mM \cdot kg^{-1} \cdot hr^{-1}$) for ΔNH_4^+, ΔHCO_3^-, and ΔH^+ for control and dilution groups (20 and 5 mM) are summarized in Table 1. Cumulative values ($mM \cdot kg^{-1}$) for ions measured during the long-term control experiment and dilution experiments are summarized in Table 2. Student's paired t-test was used to compare all successive time periods (24, 48, and 72 [week 1]; 24, 48, and 72 [week 2]) to the initial control period. Student's unpaired t-test was also used to compare values of the long-term control experiment to those obtained from the 20 mM and 5 mM dilution experiments. Cumulative values for ions measured during the long-term control experiment are shown in Figure 3. Cumulative exchanges of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ between the fish and the water over two weeks of experimentation are shown in Figure 3 (20 mM dilution group) and Figure 4 (5 mM dilution group). Experimental values are plotted against long-term controls in both Figure 3 and 4. Fish exposed to 20 mM diluted seawater lived for the entire length of the experiment. However, one fish expired after hour 60 of the second week of exposure to 5 mM diluted seawater. Plasma pH, Tco_2 (Pco_2; HCO_3^-), and $[Cl^-]$ for fish in 20 mM diluted seawater are summarized in Table 3 and shown in Figure 5.

I.A. Seawater control series

Toadfish excreted ΔNH_4^+, ΔHCO_3^-, and ΔH^+ at constant rates throughout long-term control conditions. Following a 12-hour control period, fish remained in 400 mM seawater for an additional 72 hours. Toadfish were then held in seawater for an additional 7 days. At the end of this 7 day period, fish were placed in experimental chambers for an additional 60 hours. Water samples were taken at 12-hour intervals.

The ΔNH_4^+ excretion rate at hour 24 ($.117 \pm 0.023$; $n=10$) during the first 72 hours of measurement was significantly higher ($t=3.18$; $p<0.05$; $n=10$) than the rate at hour 48 ($.630 \pm 0.005$; $n=4$) during the second 72-hour period of measurement (Table 1). There were no other significant differences between the two periods of the experiment.

Cumulative values for ΔNH_4^+ (Figure 3; Table 2) are significantly different from the initial control period at hour 24 ($t=4.81$; $p<0.001$; $n=10$), 36 ($t=4.83$; $p<0.001$; $n=10$), 48 ($t=4.99$; $p<0.001$; $n=10$), 60 ($t=5.10$; $p<0.001$; $n=10$), and 72 ($t=33.8$; $p<0.001$; $n=10$) and at hour 24 ($t=9.82$; $p<0.001$; $n=4$), 36 ($t=9.93$; $p<0.001$; $n=4$), 48 ($t=11.86$; $p<0.001$; $n=4$), 60 ($t=10.89$; $p<0.001$; $n=4$), and 72 ($t=11.75$; $p<0.001$; $n=4$) during the final 72 hours of exposure.

Excretion rates ($mM \cdot kg^{-1} \cdot hr^{-1}$) for ΔHCO_3^- during the long-term control experiment were not significantly different from the initial control value or the initial 24-hour period at any point in the experiment (Table 1). Excretion rates for ΔHCO_3^- were not significantly different at corresponding time points between the first and

second exposures. Cumulative values (Figure 1; Table 2) are significantly higher than the initial control period at hour 36 ($t=2.41$; $p<0.05$; $n=10$), 48 ($t=2.57$; $p<0.05$; $n=10$), 60 ($t=3.82$; $p<0.005$; $n=10$), and 72 ($t=4.22$; $p<0.005$; $n=10$). During the second 72-hour sampling period, cumulative values (Figure 1; Table 2) are significantly higher than the intial control period at hours 36 ($t=4.20$; $p<0.05$; $n=4$), 48 ($t=7.99$; $p<0.005$; $n=4$), 60 ($t=6.43$; $p<0.01$; $n=4$), and 72 ($t=6.65$; $p<0.01$; $n=4$).

Control ΔH^+ excretion rates ($mM \cdot kg^{-1} \cdot hr^{-1}$) were not significantly different from the inital 12-hour value (Table 1). Cumulative values (Figure 1) were significantly higher than the initial control period at hours 24 ($t=5.37$; $p<0.001$; $n=10$), 36 ($t=2.50$; $p<0.05$; $n=10$), and 48 ($t=3.63$; $p<0.005$; $n=10$).

I.B. 20 mM series

Initially, toadfish exposed to 20 mM diluted seawater took up ΔH^+ from the surrounding environment. However, after seven additional days in this salinity, net transfers returned to control levels. Following a 12-hour control period in 400 mM seawater, fish were placed in 20 mM diluted seawater for 72 hours. Toadfish were held in 20 mM diluted seawater for an additional 7 days before being returned to experimental chambers. Fluxes were them measured for an additional 60 hours.

When compared to long-term controls ΔNH_4^+ excretion rates were significantly different at hour 48 during the first sampling period ($t=3.24$; $p<0.02$; $n=8$; see Table 2). There were no significant differences between corresponding points during the first and second periods of exposure.

Cumulative ($mM \cdot kg^{-1}$) ΔNH_4^+ excretion for fish in 20 mM diluted seawater was lower than that of the control fish at all time points during the first week of exposure (Figure 3; Table 2). Only at hour 12 are exchanges significantly lower than control values ($t=3.07$; $p<0.008$; $n=8$).

Excretion rates for ΔHCO_3^- ($mM \cdot kg^{-1} \cdot hr^{-1}$) during toadfish exposure to 20 mM diluted seawater were not significantly different ($p \leq .05$; Table 1) from the initial control at any time period. Cumulative values for ΔHCO_3^+ excretion during exposure to 20 mM were significantly higher than the initial control period at hours 24 ($t=4.28$; $p<0.005$; $n=8$), 36 ($t=4.95$; $p<0.002$; $n=8$), 48 ($t=5.45$; $p<0.001$; $n=8$), 60 ($t=5.67$; $p<0.001$; $n=8$), and 72 ($t=5.67$; $p<0.001$; $n=8$). During the second period of measurement cumulative values at hours 12 ($t=3.26$; $p<0.01$; $n=6$) and 48 ($t=2.55$; $p<0.03$; $n=6$) were significantly higher than corresponding long-term control values. Excretion rates for ΔH^+ ($mM \cdot kg^{-1} \cdot hr^{-1}$) during 20 mM exposure were significantly different from the initial control period at hour 72 ($t=3.08$; $p<0.02$; $n=8$) and hour 24 ($t=4.38$; $p<0.01$; $n=6$) of the second sampling period.

Cumulative ΔH^+ excretions were all significantly lower than control values at hours 24 ($t=2.57$; $p<0.05$; $n=8$), 36 ($t=2.70$; $p<0.05$; $n=8$), 48 ($t=2.86$; $p<0.05$; $n=8$), 60 ($t=3.29$; $p<0.02$; $n=8$), and 72 ($t=3.58$; $p<0.005$; $n=8$) during the first 72 hours of sampling. Only ΔH^+ values for hour 12 ($t=4.43$; $p<0.01$; $n=6$) and 24 ($t=2.70$; $p<0.05$; $n=6$) were significantly higher than initial control values. Negative ΔH^+ values indicate that fish are either taking up ΔH^+ from their surrounding environment or losing ΔHCO_3^-.

I.C. 5 mM Series

In 5 mM diluted seawater, ΔH^+ uptake increased approximately three times that observed in the 20 mM group. After an additional four days (96 hours), net transfers were still significantlty below control excretion rates and mortality was noted. Toadfish were exposed to 5 mM diluted seawater following a 12-hour control period for 72 hours. After 4 days of additional exposure to 5 mM diluted seawater, fish were returned to experimental chambers and fluxes were measured for an additional 72 hours.

Long-term 5 mM series ΔNH_4^+ excretion rates ($mM \cdot kg^{-1} \cdot hr^{-1}$) were significantly different from the initial control period at hour 24 ($t=2.78$; $p<0.05$; $n=6$) during the first sampling period and hours 24 ($t=4.61$; $p<0.01$; $n=6$) and 48 ($t=4.62$; $p<0.01$; $n=6$) of the second sampling period (see Table 1). Experimental excretion rates for ΔNH_4^+ were significantly different from long-term control values at hour 12 ($t=3.03$; $p<0.001$; $n=6$), 24 ($t=2.92$; $p<0.01$; $n=6$), and 48 ($t=2.22$; $p<.04$; $n=6$; see Table 2). Excretion rates for ΔNH_4^+ during 5 mM exposure were not significantly different from rates found in fish exposed to 20 mM diluted seawater.

Cumulative ΔNH_4^+ values for fish in 5 mM diluted seawater were significantly lower ($p \leq .05$) than long-term controls at hour 12 ($t=3.07$; $p<0.008$; $n=6$), 24 ($t=2.93$; $p<0.01$; $n=6$), 36 ($t=2.79$; $p<0.01$; $n=6$), 48 ($t=2.67$; $p<0.02$; $n=6$), 60 ($t=2.54$; $p<0.02$; $n=6$), and 72 ($t=2.50$; $p<0.02$; $n=6$) during the first period of exposure. See Figure 4 and Table 2. During the second period of exposure, fish

in 5 mM are excreting more ΔNH_4^+ than long-term control fish, however there were no significant differences.

During exposure to 5 mM diluted seawater ΔHCO_3^- excretion rates ($mM \cdot kg^{-1} \cdot hr^{-1}$) were significantly different from the control period at hour 72 ($t=3.64$; $p<0.02$; $n=6$) during the first 72-hour sampling period and hours 24 ($t=3.10$; $p<0.05$; $n=6$) and 48 ($t=2.73$; $p<0.05$; $n=6$) of the second sampling period (see Table 1). In comparing experimental and long-term control rates, hour 24 ($t=2.39$; $p<0.03$; $n=6$) of the first sampling period and hours 24 ($t=4.93$; $p<0.001$; $n=6$), 48 ($t=4.73$; $p<0.001$; $n=6$) and 72 ($t=3.52$; $p<0.03$; $n=2$) of the second period were significantly higher than the control values (see Table 1).

Cumulative excretions of ΔHCO_3^- were significantly higher than the initial time zero measurement at hour 12 ($t=3.28$; $p<0.05$; $n=6$), 24 ($t=6.52$; $p<0.002$; $n=6$), 36 ($t=11.74$; $p<0.001$; $n=6$), 48 ($t=12.41$; $p<0.001$; $n=6$), and 60 ($t=11.77$; $p<0.001$; $n=6$) of the first measurement period and hours 24 ($t=4.64$; $p<0.01$; $n=6$), 36 ($t=6.37$; $p<0.002$; $n=6$), 48 ($t=6.38$; $p<0.002$; $n=6$), 60 ($t=6.66$; $p<0.002$; $n=6$) of the second period (Figure 4). Cumulative values at hours 24 ($t=2.36$; $p<0.03$; $n=6$), 48 ($t=2.13$; $p<0.05$; $n=6$), and 60 ($t=2.18$; $p<0.04$; $n=6$) of the first measurement period were significantly different from values obtained in the long-term control experiment (Table 2). Cumulative values of ΔHCO_3^- were significantly different from values measured during exposure to 20 mM diluted seawater at hours 24 ($t=2.72$; $p<0.02$; $n=6$), 36 ($t=2.98$; $p<0.01$; $n=6$),

48 ($t=2.35$; $p<0.04$; $n=6$), 60 ($t=2.48$; $p<0.03$; $n=6$) of the second sampling period (Table 2).

Negative ΔH^+ excretion rates ($mM \cdot kg^{-1} \cdot hr^{-1}$) occurred during exposure to 5 mM diluted seawater. A negative excretion rate indicates that the fish is uptaking $\Delta H+$ ions from the surrounding environment (or losing bicarbonate). Excretion rates of ΔH^+ were significantly lower than the initial control exposure at hours 48 ($t=2.60$; $p<0.05$; $n=6$) and 72 ($t=5.06$; $p<0.005$; $n=6$) of the first sampling period and at hour 24 ($t=3.92$; $p<0.05$; $n=6$), 48 ($t=3.74$; $p<0.02$; $n=6$), 72 ($t=2.66$; $p<0.05$; $n=3$) during the second sampling period (Table 1). Excretion rates during the first sampling period of 5 mM exposure at hours 24 ($t=5.98$; $p<0.0001$; $n=6$) and 48 ($t=5.78$; $p<0.0001$; $n=6$) and 24 ($t=5.23$; $p<0.001$; $n=6$), 48 ($t=5.51$; $p<0.0008$; $n=6$) and 72 ($t=5.20$; $p<0.007$; $n=6$) were significantly lower than corresponding long-term control values (Table 1). ΔH^+ excretion rates during exposure to 5 mM diluted seawater were significantly different from 20 mM exposure at hours 24 ($t=3.09$; $p<0.009$; $n=6$), 48 ($t=2.66$; $p<0.02$; $n=6$) and 72 ($t=3.52$; $p<0.0004$; $n=6$) of the first sampling period and hour 24 ($t=3.30$; $p<0.008$; $n=6$) of the second 72-hour sampling period.

Cumulative ΔH^+ uptake (or ΔHCO_3^- loss) is greater during the first 72 hours of exposure (Table 2; Figure 4). ΔH^+ excretion during the first 72 hours is significantly different from initial control values at hours 24 ($t=3.64$; $p<0.02$; $n=6$), 36 ($t=5.12$; $p<0.005$; $n=6$), 48 ($t=5.57$; $p<0.001$; $n=6$), 60 ($t=6.37$; $p<0.001$; $n=6$) and 72 ($t=6.46$; $p<0.001$; $n=6$). See Table 2. During the second 72-hour

sampling period, values were significantly lower than control values at hour 72 ($t=13.11$; $p<0.001$; $n=2$). ΔH^+ cumulative excretions while fish were exposed to 5 mM diluted seawater were significantly lower than corresponding long-term control values at hours 12 ($t=3.89$; $p<0.002$; $n=6$), 24 ($t=5.93$; $p<0.0001$; $n=6$), 36 ($t=6.67$; $p<0.0001$; $n=6$), 48 ($t=6.68$; $p<0.0001$; $n=6$), 60 ($t=4.17$; $p<0.001$; $n=6$) and 72 ($t=4.88$; $p<0.0004$; $n=6$) during the initial 72 hours of sampling (Table 2). During the final 72 hours of sampling, 5 mM cumulative excretions were significantly lower than long-term controls at hours 24 ($t=5.31$; $p<0.001$; $n=6$), 36 ($t=2.80$; $p<0.02$; $n=6$), 48 ($t=8.95$; $p<0.0001$; $n=6$), 60 ($t=8.85$; $p<0.0001$; $n=6$). Cumulative excretions of ΔH^+ were significantly lower than values for fish exposed to 20 mM diluted seawater at hours 12 ($t=3.40$; $p<0.005$; $n=6$), 24 ($t=5.94$; $p<0.0001$; $n=6$), 36 ($t=3.29$; $p<0.006$; $n=6$), 48 ($t=3.18$; $p<0.007$; $n=6$), 60 ($t=3.47$; $p<0.004$; $n=6$) and 72 ($t=3.58$; $p<0.003$; $n=6$) during the intial 72-hour sampling period and hours 24 ($t=3.21$; $p<0.009$; $n=6$), 36 ($t=3.00$; $p<0.001$; $n=6$), and 60 ($t=2.53$; $p<0.03$; $n=6$) during the second 72 hours of measurement. Fish kept in 5 mM diluted seawater for periods beyond this experiment did not survive.

I.D. Plasma series

See Table 3 for a summary of all plasma data measured. Changes in plasma pH, Tco_2, Pco_2, $[HCO_3^-]$ and $[Cl^-]$ over time are shown in Figure 5. Plasma pH for *Opsanus tau* during exposure to 20 mM diluted seawater was significantly lower at hour 48 ($t=3.689$; $p<.05$; $n=4$) than plasma pH during the seawater control period

(See Table 3; Figure 5). After fish were returned to seawater pH increased and was not significantly different from the initial control period. Tco_2 ($t=10.50$; $p<0.001$; $n=4$), Pco_2 ($t=12.29$; $p<0.001$; $n=4$) and $[HCO_3^-]$ ($t=10.42$; $p<0.001$; $n=4$) decreased significantly following 48 hours exposure to 20 mM diluted seawater. At 96 hours Tco_2 was not significantly different from control values, or measurements made following the fish's return to seawater. Plasma $[Cl^-]$ appeared to decrease during exposure to the lower salinity. This decrease however, was only significantly different ($t=3.22$; $p<0.05$; $n=4$) at hour 96. After fish were returned to seawater $[Cl^-]$ increased and was not significantly different from the control value.

II. Flounder series

Flounder exposed to 20 mM diluted seawater did not show any significant change in whole body excretion rates. Following a 10-hour control period, fish were placed in 20 mM diluted seawater for a total of 168 hours. Of the fish left in 20 mM seawater for an additional week, one survived. Water samples were taken for this fish an additional 72 hours.

Whole body exchange rates between the fish and the water for ΔNH_4^+, ΔHCO_3^-, and ΔH^+ are summarized in Table 4. Cumulative values for these ions over the length of the experiment are shown in Figure 6.

Excretion rates ($mM \cdot kg^{-1} \cdot hr^{-1}$) for ΔNH_4^+, ΔHCO_3^- and ΔH^+ were not significantly different from control rates at any point during exposure to 20 mM diluted seawater (Table 4). Fish remained in 20 mM for an additional week, but only one fish survived. Additional measurements for the surviving fish were made for 72

hours. Both ΔNH_4^+ and ΔHCO_3^- excretion rates were similar to control values (Table 4). This fish was returned to Frenchman's Bay.

III. Sculpin series

III.A. Sculpin 20 mM series

III.A.1. Seawater/20 mM/seawater series

Sculpin were able to withstand exposure to 20 mM diluted seawater for periods of 24 hours. During this 24 hour period fish exhibited a rapid loss of ΔHCO_3^-, but no change in plasma pH. Sculpin were placed in seawater for a 10-hour control period. Fish were them placed in 20 mM diluted seawater for 24 hours and then returned to seawater for an additional 24 hours. Whole body, plasma, and urine parameters were measured.

Sculpin were able to survive short-term exposure to 20 mM diluted seawater very well. Whole body excretion rates of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ as well as plasma and urine values for parameters measured are summarized in Table 5. Cumulative values for ΔNH_4^+, ΔHCO_3^-, and ΔH^+ are shown in Figure 8: Values for plasma and urine are also plotted in Figures 7-9.

Whole body excretions increased significantly from control values following 24 hours of exposure to 20 mM diluted seawater for ΔNH_4^+ ($t=3.75$; $p<0.005$; $n=10$), and ΔHCO_3^- ($t=3.94$; $p<0.001$; $n=10$). While the value for ΔH^+ decreased significantly from the control ($t=2.38$; $p< 0.05$, $n=10$). Plasma pH appeared to decrease during the low salinity exposure, but this decrease was not significant. Values for Tco_2 ($t=12.55$; $p<0.001$; $n=7$), Pco_2 ($t=4.41$; $p<0.01$; $n=6$), and

$[HCO_3^-]$ ($t=12.72$; $p<0.001$; $n=6$) increased significantly from control values (Table 5). Plasma Na^+ and Cl^- both decreased significantly as expected during exposure to 20 mM diluted seawater (Na^+: $t=8.31$; $p<0.001$; $n=12$; Cl^-: $t=8.4$; $p<0.001$; $n=12$). Plasma $[Ca^{++}]$ decreased significantly ($t=4.73$; $p<0.002$; $n=9$), while $[PO_4^{3-}]$ increased significantly ($t=3.06$; $p<0.05$; $n=6$). Total osmolarity also decreased significantly ($t=5.27$; $p<0.02$; $n=4$).

