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EFFECTS OF SALINITY ON CHLORIDE CELLS AND Na^+, K^+–ATPase ACTIVITY IN THE TELEOST *GILLICHTHYS MIRABILIS*

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Abstract—1. Longjawed mudsuckers, *Gillichthys mirabilis*, in 30 ppt seawater (SW) were transferred to 1.5, 30 and 60 ppt SW.

2. In the first 1–3 days after transfer, plasma chloride level and plasma osmolarity rose in the 60 ppt SW fish, and decreased in the 1.5 ppt SW fish.

3. By day 21, however, plasma chloride and osmolarity were at or near the levels seen in the controls (30 ppt).

4. Branchial and jawskin Na^+, K^+–ATPase activities were high in all salinities, and did not differ significantly among treatments.

5. The vital fluorescent stains DASPEI and anthroylouabain were used to detect mitochondria and Na^+, K^+–ATPase, respectively, in chloride cells.

6. Both stains indicated that jawskin chloride cell density did not differ among treatment groups.

7. In contrast, chloride cell size increased significantly with increasing salinity.

8. The chloride cells of fish in 60 ppt SW were noticeably angular in outline, whereas those of both the 1.5 and 30 ppt SW fish were circular.

9. The results are discussed in relation to the ion transport requirements encountered in the intertidal habitat of the mudsucker.

INTRODUCTION

The chloride cell was conclusively shown to be the cell type responsible for branchial salt secretion in seawater-adapted fish (Foskett and Scheffey, 1982). In addition to the gills, the jaw epithelium of the longjawed mudsucker *Gillichthys mirabilis* contains a relatively high density of chloride cells (Marshall and Nishioka, 1980), which appear to contribute to ion regulation in this species.

Chloride cell density and Na^+, K^+–ATPase activity are strongly correlated with chloride ion transport in teleost gills, opercular membranes, and jawskin (see Payan and Girard, 1984; Zadunaisky, 1984; Karnaky, 1986). Na^+, K^+–ATPase is located on the extensive tubular system of chloride cells, and is indirectly responsible for chloride secretion by these cells (Silva *et al.*, 1977; Epstein *et al.*, 1980). In most euryhaline teleosts, Na^+, K^+–ATPase activity is greater in seawater (SW)-adapted than in freshwater (FW)-adapted fish (Jampol and Epstein, 1970; Zaugg and MacLain, 1971; Utida *et al.*, 1971; Sargent and Thomson, 1974; Towle *et al.*, 1977; Folmar and Dickhoff, 1979; Epstein *et al.*, 1980). In *Dicentrarchus labrax* and *Crenimugil labrosus*, however, the opposite was observed, with Na^+, K^+–ATPase activity higher in FW than in SW (Lasserre, 1971). Moreover, the enzyme activity in some species, including *Platichthys flesus* and *Anguilla anguilla*, did not change upon transfer from FW to SW (Kirschner, 1969; Stagg and Shuttleworth, 1982). Interestingly, enzyme activity in the longjawed mudsucker increased after adaptation to either FW or 170% SW, but remained lowest in SW-adapted fish (Doneen, 1981).

Although osmoregulatory studies have been conducted on euryhaline teleosts, most of these have focused on species of freshwater origin, such as killifish (*Fundulus heteroclitus*), tilapia (*Oreochromis mossambicus*), rainbow trout (*Oncorhynchus mykiss*) and anadromous salmonids (e.g., *Oncorhynchus kisutch*, *Salmo salar*). Only a few marine species, such as the flounder (*Platichthys flesus*), sea bass (*Dicentrarchus labrax*), and thick-lipped mullet (*Crenimugil labrosus*) have been examined. This study investigates the effects of varying salinity upon plasma osmolarity and chloride concentration, size, density and morphology of chloride cells, and Na^+, K^+–ATPase activity of jawskin and branchial tissues in an inter-

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tidal goby, *Gillichthys mirabilis*, which is highly euryhaline.

