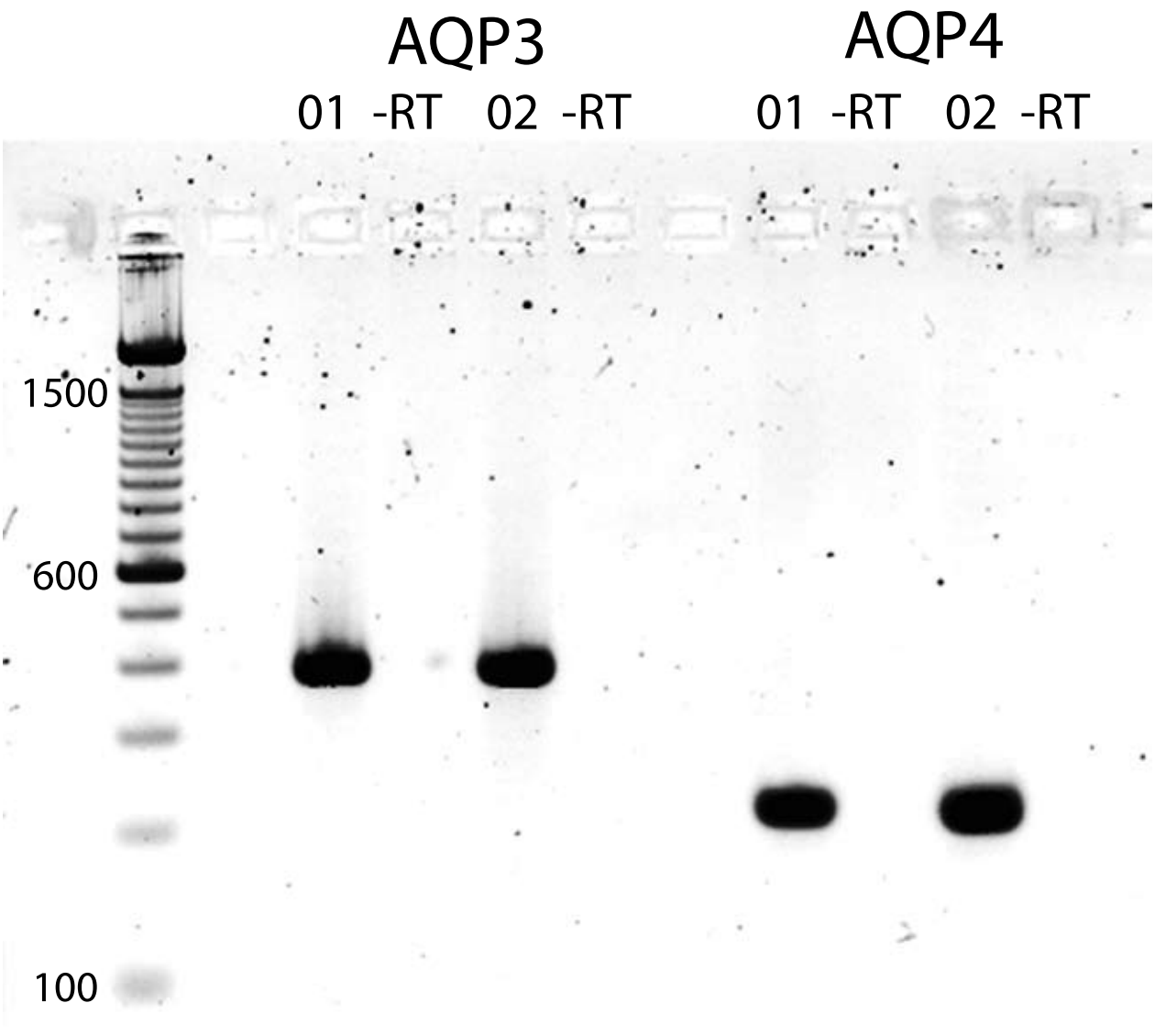

Fig. S1. Expression of EsAQP3 and EsAQP4 in hagfish slime gland tissue. Total RNA was extracted from the slime glands of two hagfish (01 and 02), and was reverse transcribed to cDNA. Target sequences 344 bp long for *EsAQP3* and 181 bp long for *EsAQP4* were amplified using reverse transcription (RT) PCR, and both homologs were found to be expressed in the slime gland. The products were run on a 1% agarose gel, and were sequenced to confirm their identity. First lane is a 100 bp DNA ladder, -RT lanes are non-reverse transcribed controls.