Upon returning fish to seawater, plasma values for Tco_2 and $[HCO_3^-]$ decreased, but remained significantly higher than control values ($t=5.37$; $p<0.0005$; $n=3$; $t=5.53$; $p<0.002$; $n=3$, respectively). Plasma $[PO_4^{3-}]$ continued to increase following exposure to 20 mM diluted seawater. This increase was significantly higher than the initial control value ($t=3.55$; $p<0.02$; $n=6$). It appeared that $[Cl^-]$ increased, but this value remained significantly below the control value ($t=3.10$; $p<0.02$; $n=10$). Total osmolarity increased during the return to seawater, but this increase remained significantly below the control value ($t=3.68$; $p<0.05$; $n=4$). During the exposure to seawater plasma $[Ca^{++}]$, $[Na^+]$ and Pco_2 returned to values similar to those seen during the initial seawater control period and were not significantly different.

Urine flow rates increased significantly during exposure to 20 mM diluted seawater and remained significantly higher than control values following the return to seawater ($t=2.23$; $p<0.05$; one tailed test, $n=5$). Urine values for $[NH_4^+]$ and $[Na^+]$ increased during exposure to 20 mM diluted seawater, but this increase was not significant until fish were returned to seawater ($t=2.78$; $p<0.05$; $n=4$). Urine

values for [Cl^-] were not significant from the control value at any point. Titratable acidity, ΔH^+, remained low during the entire experiment. It appears that the kidneys compensate for approximately 35 percent of the net ΔH^+ lost by the fish during exposure to 20 mM diluted seawater (Figure 10).

Another group of sculpin were exposed to 20 mM diluted seawater for a 48-hour time period. Rates ($mM \cdot kg^{-1} \cdot hr^{-1}$) for ΔNH_4^+, ΔHCO_3^-, and ΔH^+ are summarized in Table 6. Cumulative values for these ions are shown in Figure 11. The long-horned sculpin were able to recover from 24 hours exposure to 20 mM diluted seawater. These fish were not as tolerant to a longer 48-hour period of exposure. Rates of excretion increased significantly for ΔNH_4^+ ($t=9.43$; $p<0.01$; $n=3$), during the first 24 hours of exposure, while ΔH^+ decreased significantly ($t=5.01$; $p<0.05$; $n=3$). ΔNH_4^+ remained significantly higher than the initial control value ($t=8.48$; $p<0.02$; $n=2$) at hour 48. All whole body exchange values decreased upon the fishes return to seawater. Excretion of ΔH^+ became a negative value.

III.A.2. Intracellular pH series

Intracellular pH was not affected in fish exposed to 20 mM diluted seawater. Following 10 minutes of exercise, inducing metabolic acidosis, intracellular pH decreased. Intracellular pH was measured in control fish, fish that had been in 20 mM diluted seawater for 24 hours, and fish that had been exercised (chased in tank for 10 minutes to induce metabolic acidosis). These values are summarized in a subsection of Table 5. Intracellular pH in the control fish was found to be 7.27 $\pm$

0.01 (n=2). Following 24 hours in 20 mM diluted seawater the value was 7.22 $\pm$ 0.01 (n=2). The exercised fish had a much lower intracellular pH, 6.56 $\pm$ 0.01 (n=2).

III.A.3. Seawater/low salinity epinephrine series

Injecting sculpin with epinephrine during 20 mM exposure caused increases in ΔNH_4^+, ΔHCO_3^-, and ΔH^+. Plasma pH decreased. Two sculpin were injected intramuscularly with epinephrine ($2.5x10^{-5}$ $mM \cdot kg^{-1}$) mixed with sculpin ringers following 20 hours of exposure to 20 mM diluted seawater. Rates for whole body excretion of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ and plasma values for pH, Tco_2, Pco_2, $[HCO_3^-]$, and $[Cl^-]$ are summarized in Table 7. Cumulative values for ΔNH_4^+, ΔHCO_3^-, and ΔH^+ are shown in Figure 12.

Results from Sculpin used in this pilot experiment were very similar to fish discussed in the previous sections during exposure to 20 mM diluted seawater: ΔNH_4^+ and ΔHCO_3^- increased producing a net loss of ΔH^+ to the surrounding water. Following the injection of epinephrine, the excretion rate for ΔNH_4^+ doubled. Values for ΔHCO_3^- almost tripled. The net result was a negative loss of ΔH^+ (or excretion of ΔHCO_3^-; see Figure 12). Plasma Tco_2 increased and continued to increase following the injection. Further decreases in pH and $[Cl^-]$ were evident following the epinephrine injection (Table 7).

III.A.4. Low salinity/seawater osmolarity series

Adjusting the osmolarity of 20 mM diluted seawater to that of normal seawater did not alter the amount of ΔHCO_3^- lost (ΔH^+ taken up) from the environment. Two

sculpin were placed in water composed of 20 mM NaCl and enough mannitol (700 mM) to bring the solution to the osmolarity of seawater (900 - 1000 mOsm). Whole body excretion rates ($mM \cdot kg^{-1} \cdot hr^{-1}$) for ΔNH_4^+, ΔHCO_3^-, and ΔH^+ are shown in Table 8. Cumulative values for these ions are shown in Figure 13.

In 20 mM diluted seawater ΔNH_4^+, and ΔHCO_3^- both increased while ΔH^+ decreased (see Table 5). Exposure to a low salinity/high osmolarity environment caused similar responses in rates of excretion for the three ions measured. Excretion of ΔH^+ became a negative value indicating that fish began to lose ΔHCO_3^- to the environment.

When comparing plasma values of fish in normal 20 mM seawater to fish in 20 mm/high osmolarity, the following trends can be noticed. Values for pH, Tco_2, $[HCO_3^-]$, $[Na^+]$ and $[Cl^-]$ decreased from control values (Table 8). Values for Pco_2, Tosm, and $[PO_4^{3-}]$, increased from control values.

III.B. Sculpin seawater/100 mM diluted seawater series

Sculpin were able to adapt to 100 mM diluted seawter. Whole body excretions of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ were not significantly different from seawater control. Following a 10-hour control period, sculpin were placed in 100 mM seawater for 46 hours. Whole body and plasma parameters were measured.

Whole body excretion rates ($mM \cdot kg^{-1} \cdot hr^{-1}$) for ΔNH_4^+, ΔHCO_3^-, and ΔH^+ are shown in Table 9. Cumulative values for this experiment are shown in Figure 14. Plasma values for $[Na^+]$, $[Cl^-]$, and osmolarity are shown in Table 9.

Excretion rates during the first 22 hours in 100 mM diluted seawater for

ΔHCO_3^-, and ΔH^+, were not significantly different from initial seawater control values. ΔNH_4^+ excretion in 100 mM diluted seawater was significantly higher than seawater controls at hours 22 ($t=6.70$; $p<0.005$; $n=5$) and 46 ($t=2.98$; $p<0.05$; $n=5$). Excretion of ΔH^+ in 100 mM at hour 24 was significantly higher than ΔH^+ excretion in 20 mM diluted seawater.

Plasma $[Na^+]$ values (mM/l) did not change significantly from the seawater control period when fish were placed in 100 mM diluted seawater. Plasma $[Na^+]$ did increase significantly between hours 96 and 120 ($t=3.75$; $p<0.006$; $n=5$). Values for $[Cl^-]$ for fish in 100 mM seawater decreased significantly from seawater control values at hours 32 ($t=4.40$; $p<0.05$; $n=3$) and 96 ($t=4.51$; $p<0.05$; $n=5$). However, at hour 120 of exposure to 100 mM, $[Cl^-]$ was not significantly different. Total osmolarity was significantly different ($t=3.99$; $p<0.02$; $n=3$) at hour 32 and hour 96 ($t=4.56$; $p<0.05$; $n=5$) of exposure (see Table 9).

III.C. Sculpin 100 mM/20 mM diluted seawater series

III.C.1. Long-term 100 mM/20 mM diluted seawater series

Following eleven days of exposure to 100 mM seawater, excretion rates were not significanlty different from seawater controls. When placed in 20 mM diluted seawater following a long 100 mM adaptation, fish did begin to lose ΔHCO_3^-, but this rate was significantly lower ($5=2.613$; $p<0.02$; $n=5$; $n=10$ respectively) than the rate for fish that were transferred directly from seawater to 20 mM diluted seawater. Sculpin were placed in large aerated plastic containers of 100 mM diluted seawater for 10 days. Following a 24 hour control period in 100 mM, fish were placed in 20

mM diluted seawater for an additional 70 hours. Whole body, blood, and urine parameters were measured.

Sculpin were able to survive in 100 mM diluted seawater quite well. Whole body exchanges for ΔNH_4^+, ΔHCO_3^-, and ΔH^+ measured at the end of eleven days of 100 mM exposure, were not significantly different from values measured for fish in seawater (see Table 10 and Figure 15). Plasma values for the following ions were significantly higher than seawater control values: pH ($t=4.05$; $p<0.002$; $n=3$), Tco_2 ($t=3.61$; $p<0.004$; $n=3$), $[HCO_3^-]$ ($t=3.60$; $p<0.004$; $n=3$), and Pco_2 ($t=3.23$; $p<0.008$; $n=3$). See Table 9.

During the first 24 hours of exposure to 20 mM diluted seawater, excretion rates for ΔNH_4^+, ΔHCO_3^- and ΔH^+ decreased from control measurements taken at the end of the 11 days of 100 mM, but the change was not significant (Table 10). Values for ΔH^+ excretion were negative at hour 24 and subsequent sampling periods. Sculpin continued to lose ΔHCO_3^- through the end of the experiment. Plasma collections were made through hour 48 of 20 mM diluted seawater exposure. There were no significant differences between control period values and the 20 mM period for pH, Tco_2, $[HCO_3^-]$, Pco_2, $[Na^+]$, and $[PO_4^{3-}]$. Only $[Cl^-]$ changed significantly after 24 hours of 20 mM exposure ($t=4.82$; $p<0.01$; $n=4$). Values for $[Cl^-]$ continued to decline significantly at hour 48 ($t=4.02$; $p<0.001$; $n=4$). When comparing values obtained in 100 mM seawater longterm controls (Table 10) to seawater controls (Table 5), only $[PO_4^{3-}]$ and $[Cl^-]$ are not significantly changed.

Urine values for this dilution experiment are shown in Table 10. Urine flow rates for fish in 100 mM diluted seawater were significantly higher ($t=3.68$; $p<0.0004$; $n=3$) than seawater control values (see Table 5). At hour 24 of exposure to 20 mM diluted seawater, the flow rate increased only slightly. Urine values for $[Na^+]$ increased significantly ($t=32.07$; $p<0.0001$; $n=3$) from seawater controls. The $[Na^+]$ value decreased during the first 24 hours of 20 mM diluted seawater exposure and then began to increase. However at hour 48, these increases were not significant. Urine pH in 100 mM was not significantly different from seawater control values. Urine pH values did not change when fish were placed in 20 mM diluted seawater. Total ΔH^+ excreted during 100 mM diluted seawater exposure was not significantly different from values obtained during seawater exposure.

III.C.2. 96 hours 100 mM/20 diluted seawater series

Following 96 hours of exposure to 100 mM diluted seawater, wholebody excretions of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ were not significantly different from seawater control values. Values for ΔNH_4^+ and ΔHCO_3^- during 20mM exposure were significantly higher than values found in fish that had been pre-adapted for eleven days. Sculpin were placed in 100 mM for a period of 96 hours. During the last 18 hours of the 96-hour period, control water samples were taken. The fish were then placed in 20 mM diluted seawater for 48 hours.

Sculpin were exposed to 100 mM diluted seawater for a total of 96 hours before being placed in 20 mM diluted seawater. Excretion rates for ΔNH_4^+, ΔHCO_3^-, and ΔH^+ are shown in Table 11. Cumulative values for these ions are shown in

Figure 19. At hour 96 whole body excretion rates of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ were not significantly different from seawater control values shown in Table 5 (III.A.1.). The measurements taken after fish had been in 100 mM diluted seawater for 11 days were not significantly different from seawater controls (see Table 11). At hour 24 of exposure to 20 mM diluted seawater ΔHCO_3^- excretion rates increased significantly from control period values ($t=4.74$; $p<0.02$; $n=4$). At hour 48 ΔNH_4^+, ΔHCO_3^-, and ΔH^+ were not significantly different from the initial control value (Table 11).

III.C.3. Low salinity/100 mM osmolarity series

ΔHCO_3^- increase three times the seawater control value. Plasma measurements were very similar to those found in experiment. Two sculpin were exposed to 100 mM diluted seawater for 11 days. These fish were then placed in 20 mM seawater with mannitol added to make the total osmolarity of the solution 220 mOsm, the approximate osmolarity of 100 mm seawater.

Two sculpin were placed in 100 mM diluted seawater for 11 days prior to being placed in 20 mM seawater with an osmolarity of approximately 220 mOsm. This is the osmolarity of 100 mM diluted seawater. Results from this experiment were compared to the long-term 100 mM to 20 mM diluted seawater series. See this data in Table 10. Results for this pilot experiment are summarized in Table 12 and Figure 20.

Ion excretions for ΔNH_4^+ and ΔH^+ decreased when fish went from 100 mM diluted seawater to 20 mM/220 mOsm seawater. Excretion of ΔHCO_3^- increased by

three times. Plasma pH did not change. Plasma Tco_2, $[HCO_3^-]$, and Pco_2 increased, while $[Na^+]$ and $[Cl^-]$ both decreased. When comparing this group of fish to the fish that were placed in 100 mM seawater for 11 days (Table 5; see previous section), ΔNH_4^+, ΔHCO_3^-, and ΔH^+ appeared lower.

III.D. Sculpin acetazolamide series

Acetazolamide, a carbonic anhydrase inhibitor, caused a 50% increase in urinary Tco_2. Concurrently plasma pH also decreased. Three fish were injected with acetazolamide following a 24 hour sampling period while the fish were in seawater. Urine and blood samples were taken.

Urine flow rates decreased a slight amount following the injection of acetazolamide, but then continued to fluctuate. Values for urine pH were variable (see Table 13) following the initial injection of acetazolamide. A 50% increase in urine Tco_2 occurred 12 hours after the first injection of acetazolamide. Total acid excretion remained very small.

Corresponding plasma pH values for the first 6.5 hours following the injection of acetazolamide, began to decrease at hour 0.5. Values for plasma Tco_2 remained relatively constant.

DISCUSSION

I. Toadfish series

In *Opsanus tau* the key to successful living in dilute environments may be their ability to regulate the transfers of ΔNH_4^+ and ΔHCO_3^- as well as Na^+ and Cl^- between the fish and surrounding environment. Excretion of ΔNH_4^+ remained constant during long-term exposure to the experimental procedures. Significant differences appeared only during the initial period of 20 mM diluted seawater exposure at hour 48, and the first 48 hours of exposure to 5 mM diluted seawater (Table 1; Figure 3). Loss of ΔNH_4^+ increased following long-term exposure to 5 mM diluted seawater (Figure 4). At hours 24-48, during the second experimental period, fish did begin to increase ammonia excretion. Four fish expired. This indicates that toadfish are able to control the amount of ΔNH_4^+ excretion, but this can be limited by the medium.

Excretion of ΔHCO_3^+ was only significantly different from the long-term control values during exposure to 5mM diluted seawater at hour 24. Significant differences between the paired control value occur at hours 72, values during the second week of exposure were significantly different from this control at hours 24 and 48 (Table 1). *Opsanus tau* did not tolerate 5 mM seawater as well as 20 mM seawater. It has been shown in our lab by Julie Walton that toadfish can live in distilled water for up to 5 days. It is evident that under these severe conditions the

toadfish cannot control the amount of ΔNH_4^+ and ΔHCO_3^+ lost to the environment. If these ions are being lost to the environment it is presumed that others are being lost as well.

From preliminary plasma data it appears that toadfish are able to regulate Cl^- loss during exposure to low salinities. Values for $[Cl^-]$ were only significantly different from control values following 96 hours of exposure to 20 mM diluted seawater. Claiborne and Heisler (1984, 1986) found that Cl^- influx can be linked to HCO_3^- efflux in the carp, Cyprinus carpio. This species is able to reverse the direction of this exchange during exposure to hypercapnic conditions. Kormanik and Evans (1979) found the same mechanism to be present in the Gulf toadfish, Opsanus beta. Living in estuaries where conditions are constantly changing, it would be to the oyster toadfish's advantage to have a reversible mechanism such as this. If $[HCO_3^-]$ in the environment is decreased, such as it is when the salinity is decreased, then NaCl loss to the environment would become slower and loss of ions by the fish would be prevented (Kormanik and Evans, 1979).

Plasma pH remained constant during exposure to 20 mM diluted seawater. $[Cl^-]$ also remained unchanged. A larger group of fish and a more refined sampling technique would be required to demonstrate what changes take place in the plasma of the toadfish during exposure to low salinities. Cannulation techniques used on the long-horned sculpin (Walton and Claiborne, 1987) did not work for *Opsanus tau*. Other methods, such as chronic cannulation of an artery in the swim bladder or caudal artery were attempted but not successfully.

II. Flounder low salinity series

Flounder are considered to be as euryhaline as is the toadfish (Evans, 1979). The winter flounder (*Pseudopleuronectes americanus*) used in our study did not appear to be as adaptable as the toadfish. This may have been due to several reasons. First of all, this species was not studied as extensively as the toadfish. Secondly, these fish did not appear as healthy upon arrival to the lab. Therefore without further study these data must be considered preliminary. Following 178 hours (approximately 7 days) of exposure to 20 mM diluted seawater ΔNH_4^+ and ΔH^+ excretion were significantly different from control values. All but one fish left in 20 mM diluted seawater died. This fish continued to do well and was eventually released (see Figure 6). In previous studies the flounder, *Platicthhys flesus*, was transferred from seawater to freshwater. This resulted in a 90% reduction of Na^+ efflux from the gills (Motais et. al., 1972). These authors believed that following long-term exposure to freshwater, the gills become less permeable to Na^+ and Cl^- due to structural modifications. In our study, the one surviving fish was indeed able to make renal/branchial adjustments. A larger group of healthy fish, and further investigation is needed.

III. Sculpin series

III.A. Sculpin 20 mM series

III.A.1. Seawater/20 mM/seawater series

Exposure to low salinity seawater induces a rapid loss of ΔHCO_3^- to the water even though plasma pH remains stable and plasma Tco_2 increases by 50%. When the

long-horned sculpin is exposed to low salinities, plasma ion concentrations decreased by approximately 25% over 24 hours, due to the loss of these ions to the dilute medium (Claiborne, et al. 1990 and Evans, 1984). In contrast, plasma [PO_4^{3-}] concurrently increased. It is also likely that [PO_4^{3-}] was being lost to the water during this time. The origin of the [PO_4^{3-}], and the measured net loss of base to the water, may be the demineralization of the bone compartment. Plasma [Ca^{++}] decreased by 0.4 mM during exposure to 20 mM diluted seawater, the appearance of 2.3 $mM \cdot kg^{-1}$ in the water may indicate that a large fraction of the Ca^{++} was derived intracellularly (Claiborne et al., 1990). If the Ca^{++} had originated soley from the extracellular fluid, plasma [Ca^{++}] should have decreased by more than 11 mM. This information is based on an estimate of 20% extracellular space; see similar calculation for proton loads by Claiborne and Evans, 1988; Claiborne et al, 1990). This value is quite implausible considering that control plasma [Ca^{++}] was 2.2 mM. It has been previously demonstrated that Ca^{++} and PO_4^{3-} were the two major mineral salts in the bone of the catfish (*Ictalurus punctatus*; Cameron, 1985). While Cameron found no contribution of the bone compartment to acid-base regulation during hypercapnia, our indirect evidence would suggest that bone demineralization is a source of the observed acid base alterations during exposure to low salinities. Whether the bone contribution is an adaptive response to the dilution stress, an effect secondary to the diffusive loss of other ions, or a pathological condition which ultimately leads to the expiration of the animal, remains to be determined (Claiborne et. al. 1990).

