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N-acetylhistidine, a novel osmolyte in the lens of Atlantic salmon (*Salmo salar* L.)

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Rhodes JD, Breck O, Waagbo R, Bjerkas E, Sanderson J. *N*-acetylhistidine, a novel osmolyte in the lens of Atlantic salmon (*Salmo salar* L.). *Am J Physiol Regul Integr Comp Physiol* 299: R1075–R1081, 2010. First published July 21, 2010; doi:10.1152/ajpregu.00214.2010.—Volume homeostasis is essential for the preservation of lens transparency and this is of particular significance to anadromous fish species where migration from freshwater to seawater presents severe osmotic challenges. In Atlantic salmon (*Salmo salar* L.), aqueous humor (AH) osmolality is greater in fish acclimated to seawater compared with young freshwater fish, and levels of lens *N*-acetylhistidine (NAH) are much higher in seawater fish. Here we investigate NAH as an osmolyte in the lenses of salmon receiving diets either with or without histidine supplementation. In the histidine-supplemented diet (HD) histidine content was 14.2 g/kg, and in the control diet (CD) histidine content was 8.9 g/kg. A transient increase in AH osmolality of 20 mmol/kg was observed in fish transferred from freshwater to seawater. In a lens culture model, temporary decreases in volume and transparency were observed when lenses were exposed to hyperosmotic conditions. A positive linear relationship between extracellular osmolality and lens NAH content was also observed, whereas there was no change in lens histidine content. Hypoosmotic exposure stimulated [^{14}C]-histidine efflux by 9.2- and 2.6-fold in CD and HD lenses, respectively. NAH efflux, measured by HPLC, was stimulated by hypoosmotic exposure to a much greater extent in HD lenses. In vivo, lens NAH increased in response to elevated AH osmolality in HD but not CD fish. In conclusion, NAH has an important and novel role as a compatible osmolyte in salmon lens. Furthermore, it is the major osmolyte that balances increases in AH osmolality when fish move from freshwater to seawater. A deficiency in NAH would lead to a dysfunction of the normal osmoregulatory processes in the lens, and we propose that this would contribute to cataract formation in fish deficient in histidine.

histidine

PREVALENCE OF CATARACT IN farmed Atlantic salmon (*Salmo salar* L.) has increased in recent years (3) and this has been linked to the removal of a rich source of dietary histidine (blood meal) (7). Several recent studies have investigated the possible causes of cataract in farmed salmon (6, 9, 30), although the mechanisms involved remain to be elucidated.

Osmotic stress has been shown to contribute to cataract formation in multiple species, including man (19). Efficient volume regulation is crucial for the maintenance of lens transparency and disruption to the tightly packed and highly ordered cells and proteins that make up the lens will cause a loss of lens transparency. In fish there are numerous examples where osmotic factors have been shown to play a role in cataract development (20), and the anadromous life cycle of the Atlantic salmon exposes the fish to particularly severe osmotic stress. The early part of salmon development is spent in freshwater as parr followed by transformation to smolts before entering the sea. Despite the physiological changes associated with parr-smolt transformation, a preadaption for life in seawater, reversible osmotic cataracts, are commonly described immediately after seawater transfer (9, 22), and irreversible cataracts, which also develop soon after transfer, are often seen in seawater fish (7, 9, 31). There is additional evidence that osmotic stress may cause cataract in farmed salmon since the appearance of cataract was shown to be increased by environmental fluctuations in salinity (4). Furthermore, spontaneous cataracts have been reported in seawater fish farms following heavy rain (5).