MATERIALS AND METHODS

Animals and sampling

Adult longjawed mudsuckers (10–25 g) of both sexes were obtained from hypersaline ponds in San Francisco Bay, kept in the laboratory in 40 l tanks with recirculating artificial SW (Instant Ocean, 30 ppt) at $12 \pm 1°C$ and charcoal filtration. The photoperiod was 12L:12D and fish were fed brine shrimp on alternate days. Fish were randomly separated into three tanks, and acclimated to 100% SW (30 ppt) for at least 2 weeks. The water in each tank was then removed and replaced with fresh 1.5, 30, or 60 ppt water. Fifty percent of the water was changed every other day. Groups were sampled six times: 0, 8 hr; 1, 3, 7, and 21 days after exposure to new salinity. The animals were anesthetized in 0.3 g MS-222/l water. Fish were bled from the caudal blood vessels into heparinized syringes. Blood was immediately centrifuged, and plasma was removed and frozen at −80°C.

All eight gill arches from each animal were removed and the gill filaments were dissected from the ceratobranchials. Jawskin was collected in the manner of Marshall (1981): epithelium was removed from the triangular region enclosed by the jaw symphysis, the tip of the elongated premaxillae and the side of the head. For measurement of Na^+, K^+–ATPase activity, gill filaments and jawskin were placed in SEI buffer (0.3 M sucrose, 0.02 M Na_2 EDTA, and 0.05 M imidazole, pH 7.3), frozen immediately on dry ice, and maintained at −80°C until assayed.

Plasma analysis

Osmolarity was measured in duplicate 10 μl plasma samples from each fish using a Wescor 5100C Vapor Pressure Osmometer. Chloride concentration was measured in 5 μl plasma samples in a Buchler–Cotlove chloridometer.

Gill and jawskin Na^+, K^+*–ATPase activity*

Na^+, K^+–ATPase activity was measured by the method of McCormick and Bern (1989). Five to 10 min before assay, the tissue was rapidly thawed, removed from SEI buffer, and homogenized in 85 μl SEI buffer with 0.1% Na deoxycholate in a 200 μl capacity ground glass homogenizer (Wheaton no. 357848). The homogenate was centrifuged at 5000 ***g*** for 30 sec to remove cartilage and insoluble tissue. The supernatant was removed and assayed for Na^+, K^+–ATPase activity and protein content.

Ten μl of tissue homogenate were placed in a final volume of 1 ml assay mixture containing 50 mM imidazole, 1 U/ml lactate dehydrogenase (LDH), 2.5 U/ml pyruvate kinase (PK), 2 mM phosphoenolpyruvate (PEP), 0.05 mM NADH, 0.5 mM ATP, 0.4 mM KCN, 60 mM NaCl, 2.5 mM $MgCl_2$, and 2 mM KCl (pH 7.5). A duplicate cuvette run simultaneously contained 0.5 mM ouabain. The assay mixture was mixed, and the change in absorbance (at 340 nm) was recorded with a Perkin–Elmer recording spectrophotometer. The assay mixture was maintained at 25°C before assay, and the cuvettes were kept at this temperature during the assay in a water-jacketed cuvette holder. ADP production was calculated from the linear rate between 3 and 10 min. Protein content of the homogenate was measured by the method of Larson *et al.* (1986) with the use of bovine serum albumin as standard. Na^+, K^+–ATPase was calculated as the difference in ouabain-sensitive and -insensitive ATP hydrolysis and expressed as μmol ADP/mg protein/hr. The ATP hydrolysis not inhibited by ouabain is reported as residual ATPase.