As might be adaptive for a teleost in a dilute medium, urine flow rate doubled following exposure to 20 mM diluted seawater (see Table 5). While [Na^+] and ΔNH_4^+ loss increased significantly, and [Cl^-] excretion remained relatively constant, all of these losses amounted to less than 1% of the measured gill transfers for these ions (Claiborne and Evans, 1988). Net whole body H^+ excretion in the seawater sculpin is 96 umol·kg^{-1}hr^{-1} and is reversed to an uptake (or loss of ΔHCO_3^-) of -18 umol·kg^{-1}hr^{-1} when fish are exposed to 20 mM diluted seawater (Walton and Claiborne, 1987). The net [H^+] excretion in the urine measured under these same conditions was 3.4 and 6.6 umol·kg^{-1}hr^{-1}, respectively (Figure 9). Renal loss of [H^+] remained low, but could compensate for a calculated ~35% of the net ΔHCO_3^- lost by the fish during low salinity exposure. Maren et al. (1992), showed that the long-horned sculpin was able to excrete 28% if an injected acid load. Therefore the contribution of the kidney to pH regulation in these animals is less than that measured in freshwater species (Cameron and Kormanik, 1982), but still may assist the animal in times of acid-base imbalance.

III.A.2. Sculpin intracellular pH series

Control intracellular pH was almost identical to the values obtained for sculpin exposed to 20 mM diluted seawater for 23 hours. A decrease in pH or an increase in intracellular [H^+] levels only occurred due to metabolic acidosis that was brought about by exercise. This evidence would lead us to believe that [HCO_3^-] is not coming from intracellular space. Evidence indicates that when animals are in low salinity,

intracellular fluid acid-base balance is not being effected by low salinity. When an animal is exercised, metabolic acidosis occurs and this is shown in pH measurements.

III.A.3. Seawater/low salinity epinephrine series

Prolactin and epinephrine are each known to play important roles in ion balance in lower salinities (Evans, 1979). In this particular experiment, plasma pH remained very similar to the control level, dropping only slightly following the epinephrine injection (see Table 7 and Figure 12). It is interesting that total excretions into the surrounding environment increased greatly following the injection. In this case epinephrine did not help the fish make adjustments to decrease the amount of ΔHCO_3^- being lost to the water.

III.A.4. Low salinity/seawater osmolarity series

The question asked in this experiment was: What factors may contribute to the release of ΔHCO_3^- in low salinities? It has been shown that low salinity (20 mM) causes the sculpin to release ΔHCO_3^- to the surrounding medium (Walton and Claiborne, 1987 and the present data). Changing the osmolarity of the surrounding medium did not seem to help the sculpin. The ΔHCO_3^- loss increased by almost four times (Figure 13). The plasma pH also decreased (Table 8). This did not happen in normal 20 mM diluted seawater. The addition of mannitol to the water caused plasma pH levels to decrease. One fish expired before being returned to seawater. Another possible control for this experiment may have to add mannitol directly to seawater in order to see if the presence of mannitol in normal seawater would have directly affected the fish.

Sculpin seawater/100 mM diluted

seawater series

The sculpin proved to be more euryhaline than expected. These fish survived gradual dilutions much better than when placed directly into very dilute environments, such as 20 mM seawater. Unlike sculpin going from seawater directly to 20 mM diluted seawater, sculpin transferred to 100 mM exhibited ion excretions for ΔHCO_3^-, and H^+ that were not significantly different from control values. When comparing the first 22 hours of exposure in 100 mM (Table 9) to the first 24 hours in 20 mM (Table 5), ΔHCO_3^- excretions were significantly lower in the 100 mM diluted seawater ($p \leq 0.05$ $n=5$, $n=10$ respectively). It is also interesting to note that ΔNH_4^+ at hour 22 of 100 mM diluted seawater exposure is significantly higher than at hour 24 following 11 days of 100 mM exposure. The value for $[Na^+]$ did not decrease significantly following 11 days of exposure to 100 mM diluted seawater. Evans (1977) found that ammonia efflux was at least partially dependent on external Na^+. In order to maintain internal Na^+ balance the sculpin may be employing a Na^+/NH_4^+ exchange mechanism. Evans (1980) calculated that the Na^+/NH_4^+ exchange accounted for 10% of the Na^+ load that Opsanus beta must excrete in order to maintain Na^+ balance in seawater. In dilute salinities marine teleost are faced with a problem of needing to conserve Na^+ ions. Evidence has indicated that the principle form of ammonia excretion is the passive diffusion of NH_3 (Claiborne and Evans, 1988). More recently it has been shown that the long-horned sculpin has the ability to excrete ammonia in conditions where external ammonia was high, suggesting a role

for the excretion of ΔNH_4^+ (Claiborne and Evans, 1988). Our evidence supports a definite role for ΔNH_4^+ excretion. This mechanism may indeed be the method the sculpin can use in order to maintain Na^+ balance in low salinities. It may take long-term exposure for the fish to make the necessary adjustments.

Values for ΔHCO_3^- and ΔH^+ were not significantly different (Tables 9 and 10). Excretion of Cl^- did decrease significantly ($p \leq 0.05$, $n=3$) from the initial control value but remained significantly higher than the 24 hour Cl^- value for fish placed directly in 20 mM diluted seawater. Sculpin were able to control the amount of Cl^- lost following long-term exposure to 100 mM diluted seawater. Plasma T_{osm} also decreased significantly from the paired control value ($p \leq 0.05$, $n=3$), and also remained higher than the 20 mM value ($p \leq 0.05$, $n=4$). This more gradual entry into diluted environments may allow the fish to make certain adjustments in gill permeability that may slow down the loss of ions to the surrounding environment.

Sculpin long-term 100 mM/20 mM diluted seawater series

Sculpin were able to survive 100 mM diluted seawater for a period of at least 11 days. Loss of acid-base relevant ions to the surrounding environment took place much more slowly in 20 mM diluted seawater following the long-term 100 mM exposure (Table 10, Figure 15). When comparing the two 24-hour periods in 20 mM water (see Table 5 and Figure 7; Table 10 and Figure 16), excretion rates for ΔNH_4^+ and ΔHCO_3^- were both significantly less than values from fish that were placed directly into 20 mM diluted seawater (see Tables 5 and 10). Values for plasma pH,

Tco_2, and $[HCO_3^-]$ were all significantly higher for the long-term 100 mM exposed fish. Exposure to 100 mM diluted seawater allowed the fish to live in 20 mM for up to 72 hours. Between hours 48 and 72, three fish did expire. At hour 48, fish in 20 mM diluted seawater had ion excretions for ΔNH_4^+ and ΔHCO_3^- that were significantly higher than initial control values, however only ΔHCO_3^+ was significantly higher than the 24 hour "direct to 20 mM diluted" seawater value. Cl^- remained significantly lower from the initial 100 mM control ($p \leq 0.05$ $n=4$). The Cl^- value continued to decrease while all other parameters measured were relatively constant when compared to the initial 100 mM control period. Values for $[Ca^{++}]$ decreased only slightly.

Sculpin may be getting help from their kidneys when entering a dilute medium (See Table 5; Figure 10). The increase in flow rate indicates that the fish is retaining more water as the fish continues to drink seawater. In order to maintain proper ion balance in the body, excess water is excreted into the bladder by the kidneys back into the dilute medium. In 20 mM diluted seawater, the kidneys compensated for 35% of the total ΔH^+ being lost to the water. In 100 mM diluted seawater the kidneys compensate for a total of 3% of the total acid load.

Sculpin 96 hours 100 mM/20 mM diluted seawater series

Sculpin adapted for only 96 hours had excretion rates for ΔNH_4^+ and ΔH^+ that were significantly higher ($P \leq 0.05$) higher than initial values taken for sculpin that had been in 100 mM diluted seawater for 11 days. Loss of [ΔHCO_3^-] was not significantly changed. It appears that over the longer period of 11 days, sculpin are able to begin to make necessary changes possibly in gill permeability that slow down or reverse the loss of acid-base related ions.

Sculpin low salinity/100 mM osmolarity series

Changing osmolarity during exposure to a low NaCl environment did not help the sculpin maintain the normally expected excretion rates of ΔHCO_3^- (see Table 10). Excretion of ΔHCO_3^- and ΔH^+ are both higher during exposure to the higher osmolarity environment. A better method of increasing osmolarity may be needed so it can be clearly determined that the additional substance is what caused this unexpected increase in excretions.

Sculpin acetazolamide series

Various authors had accepted earlier works that sculpin had a fixed urinary pH of 6.25 (Hodler et al. 1955). Hodler et al. (1955) also reported that there was no change in urine pH following an attempt to inhibit carbonic anhydrase. The widely accepted doctrine emerged that all marine fish had a fixed urinary pH of about 6, and that this value could not be altered by acid-base changes for by acetazolamide, since the kidneys of marine teleost lack carbonic anhydrase (Maren et al., 1990). These

ideas can be confirmed for the elasmobranch (Maren et al., 1990). Hickman and Trump (1969), had reported that urinary pH in the Southern flounder, Paralichthys lethostigma ranged from 5.7 to 8.2. Experiments involving the effects of salinity changes on acid-base parameters in urine have previously been discussed (see Tables 5 and 10). Our experiments have yielded urine pH values ranging from 6.2 to 7.6 in the longhorn sculpin. These values have also been confirmed by Maren et al.(1990). However, in Maren's work control plasma pH values are reported to be 7.5 ± 0.02 ($n=11$). In our work we have found plasma pH to be 7.8 normal resting conditions for the long-horned sculpin. Blood sampling techniques used by Maren differ from those used by our group (Walton and Claiborne, 1987). Maren et al. (1992) also reported no change in urine Tco_2. In our study, urine Tco_2 decreased immediately upon injection of acetazolimide, them increased 5 fold at hour 2.5. Taking into account the higher, more stable pH values Maren may have indeed seen the same increase in urine Tco_2 that we found (See Table 13). The total $[H^+]$ excreted by the kidneys is a significant amount that allows the fish to maintain acid-base balance.

Summary

Opsanus tau and *Pseudopleuronectes americanus* were able to survive drastic changes in salinity much better than *Myoxocephalus octodecimspinosus*. The euryhaline toadfish and flounder were able to control the amount of ions lost to the surrounding environment during low salinity exposure. When ΔHCO_3^- loss cannot be controlled this may lead to the mortality of the fish. Even when sculpin begin to lose large amounts of ΔHCO_3^-, plasma pH is maintained. The "stenohaline" sculpin can

survive very long periods of time in 100 mM diluted seawater and should therefore be considered somewhat Euryhaline. It is intriguing that following the long-term exposure to 100 mM diluted seawater that the sculpin can survive in 20 mM diluted seawater for longer than 24 hours. This long-term exposure may change the fish's gill permeability, exchange mechanisms may be activated or reversed due to the change in ionic composition of the environment, and hormones may be activated in order to help the fish adjust to the low salinity environment.

Maintaining a constant pH in the body fluids at a given temperature is one of the most important tasks of regulatory systems for homeostasis in any animal (Heisler, 1984). In the long-horned sculpin, the plasma pH value is maintained even during long periods of exposure to diluted seawater before the fish eventually dies. Loss of PO_4^{3-} increases during exposure to low salinities as does ΔHCO_3^- loss. Plasma Ca^{++} decreases. Evidence indicates that the buffering capabilities of this species are limited in dilute salinities, but they may demineralize bone in order to buffer the acid load caused by exposure to dilute salinities. Whether the bone contribution is an adaptive response to the dilution stress, an effect secondary to the diffusive loss of other ions, or a pathological condition which leads to the expiration of the animal, remains to be determined.

In teleost fish, the gills are normally considered to be the main site of acid-base regulation, while the role of the kidney has been considered insignificant. During exposure to low salinities (20 mM) the kidneys can ameliorate the net acid

load by 35%. This is a small, but significant, contribution by the kidney to aid the sculpin in maintaining acid-base balance.

Conclusions

The ability of certain species of fish to be either euryhaline or stenohaline seems to be determined in their ability to control ion loss and acid-base balance in a particular environment. Loss of ΔHCO_3^- and loss of Cl^- can be prevented by the toadfish. A Na^+/H^+ exchange mechanism may be in place. In extreme conditions when fish excrete excess H^+, taking up a Na^+ in return would be beneficial to the fish's internal ionic and acid-base balance. It is also possible that a branchial Cl^-/HCO_3^- exchange is likely to contribute to the success of this and other euryhaline species in dilute environments. In the sculpin, bone demineralization may contribute to this species' ability to maintain a constant Ph when other acid-base parameters are out of balance in low salinities. In future studies, more extensive blood data for the toadfish will contribute to a greater understanding of plasma acid-base changes. It is also a possibility that the buffering of ΔH^+ could be taking place somewhere else in the fish's body, possibly in the muscle tissue. By measuring Ca^{++} in the muscle tissue, we may find that the muscle is also contributing to the maintenance of a constant plasma pH.

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Table 1. Control and dilution group rate values ($mM \cdot Kg^{-1} \cdot hr^{-1}$) for ion transfer parameters in *Opsanus tau* during exposure to 20 mM and 5mM diluted seawater.

	Control (seawater)				20 mM (diluted seawater)			5 mM (diluted seawater)		
	time	mean	SE	n	mean	SE	n	mean	SE	n
ΔNH4+										
	0-12	0.123	0.025	10	0.069	0.051	8	0.022	0.008	6#
	12-24	0.117	0.023	10	0.079	0.020	8	0.027	0.009	6*#
	24-48	0.107	0.022	10	0.085	0.019	8*	0.041	0.010	6#
	48-72	0.108	0.020	10	0.079	0.018	8	0.051	0.012	6
	0-24	0.052	0.003	4	0.073	0.013	6	0.074	0.012	6*
	24-48	0.630	0.005	4	0.075	0.015	6	0.077	0.012	6*
	48-72	0.056	0.005	4	0.056	0.013	3	0.060	0.010	2
ΔHCO3-										
	0-12	0.053	0.042	10	0.128	0.022	8	-0.078	0.154	6
	12-24	0.048	0.030	10	0.126	0.024	8	0.152	0.025	6#
	24-48	0.079	0.027	10	0.126	0.022	8	0.141	0.020	6
	48-72	0.155	0.147	10	0.110	0.021	8	0.136	0.023	6*
	0-24	0.029	0.014	4	0.071	0.013	6	0.116	0.011	6*#+
	24-48	0.041	0.014	4	0.087	0.015	6	0.120	0.010	6 * #
	48-72	0.035	0.015	4	0.075	0.023	3	0.117	0.010	2 #
ΔH+										
	0-12	0.069	0.038	10	-0.059	0.011	8#	-0.133	0.206+	5 # +
	12-24	0.068	0.020	10	-0.048	0.011	8#	-0.125	0.025	6 # +
	24-48	0.029	0.015	10	-0.041	0.016	8#	-0.100	0.014	6 * # +
	48-72	-0.046	0.053	10	-0.031	0.009	8*	-0.087	0.014	6 * +
	0-24	0.023	0.015	4	0.002	0.013	6*	-0.042	0.003	6 * # +
	24-48	0.022	0.010	4	-0.012	0.021	6	-0.043	0.007	6 * #
	48-72	0.021	0.010	4	-0.019	0.014	3	-0.057	0.001	2 * #

* Significantly different from initial control (0) period for individual experiments ($p < .05$). Paired t-test.

Significantly different from corresponding longterm control values ($p < .05$). Unpaired t-test.

+ Significantly different from corresponding 20 mM (diluted seawater) values ($p < .05$). Unpaired t-test.

Table 2. Control and dilution group cumulative values (mM.Kg-1) for ion transfer parameters in *Opsanus tau* during exposure to 20 mM and 5mM diluted seawater.

Control (seawater)				20 mM (diluted seawater)			5 mM (diluted seawater)		
	time	mean	SE	N	mean	SE	N	mean	SE	N
ΔNH_4^+										
	0	0.000	0.000	10	0.000	0.000	8	0.000	0.000	6
	12	1.471	0.294	10	0.831	0.230	8#	0.269	0.094	6#
	24	2.797	0.548	10*	1.888	0.487	8*	0.641	0.219	6#
	36	4.096	0.798	10*	2.861	0.695	8*	1.098	0.335	6#
	48	5.371	1.051	10*	3.937	0.944	8*	1.620	0.414	6*#
	60	6.702	1.295	10*	4.788	1.140	8*	2.266	0.547	6*#
	72	7.968	1.517	10*	5.831	1.372	8*	2.841	0.674	6*#
	0	0.000	0.000	4	0.000	0.000	6	0.000	0.000	6
	12	0.642	0.037	4	0.973	0.176	6	0.901	0.155	6*
	24	1.247	0.076	4*	1.747	0.321	6*	1.784	0.280	6*
	36	2.034	0.164	4*	2.691	0.500	6*	2.685	0.423	6*
	48	2.768	0.188	4*	3.563	0.669	6*	3.625	0.559	6*
	60	3.473	0.269	4*	4.665	0.865	6*	4.658	0.792	6*
	72	4.106	0.306	4*	4.056	1.089	3	4.100	0.965	2*
ΔHCO_3^-										
	0	0.000	0.000	10	0.000	0.000	10	0.000	0.000	10
	12	0.637	0.510	10	1.539	0.266	8	1.870	0.246	6
	24	1.162	0.728	10	3.033	0.566	8*	3.651	0.592	6*#
	36	2.353	0.986	10*	4.479	0.825	8*	5.390	0.769	6*#
	48	3.048	1.266	10*	6.059	1.066	8*	7.039	1.029	6*#
	60	5.622	1.415	10*	7.310	1.256	8*	8.792	1.276	6*
	72	6.758	1.629	10*	8.702	1.511	8*	10.312	1.521	6*
	0	0.000	0.000	4	0.000	0.000	6	0.000	0.000	6
	12	-0.053	0.326	4	1.085	0.188	6#	1.407	0.161	6#
	24	0.688	0.345	4	1.705	0.304	6	2.791	0.258	6*#+
	36	1.299	0.268	4	2.607	0.440	6*	4.309	0.363	6*#+
	48	1.675	0.349	4	3.800	0.628	6*#	5.671	0.491	6*#+
	60	2.406	0.448	4	4.684	0.770	6*	7.173	0.645	6*#+
	72	2.512	0.688	4	5.846	1.786	3	7.964	1.320	2*#
ΔH^+										
	0	0.000	0.000	10	0.000	0.000	8	0.000	0.000	6
	12	0.834	0.456	10	-0.708	0.134	8#	-1.600	0.247	6#+
	24	1.635	0.489	10*	-1.145	0.272	8*#	-3.010	0.596	6*#+
	36	1.748	0.544	10*	-1.618	0.440	8*#	-4.292	0.739	6*#+
	48	2.323	0.718	10*	-2.121	0.598	8*#	-5.419	0.897	6*#+
	60	1.081	1.267	10	-2.521	0.665	8*#	-6.527	1.004	6*#+
	72	1.210	1.189	10	-2.871	0.718	8*#	-7.471	1.140	6*#+
	0	0.000	0.000	4	0.000	0.000	6	0.000	0.000	6
	12	0.696	0.338	4	-0.111	0.145	6*#	-1.433	0.951	6
	24	0.559	0.352	4	0.042	0.317	6*	-1.007	0.078	6#+
	36	0.735	0.305	4	0.084	0.543	6	-1.624	0.169	6#+
	48	1.093	0.281	4	-0.237	0.798	6	-2.046	0.217	6#
	60	1.067	0.418	4	-0.019	0.972	6	-2.520	0.188	6#+
	72	1.594	0.498	4	-1.790	1.251	3#	-3.864	0.355	2*

* Significantly different from initial control (0) period ($p < .05$). Paired t-test.