Feeding trials have shown that supplementing the diet of Atlantic salmon with histidine significantly reduced the incidence of cataract (4, 6, 7). Interestingly, it has been shown that *N*-acetylhistidine (NAH), a histidine metabolite found in the lenses of poikilothermic vertebrates (2), increased more than 10-fold in the lenses of salmon fed a histidine-supplemented diet (HD) following transfer from fresh to seawater (7). In fish fed the control diet (low histidine; CD) this increase did not occur, although total amino acid concentration did increase by a similar amount. The increase in NAH concentration coincided with a rise in the osmolality of the aqueous humor (AH) following seawater transfer, suggesting that NAH is acting as an osmolyte in the salmon lens. A alternative role for NAH as a molecular water pump, however, has also been proposed (2). Given the link between osmotic stress and cataract, coupled with the necessity for salmon to be able to adapt to changes in environmental osmolality, we have hypothesized that reduced levels of NAH cause increased susceptibility to osmotic trauma in the lenses of fish deficient in dietary histidine (8). In a recent study, we have gone some way to test this hypothesis by showing that NAH efflux was stimulated when cultured salmon lenses were exposed to hypoosmotic conditions for up to 4 days (29). In the research presented here, we have investigated in detail the role of histidine and its major metabolite, NAH, in osmoregulation in the salmon lens. In particular, we show that there is a rapid increase in the efflux of [^{14}C]-histidine metabolites in response to a hypoosmotic stimulus. We also show that lens NAH, but not histidine content, is positively correlated to the osmolality of the external medium both in vitro and in vivo. These findings lead us to conclude that NAH is the major osmolyte in the salmon lens.

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MATERIALS AND METHODS

This study complied with the guidelines of the Norwegian Regulation on Animal Experimentation, and the protocol was approved by the competent person at the laboratory unit at the Institute of Marine Research (Bergen, Norway) and the National Animal Research Authority.

Materials. Radioactive isotopes were from Amersham Pharmacia Biotech. Liquid scintillation was performed using Optiphase "Supermix" (Wallac Scintillation Products). All other chemicals were from Sigma-Aldrich (St. Louis, MO).

Fish culture and seawater challenge test. Atlantic salmon parr (Mowi strain) were randomly selected into two outdoor tanks located at the Institute of Marine Research, Bergen, Norway. They were maintained in freshwater for 4 mo after which they were transferred to seawater. They were fed either a control diet (CD) based on Norse LT 94 with a natural histidine concentration of 8.9 g/kg or a diet supplemented with crystalline histidine giving a concentration of 14.2 g/kg [histidine supplemented diet (HD)]. Fish were killed, and samples collected as described by Breck et al. (7).

Seawater challenge tests were conducted prior to seawater transfer. Fish from each dietary group were transferred from freshwater to seawater. In another experiment, seawater-acclimated post-smolts, were exposed to diluted (50%) seawater for 48 h. Samples of AH were collected from killed fish using a hypodermic needle inserted into the anterior chamber of the eye. The lens was removed for analysis. AH osmolality (mmol/kg) was measured using a vapor pressure osmometer (Vapro; Wescor).

Lens culture and viability measurements. The lens was dissected free of the eye and placed into culture medium containing penicillin (200 U/ml) and streptomycin (200 μg/ml) at ~4°C for 1 h. Lenses were transferred to 12-well plates containing 2 ml/well control culture medium (300 mmol/kg). The culture medium consisted of MEM (pH 7.3) without $NaHCO_3$ and including HEPES (10 mM) and gentamycin (50 μg/ml). Osmolality was adjusted by the addition of NaCl or dilution with water. Medium osmolality (mmol/kg) was measured using a vapor pressure osmometer (Vapro; Wescor). Lenses were cultured at 4°C.

To assess transparency, lenses were illuminated from below and images captured by a charge-coupled device camera (UVP) as previously described (13). Lens diameter was analyzed using Adobe Photoshop 6.0. Teleost lenses are close to spherical in shape (1), and therefore changes in the diameter will be proportional to changes in lens volume. All lenses were cultured in control medium (300 mmol/kg) for 1 wk before starting experiments, and only clear lenses were used.

Lens membrane potential (V_m) was measured as previously described (26). Lenses were placed into a plastic chamber (1 ml) and perifused with an artificial AH (~10°C) at 1 ml/min. Artificial AH has the following composition (in mM): 130 NaCl, 5 KCl, 5 $NaHCO_3$, 1 $CaCl_2$, 0.5 $MgCl_2$, 5 glucose, 20 HEPES. pH was adjusted to 7.25 with NaOH. A glass micropipette filled with 2 M KCl (1–6 MΩ) inserted into a cortical fiber via the posterior of the lens measured V_m with reference to a low-resistance electrode in the bath. Lens cells are electrically very well coupled (16), and thus V_m is a measure largely of the voltage across the plasma membrane of the outermost cells. Electrical measurements were recorded using a two-channel, high-impedance amplifier (Firbank Electronics, Norwich, UK) in conjunction with an analog-to-digital converter (Handyscope, TiePie Engineering, Leeuwarden, The Netherlands) for storage and analysis on computer.