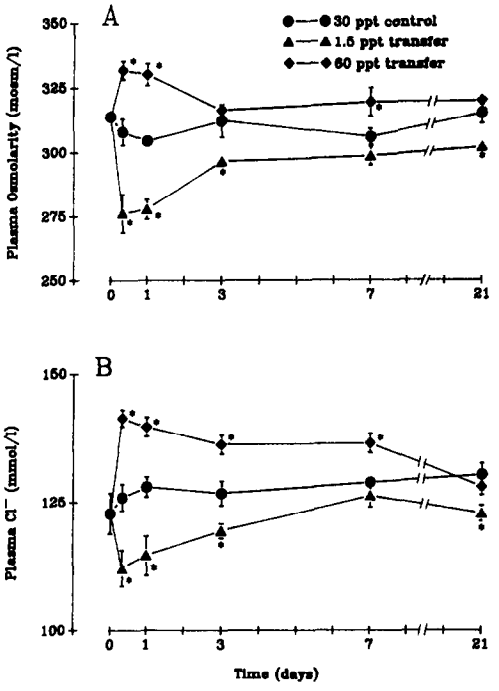
Fluorescent staining of chloride cells

Animals were killed by decapitation and pithing, and jawskin was collected as described above. Dimethylaminostyrylethylpyridiniumiodine (DASPEI), a mitochondrion-specific fluorescent stain (Bereiter-Hahn, 1976), was used to stain chloride cells for measurement of density and size. Membranes were incubated in 25 ml Ringer solution (in mM: 140 NaCl, 15 $NaHCO_3$, 1 $MgSO_4$, 2 $CaCl_2$, 1 NaH_2PO_4, 4 KCl and 5.5 glucose, pH 7.8) containing 2 μM DASPEI for 60 min.

The tissues were placed on glass slides (apical surface up), covered with glass cover slips, and viewed with a Zeiss standard RA microscope modified for epifluorescence with a 100 W Ploemopak illuminator. A 450–490 bandpass excitation filter, a 510 chromatic beam splitter, and a 520 longpass filter were used to examine DASPEI staining (see McCormick, 1990). Randomly-selected fields were exposed for 12 sec and photographed through a 10× objective.

Jawskin samples were also treated with anthroylouabain (AO), a fluorescent stain specific for Na^+, K^+–ATPase (Fortes, 1977; McCormick, 1990). The tissues were rinsed in low-potassium Ringer solution (LKTR; Ringer solution as above but with 0.1 mM KCl) at room temperature for 1 min and then placed in 4 ml LKTR with 2 μM AO at room temperature for 1 hr. The tissue was then removed and rinsed twice in 2 ml ice-cold LKTR for 3 min. AO-stained tissues were prepared and viewed as above, except that a 364 nm excitation filter, a 395 chromatic beam splitter, and a 420 longpass filter were used. Photomicrographs of randomly-selected AO-stained cells were taken after exposure for 20 sec at 10× magnification.

DASPEI-positive chloride cell sizes were measured from micrographs by the method of McCormick (1990). Micrographs were projected onto a digitizing pad, and cell size was determined by tracing the cell



F g. 1. The effects of salinity on plasma osmolarity (A) and plasma chloride concentration (B) in *G. mirabilis*. Fish initially in 30 ppt SW were transferred to 1.5 and 60 ppt on day 0. Values are given as mean ± SEM of 6–8 fish per group per sampling date. Points marked with an asterisk are significantly different from the control (30 ppt) group ($P \leq 0.05$, two-way ANOVA followed by Student–Newman–Keuls test).

outline from which the cross-sectional area in μm^2 was measured (Sigmascan, Jandel Scientific, Corta Madera, CA). For calibration, the micrograph of a micrometer slide at the same magnification was used. At least 60 cells per fish were measured. Densities of both DASPEI- and AO-stained chloride cells were calculated. A single average value of fluorescent cells was determined for each set of micrographs (three per fish), and expressed as cells/cm^2.

Statistical analysis

Statistical comparisons were made with two-way- and one-way-analysis of variance tests, followed by Student–Newman–Keuls tests with the use of CRISP software (CRUNCH, Berkeley, CA). Differences were deemed statistically significant when $P \leq 0.05$.

RESULTS

Plasma osmolarity and chloride ions

Plasma osmolarity of the control group fluctuated slightly during the 21 day period (Fig. 1A). Plasma osmolarity of the 1.5 ppt transfer group dropped 8 hr after transfer, and was significantly lower than SW control values at all time points ($P \leq 0.01$), except on day 7 (Fig. 1A). Plasma osmolarity of the 60 ppt transfer group increased significantly after the transfer ($P \leq 0.01$), and was significantly higher than that of the SW control on days 1 and 7 ($P \leq 0.05$).

Plasma chloride concentration of the control group remained relatively stable. Plasma chloride ion concentration of the 1.5 ppt fish dropped significantly after transfer ($P \leq 0.01$), and increased until it was not signficantly different from the SW control group on day 7 (Fig. 1B). On day 21, however, the plasma chloride concentration of the 1.5 ppt fish dropped slightly, becoming significantly lower than the SW control ($P \leq 0.05$). Plasma chloride concentration of the 60 ppt fish peaked immediately after the transfer ($P \leq 0.01$) and slowly dropped, but remained significantly different ($P \leq 0.01$) from SW controls until day 21.