Significantly different from corresponding longterm control value ($p < .05$). Unpaired t-test.

+ Significantly different from corresponding 20 mM (diluted seawater) values ($p < .05$). Unpaired t-test.

Table 3. Plasma pH, Tco2, P_{CO_2}, [HCO_3^-] and [Cl^-] measured in *Opsanus tau* during exposure to 20 mM diluted seawater. Time zero represents values obtained during control conditions.

	time	mean	SE	n
pH				
	0	7.744	0.023	4
	48	7.529	0.077	4*
	96	7.364	0.099	4*
	120	7.803	0.037	3
T_{CO_2}				
	0	9.010	0.982	4
	48	2.313	0.418	4*
	96	4.128	1.552	4
	120	9.313	1.321	3
P_{CO_2}				
	0	3.886	0.521	4
	48	1.636	0.371	4*
	96	4.454	2.304	4
	120	3.455	0.413	3
HCO_3^-				
	0	8.844	0.963	4
	48	2.236	0.408	4*
	96	3.919	1.460	4
	120	9.165	1.308	3
Cl^-				
	0	138	6.652	4
	48	131	5.260	4
	96	129	6.305	4*
	120	150	10.837	3

* Significantly different from initial control (0) period ($p < .05$). Paired t-test.

Table 4. Control (hour 10) and dilution group rate values ($mM\,Kg^{-1}\,hr^{-1}$) for ion transfer parameters in *Pseudopleuronectes americanus* during exposure to 20 mM diluted seawater.

Cummulative values (mM/kg)	time	mean	SE	n	Excretion rates (mM/kg/hr) time	mean	SE	n
ΔNH_4^+								
	0	0.000	0.000	4				
	10	2.969	0.612	4	0-10	0.297	0.061	4
	34	8.655	1.146	4 *	10-34	0.237	0.026	4
	82	19.250	3.269	4 *	34-82	0.221	0.046	4
	130	26.886	5.471	4 *	82-130	0.159	0.061	4
	178	36.067	10.069	3	130-178	0.132	0.065	3
ΔHCO_3^-								
	0	0.000	0.000	4				
	10	1.504	0.339	4	0-10	0.151	0.034	4
	34	6.961	1.274	4	10-34	0.227	0.060	4
	82	17.796	3.982	4 *	34-82	0.226	0.057	4
	130	34.695	8.027	4 *	82-130	0.352	0.091	4
	178	56.038	11.869	3 *	130-178	0.323	0.089	3
ΔH^+								
	0	0.000	0.000	4				
	10	1.466	0.766	4	0-10	0.147	0.077	4
	34	1.695	0.475	4	10-34	0.010	0.040	4
	82	1.455	0.774	4	34-82	-0.005	0.011	4
	130	-7.809	3.194	4	82-130	-0.193	0.053	4
	178	-19.972	2.862	3 *	130-178	-0.191	0.029	3

Measurements made 1 week following hour 168 for 1 fish. Cummulative values (mM/kg). Excretion rates (mM/kg/hr)

	ΔNH_4^+	ΔHCO_3^-	ΔH^+		ΔNH_4^+	ΔHCO_3^-	ΔH^+
24	4.27	4.26	0.1	24	0.204	0.193	-0.011
48	10.12	8.18	1.2	48	0.298	0.191	0.107
72	15.44	16.26	0.1	72	0.148	0.136	0.013

* Significantly different ($p < .05$) from initial control period (10). Paired Student's t-test

Table 5. Control and dilution group rate values (mM.Kg-1.hr-1) for ion transfer parameters, as well as plasma and urine parameters, measured in *Myoxocephalus octodecimspinosus* during exposure to 20 mM diluted seawater for 24 hours before being returned to seawater.

Whole body exchange rates

	seawater control			diluted seawater			Returned to seawater		
time	0			24			46		
Ion	mean	SE	n	mean	SE	n	mean	SE	n
ΔNH_4^+	0.186	0.0193	10	0.2684	0.0227	10*	0.2387	0.027	7
ΔHCO_3^-	0.092	0.044	10	0.2516	0.0257	10*	0.1719	0.0472	7
ΔH^+	0.0955	0.0327	10	0.0168	0.0168	10*	0.0669	0.0406	7
Plasma									
Ca^{++}	2.19	0.28	9	1.8	0.3	9*	2.61	0.35	6
PO_4^{3-}	1.91	0.13	6	2.22	0.18	6*	2.46	0.18	6
Na^+	178	4.24	12	133	3.3	12*	174	7.36	10
Cl^-	162	5.26	12	116	3.77	12*	148	3.62	10
Tosm	378	27.01	4	242	9.87	4*	312	9.46	4
pH	7.846	0.029	7	7.78	0.043	6	7.957	0.008	3
Tco_2	5.143	0.414	7	7.81	0.525	6*	6.204	0.276	3
Pco_2	1.506	0.0877	7	2.84	0.301	6*	1.438	0.044	3
HCO_3^-	5.058	0.412	7	7.653	0.523	6*	6.124	0.273	3
Urine									
pH	7.18	0.14	9	6.93	0.18	5	6.71	0.18	4
Na^+	178	6.9	9	45.1	20.8	5	45.6	13.1	4
Cl^-	19.7	8.9	6	61.1	27.3	3	40.2	9.2	4
H^+	3.38	1.3	5	6.64	3.41	5	5.03	1.69	4
ΔNH_4^+	0.02	0.001	9	0.032	0.001	5	0.051	0.017	4
Flow	0.261	0.043	9	0.575	0.231	5*	0.503	0.119	4
Intracellular pH									
pH	7.27	0.0008	2	7.22	0.013	2			
Intracellular pH for exercised fish									
pH	6.56	0.01	2						

*Significantly different ($p < 0.05$) from the intial control period (0). Paired t-test.

Table 6. Control and dilution group rate values (mM.Kg^{-1}.hr^{-1}) for ion transfer parameters in *Myoxocephalus octodecimspinosus* during exposure to 20 mM diluted seawater for 46 hours before being returned to seawater.

Whole body exchange rates

	seawater control			diluted seawater			returned to seawater					
time	0			24			46			70		
ion	mean	SE	n	mean	SE	n	mean	SE	n	mean	SE	n
ΔNH_4^+	0.2110	0.0210	3	0.3287	0.0203	3*	0.3926	0.0409	2	0.2760	0.0290	2*
ΔHCO_3^-	0.0020	0.0200	3	0.3104	0.0384	3*	0.2991	0.0021	2	0.2822	0.1399	2
ΔH^+	0.2100	0.0400	3	0.0183	0.0186	3*	0.0936	0.0390	2	-0.0252	0.1110	2

*Significantly different from initial control period ($p < 0.05$). Paired t-test.

Table 7. Dilution group rate values (mM.Kg^{-1}.hr^{-1}) for ion transfer parameters, as well as plasma parameters, measured in *Myoxocephalus octodecimspinosus* injected with epinephrine, during exposure to 20 mM diluted seawater for 24 hours. Values obtained in this experiment were compared to control values obtained in the experiment described in Table 5.

Whole body exchange rates

	seawater control			diluted seawater			after injection		
time	0			20			28		
Ion	mean	SE	n	mean	SE	n	mean	SE	n
ΔNH_4^+	0.186	0.0193	10	0.256	0.00518	2	0.5273	0.0448	2
ΔHCO_3^-	0.092	0.044	10	0.2286	0.0224	2	0.7344	0.0214	2
ΔH^+	0.0955	0.0327	10	0.0273	0.0275	2	-0.207	0.0234	2
Plasma									
pH	7.778	0.0355	2	7.881	0.008	2	7.764		1
Tco_2	3.92	0.575	2	5.42	0.395	2	6.673		1
Pco_2	1.223	0.108	2	1.516	0.0805	2	2.487		1
HCO_3^-	3.275	0.005	2	5.386	0.465	2	6.537		1
Cl^-	167	4.3	2	139	1.55	2	137		1

Table 8. Control and dilution group rate values (mM.Kg^{-1}.hr^{-1}) for ion transfer parameters, as well as plasma parameters, measured in *Myoxocephalus octodecimspinosus* during exposure to 20 mM diluted seawater with seawater osmolarity for 24 hours.

Whole body exchange rates

	seawater control			20 mM diluted seawater with mannitol			Returned to seawater		Normal 20 mM diluted seawater values		
time	0			20			27				
Ion	mean	SE	n	mean	SE	n	mean	n	mean	SE	n
ΔNH_4^+	0.186	0.0193	10	0.2097	0.0273	2			0.2684	0.023	10
ΔHCO_3^-	0.092	0.044	10	0.405	0.372	2			0.2516	0.026	10
ΔH^+	0.0961	0.0327	10	-0.1952	0.345	2			0.0185	0.0186	10
Plasma											
pH	7.828	0.004	2	7.203	0.252	2	7.621	1	7.781	0.043	6
Tco_2	5.03	1.213	2	2.463	1.296	2	5.429	1	7.81	0.525	6
Pco_2	1.608	0.403	2	2.957	0.017	2	2.874	1	2.836	0.301	6
HCO_3^-	4.941	1.191	2	2.300	1.295	2	5.27	1	7.653	0.523	6
Na^+	169	0	2	94		1	197	1	133	3.3	12
Cl^-	162	2	2	134.5	20.5	2	178	1	116	3.77	12
Tosm	336.5	0.5	2	374		1	499	1	242	9.87	4
PO_4^{3-}	2.12	0.11	2	3.54		1	2.86	1	2.22	0.18	6

Table 9. Control and dilution group rate values (mM.Kg^{-1}.hr^{-1}) for ion transfer parameters, as well as plasma parameters, measured in *Myoxocephalus octodecimspinosus* during exposure to 100 mM diluted seawater.

Whole body exchange rates

	seawater control			100 mM diluted seawater								
Time	0			32			96			120		
Ion	mean	SE	n	mean	SE	n	mean	SE	n	mean	SE	n
ΔNH_4^+	0.1968	0.0245	5	0.274	0.0333	5*	0.2717	0.0331	5* **	0.2071	0.0387	4
ΔHCO_3^-	0.1599	0.0759	5	0.1156	0.0461	5$^+$	0.157	0.0143	5	0.1973	0.0203	4
ΔH^+	0.0367	0.0733	5	0.0629	0.0462	5	0.1146	0.0253	5**	0.0097	0.0387	4
Plasma												
Na^+	172	22.4	5	168	2.06	3	167	4.39	5	185	1.95	5**
CL^-	166	4.28	5	147	2.00	3*	149	2.809	5*	166.6	0.71	5**
Tosm	351	7.31	5	311	2.03	3*	309	4.03	5*	331	1.061	5* **

*Significantly different from the control value ($p < 0.05$). Paired t-test.

**Significantly different from the 96 hour value ($p < 0.05$). Unpaired t-test.

$^+$Significantly lower thatn the 24 hour value in the seawater\20mM experiment (see Table 5; $p < 0.05$). Unpaired t-test.

$^{++}$Significantly lower thatn the 24 hour value in the seawater\20mM experiment (see Table 5; $p < 0.05$). Unpaired t-test.

Table 10. Control and dilution group rate values (mM.Kg^{-1}.hr^{-1}) for ion transfer parameters, as well as plasma and urine parameters, measured in *Myoxocephalus octodecimspinosus* during exposure to 100 mM diluted seawater for 11 days before being transferred to 20 mM diluted seawater.

Whole body exchange rates

	100 mM control			20 mM diluted seawater								
time 0	24			48			72					
Ion	mean	SE	n	mean	SE	n	mean	SE	n	mean	SE	n
ΔNH_4^+	0.1292	0.0255	5	0.1265	0.0175	5^{++}	0.2413	0.054	5	0.1881	0.058	2
ΔHCO_3^-	0.1069	0.024	5	0.1436	0.027	5^{++}	0.3447	0.03	5^{++}	0.167	0.067	2
ΔH^+	0.022	0.0168	5	-0.017	0.0202	5	-0.1035	0.075	5	0.021	0.047	2
Plasma												
Ca^{++}	1.3	0.0162	3^+	1.25	0.115	3	0.84	0.05	3*			
$PO4^{3-}$	2.12	0.1395	3	2.52	0.237	4	2.841	0.311	4			
Na^+	147.7	4.09	3^+	146	7.77	3	134.03	7.93	3			
Cl^-	146.3	2.43	6	133	8.935	4*	118.225	9.8	4*			
pH	8.013	0.029	6^+	7.9	0.0268	4^{++}	7.983	0.027	4^{++}			
Tco_2	13.185	2.376	6^+	11.98	2.071	4^{++}	14.022	2.399	4^{++}			
Pco_2	2.561	0.3404	6^+	2.59	0.3637	4	3.054	0.573	4			
HCO_3^-	13.043	2.36	6^+	11.80	2.052	4^{++}	13.854	2.37	4^{++}			
Urine												
pH	7.66	0.21	3	7.77	0.12	2	7.74	0.3	2			
Na^+	76.01^+	6.14	3^+	66.88	44.3	2	83.39	17.54	2			
Cl-	53.82^+	82	3^+	50.9	17.8	2	78.83	27.6	2			
H^+	1.47	0.56	3	0.458	0.102	2	1.125	1.26	2			
ΔNH_4^+	0.0183	0.001	3	0.0519	0.001	2	0.0114	0.102	2			
Flow Rate	0.6161^+	0.112	3^+	0.687	0.3316	2	0.901	0.349	2			

* Significantly different from initial control (0) period for individidual experiment ($p < 0.05$). Paired t-test.

$^+$ Significantly different from corresponding seawater control values ($p < 0.05$). See Table 5. Unpaired t-test

$^{++}$ Significantly different from corresponding 20 mM value in seawater\20mM experiment (See Table 5; $p < 0.05$). Unpaired t-test.

Table 11. Control and dilution group rate values (mM.Kg^{-1}.hr^{-1}) for ion transfer parameters in *Myoxocephalus octodecimspinosus* during exposure to 100 mM diluted seawater for 96 hours before being transferred to 20 mM diluted seawater.

Whole body exchange rates

	100 mM control			20 mM diluted seawater					
time	0			24			48		
Ion	mean	SE	n	mean	SE	n	mean	SE	n
ΔNH_4^+	0.1973	0.0203	4	0.3597	0.0327	4 * ++	0.2345	0.0132	4
ΔHCO_3^-	0.2071	0.0387	4	0.3482	0.0508	4 * ++	0.0528	0.0218	4 ++
ΔH^+	-0.0097	0.0387	4	0.0115	0.0299	4	0.0819	0.0129	4 *

*Significantly different from control values ($p < 0.05$)

++Significantly different from corresponding values for fish that had been exposed to 100 mM diluted seawater for 11 days ($p < 0.05$). Unpaired t-test.

Table 12. Control and dilution group rate values (mM.Kg^{-1}.hr^{-1}) for ion transfer parameters, as well as plasma parameters, measured in *Myoxocephalus octodecimspinosus* during exposure to 100 mM diluted seawater for 11 days before being transferred to 20 mM diluted seawater with 100 mM osmolarity.

Whole body exchange rates

	100 mM control			20 mM seawater 100 mM osmolarity					
time	0			8			20		
Ion	mean	SE	n	mean	SE	n	mean	SE	n
ΔNH_4^+	0.1292	0.026	5	0.116	0.0378	2	0.1098	0.003	2
ΔHCO_3^-	0.1069	0.024	5	0.316	0.093	2[+]	0.1263	0.037	2
ΔH^+	0.022	0.017	5	-0.1997	0.0553	2[+]	-0.0165	0.040	2
Plasma									
pH	7.975	0.019	2	8.01	0.0385	2	7.97	0.010	2
Tco_2	9.12	0.92	2	15.55	2.145	2	15.76	1.005	2
Pco_2	2.035	0.295	2	2.47	0.11	2	3.44	0.135	2
HCO_3^-	9.005	0.905	2	15.41	0.214	2	15.54	0.98	2
Na^+	152	8.8	2	154	6.35	2	136	8.85	2
Cl^-	147	6.7	2	138	7.35	2	122	2.8	2

[+] Value higher here than in 20 mM diluted seawater.

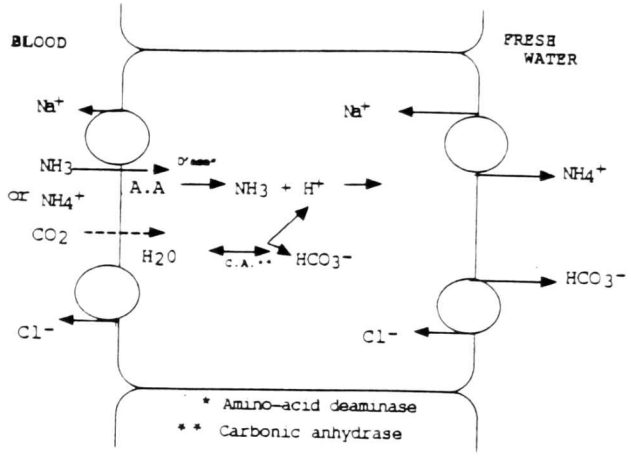
Table 13. **Plasma** and urine values for parameters measured in *Myoxocephalus octodecimspinosus* during exposure to seawater following injections of acetazolamide.

	Control	Following Injection				
time	0	0.5	2.5	4	6.5	7.0
ion						
Plasma						
n	2	2	2	2	2	
pH	7.717	7.69	7.45	7.45	7.39	
Tco_2	5.94	5.03	5.37	5.32	4.74	
Pco_2	2.53	2.25	4.36	4.15	4.39	
HCO_3	-5.8	4.9	5.13	5.08	4.49	
Urine						
n	3	2	2	2	1	1
pH	7.173	0.03	7.315	7.252	7.606	
Tco_2	1.83	0.573	3.16	2.89	4.25	
H^+	1.321	0.548	1.756	2.46	0.315	
ΔNH_4^+	0.012	0.006	0.0102	0.0218	0.0047	0.0029
Flow Rate	0.339	0.086	0.288	0.314	0.2384	0.3149

$$H_2O + CO_2 <=====> H_2CO_3 <=====> H^+ + HCO_3^-$$

Figure 1. Carbon dioxide - bicarbonate buffering system.

A.



B.

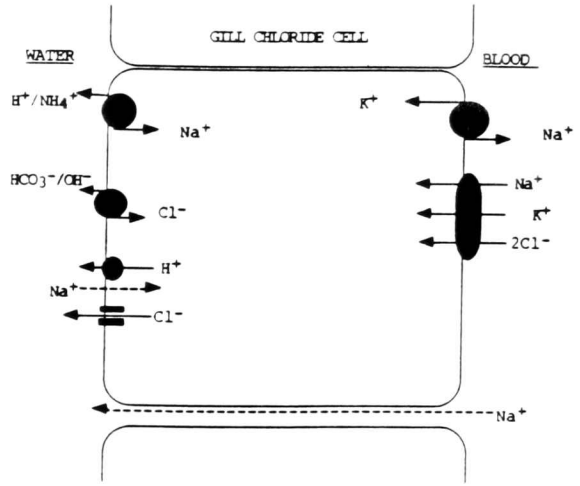


Figure 2. A. Proposed model for [Na^+] and [Cl^-] uptake by the freshwater fish branchial epithelium cell (Maetz and Garcia-Romeu, 1964); B. Proposed model for [Na^+] and [Cl^-] extrusion by the seawater fish branchial chloride cell (Silva, et. al., 1977).

Figure 3. Cumulative transfers of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ in *Opsanus tau* during exposure to 20 mM seawater. Control group was maintained in seawater for the length of the experiment (mean $\pm$ SE, n=10 for week 1, n=4 for week 2). Week 1 indicates the first 60 hours in 20 mM seawater (mean $\pm$ SE; n=8). Week 2 indicates an additional 60 hours of experimentation after being maintained in 20 mM seawater for 7 days (mean $\pm$ SE; n=6; shown at hour 108-168).