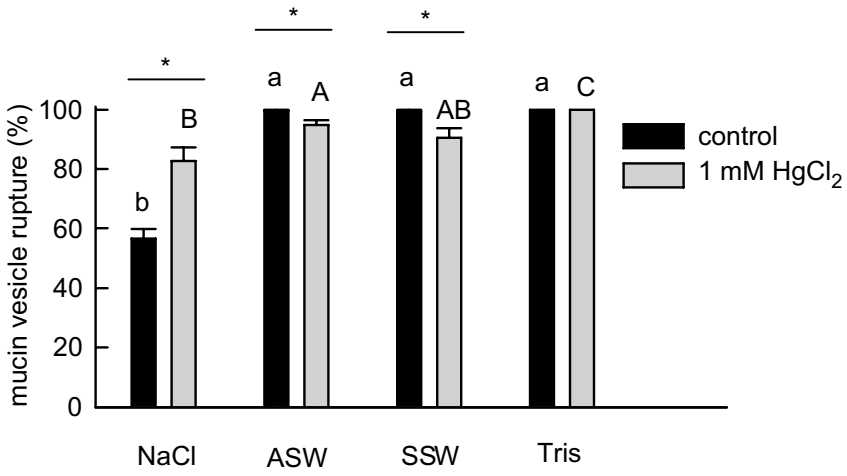

Fig. S2. The effect of $HgCl_2$ on mucin vesicle rupture. Vesicles treated with 1 mM $HgCl_2$ exhibited significantly higher percent rupture in 545 mM NaCl (Ca^{2+}-free) than in control NaCl, but in artificial seawater (ASW) and simplified seawater (SSW, 10 mM $CaCl_2$ + 535 mM NaCl), significantly fewer vesicles ruptured over the exposure period (2 min). $HgCl_2$ did not affect vesicle rupture in a very hypotonic solution (5 mM Tris). Error bars are s.e.m.. Asterisks (*) indicate significant differences between control and $HgCl_2$ treatments within each solution treatment. Lowercase letters indicate significant difference ($p < 0.05$) among control treatments, and uppercase letters indicate significant differences ($p < 0.05$) among solutions containing mercury. N = 6.

Movie 1. Hagfish slime mucin vesicles treated with 0.1% Triton X-100 prior to exposure to simplified seawater (SSW, 10 mM $CaCl_2$ + 535 mM NaCl). Videos were recorded at ~14 frames per second using a monochrome digital camera (Q-imaging Retiga Exi Fast1394) connected to a Nikon Eclipse 90i microscope (Nikon Instruments, Inc., Melville, NY). Videos were processed using NIS-Elements A.R. 3.0 software (Nikon Instruments, Inc.).

Movie 2. Hagfish slime mucin vesicles exposed to artificial seawater (ASW). Videos were recorded at ~14 frames per second using a monochrome digital camera (Q-imaging Retiga Exi Fast1394) connected to a Nikon Eclipse 90i microscope (Nikon Instruments, Inc., Melville, NY). Videos were processed using NIS-Elements A.R. 3.0 software (Nikon Instruments, Inc.).

Movie 3. Hagfish slime mucin vesicles treated with 1 mM $HgCl_2$ prior to exposure to artificial seawater (ASW). Videos were recorded at ~14 frames per second using a monochrome digital camera (Q-imaging Retiga Exi Fast1394) connected to a Nikon Eclipse 90i microscope (Nikon Instruments, Inc., Melville, NY). Videos were processed using NIS-Elements A.R. 3.0 software (Nikon Instruments, Inc.).

Table S1. Primers used to obtain full length coding sequence for *EsAQP3* and *EsAQP4*. Interspecies primers were developed using published AQP4 from *Eptatretus burgeri* (NCBI accession BAE93686.1).

Interspecies primers for *AQP4*	
5'-GTTCGCAAAGCCACTCC-3',	F1_E.stouti
5'-CCCTGTCTGAACAAATGAGC-3',	R1_E.burgeri
5'-TCTTCTGAGAAAGGACAGTCG-3',	F2_E.burgeri
5'-CCATTCTCACTCTGGATTTGC-3'.	R2_E.burgeri
AQP3 full length primers	
F: 5'-CTGAGTACCTGATACCTCCTGA-3'	
R: 5'-GATGATAGATGCAGGACGAAGG-3'	
AQP4 full length primers	
F: 5'-CATGCCGCAAATAAGCAGAC-3'	
R: 5'-TCT TCTCCCTGTATGAACAAATGA-3'	

Table S2. *EsAQP3* and *EsAQP4* primers used in the detection of expression of these genes in the hagfish slime gland via RT-PCR.

RT-PCR primers	
5'-GCGGGTCCTCGCACAGCGAATGCGC-3'	F_EsAQP3
5'-CCGCGGTCTCCATGCTCCAGGGT-3'	R_EsAQP3
5'-CGACCTACACATCGCACTTG-3'	F_EsAQP4
5'-ACGAGGTTGTGAAGGGTGAC-3'	R_EsAQP4