Gill and jawskin Na^+, K^+*-ATPase activity*

After 21 days, there was no significant difference in gill Na^+, K^+-ATPase activity among fish in 1.5, 30 or 60 ppt (Table 1). There was no consistent pattern of change or difference among the groups in gill Na^+, K^+-ATPase activity measured from days 1–21.

Table 1. Gill and jawskin Na^+, K^+-ATPase activity and jawskin chloride cell density and size after acclimation for 21–23 days to 1.5, 30 and 60 ppt SW

	1.5 ppt SW	30 ppt SW	60 ppt SW
Na^+, K^+-ATPase*			
Gill	32.01 ± 0.78	26.65 ± 3.41	34.25 ± 3.44
Jawskin	3.42 ± 0.80	4.14 ± 1.34	2.93 ± 0.94
Residual ATPase*			
Gill	4.57 ± 0.82	4.38 ± 1.47	3.64 ± 0.78
Jawskin	3.26 ± 0.73	3.53 ± 1.66	3.61 ± 2.19
Cl cell density†			
DASPEI	38,226 ± 5109	35,100 ± 10,445	33,032 ± 5365
Anthroylouabain (AO)	32,902 ± 1891	29,273 ± 4645	36,608 ± 5672
Cl cell size‡			
DASPEI	265.0§ ± 4.6	395.6 ± 7.4	509.4§ ± 9.4

Residual ATPase refers to ATP hydrolysis not inhibited by ouabain. Values are mean ± SE.

*$N = 7$ per group. Values are given in μmol ADP/mg protein/hr.

†Values are given in cells/cm^2. Three micrographs per fish ($N = 4$) in each group were measured for chloride cell density.

‡Values are given in μm^2. At least 60 cells per fish and 4 fish per group were measured ($N = 240$).

§Values are significantly different from 30 ppt group ($P \leq 0.01$).