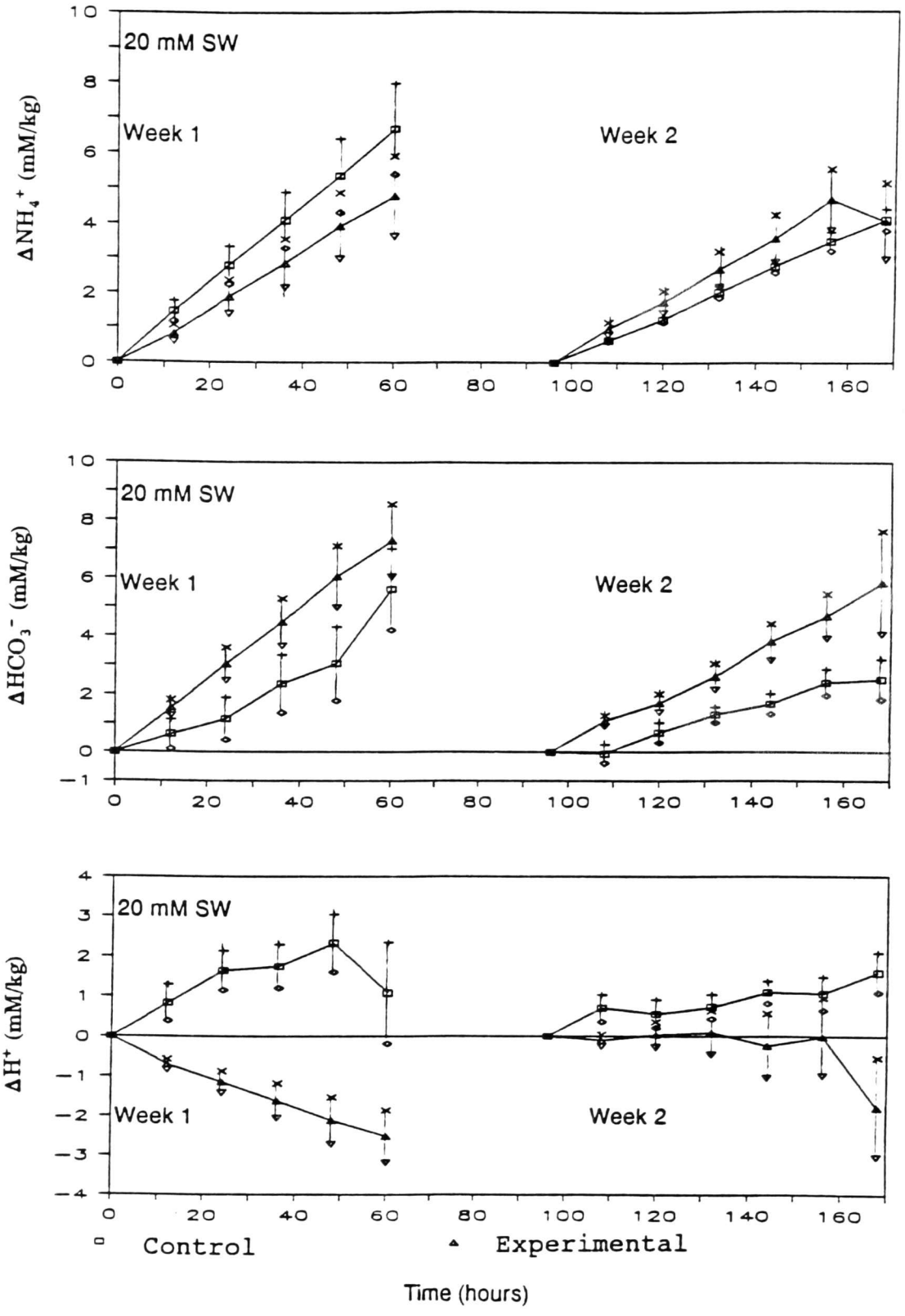

20 mM SW
Week 1
Week 2
ΔNH4+ (mM/kg)
20 mM SW
Week 1
Week 2
ΔHCO3- (mM/kg)
20 mM SW
Week 1
Week 2
ΔH+ (mM/kg)
Control
Experimental
Time (hours)

Figure 4. Cumulative transfers of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ in *Opsanus tau* during exposure to 5 mM seawater. Control group was maintained in seawater for the length of the experiment (mean $\pm$ SE, n=6 for week 1, n=6 for week 2). Week 1 indicates the first 60 hours in 5 mM seawater (mean $\pm$ SE; n=8). Week 2 indicates an additional 60 hours of experimentation after being maintained in 5 mM seawater for 4 days (mean $\pm$ SE; n=6; shown at hour 108-168).

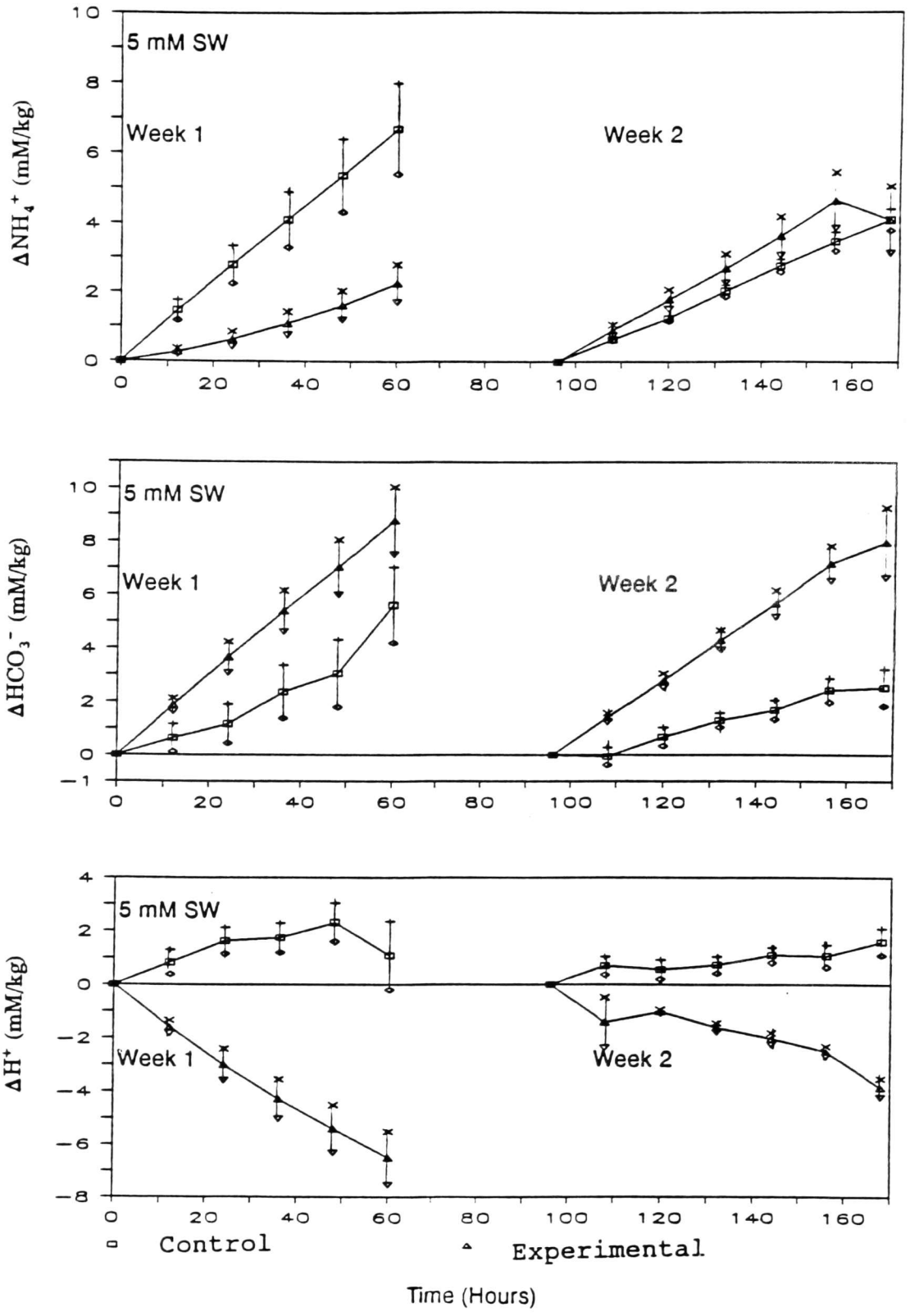

5 mM SW
Week 1
Week 2
ΔNH_4^+ (mM/kg)
5 mM SW
Week 1
Week 2
ΔHCO_3^- (mM/kg)
5 mM SW
Week 1
Week 2
ΔH^+ (mM/kg)
Control
Experimental
Time (Hours)

Figure 5. Plasma pH, Tco_2 (Pco_2 and $[HCO_3^-]$) and $[Cl^-]$ measured in *Opsanus tau*. Parameters measured at time zero (fish in 400 mM seawater; mean $\pm$ SE, n=4), during a 72 hour exposure to a dilute salinity (20 mM; mean $\pm$ SE, n=4), and finally an additional 24 hours in seawater (SW; mean $\pm$ SE, n=3 at hours 120-144).

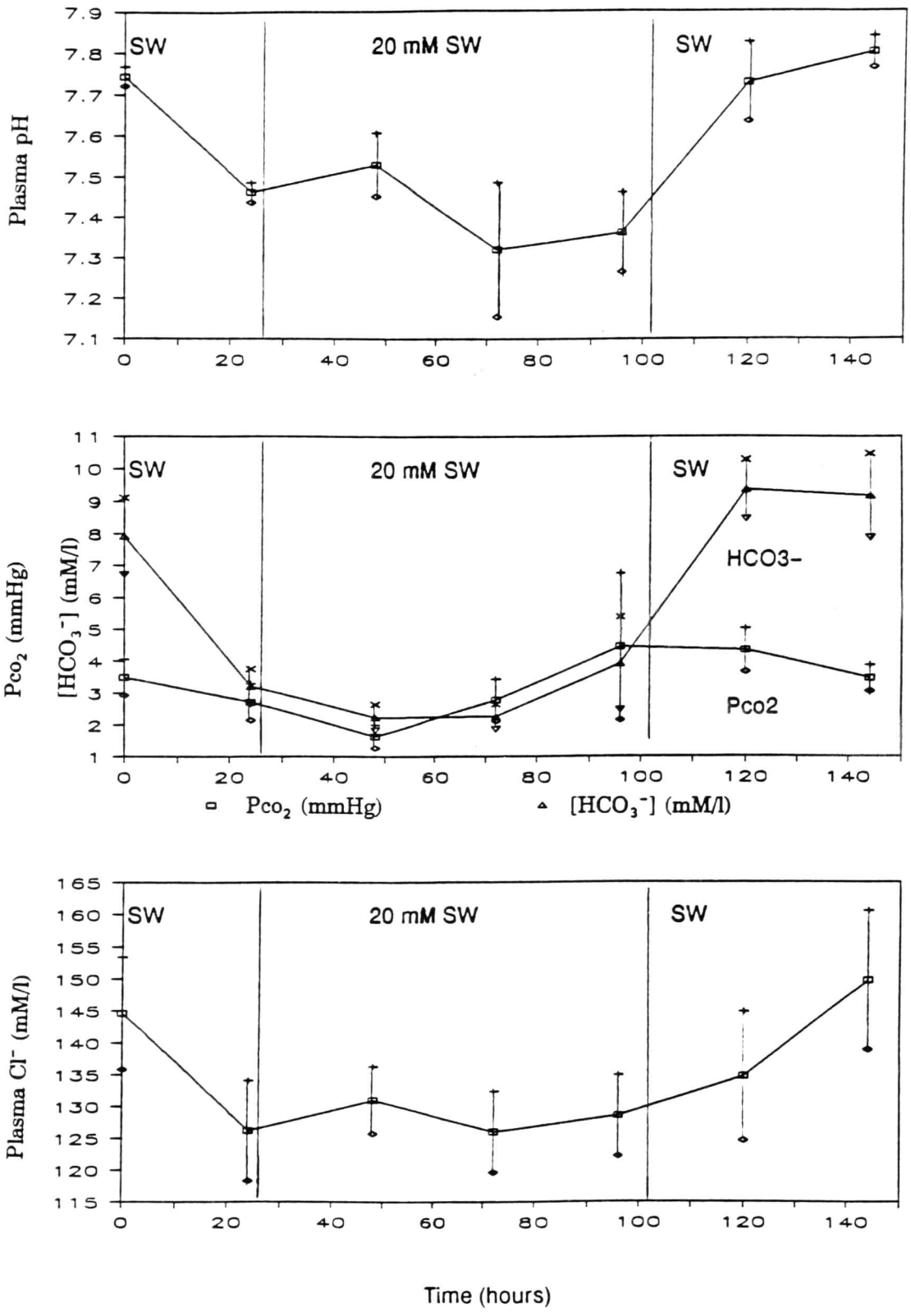

Plasma pH
SW
20 mM SW
SW
Pco2 (mmHg)
[HCO3-] (mM/l)
HCO3-
Pco2
Pco2 (mmHg)
[HCO3-] (mM/l)
Plasma Cl- (mM/l)
Time (hours)

Figure 6. Cumulative transfers of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ in *Pseudopleuronectes americanus* exposed to 20 mM seawater: Control ($\sim$480 mM, mean $\pm$ SE, n=4) and experimental salinity (20 mM, mean $\pm$ SE, n=4 hours 12-158; n=3 at hour 168) were measured as water [Cl^-] in mM NaCl.

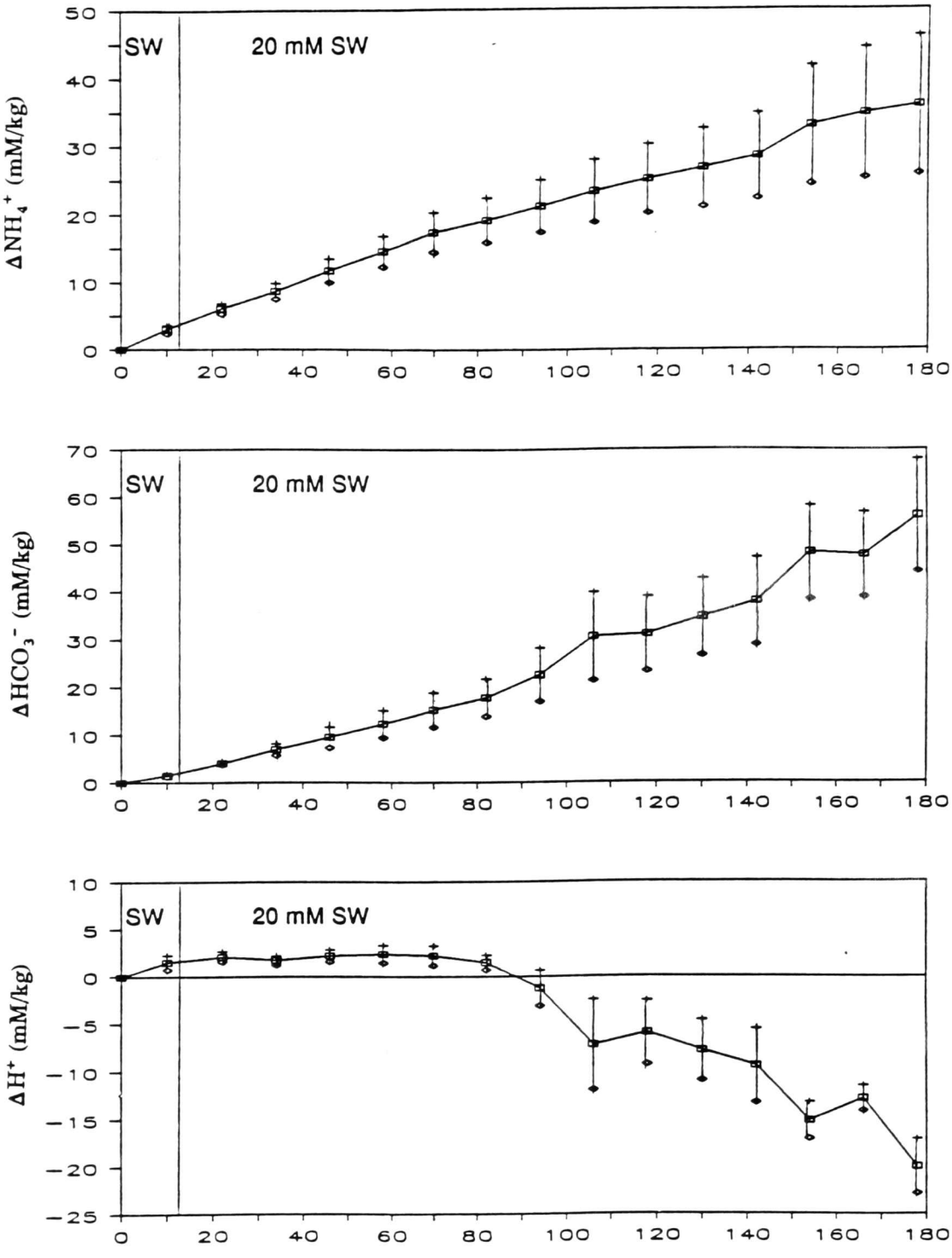

SW
20 mM SW
ΔNH4+ (mM/kg)
ΔHCO3- (mM/kg)
ΔH+ (mM/kg)
Time (hours)

Figure 7. Cumulative transfers of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ in *Myoxocephalus octodecimspinosus* exposed to 20mM seawater: Control ($\sim$480 mM, mean $\pm$ SE, n=10) and experimental salinity (20 mM, mean $\pm$ SE, n=10 at hours 0-34, n=7 at hour 56-70, and n=3 at hour 80) were measured as water [Cl^-] in mM NaCl.

