MOLECULAR IDENTIFICATION AND CLONING OF THE FIRST FULL-LENGTH FISH NHE2-LIKE SEQUENCE FROM THE GILLS OF THE LONG-HORNED SCULPIN, *MYOXOCEPHALUS OCTODECIMSPINOSUS*

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The regulation of internal pH and ion balance are equally important in the maintenance of physiological homeostasis. Acid-base balance in fishes is challenged by both internal and environmental factors (Claiborne, The Physiology of Fishes, 2nd Edition, ed. D. Evans, Boca Raton, CRC Press 177-198, 1998). The predominant site of acid-base regulation is the branchial epithelium. We have proposed that the sodium/hydrogen antiporter (NHE) is involved in ion regulation and acid excretion in marine species (Claiborne, et al., J. Exp. Biol. 202:315-324, 1999). NHE2 is thought to be an apically located, epithelial specific isoform in mammals (Malakooti et al., Am. J. Physiol. 277:G383-G390, 1999). In the present study our objective was to detect a homologous form of NHE2 mRNA in gill tissues of the sculpin (*Myoxocephalus octodecimspinosus*). Changes in the expression of this mRNA following acidosis were then measured *via* relative RT-PCR.

3'/5' Race (Clontech SMART™ RACE cDNA Amplification Kit) was used to isolate the complete sequence of the 5' end or the 3' end of the sculpin gill NHE sequence. The protocol was followed according to the directions provided by Clontech. Gene specific primers (GSP), NHE2 reverse primer for the 3' end and NHE2 forward primer for the 5' reactions were used. The GSP primers were designed against the conserved regions of the NHE2 partial sequence previously obtained (Claiborne et. al., *Op. Cit.*).

The full-length sculpin cDNA contains a 2277 nucleotide open reading frame encoding a protein of ~760 amino acids. Genbank BLAST comparisons of the sculpin gill NHE amino acid sequence with all known sequences revealed the highest homology to rat NHE2 (66%; Figure 1). In addition, the sculpin gill NHE shares several structural features with the NHE family. The primary structure of the sculpin NHE revealed a high degree of similarity in hydrophobic and hydrophilic regions, as well as predicted secondary structure. Relationship tree analysis (not shown) also indicates that the sculpin cDNA is phylogenetically most similar to the mammalian NHE2 isoform family.

Semi-quantitative PCR (Ambion's QuantumRNA Classic 18S Internal Standard kit) was used to determine the mRNA expression of the sculpin NHE2-like isoform under acidic conditions. Sculpin were infused with an HCl load (2 mM kg^{-1}; according to the methods of Claiborne et al., J. Exp. Zool. 279: 509-520, 1997) in order to study the time course of branchial mRNA expression. Fish were sacrificed at hour 0, 2, or 6 post-infusion and gill total RNA was isolated. In order to quantify the expression levels of NHE in the gill tissue, sculpin specific NHE2 primers were designed to produce a band of approximately 870 bp. These primers (NHE2F and SCULP2-R689) were used in combination with the 18S internal standard primers supplied with the kit. While preliminary (n=2 animals for each time period), our data may indicate an increase in transcription of NHE2 mRNA occurs in the gill tissue in response to acidic conditions (Figure 2). These data agree with our *in vivo* measurements of net H^+ transfers

in this species as acid efflux to the water increased within two hours of the infusion and peaked at hour 6 (Claiborne et al., *Op. Cit.*). The results imply that an increase in transcription and synthesis of *de novo* NHE2 may be partially responsible for the observed "whole animal" compensatory transfers observed following acidosis. This research was supported by NSF IBN 9808141 to J.B.C. and A.I.M.S.