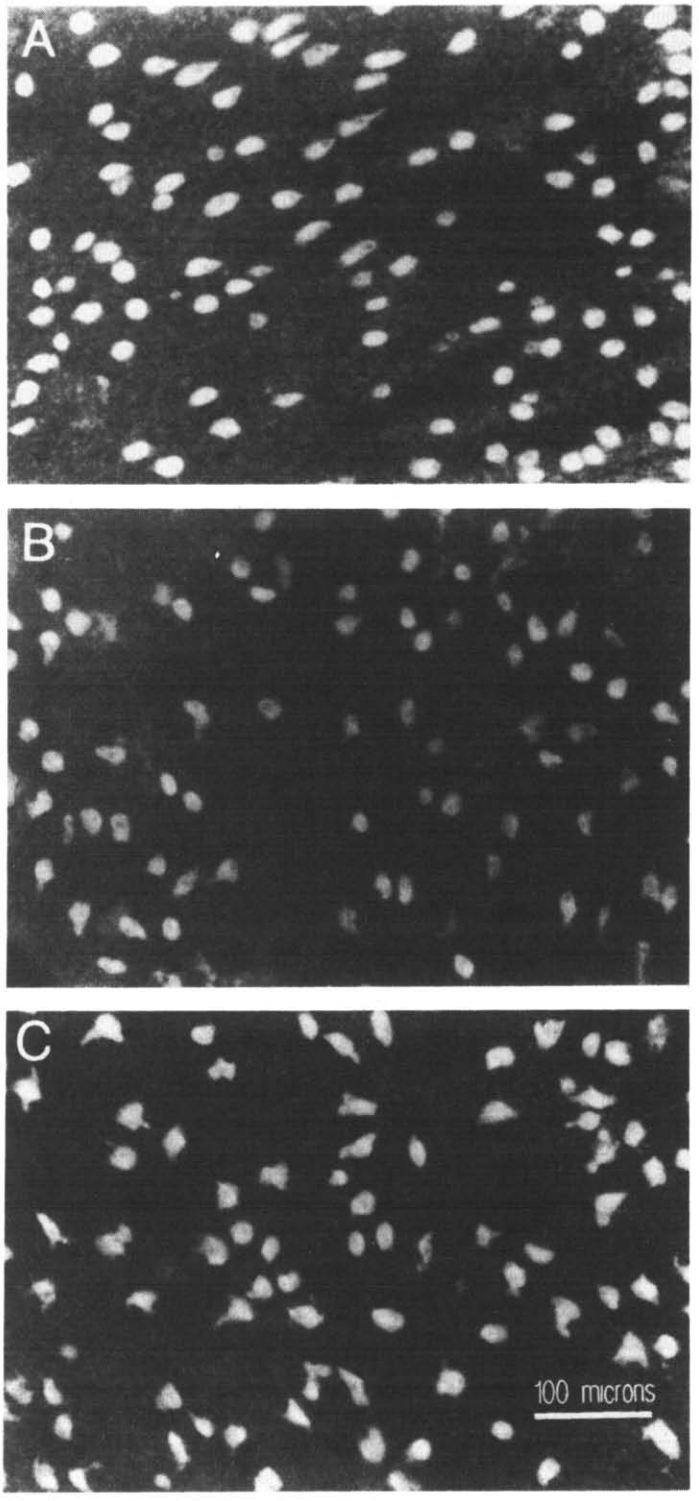


Fig. 2. DASPEI-staining chloride cells in jawskin of *G. mirabilis* in 1.5 ppt (A), 30 ppt (B) and 60 ppt (C) seawater. Note the angular outline of many cells in fish adapted to 60 ppt (C). Fish were kept in their respective salinities for 21–23 days. Bar = 100 μm.

Jawskin Na^+, K^+–ATPase activity was lower (by an order of magnitude) than that of the gill. Jawskin Na^+, K^+–ATPase activity ranged from 2.93 ± 0.94 μmol ADP/mg protein/hr in 60 ppt to 4.14 ± 1.34 μmol ADP/mg protein/hr in 30 ppt, but there were no significant differences among treatment groups (Table 1). Residual ATPase activity in gill and jawskin was unaffected by salinity.

Chloride cells

Mean chloride cell size (cross-sectional area) increased with increasing salinity ($P \leq 0.01$; Table 1 and Fig. 2). Chloride cells of fish in 1.5 ppt were 33% smaller than those of fish in 30 ppt, whereas chloride cells of fish in 60 ppt were 29% larger.

There was no significant effect of salinity on chloride cell density (Table 1). The densities of DASPEI- and AO-stained cells were similar, ranging from 29 956 to 43,548 cells/cm^2 (DASPEI), and from 29 273 to 35,858 cells/cm^2 (AO).

Chloride cell morphology was noticeably different among the various treatment groups (Fig. 2). The chloride cells of the 60 ppt fish were angular in outline, compared with the more rounded outline of chloride cells in the 30 and 1.5 ppt groups. The cells of the 1.5 ppt group appeared more uniformly circular than those of the 30 ppt group.

DISCUSSION

Measurements of plasma osmolarity and chloride concentration show that the longjawed mudsucker is capable of adapting to major salinity changes within 3 to 7 days. This ability would be of definite advantage to this species, which usually inhabits relatively shallow mudflats subject to extreme tidal changes, precipitation and evaporation that result in rapid alterations in salinity.

Although gill Na^+, K^+–ATPase activity was 20–28% higher in the 1.5 and 60 ppt groups than in the 30 ppt group, this difference was not statistically significant. Doneen (1981) found that gill Na^+, K^+–ATPase activity was significantly elevated in FW and in 1.5 ppt SW-adapted animals, compared with the 30 and 50 ppt SW-adapted fish. Difference in membrane purification between the two studies could be partially responsible for these differences. Na^+, K^+–ATPase activity in crude gill homogenates of the longjawed mudsucker in this study were 5- to 10-fold higher than reported by Doneen (1981). Furthermore, our gill enzyme activities were about 10-fold higher than in *C. labrosus* and *D. labrax* (Lasserre, 1971), *P. flesus* (Stagg and Shuttleworth, 1982) and *Oncorhynchus kisutch* (McCormick and Bern, 1989), and 50% higher than in SW-adapted *F. heteroclitus* (Epstein *et al.*, 1967). The high levels of gill Na^+, K^+–ATPase activity in the longjawed mudsucker correlate with a high density of chloride cells in the gill epithelium (McCormick, unpublished results).