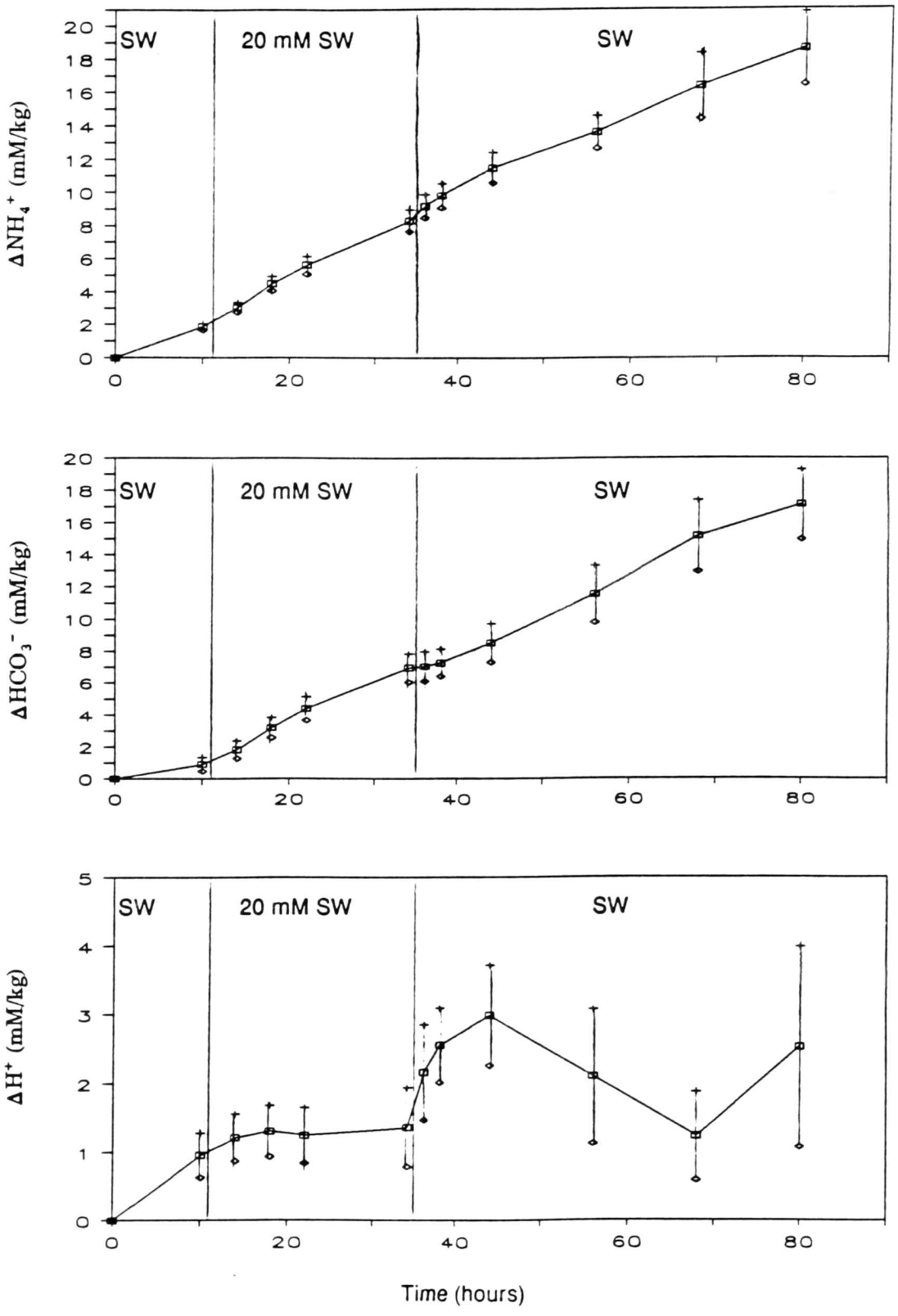

SW
20 mM SW
SW
ΔNH_4^+ (mM/kg)
ΔHCO_3^- (mM/kg)
ΔH^+ (mM/kg)
Time (hours)

Figure 8. Plasma pH and Tco_2 (Pco_2 and $[HCO_3^-]$) measured in *Myoxocephalus octodecimspinosus*. Parameters measured at time zero (fish in 400 mM seawater, mean $\pm$ SE, n=7), during a 24 hour exposure to a dilute salinity (20 mM, mean $\pm$ SE, n=6 at hour 24), and finally an additional 24 hours in seawater (SW, mean $\pm$ SE, n=5 hours 26-34, n=3 at hour 46).

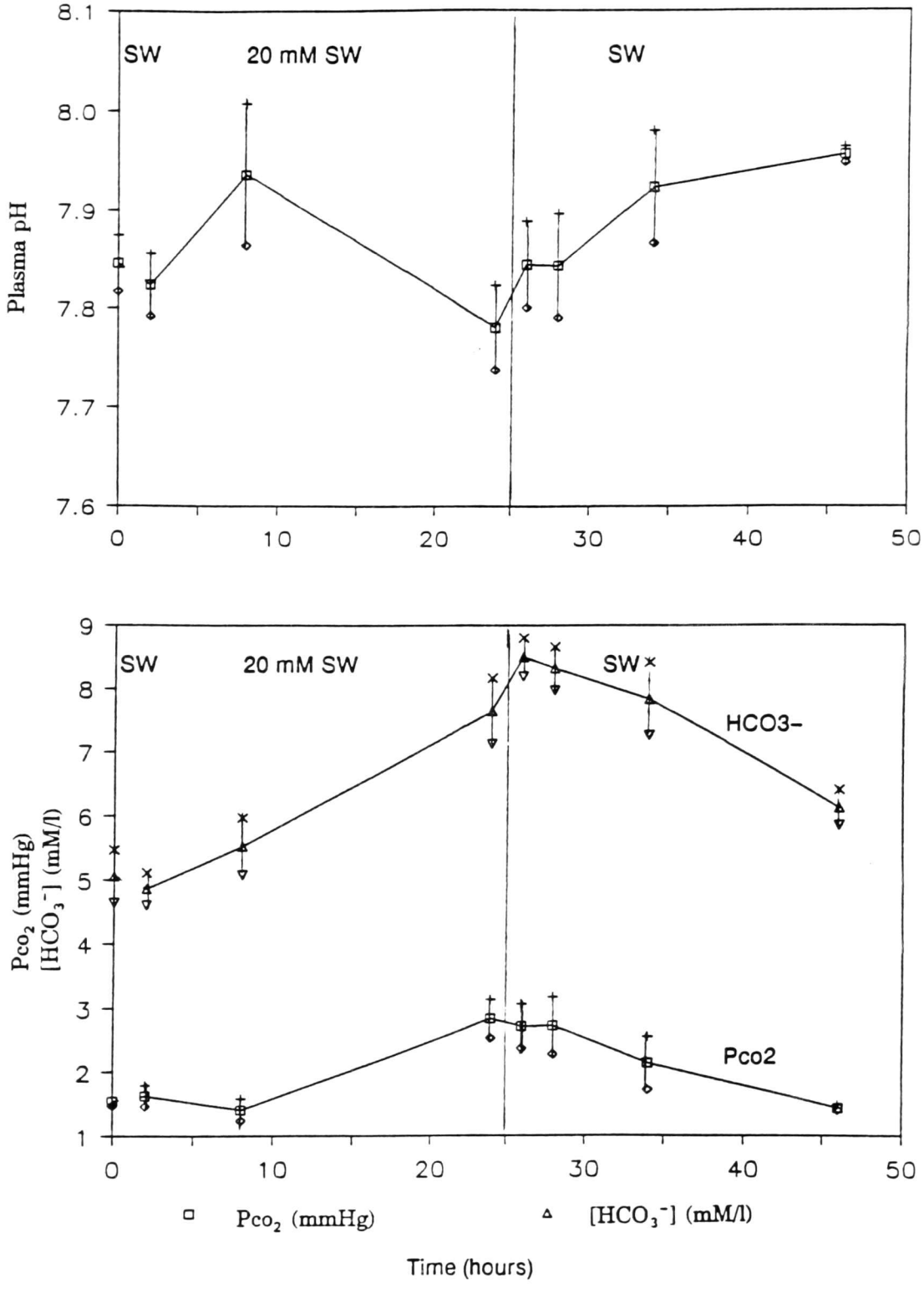

SW
20 mM SW
SW
Plasma pH
8.1
8.0
7.9
7.8
7.7
7.6
0
10
20
30
40
50
SW
20 mM SW
SW
HCO3-
Pco2
Pco_2 (mmHg)
$[HCO_3^-]$ (mM/l)
Time (hours)

Figure 9. Plasma [Na^+], [Cl^-], [Ca^{++}], and [PO_4^{3-}] measured in *Myoxocephalus octodecimspinosus*. Parameters measured at time zero (fish in 400 mM seawater), during a 24 hour exposure to a dilute salinity (20 mM), and finally an additional 24 hours in seawater (SW, mean $\pm$ SE; Plasma [Na^+] and [Cl^-]: n=12 hours 0-34, n=10 at hour 46;[Ca^{++}]: n=9 hours 0-34, n=6 at hour 46; [PO_4^{3-}]: n=6 for hours 0-46.

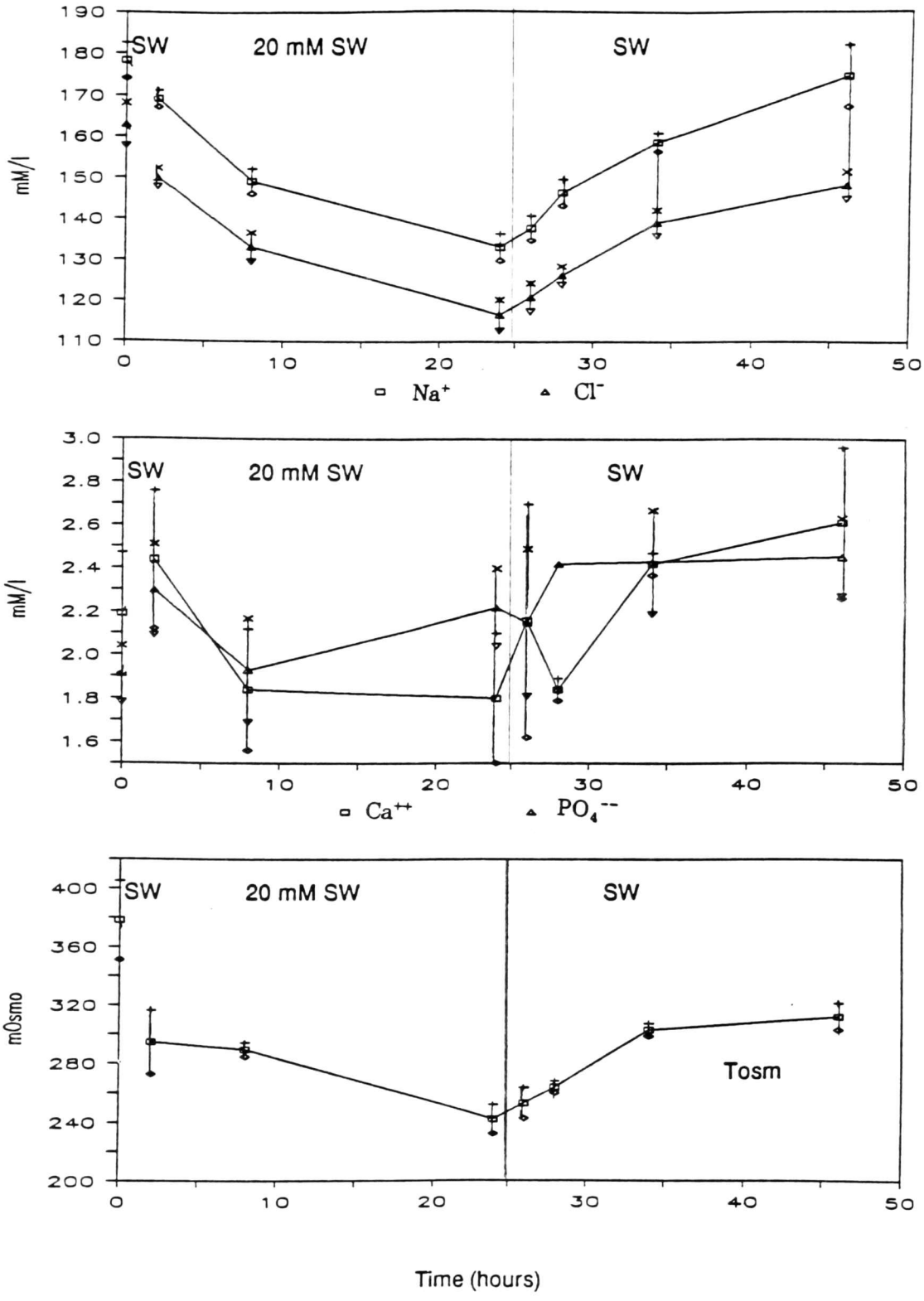

SW
20 mM SW
SW
mM/l
Na+
Cl-
Ca++
PO4--
mOsmo
Tosm
Time (hours)

Figure 10. Renal ΔH^+ excretion compared to total whole body ΔH^+ excretion by *Myoxocephalus octodecimspinosus* in seawater and dilute 20 mM seawater. Negative values indicate an acid uptake or ΔHCO_3^- loss (mean $\pm$ SE, n=5 in first two periods, SW and 20 mM; n=4 in second seawater period).

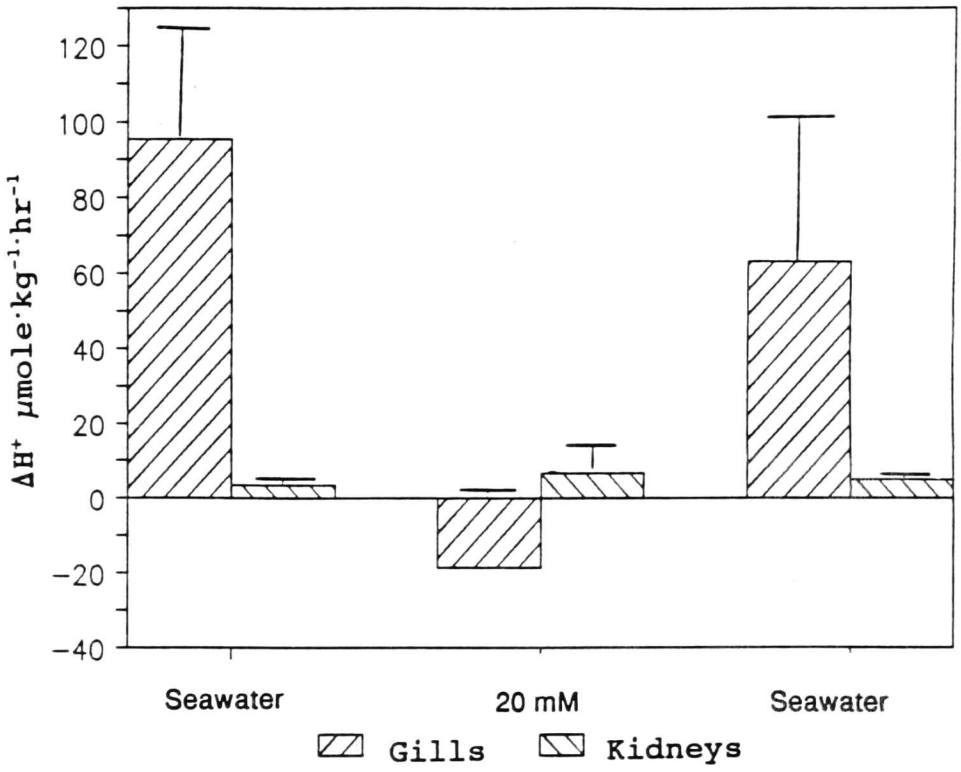

120
100
80
60
40
20
0
−20
−40
ΔH⁺ μmole·kg⁻¹·hr⁻¹
Seawater
20 mM
Seawater
Gills
Kidneys

Figure 11. Cumulative transfers of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ in *Myoxocephalus octodecimspinosus* exposed to 20 mM seawater: Control (~ 480 mM, mean $\pm$ SE, n=3) and experimental salinity (20 mM, mean $\pm$ SE, n=3 hours 10-46, n=2 at hours 56-80) were measured as water [Cl^-] in mM NaCl. Transfers measured at time zero (fish in 480 mM seawater), during a 48 hour exposure to a dilute salinity (20 mM), and finally an additional 24 hours in seawater (SW).

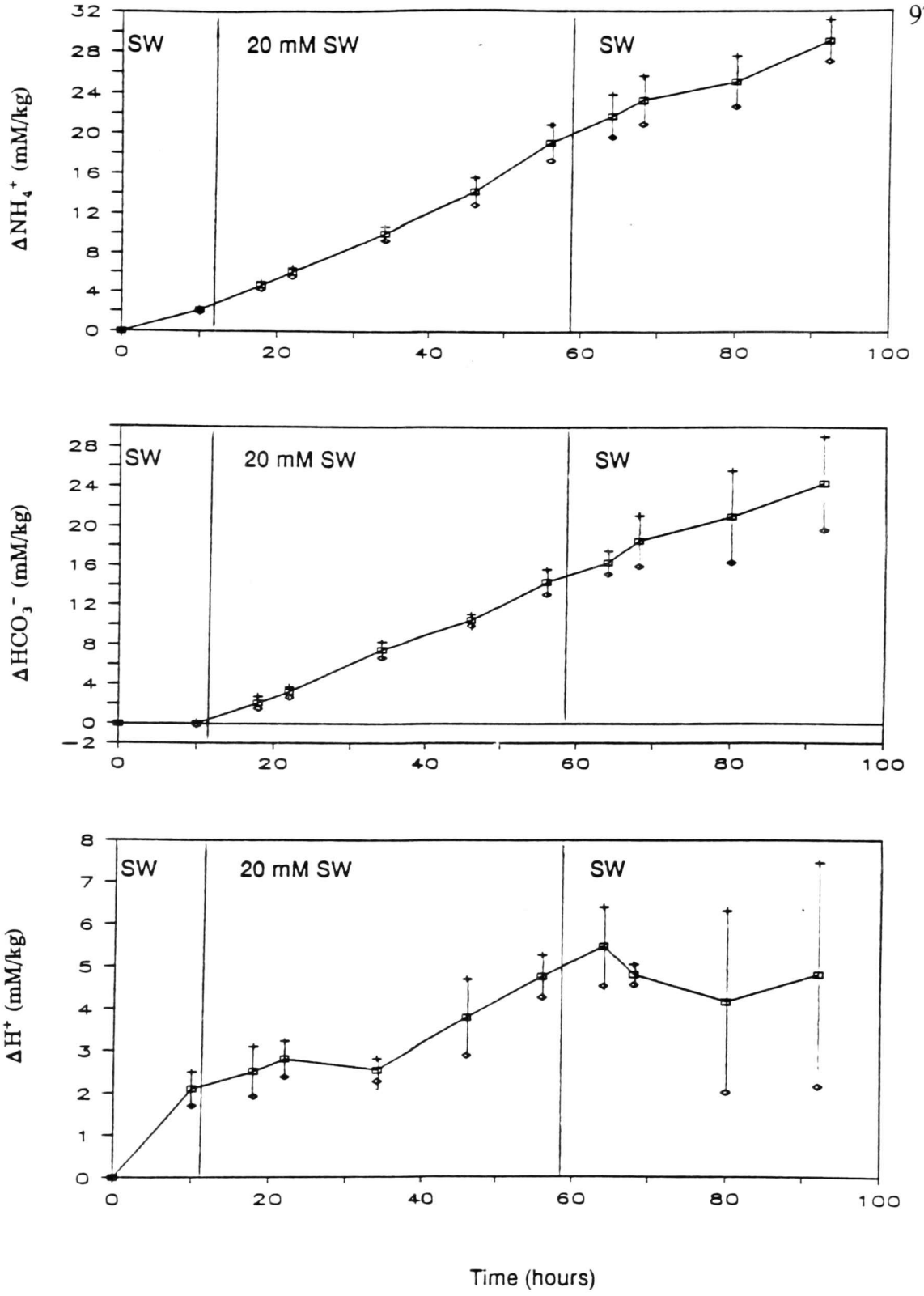

SW
20 mM SW
SW
ΔNH4+ (mM/kg)
ΔHCO3- (mM/kg)
ΔH+ (mM/kg)
Time (hours)

Figure 12. Cumulative transfers of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ in *Myoxocephalus octodecimspinosus* measured at time zero (mean $\pm$ SE, n=2, fish in 480 mM seawater), during a 24 hour exposure to a dilute salinity (20 mM, mean $\pm$ SE, n=2) before being injected with epinephrine, and after being injected with epinephrine. Control ($\sim$480 mM) and experimental salinity (20 mM) were measured as water [Cl^-] in mM NaCl.