Radioactive amino acid incorporation into protein was measured using methods described by Sanderson et al. (27). Briefly, lenses were incubated at 4°C for 24 h in medium labeled with a [U-^{14}C] protein hydrolysate (0.25 μCi/ml) containing 16 amino acids (CFB104; Amersham Biosciences, UK). Lenses were homogenized in extraction buffer and centrifuged to separate soluble from insoluble proteins. The soluble fraction was treated with TCA (5%) and centrifuged to separate the protein from the free amino acid pool. The pellets were washed twice by resuspension and centrifugation and then dissolved in NaOH (250 mM) and added to 10 ml of scintillation fluid for counting (27).

Viability of salmon lenses in organ culture. Salmon lenses cultured in control medium (300 mmol/kg, 4°C) remained transparent, and no significant volume changes were seen for the duration of the experiments reported here (see Fig. 2). Lenses cultured for longer periods (>4 wk) also remained clear and showed no visible signs of deterioration (data not shown). The culture medium was serum free, and it was found that no supplements were necessary for the maintenance of lens clarity. Postmortem deterioration and dissection trauma caused opacity in some of the lenses, but this was short lived and the majority of these lenses rapidly regained their transparency during the first 2–3 days in culture. Experiments were therefore initiated after 1 wk of preculture in control medium, and this represents the start time of the experiments. As a measure of viability, lens V_m was recorded in lenses cultured for up to 2 wk, and over this period V_m remained relatively constant at approximately −80 mV. As a further indication of viability, the capacity of lenses to synthesize proteins in culture was measured. No significant decline in protein synthesis was observed over 2 wk of culture. The level of incorporation of a ^{14}C-amino acid mixture into the water-soluble protein fraction, expressed as a fraction of the amino acid pool, was 14 ± 1% on the day of dissection and 12 ± 3% 2 wk later. Both of the above methods indicate that the cultured lenses remained in a viable state for the duration of the experiments.

Histidine efflux. Lenses were incubated in control medium (300 mmol/kg) containing [^{14}C]-histidine (CFB140; 0.25 μCi/ml) for ~24 h. Lenses were washed in control medium (3 × 30 min). Efflux experiments were carried out in 12-well culture plates with 2 ml medium/well. Basal efflux was determined from three periods (15 min each) in control medium, followed by four periods in hypoosmotic medium (200 mmol/kg) and finally two periods in control medium. At the end of the experiment, medium in each well was added to scintillation fluid, and the radioactivity was measured. To release lens-free [^{14}C]-histidine, lenses were placed into 1-ml cold TCA (5%) overnight (4°C) prior to counting. Rate constants were calculated as previously described (14):

$$k = \frac{\ln(A_{t_1}) - \ln(A_{t_2})}{t_2 - t_1}$$

where k is the efflux rate constant and A_{t1} and A_{t2} are the activities in the lens (counts/min) at time t_1 and t_2, respectively.

NAH and histidine determination by HPLC. Measurement of free histidine and NAH was by isocratic reverse-phase HPLC on a 4.6-mm ID × 250-mm column with silica-based packing (Zorbax model SB-C18; Agilent Technologies). Detection was by UV (210 nm) absorbance (Waters). These methods have been described previously in detail (7) .

Statistical analysis. Differences between means were tested for using Student's *t*-tests, and the level of significance was $P \leq 0.05$. Data are reported as means ± SE.

RESULTS

AH osmolality is life cycle and environment dependent. To fulfill the requirements of the life cycle, young farmed salmon smolts are transferred from freshwater to seawater. The osmolality of AH from salmon parr (before smoltification) living in freshwater was 309.8 ± 1.9 mmol/kg (means ± SE; $n = 6$). After several months in seawater, however, AH osmolality had significantly increased by 14.3 mmol/kg to 324.1 ± 3 mmol/kg (means ± SE; $n = 6$; $P \leq 0.01$).