Our results show that enzyme activity in the jawskin was much lower (about one-tenth) than in the gills. As with the gills, jawskin Na^+, K^+–ATPase activity was not affected by differences in salinity. Nonetheless, the jawskin Na^+, K^+–ATPase activity in the longjawed mudsucker is comparable to this activity in the gills of other species (see above).

Owens *et al.* (1977) examined sodium transport in the bladder of the longjawed mudsucker. Rates of net sodium movement in bladders of fish adapted to 1.5, 10 and 30 ppt SW were not different. In fish transferred to 60 and 90 ppt SW, however, rates of net bladder sodium movement were elevated, but significantly so only in the 90 ppt SW fish. These results correspond with our finding of relatively unchanged Na^+, K^+–ATPase activity in the gill and jawskin of the longjawed mudsucker exposed to various salinities.

Chloride cells responsible for chloride transport are located on many epithelial surfaces of the longjawed mudsucker (Marshall and Nishioka, 1980). Chloride cells have also been found in the opercular membrane of *F. heteroclitus* (Degnan *et al.*, 1977; Karnaky and Kinter, 1977), *O. mossambicus* (Foskett *et al.*, 1981), and in very low density in *Oncorhynchus kisutch* (Richman *et al.*, 1987). The proportion of chloride secretion from the opercular membrane or skin, as opposed to that from the gills, remains to be investigated.

Jawskins of longjawed mudsuckers adapted to 1.5, 30 or 60 ppt for 21–23 days were stained with AO or DASPEI, and chloride cell size and densities measured. McCormick (1990) has shown that the same cells are stained by these two methods. Although individual variation was high, there was no significant variation in chloride cell densities among the three groups (Table 1). The densities are comparable with those reported by Marshall and Nishioka (1980), who obtained chloride cell densities ranging from 10,000 to 100,000 cells/cm^2. Our measurements also correspond with those of McCormick (1990), who found a chloride cell density of 29,270 $\pm$ 9290 cells/cm^2 in longjawed mudsucker jawskin (30 ppt). Moreover, these stains confirm the presence of Na^+, K^+–ATPase-rich chloride cells in longjawed mudsucker jawskin, even when the fish are exposed to hypoosmotic conditions. These findings contrast with studies of tilapia, where AO-positive cells are not present in the opercular membrane of freshwater-adapted fish (McCormick, 1990).

Na^+, K^+–ATPase, concentrated in chloride cells (Langdon and Thorpe, 1984; Karnaky, 1986), is believed to be intimately involved in branchial chloride secretion (Towle, 1981). Thus, increased levels of Na^+, K^+–ATPase activity are expected, and do occur, in some SW-adapted fish. Conversely, in low salinity media where chloride uptake, not secretion, prevails, decreased levels of Na^+, K^+–ATPase activity are often found in euryhaline teleosts (see Towle, 1981). However, these differences are not universal among teleosts. High gill Na^+, K^+–ATPase activity in FW has been observed in *D. labrax* and *C. labrosus* (Lasserre, 1971), euryhaline species of marine origin. The strategies for gill Na^+, K^+–ATPase regulation that exist among teleosts (Towle, 1981; McCormick *et al.*, 1989), probably reflects differences in recent evolutionary origin (freshwater vs seawater) and the frequency and magnitude of salinity change they are likely to encounter.

There are two adaptive explanations, not mutually exclusive, for the presence of Na^+, K^+–ATPase-rich cells in 1.5 ppt in longjawed mudsuckers. First, the AO-positive cells may be the site of gill ion uptake in hypoosmotic media, and Na^+, K^+–ATPase is involved in this uptake. Second, Na^+, K^+–ATPase, though not necessarily active, may be maintained for future use when fish again encounter a hyperosmotic medium and ion secretion is again necessary. The latter may reflect the normal physiological ecology of the longjawed mudsucker, which is likely to experience frequent salinity changes in its intertidal habitat.

Notably, chloride cell size was significantly larger in fish in 30 and 60 ppt than those in 1.5 ppt. This increased size may be related to the greater rate of net ion transport required in seawater. Since chloride cell densities are similar in the three media, it is likely that individual chloride cells need to be more active as ion-pumping requirements increase. This increased activity may involve chloride cell hypertrophy.

