(which is predominantly K^+-dependent) or intracellular Cl^- activity in Ilyanassa eggs. In the presence of 15 mM procaine in sea water, both the membrane potential and Cl^- activity remain unchanged as the eggs form the first phase of polar lobe constriction and then remain inhibited for several hours. Examination of procaine-, and tetracaine-treated eggs by electron microscopy is in progress.

We conclude that, because the local anesthetics above display effective concentrations in proportion to their lipid solubilities, the drugs may be exerting their effects on constricting Ilyanassa eggs by interacting with their membranes. Moreover, the microelectrode data suggest that, however the drugs are acting, they are not causing major changes in the activities of the predominant intracellular cation and anion, K^+ and Cl^-, respectively. Supported in part by NIH HD07193.

THE EFFECTS OF BRACKISH WATER ON URINARY EXCRETION OF IONS IN THE MARINE SCULPINS MYOXOCEPHALUS SCORPIUS (L.) AND MYOXOCEPHALUS OCTODECIMSPINOSUS (MITCHILL)

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Many North Atlantic teleost fishes (e.g. M. scorpius) which are generally considered to be stenohlaline form resident populations in the brackish waters of the Baltic Sea (approximately 20% sea water). In this hyposomotic environment M. scorpius has a substantial urine flow (approximately 150 $\mu l.100g^{-1}.hr^{-1}$ vs approximately 50 $\mu l.100g^{-1}.hr^{-1}$ for the typical marine teleost; Evans, in Comparative Physiology of Osmoregulation in Animals, ed. by G.M.O. Maloiy, pp. 305-390, 1979), but also urinary Na and Cl concentrations nearly iso-ionic to the blood. In addition, the urine is nearly isosmotic to the blood (Oikari, Ann. Zool. Fennici 14, 162-172, 1977). One might propose that the substantial urine concentrations of NaCl were secondary to a reduced residence time in the urinary bladder since it has been shown that the bladder is the site of substantial reabsorption of both Na and Cl (Evans, op. cit.). However, it was found that the high urinary levels of Na and Cl in M. scorpius in 20% sea water were present whether the urine was collected via indwelling catheter or from bladders 12 hours after the urinary papilla had been ligated (Oikari, op. cit.). It therefore appeared that bladder residence time was not a variable controlling final urinary Na and Cl concentrations in M. scorpius in 20% sea water. The present study was initiated to determine the urinary constituents in M. scorpius collected from marine populations as well as those of M. octodecimspinosus in sea water and 25% sea water to determine if any species differences exist in the renal handling of Na and Cl in hyposmotic salinities.

Sculpins of mixed sexes were held in running sea water (945 mOs/kg) at ambient temperatures (13.5-16.5°C) for 5-10 days before experiments or transfer to reduced salinities. Specimens of M. scorpius weighed from 430 to 515 g and M. octodecimspinosus from 135 to 390 g. Catheterization was performed under MS 222 anesthesia (0.03%) using specially flared and perforated PE 50 tubing (Oikari, op. cit.). Two collection periods (each 10-12 hours long) were used: 10-25 hours after catheterization (termed Period I) or 2-3 days after catheterization (termed Period II). Bladder urine was collected either after stunning free-swimming fish (termed residual) or 10-12 hours after the urinary papilla had been ligated under MS 222 anesthesia.

Exposure of fish to reduced salinities (for 12 days altogether) was performed gradually, by the replacement of at least 50% of the water (total 45 liters) of the aquarium at least three times per day. The salinity reduction followed the sequence: 3 days in 50% sea water, 3 days in 30% sea water and 4 days in 25-28% sea water, followed by 25% sea water (240 mOS/kg) for the last 2 days. Fish were fed ad lib. with small pieces of fish flesh and no mortality was observed. Blood samples were collected from caudal vessels with heparized syringes and the plasma was immediately

separated by centrifugation and frozen. Thawed samples were analysed for osmolality (Wescor vapor pressure osmometer), for Na and K (flame photometry), Mg (atomic absorption) and Cl (amperometric titration).

Table 1 shows that both species are able to regulate their plasma Na, Cl, K and Mg concentrations in reduced

Table 1.--Effects of environmental salinity on plasma composition of sculpins

	A. 100% SW Atlantic	20% SW Baltic[c]	B. 100% SW Atlantic	25% SW Atlantic
Cl^-, mmol/l	166 ± 2 (3)	158 ± 15 (7)	167 ± 7 (3)	155 ± 5 (5)
Na^+, -"-	172 ± 4 (3)	166 ± 16 (7)	170 ± 4 (3)	164 ± 7 (5)
K^+, -"-	3.1 ± 0.3 (3)	2.8 ± 0.9 (7)	3.2 ± 0.4 (3)	3.9 ± 0.3 (5)
Mg^{2+}, -"-	1.3 ± 0.1 (3)	1.0 ± 0.1 (7)	1.3 ± 0.4 (3)	1.2 ± 0.2 (5)

[A] M. scorpius from the Atlantic Ocean (MDI, Maine) and from the Baltic Sea (Tvarminne, Finland).