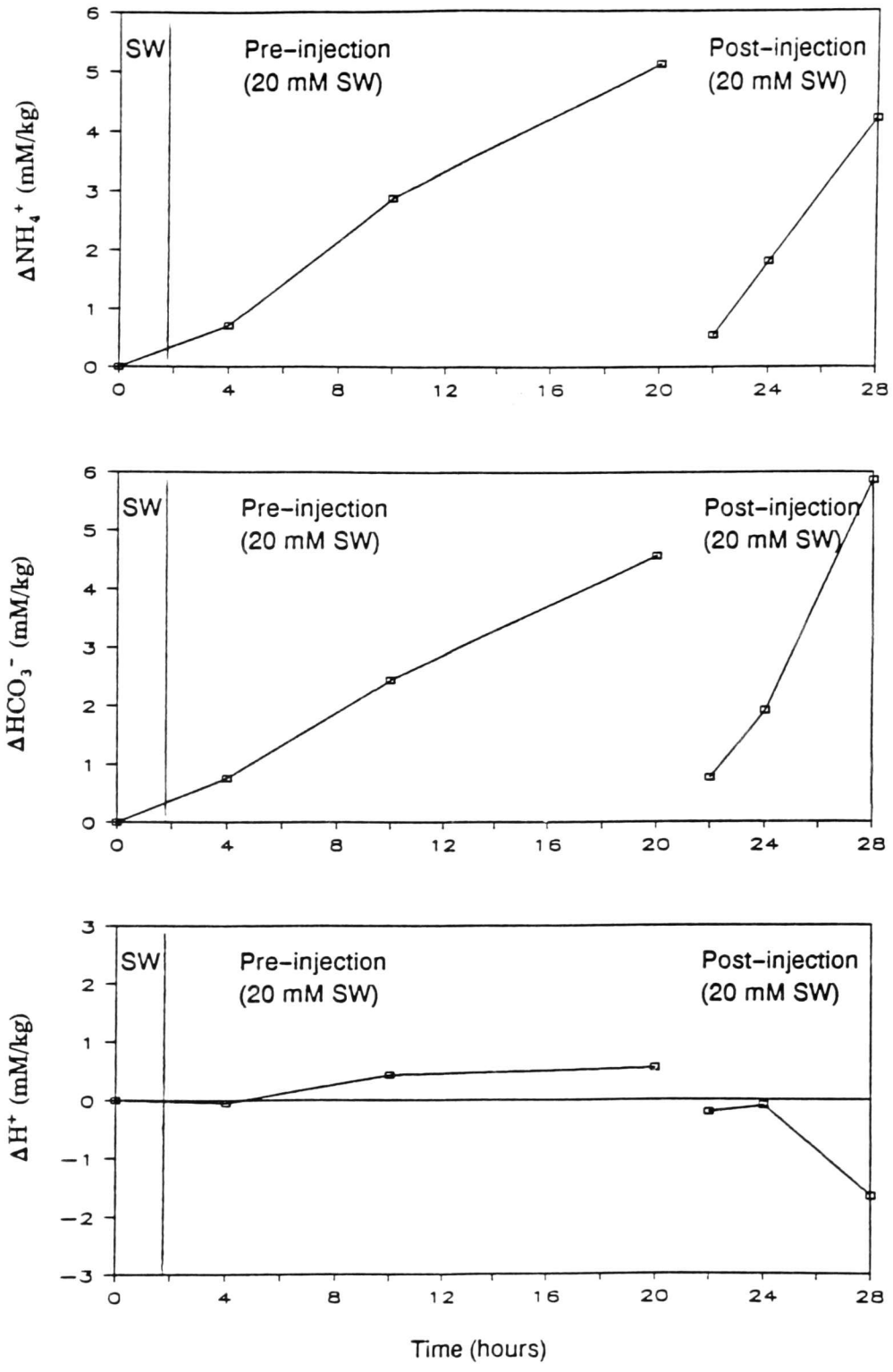

SW
Pre-injection
(20 mM SW)
Post-injection
(20 mM SW)
ΔNH4+ (mM/kg)
ΔHCO3- (mM/kg)
ΔH+ (mM/kg)
Time (hours)

Figure 13. Cumulative transfers of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ in *Myoxocephalus octodecimspinosus* exposed to 20mM seawater with control seawater osmolarity: Control (~480 mM) and experimental salinity (20 mM) were measured as water [Cl^-] in mM NaCl (mean $\pm$ SE, n=2).

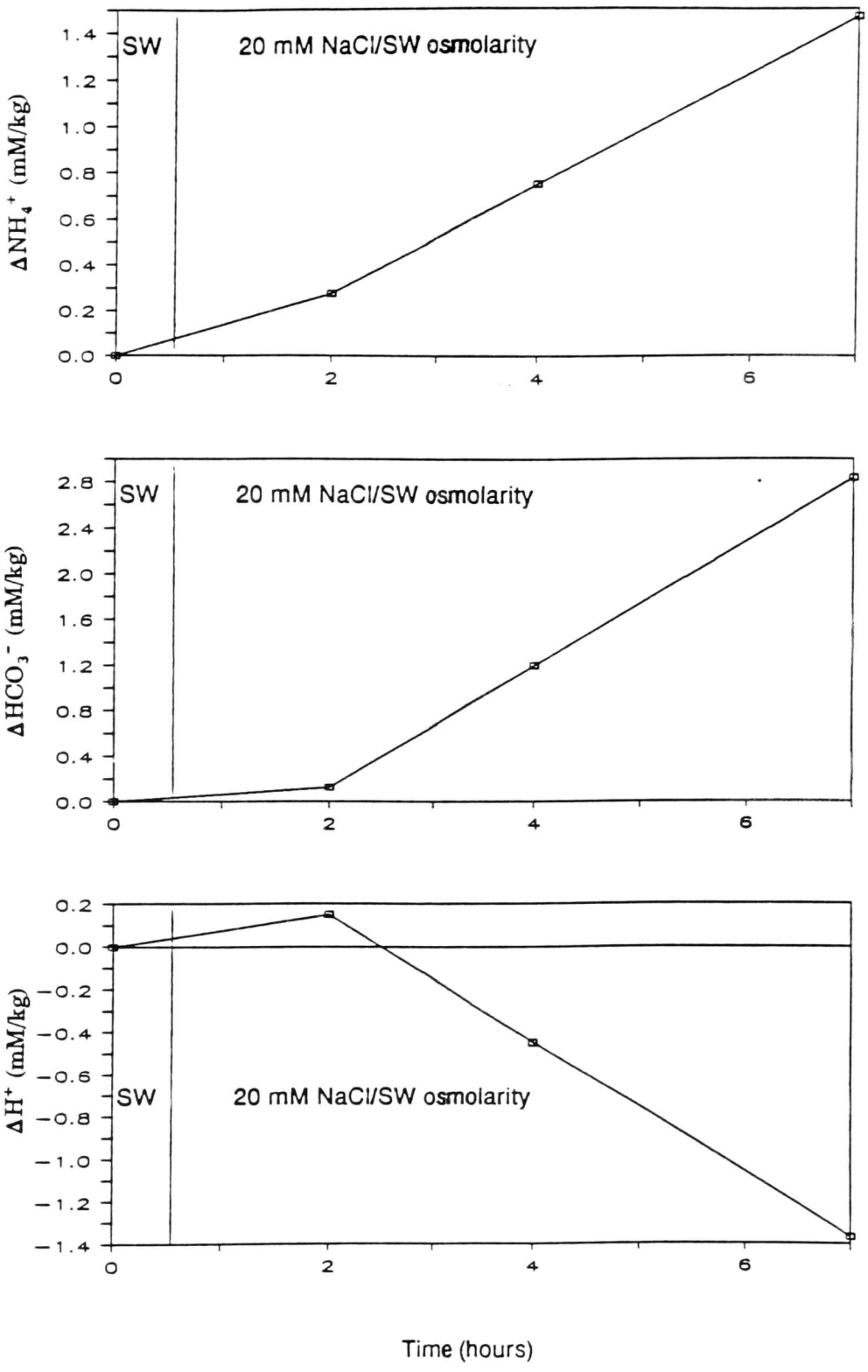

SW
20 mM NaCl/SW osmolarity
ΔNH4+ (mM/kg)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
SW
20 mM NaCl/SW osmolarity
ΔHCO3- (mM/kg)
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0
2
4
6
SW
20 mM NaCl/SW osmolarity
ΔH+ (mM/kg)
0.2
0.0
−0.2
−0.4
−0.6
−0.8
−1.0
−1.2
−1.4
0
2
4
6
Time (hours)

Figure 14. Cumulative transfers in *Myoxocephalus octodecimspinosus* exposed to 100 mM seawater: Control (~ 480 mM) and experimental salinity (100 mM) were measured as water [Cl^-] in mM NaCl (mean $\pm$ SE, n=5).

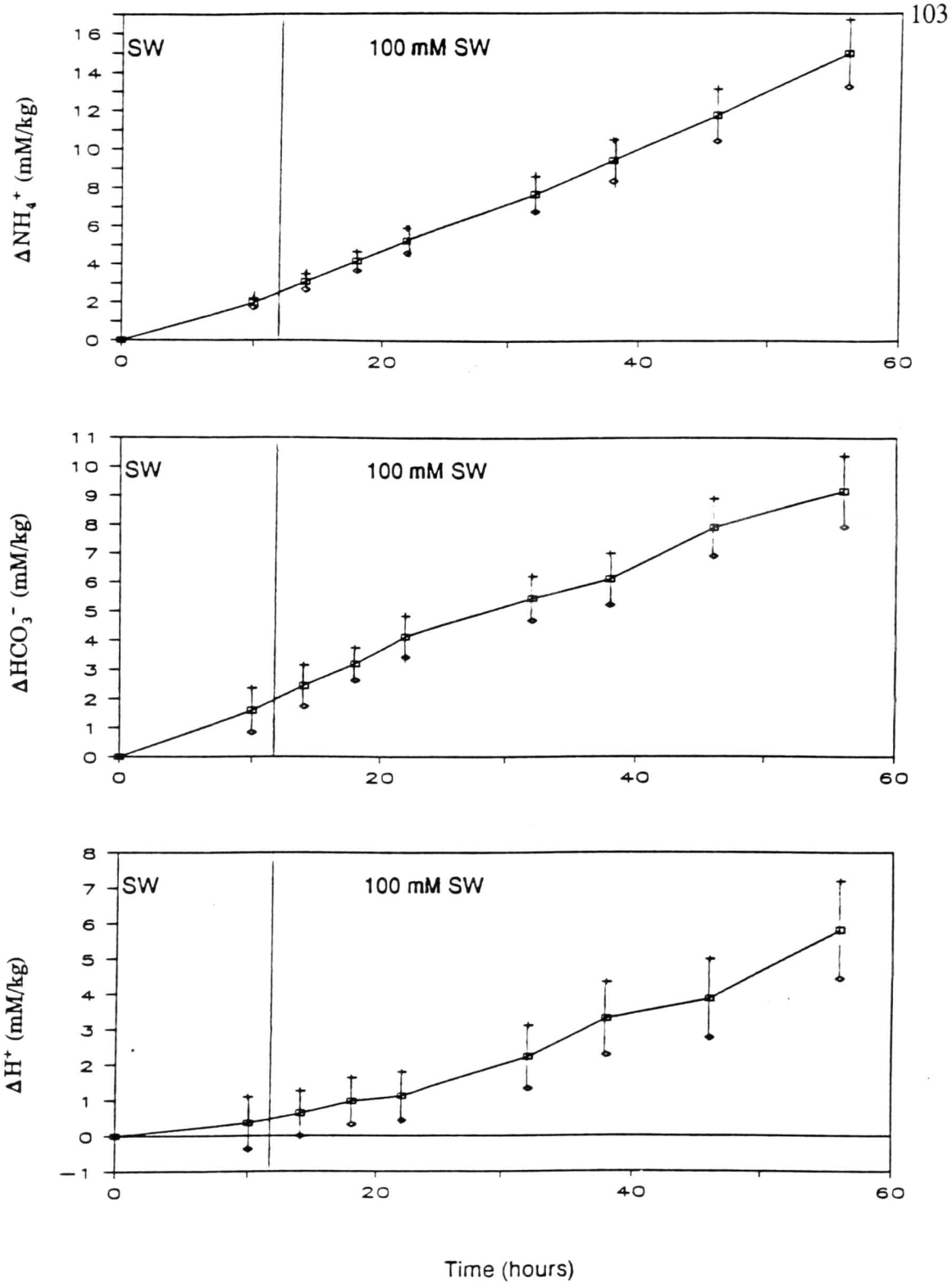

SW
100 mM SW
ΔNH4+ (mM/kg)
ΔHCO3- (mM/kg)
ΔH+ (mM/kg)
Time (hours)

Figure 15. Cumulative transfers of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ in *Myoxocephalus octodecimspinosus* exposed to 100 mM seawater for 11 days before being transferred to 20 mM diluted seawater: Control ($\sim$ 100 mM) and experimental salinity (20 mM) were measured as water [Cl^-] in mM NaCl (mean $\pm$ SE, n=5).

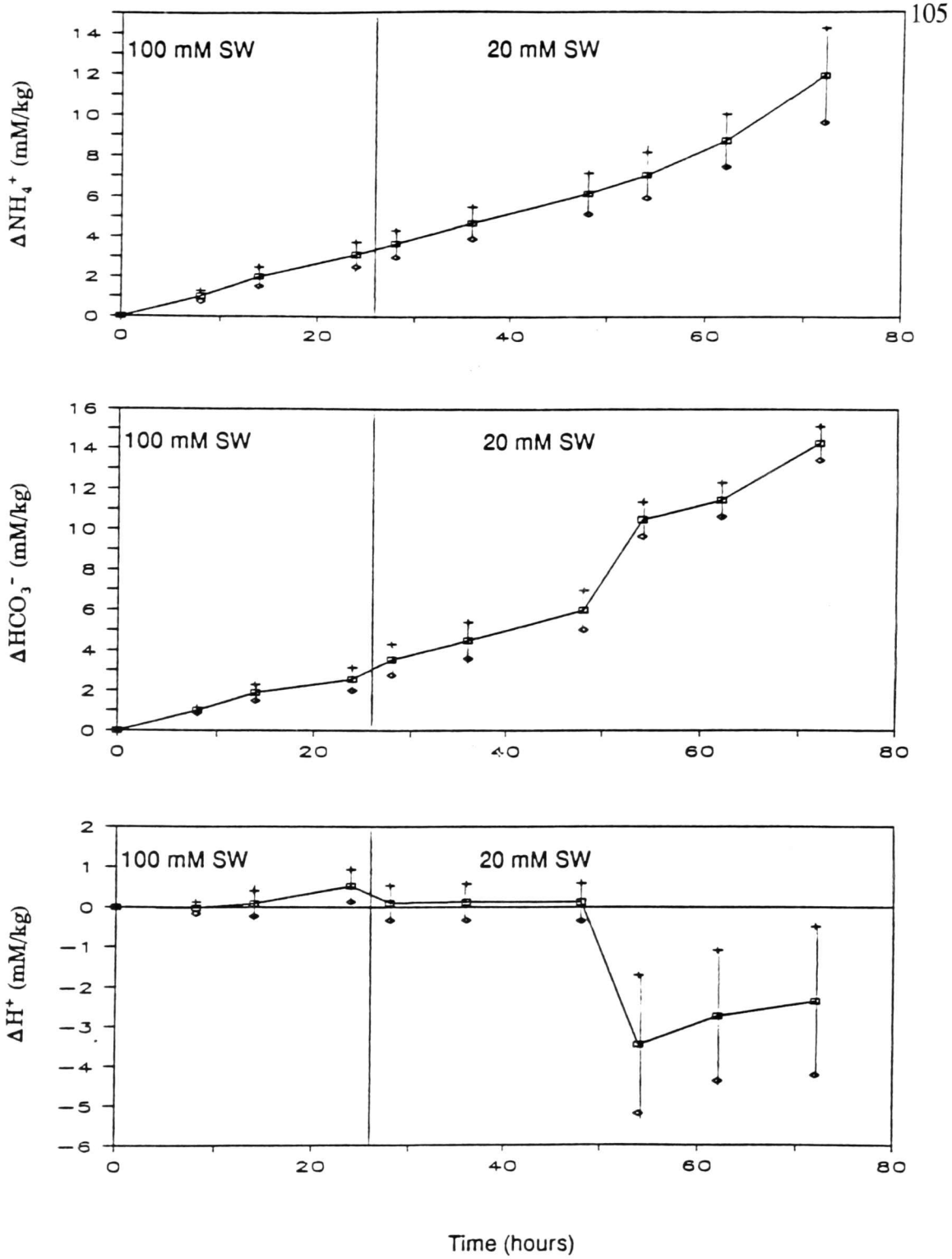
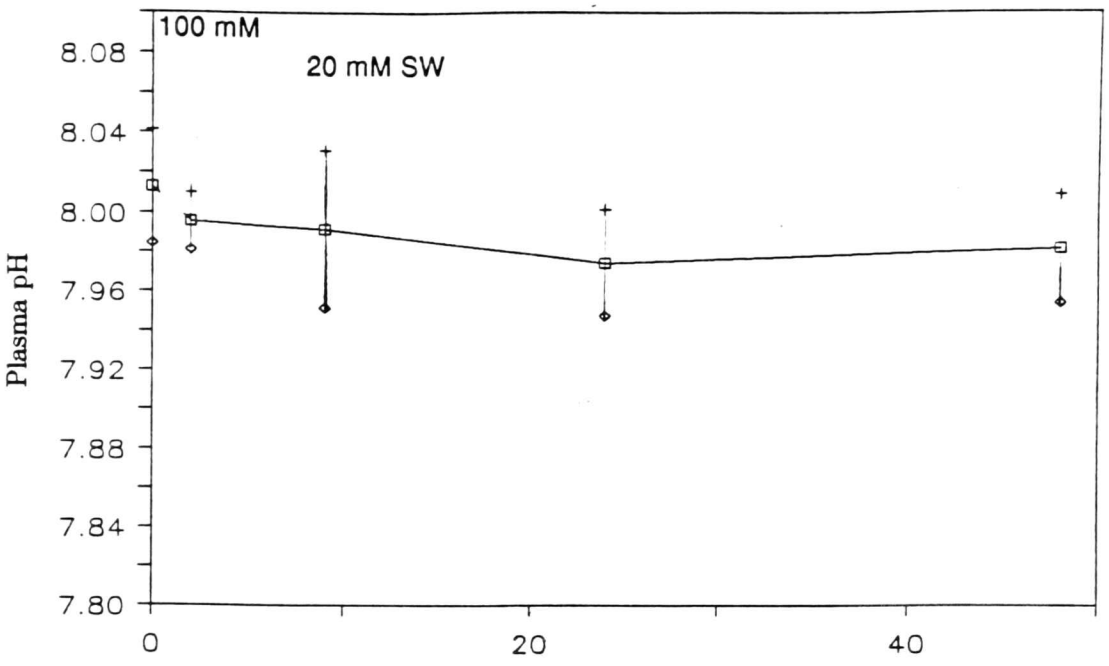

100 mM SW
20 mM SW
ΔNH4+ (mM/kg)
ΔHCO3- (mM/kg)
ΔH+ (mM/kg)
Time (hours)

Figure 16. Plasma pH, Pco_2 and $[HCO_3^-]$ measured in *Myoxocephalus octodecimspinosus*. Parameters measured at time zero (fish in 100 mM seawater for 11 days) and during a 48 hour exposure to a dilute salinity (20 mM, mean $\pm$ SE, n=6 at hour 0, n=4 for remaining time points for both parameters.