Chloride cell morphology was noticeably different among the various transfer groups. The chloride cells of the 60 ppt fish were angular in outline, while those of both the 1.5 and 30 ppt fish were more circular. This observation corresponds to the finding of Marshall and Nishioka (1980) that longjawed mudsuckers in 60 ppt possessed some chloride cells that branched proximal to the nucleus and attached to the basal lamina at several points. This branching may increase surface area of the chloride cells, and thus facilitate increased chloride-secretory rates at high salinity.

In conclusion, the longjawed mudsucker seems to be an efficient osmoregulator in a wide range of salinities. Its rapid ion-regulatory response, high branchial and jawskin Na^+, K^+–ATPase activity and chloride cell density make this fish different from other species, in that some of the morphological and biochemical characteristics usually associated with seawater adaptation are apparently maintained in both high and low salinities for at least 21 days. This may reflect, in part, adaptation to the intertidal habitat and the marine origin of this species.

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REFERENCES

Bereiter-Hahn J. (1976) Dimethylaminostryrylmethylpyridiniumiodine (DASPEI) as a fluorescent probe for mitochondria *in situ*. *Biochim. biophys. Acta* **423,** 1–14.

Degnan K. J., Karnaky K. J. Jr and Zadunaisky J. A. (1977) Active chloride transport in the *in vitro* opercular skin of a teleost (*Fundulus heteroclitus*), a gill-like epithelium rich in chloride cells. *J. Physiol.* (London) **271,** 155–191.

Doneen B. A. (1981) Effects of adaptation to seawater, 170% seawater and to fresh water on activities and subcellular distribution of branchial Na^+, K^+–ATPase, low- and high-affinity Ca^{2+}–ATPase, and ouabain-insensitive ATPase in *Gillichthys mirabilis*. *J. comp. Physiol.* **145,** 51–61.

Epstein F. H., Katz A. I. and Pickford G. E. (1967) Sodium- and potassium-activated adenosine triphosphatase of gills: role in adaptation of teleosts to salt water. *Science* **156,** 1245–1247.

Epstein F. H., Silva P. and Kormani G. (1980) Role of Na-K–ATPase in chloride cell function. *Am. J. Physiol.* **238,** R246–250.

Folmar L. C. and Dickhoff W. W. (1979) Plasma thyroxine and gill Na^+-K^+-ATPase changes during seawater acclimation of coho salmon, *Oncorhynchus kisutch*. *Comp. Biochem. Physiol.* **63A,** 329–332.

Fortes P. A. G. (1977) Anthroylouabain: a specific fluorescent probe for the cardiac glycoside receptor. *Biochemistry* **16,** 531–540.

Foskett J. K., Logsdon C. D., Turner T., Machen T. E. and Bern H. A. (1981) Differentiation of the chloride extrusion mechanism during seawater adaptation of a teleost fish, the cichlid *Sarotherodon mossambicus*. *J. exp. Biol.* **93,** 209–224.

Foskett J. K. and Scheffey C. (1982) The chloride cell: definitive identification as the salt-secretory cell in teleosts. *Science* **215,** 164–166.

Jampol L. M. and Epstein F. H. (1970) Sodium–potassium-activated adenosine triphosphatase and osmotic regulation by fishes. *Am. J. Physiol.* **218,** 607–611.

Karnaky K. J. (1986) Structure and function of the chloride cell of *Fundulus heteroclitus* and other teleosts. *Am. Zool.* **27,** 209–224.

Karnaky K. J. Jr and Kinter W. B. (1977) Killifish opercular skin: a flat epithelium with a high density of chloride cells. *J. exp. Zool.* **199,** 345–354.

Kirschner L. B. (1969) Short communication. ATPase activity in gills of euryhaline fish. *Comp. Biochem. Physiol.* **29,** 871–874.

Langdon J. S. and Thorpe J. E. (1984) Response of the gill Na^+-K^+–ATPase activity, succinic dehydrogenase activity and chloride cells to seawater adaptation in Atlantic salmon, *Salmo salar* L., parr and smolt. *J. Fish Biol.* **24,** 323–331.

Larson E., Howlett B. and Jagendorf A. (1986) Artificial reductant enhancement of the Lowry method for protein determination. *Analyt. Biochem.* **155,** 243–248.

