TRANSCRIPTS HOMOLOGOUS TO Na/H ANTIPORTER ISOFORM, NHE-1, IN mRNA FROM THE LONG HORNED SCULPIN (MYOXOCEPHALUS OCTODECIMSPINOSUS) AND WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS)

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Marine fishes face the potentially competing demands of pH homeostasis and maintenance of electrolyte balance in a hypertonic environment. Net salt uptake is a continual challenge in a marine environment and many proposed regulators of pH facilitate the outward movement of acid/base equivalents in exchange for inward ion movement. Branchial ion fluxes are thought to be critical for both processes, but the protein mediators of these fluxes and their relative importance have not been well defined. Na/H exchange activity has been hypothesized to be an important component in the regulation of both branchial and renal ion fluxes and pH (Claiborne and Perry, Bull. MDIBL 31:54-56, 1992; Evans, in "Fish Physiology", eds. W.S. Hoar and D.J. Randall, Vol Xb, pp. 239-283, 1984). To determine if mRNA transcripts homologous to cloned Na/H exchangers could be detected in marine fish, we isolated poly A $^{(+)}$ mRNA and total RNA from flounder and sculpin tissues and used Northern blot analysis to probe for transcripts homologous to an NHE-1 isoform of the Na/H exchanger.

Total RNA was isolated from tissues by homogenization in guanidinium isothiocyanate and ultracentrifugation through a CsCl gradient according to Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Lab. Press, 1989) except that an additional 2.4 M CsCl cushion was added on top of the 5.7 M CsCl layer. Poly A $^{(+)}$ mRNA was isolated via the Invitrogen Fast Track mRNA isolation kit according to the manufacturer's protocol. Northern blot analysis was performed by electrophoresis of the RNA through a 2M formaldehyde agarose gel buffered with HEPES. RNA was then transferred to Nytran membranes and hybridized with a ^{32}P-labelled cDNA probe encoding the putative membrane spanning domain of the human NHE-1 Na/H exchanger (Sardet et al., Cell 56: 271, 1989). Hybridization was carried out in the presence of 50% formamide at 42°C and filters were washed under conditions of low to moderate stringency in 0.5 X SET (5mM Tris, 2.5 mM EDTA, 0.5% SDS, 0.1% Na-pyrophosphate) at 42°C. Filters were then exposed to x-ray film for times varying from 1 to 5 days. As a control for the amount of RNA loaded, the same filters were stripped of Na/H exchanger probe and hybridized with a ^{32}P-labelled cDNA probe encoding a portion of rat 28s ribosomal RNA. Filters were washed again in 0.5 X SET at 46°C and exposed to film overnight.

As shown in Figure 1, a band at approximately 7.5 kb could be detected in total RNA from flounder gill, small intestine, kidney, and heart, indicating hybridization to transcripts similar to the human NHE-1. Northern blots of flounder mRNA from gill, kidney, small intestine, and bladder yielded similar results. No hybridization signal was detected in flounder liver and skeletal muscle (Figure 1), even after a 5 day exposure. Hybridization with the 28s rRNA probe revealed approximately equivalent amounts of intact 28s rRNA in all lanes (not shown), indicating that the lack of hybridization signal in liver and skeletal muscle was not due to degradation of the RNA.

Figure 2 shows a Northern blot of total RNA isolated from sculpin organs. A transcript at approximately 8.0 kb is evident in sculpin gill, small intestine, kidney, RBC, and bladder, a pattern similar to that observed for flounder tissues. However, after a 5 day exposure, a faint signal could also be detected in sculpin liver but not in skeletal muscle. A possible second transcript at approximately 3.3 kb was observed in sculpin gill, small intestine, kidney, RBC, and bladder total RNA. The 3.3 kb transcript was also seen in mRNA isolated from the same tissues, suggesting that this signal was not due to nonspecific binding to ribosomal RNA. Smaller molecular weight transcripts have been reported for Northern blots of rat RNA and are thought to represent incomplete transcripts or alternatively spliced products (Orlowski, et al., J. Biol. Chem: 267 (13): 9331,1992). Hybridization of the blot shown in Figure 2 with the 28s rRNA probe revealed approximately equivalent amounts of RNA loaded in all lanes.

The results presented here suggest that transcripts similar to NHE-1 are expressed in several flounder and sculpin tissues. Unlike other NHE-1 isoforms, however, flounder and sculpin transcripts do not appear to be expressed in skeletal muscle. The apparent molecular weight of the expressed transcripts differs from NHE-1 transcripts expressed in mammalian tissues, 7.5 - 8.0 kb versus 4.9 - 5.2 kb, and may reflect species specific differences in the noncoding or coding regions of the mRNA.