[B] M. octodecimspinosus from MDI kept in normal and diluted sea water.

[C] Mean ± SD (Number of fish) from Oikari, Marine Biol. 44:347, 1978.

in salinity. In sea water (Table 2) M. scorpius is able to reabsorb both Na and Cl from and secrete substantial quantities of Mg into the urine while M. octodecimspinosus seemingly does not reabsorb Cl to any great extent, but reabsorbs nearly

Table 2.--Effects of environmental salinity on urine composition of sculpins (from fish with ligated papilla for 10–12 h)

	A. 100% SW Atlantic	20% SW Baltic[c] P	B. 100% SW Atlantic	25% SW Atlantic P
Osm , mOs/kg	326 ± 7 (3)	330 . (10)	334 ± 6 (3)	306 ± 4 (5)[d]
Cl^- , mmol/l	51 ± 68 (3)	164 ± 3 (4)[d]	160 ± 36 (3)	108 ± 26 (5)
Na^+ , -"-	23 ± 26 (3)	174 ± 7 (4)[e]	1.3 ± 2.3 (3)	155 ± 6 (5)[e]
K^+ , -"-	2.0 ± 1.1 (3)	3.0 ± 0.7 (4)	2.3 ± 0.9 (3)	2.0 ± 0.6 (5)
Mg^{2+}, -"-	118 ± 20 (3)	10 ± 4 (4)[e]	149 ± 23 (3)	17 ± 6 (5)[e]

[A] M. scorpius

[B] M. octodecimspinosus - For details, see Table 1.

[C] from Oikari, Ann. Zool. Fennici 14:166, 1977 and 15:56, 1978.

[D] $p < 0.01$

[E] $p < 0.001$, (Student's t-test).

all of the Na and secretes large quantities of Mg into the urine. These data are from ligated fish and it appears that at least in M. octodecimspinosus, residence time in the bladder is an important variable for Na reabsorption and final Mg concentration. Table 3 shows that catheter and residual urine both have substantially more Na and less Mg than the urine from ligated fish. This inverse relationship in Na vs Mg concentrations, here noted to be r = -0.90 for 23 observations in catheter and ligated urines, suggests that the two transport mechanisms were functionally linked (Natochin and Gusev, Comp. Biochem. Physiol. 37: 107-111, 1970), but other results (Beyenbach and Kirschner, Am. J. Physiol. 229:389-393, 1975), indicate that there is no linkage and that Mg concentrations changes are secondary to the water reabsorption from the bladder, which is linked to NaCl uptake.

Table 3.--Effects of different sample collection techniques on urine ionic concentrations (mmol/l) of M. octodecimspinosus in 100% sea water (mean ± SD; number of fish in parentheses)

	Na^+	Cl^-	Mg^{2+}
Catheter urine I:	65 ± 26 (8)	151 ± 26 (8)	76 ± 18 (7)
II:	41 ± 23 (9)	117 ± 38 (9)	87 ± 28 (9)
Bladder urine - residual	59 ± 36 (4)	131 ± 19 (4)	-----
- ligated	1.3 ± 2.3 (3)	160 ± 36 (3)	149 ± 23 (3)

Acclimation of M. octodecimspinosus to 25% sea water results in a substantial increase in urinary concentrations of Na, but a reduction in both Cl and Mg levels. This increase in Na excretion, and reduction in Mg excretion is the same as previously noted in M. scorpius (Table 2 and Oikari, op. cit.). While M. scorpius also increased urine Cl levels in 20% sea water, M. octodecimspinosus reduced it slightly (although not statistically significantly) in 25% sea water. The causes for the apparent discrepancy in Cl data are unknown but it is clear that both species are unable to reabsorb significant quantities of Na from the urine in these hyposmotic salinities. Since these fish were ligated (so that the urine was in contact with the urinary bladder epithelium for periods up to 12 hours) this natriuresis doesn't reflect reduced residence times in the bladder, secondary to the diuresis associated with acclimation to hyposmotic salinities. Therefore the causes for this "failure" in Na balance in 20-25% sea water are unknown, but it is possible to state that low environmental salinity is the primary factor causing natriuresis in marine sculpins. It is interesting to note that M. quadricornis (which is able to enter fresh water) is able to reabsorb both Na and Cl from the urine (and produce a distinctly hyposmotic urine) in 20% sea water (Oikari, op. cit.). Which morphological or physiological differences in kidneys of these three species account for the difference in reabsorptive ability remains to be seen.