100 mM
20 mM SW
Plasma pH
8.08
8.04
8.00
7.96
7.92
7.88
7.84
7.80
0
20
40

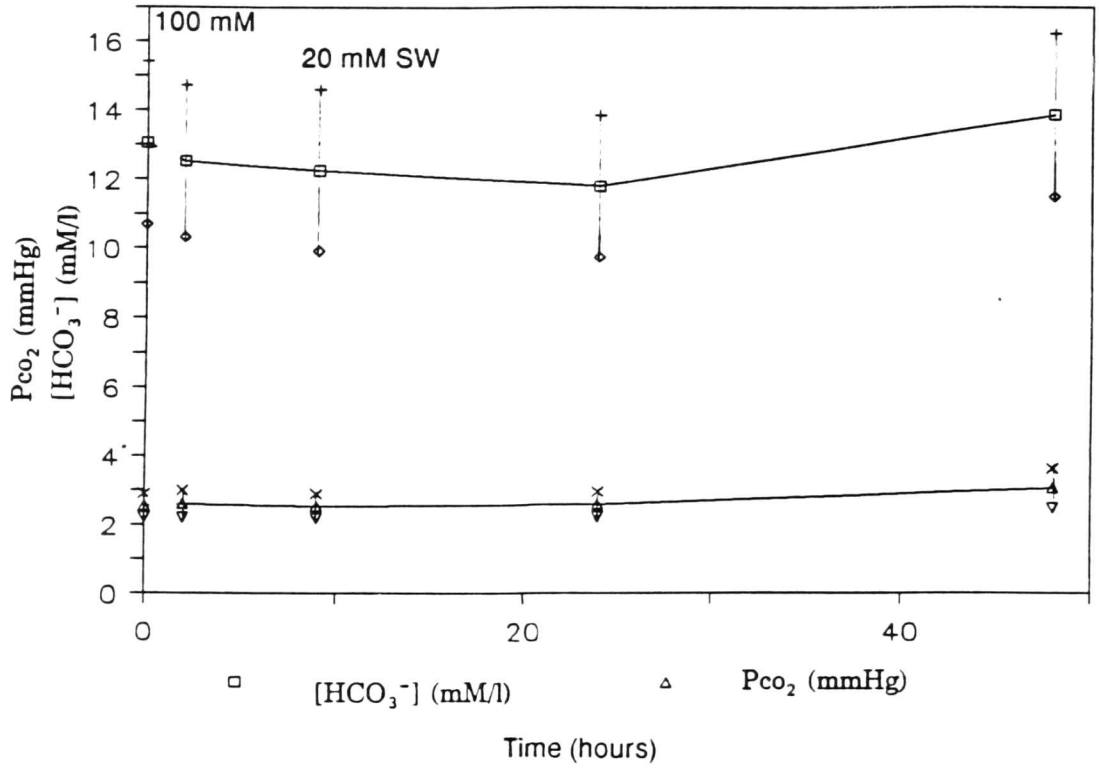

100 mM
20 mM SW
Pco2 (mmHg)
[HCO3-] (mM/l)
16
14
12
10
8
6
4
2
0
0
20
40
[HCO3-] (mM/l)
Pco2 (mmHg)
Time (hours)

Figure 17. Plasma [Na^+] and [Cl^-], measured in *Myoxocephalus octodecimspinosus*. Parameters measured at time zero (fish in 100 mM seawater for 11 days) and during a 48 hour exposure to a dilute salinity (20 mM, mean $\pm$ SE; [Na^+]: n=3; [Cl^-]: n=6 at hour 0, n=4 at hours 24-48).

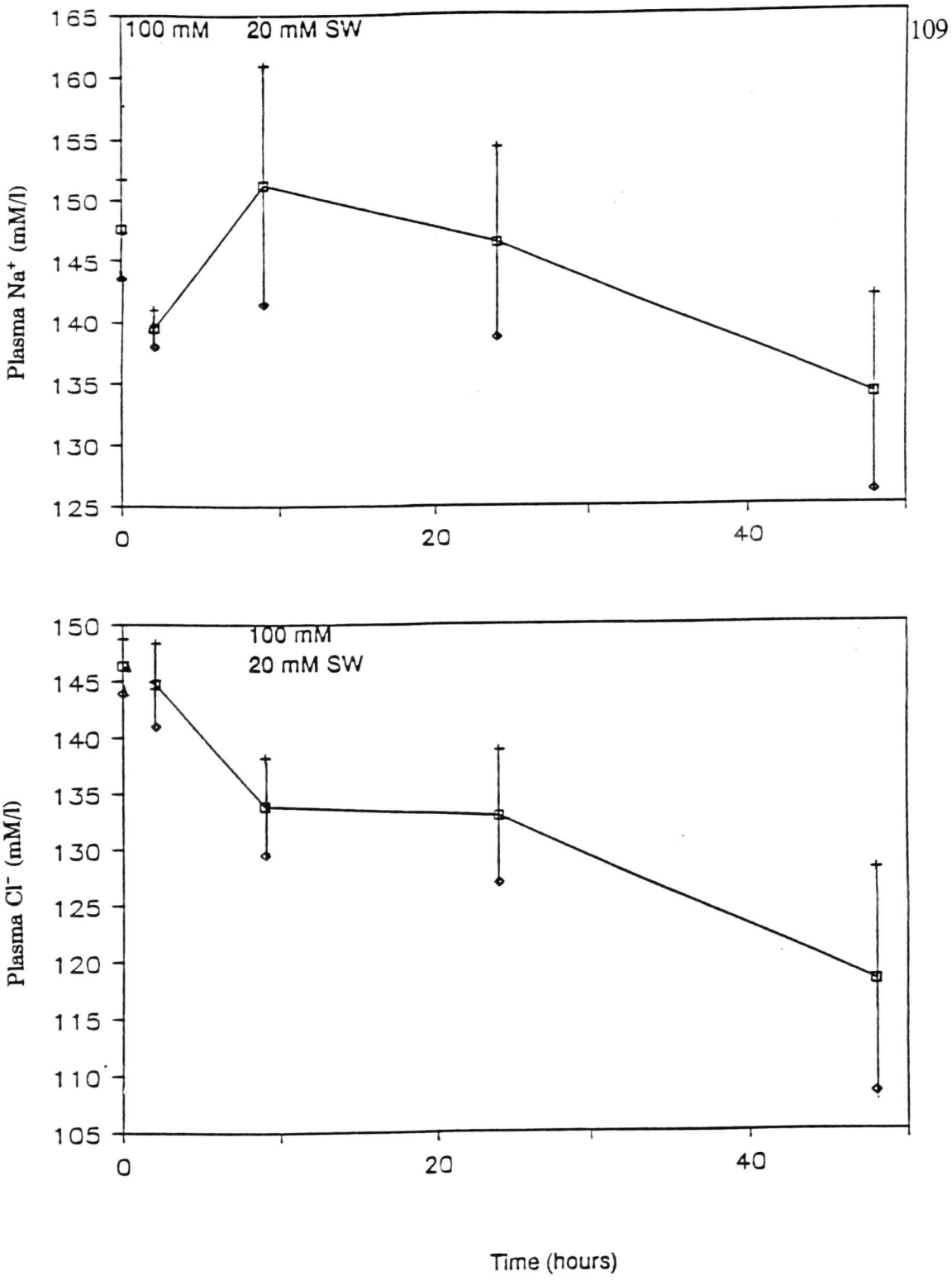

100 mM
20 mM SW
Plasma Na+ (mM/l)
165
160
155
150
145
140
135
130
125
0
20
40
100 mM
20 mM SW
Plasma Cl- (mM/l)
150
145
140
135
130
125
120
115
110
105
0
20
40
Time (hours)

Figure 18. Plasma [Ca^{++}] and [PO_4^{3-}], measured in *Myoxocephalus octodecimspinosus*. Parameters measured at time zero (fish in 100 mM seawater for 11 days) and during a 48 hour exposure to a dilute salinity (20 mM, mean $\pm$ SE; [Ca^{++}]: n=3; [PO_4^{3-}]: n=3 at hour 0, n=4 at hours 24-48).

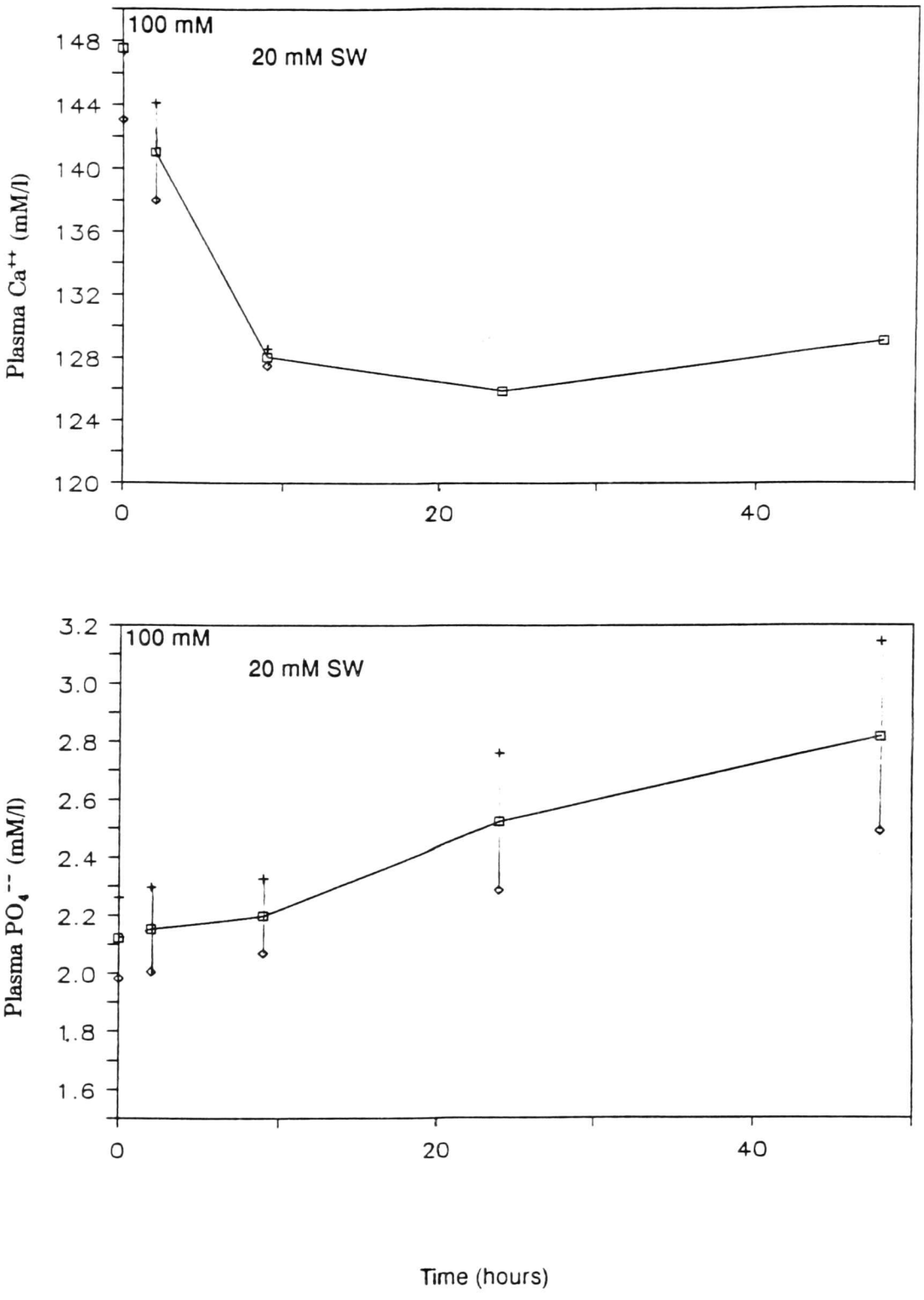

100 mM
20 mM SW
Plasma Ca^{++} (mM/l)
148
144
140
136
132
128
124
120
0
20
40
100 mM
20 mM SW
Plasma PO_4^{--} (mM/l)
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
0
20
40
Time (hours)

Figure 19. Cumulative transfers of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ in *Myoxocephalus octodecimspinosus* exposed to 100 mM seawater for 96 hours before being transferred to 20 mM diluted seawater: Control ($\sim$100 mM) and experimental salinity (20 mM) were measured as water [Cl^-] in mM NaCl (mean $\pm$ SE, n=4).

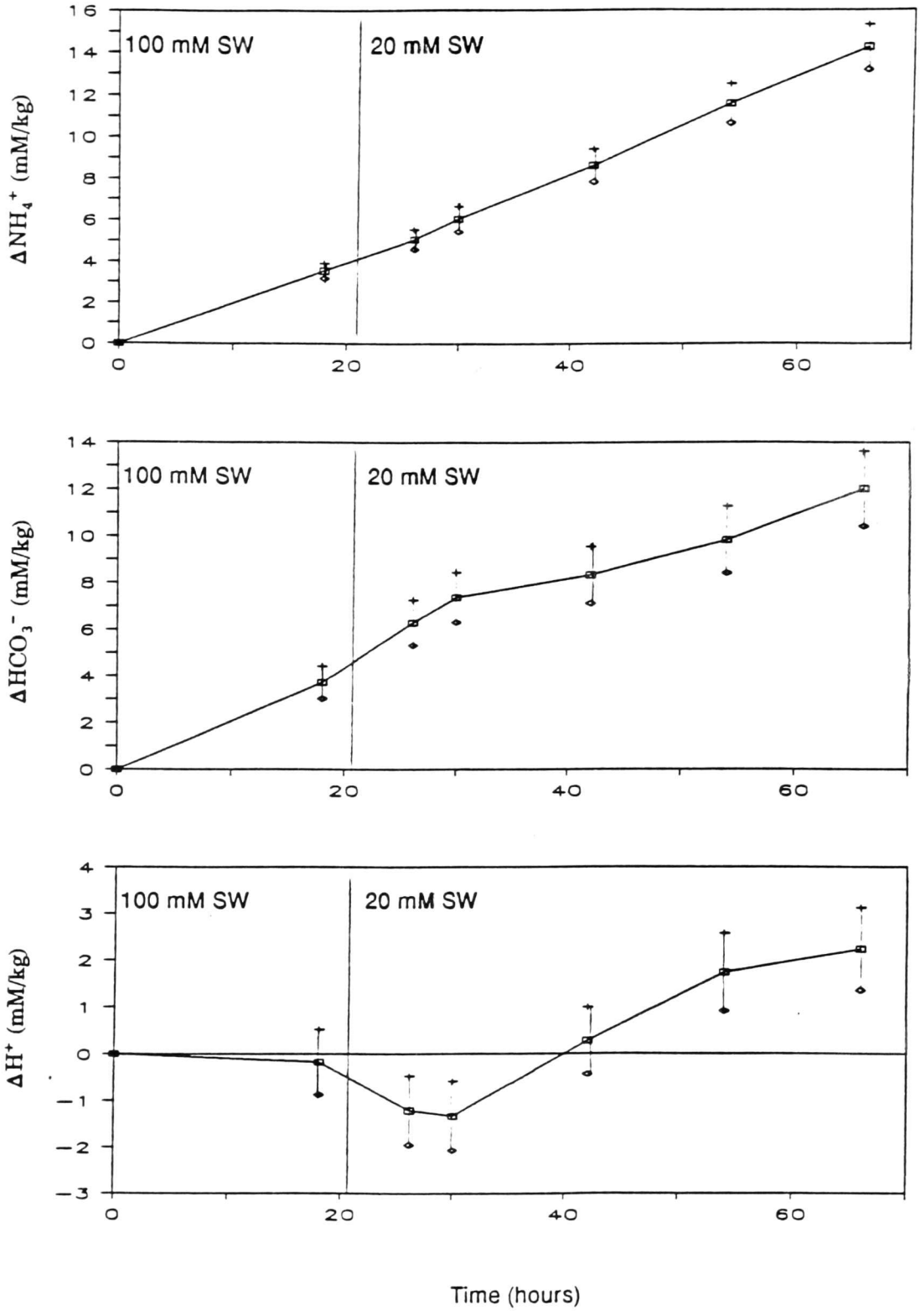
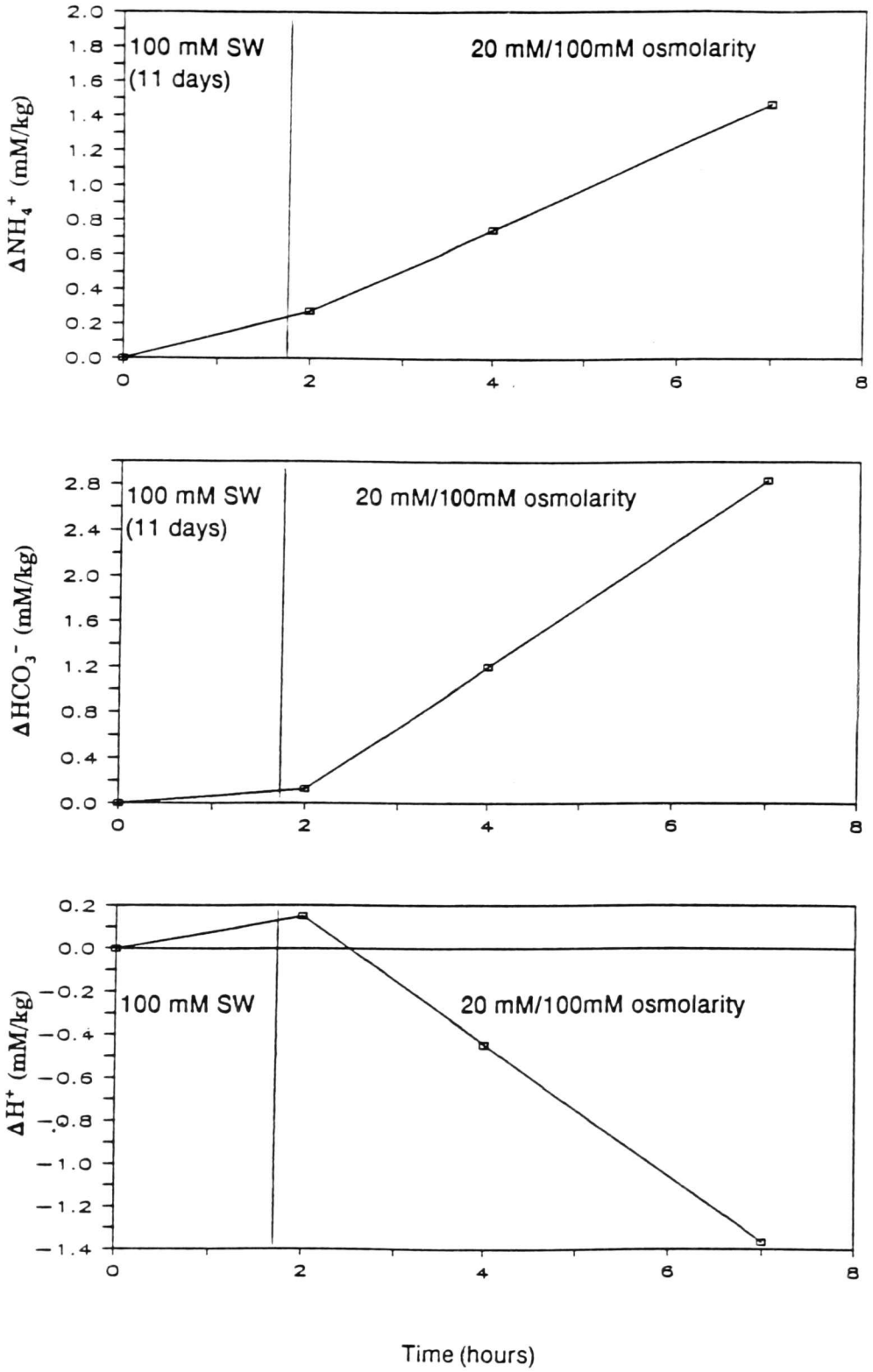

100 mM SW
20 mM SW
ΔNH4+ (mM/kg)
ΔHCO3- (mM/kg)
ΔH+ (mM/kg)
Time (hours)

Figure 20. Cumulative transfers of ΔNH_4^+, ΔHCO_3^-, and ΔH^+ in *Myoxocephalus octodecimspinosus* exposed to 100 mM diluted seawater for 11 days before being transferred to 20 mM diluted seawater with 100 mM osmolarity: Control ($\sim$100 mM, mean $\pm$ SE, n=5) and experimental salinity were measured as water $[Cl^-]$ in mM NaCl (mean $\pm$ SE, n=2 for remaining time points).


100 mM SW
(11 days)
20 mM/100mM osmolarity
ΔNH4+ (mM/kg)
100 mM SW
(11 days)
20 mM/100mM osmolarity
ΔHCO3- (mM/kg)
100 mM SW
20 mM/100mM osmolarity
ΔH+ (mM/kg)
Time (hours)