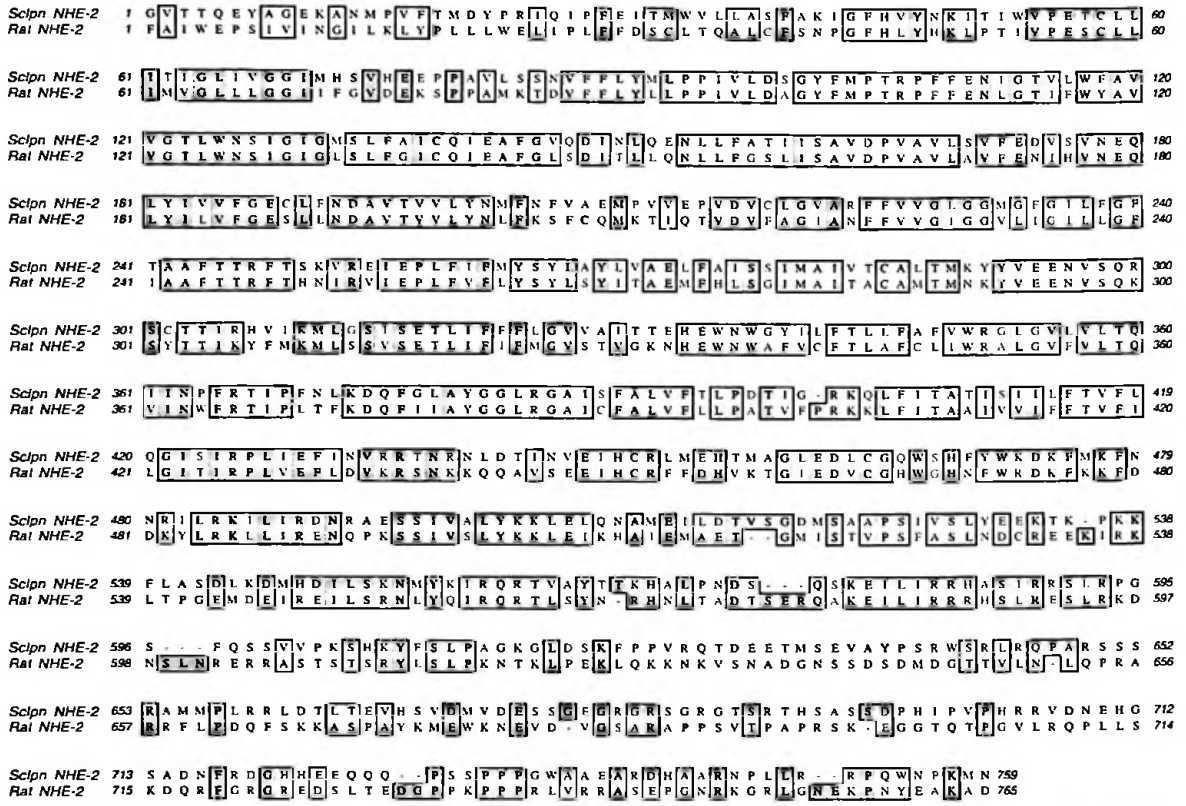

Figure 1. Amino acid homology of the sculpin sequence to rat NHE2. The rat sequence has been truncated to show only the portion which overlaps with the sculpin open reading frame. Shaded amino acid residues are identical, boxed are homologous. Overall homology is 66%.

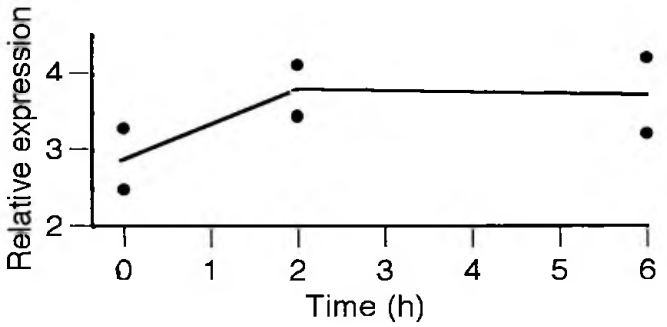

Figure 2. Relative levels of NHE2 mRNA for individual fish following acidosis (n=2 for each time period). Relative expression was determined as the ratio of the CPM for the NHE2 band and the internal standard (18S) band (n=6 for each fish). Line represents mean of each pair at each sample time.