Despite the inability to reabsorb significant quantities of Na or Cl from the bladder urine in reduced salinity, it is evident (Table 1) that M. octodecimspinosus does not face a significant (and unmanageable) loss of either ion. The few data on (at least) Na efflux from marine fish in brackish water indicate that it is approximately 15% of the efflux in sea water (Evans, op. cit.). The in vivo efflux of Na from M. octodecimspinosus is of the order of 1500 μmoles.$100g^{-1}$.hr^{-1} (Claiborne and Evans, this bulletin); 15% of this would be 225 μmoles.$100g^{-1}$.hr^{-1}. If M. octodecimspinosus has a urine flow in 25% sea water approximately equivalent to that described for M. scorpius in 20% sea water (150μl.$100g^{-1}$.hr^{-1}, Oikari, op. cit.), given the measured Na concentration (155 μmoles/l), the urinary Na efflux is of the order of

23 μmoles.$100g^{-1}$.hr^{-1}. This represents about 10% of the calculated Na efflux. Since diffusional Na efflux is still probably appreciable in 25% sea water, and also marine teleosts have been shown to have Na uptake mechanisms functioning for both NH_4 and H extrusion (Evans, Am. J. Physiol. 238, R224-R230, 1980; Evans, J. Exp. Biol. 70, 213-220, 1977), it seems unlikely that this urinary loss of Na (although fairly high in its rate) is a significant problem.

In summary, acclimation of two species of sculpin to hyposmotic salinities is followed by a cessation of (substantial) Na reabsorption from the bladder and the production of a nearly blood-isosmotic and-ionic urine, despite the need to excrete excess water and reduce salt loss. This "malfunction" is probably not due to experimental reduction of residence time of the urine in the urinary bladder. However it appears that it does not produce a substantial (and unbalanced) increase in at least Na loss. This research was supported by grants from the Herman Rosenberg Foundation (University of Helsinki) to A.O. and NSF (PCM80-08366) to DHE.

ACID AND AMMONIA EXCRETION BY SQUALUS ACANTHIAS AND MYXINE GLUTINOSA: EFFECT OF HYPERCAPNIA, ACID INJECTION AND NA-FREE SEA WATER

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In the past few years it has become apparent that marine teleost fish possess a branchial ammonia extrusion system which couples the efflux of NH_4 to the uptake of sea water Na (Evans, Am. J. Physiol. 238, R224-R230, 1980). Preliminary investigations indicated that the marine elasmobranch, Raja erinacea, did not possess this system but did extrude unwanted H in exchange for sea water Na (Evans et al., Bull. MDIBL 18, 64-65, 1978). Since evidence exists for both Na/NH_4 and Na/H ionic exchange in another elasmobranch, Scyliorhinus canicula, (Payan and Maetz, J. Exp. Biol., 58, 487-502, 1973; Bentley et al., J. Exp. Biol. 64, 629-637, 1976) it was appropriate to investigate the presence of these ionic exchange systems in Squalus acanthias. Since these systems are presumably relics of a fresh water ancestory (Evans, Comp. Biochem. Physiol. 51A, 491-495, 1975) it was of interest to test for their presence in the agnathan hagfish Myxine glutinosa, which is a modern representative of a group which has probably never entered the fresh water environment (Hardisty, The Biology of Cyclostomes, Chapman and Hall, 1979).

S. acanthias pups were removed from sacrificed pregnant females and M. glutinosa were kindly supplied by Dr. Bruce Sidell, University of Maine, Orono, (after collection near St. Andrews, N.B.). Both species were kept unfed in running sea water at 12 - 15°C. The rate of acid extrusion was determined by a modification of the technique of Heisler et al. (Bull. Europ. Physiopath. Resp. 12, 77-85, 1978), where 5 ml samples of an efflux bath (200 mls) are equilibrated with 1% CO_2 (in oxygen) while the pH is recorded on a Radiometer Titragraph Recorder (attached to a Radiometer Model PHM 62 pH meter) to the nearest 0.0025 pH units. Since the P_{CO_2} is maintained constant during the analysis, according to the Henderson-Hasselbalch equation the pH is directly proportional to H ion concentration. During the course of the experiment the change in the experimental bath pH is compared to that of a control. The flux (u moles.$100g^{-1}$.hr^{-1}) for a given experimental time period is then calculated by converting the change in pH differential between the control and experimental baths into a H ion concentration and correcting for the buffer capacity of the solutions and the weight of the fish. The buffer capacity for the control and experimental baths are determined directly by titrating 5 ml samples of the solutions before and after the experiment with either HCl or NaOH solutions. In some cases, samples of the experimental bath were removed, frozen, shipped to Miami and analyzed for ammonia via the method of Solorzano (Limn. & Oceanog. 14, 799-801, 1969). Calculations of fluxes were performed on a Univac 1100 computer using programs written by J.B. Claiborne. In most experiments the fish were made hypercapnic by bubbling 5% CO_2 in air (produced with a Wostoff Gas Mixing pump) into the experimental baths. In other experiments, nor-