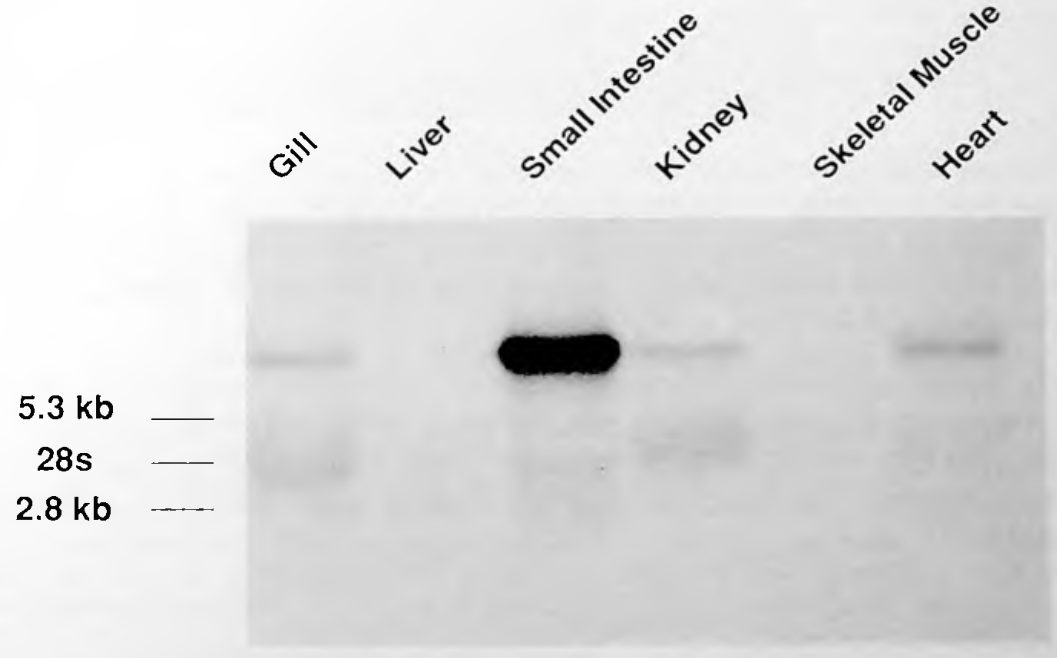

Figure 1: Northern blot of total RNA isolated from flounder tissues. 20 µg of each RNA sample was loaded per lane. Transcripts at approximately 7.5 kb are visible in RNA from gill, small intestine, kidney, and heart. Positions of molecular weight markers and 28s rRNA are marked (bars). A 24 hr exposure is shown.

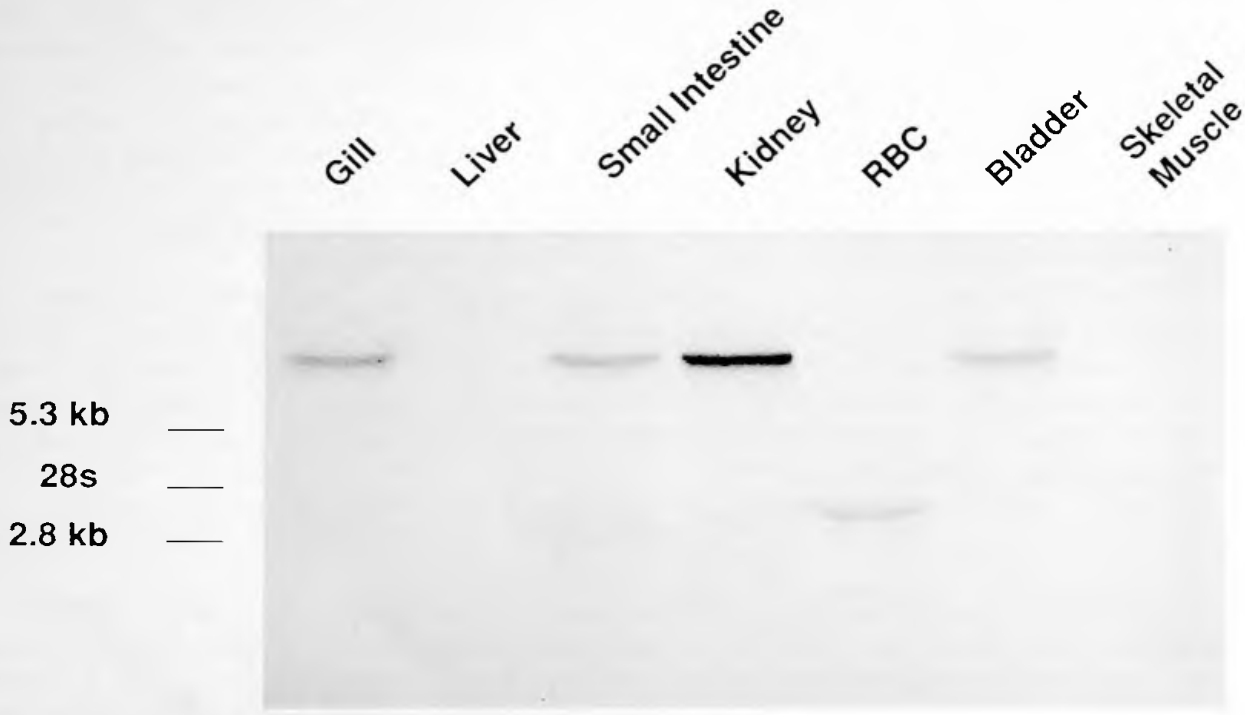

Figure 2: Northern blot of total RNA isolated from sculpin tissues. 20 µg of each RNA sample was loaded per lane. Transcripts at approximately 8.0 kb are apparent in gill, small intestine, kidney, RBC, and bladder lanes. Positions of molecular weight markers and 28s rRNA are marked (bars). A 24 hr exposure is shown.

This research was supported by NIH DK 29786 (DCD), NIH Training Grant 5T32GM08322 (SPH), NIH EHS-P30-ES03828 (Membrane Toxicity Studies), the American Heart Association Summer Research Fellowship (SPH), NSF DCM 86-02905 (JBC) and a Hearst Foundation Stipend (JBC).