Lasserre P. (1971) Increase of ($Na^+ + K^+$)-dependent ATPase activity in gills and kidneys of two euryhaline marine teleosts, *Crenimugil labrosus* and *Dicentrarchus labrax* during adaptation to fresh water. *Life Sci.* **10,** 113–119.

Marshall W. S. (1981) Sodium dependency of active chloride transport across isolated fish skin (*Gillichthys mirabilis*). *J. Physiol.* **319,** 165–178.

Marshall W. S. and Nishioka R. S. (1980) Relation of mitochondria-rich chloride cells to active chloride transport in the skin of a marine teleost. *J. exp. Zool.* **214,** 147–156.

McCormick S. D. (1990) Fluorescent labeling of Na^+, K^+–ATPase in intact cells by use of a fluorescent derivative of ouabain: Salinity and teleost chloride cells. *Cell Tissue Res.* **260,** 529–533.

McCormick S. D. and Bern H. A. (1989) *In vitro* stimulation of Na^+, K^+–ATPase activity and ouabain binding by cortisol in coho salmon gill. *Am. J. Physiol.* **256,** R707–715.

McCormick S. D., Moyes C. D. and Ballantyne J. S. (1989) Influence of salinity on the energetics of gill and kidney of Atlantic salmon (*Salmo salar*). *Fish Physiol. Biochem.* **6,** 243–254.

Owens A., Wigham T., Doneen B. and Bern H. A. (1977) Effects of environmental salinity and of hormones on

urinary bladder function in the euryhaline teleost, *Gillichthys mirabilis*. *Gen. comp. Endocr.* **33,** 526–530.

Payan P. and Girard J. P. (1984) Branchial ion movements in teleosts: the roles of respiratory and chloride cells. In *Fish Physiology* (Edited by Hoar W. S. and Randall D. J.), Vol. XB, pp. 39–63. Academic Press, New York.

Richman N. H. III, Tai de Diaz S., Nishioka R. S., Prunet P. and Bern H. A. (1987) Osmoregulatory and endocrine relationships with chloride cell morphology and density during smoltification in coho salmon (*Oncorhynchus kisutch*). *Aquaculture* **60,** 254–285.

Sargent J. R. and Thomson A. J. (1974) The nature and properties of the inducible sodium-plus-potassium ion-dependent adenosine triphosphatase in the gills of eels (*Anguilla anguilla*) adapted to fresh water and seawater. *Biochem. J.* **144,** 69–75.

Silva P., Solomon R., Spokes K. and Epstein F. H. (1977) Ouabain inhibition of gill Na-K-ATPase: relationship to active chloride transport. *J. exp. Zool.* **199,** 419–426.

Stagg R. M. and Shuttleworth T. J. (1982) Na^+, K^+ ATPase, ouabain binding and ouabain-sensitive O_2 consumption in gills from *Platichthys flesus* adapted to seawater and fresh water. *J. comp. Physiol.* **147,** 93–99.

Thomson A. J. and Sargent J. R. (1977) Changes in the levels of chloride cells and $(Na^+ + K^+)$-dependent ATPase in the gills of yellow and silver eels adapting to seawater. *J. exp. Zool.* **200,** 33–40.

Towle D. W. (1981) Role of $Na^+ + K^+$ ATPase in ionic regulation by marine and estuarine animals. *Mar. Biol.* **2,** 107–122.

Towle D. W., Gilman M. E. and Hempel J. D. (1977) Rapid modulation of gill Na and K dependent ATPase activity during acclimation of the killifish *Fundulus heteroclitus* to salinity change. *J. exp. Zool.* **202,** 179–186.

Utida S., Kamiya M. and Shirai N. (1971) Relationship between the activity of Na, K-activated adenosine triphosphatase and the number of chloride cells in eel gills with special reference to seawater adaptation. *Comp. Biochem. Physiol.* **38A,** 443–447.

Zadunaisky J. A. (1984) The chloride cell: the active transport of chloride and the paracellular pathways. In *Fish Physiology*. (Edited by Hoar W. S. and Randall D. J.), Vol. XB, pp. 129–176. Academic Press, New York.

Zaugg W. S. and McLain L. R. (1971) Gill sampling as a method of following biochemical changes: ATPase activities altered by ouabain injection and salt water adaptation. *Comp. Biochem. Physiol.* **38B,** 502–506.