To investigate whether changes in osmolality of the external environment alter the osmotic environment of the lens, we

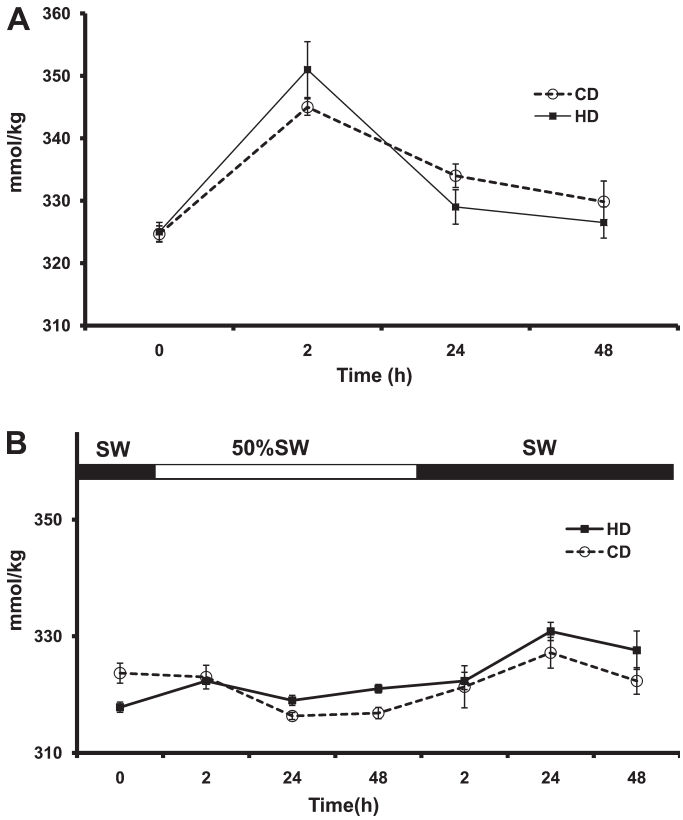

Fig. 1. Aqueous humor (AH) osmolality in salmon fed either control (CD) or histidine-supplemented (HD) diet exposed to hyper- and hypoosmotic environments. *A*: AH in freshwater (FW) smolts was sampled before transfer and at 2, 24, and 48 h after transfer to seawater (SW) ($n = 15$). *B*: AH osmolality in salmon acclimated to SW was sampled before, during, and after transfer from 100% SW to 50% SW ($n = 9$). Error bars, means ± SE.

performed two osmotic-challenge tests. To measure the acute effect of transfer into seawater on the osmolality of the AH (hyperosmotic challenge), salmon smolts were transferred from freshwater to seawater, and samples of AH were taken over a 48-h period (Fig. 1*A*). AH osmolality in freshwater smolts ($t = 0$) was similar to AH osmolality from fish that had been in seawater for several months (see above), indicating that preadaption of AH osmolality may occur as part of parr-smolt transition. After 2 h in seawater, the osmolality of the AH rose by 20 mmol/kg and after 24 h had returned to a level that was slightly, though not significantly, greater than that recorded in freshwater. The histidine content of the diet did not influence the changes in AH osmolality, such that AH from CD and HD fish showed similar changes.

We tested the effect of hypoosmotic challenge by transferring post-smolts, acclimated to seawater for 100 days, to diluted (50%) seawater for 48 h before returning them to pure seawater (Fig. 1*B*). Exposure to diluted seawater had relatively little effect on the osmolality of AH, although in CD fish there was a small decrease in osmolality. There was a slight overshoot in osmolality when fish of both dietary groups were returned to 100% seawater, which was greater than the observed decrease in 50% seawater. By 48 h in seawater, a recovery was seen in both groups.

Cultured lens transparency, volume, histidine, and NAH respond to medium osmolality. The effect of hyperosmotic stress was investigated by measuring changes in lens clarity and volume of salmon lenses in culture. We have shown that, using these culture conditions, the lenses remain viable over the culture period (see MATERIALS AND METHODS). In a pilot study (data not shown), it was found that opacification and volume changed maximally after 2 h in hyperosmotic (500 mmol/kg) conditions followed by recovery over the following 24 h. Lenses were therefore monitored at 2-h and 24-h intervals for 6 days. No significant change in transparency was observed in lenses cultured under control (300 mmol/kg) conditions. In contrast, there was a reduction in transparency (opacification), seen as a darkening of the image, when lenses were exposed to hyperosmotic conditions, and this increased with increasing osmolality (Fig. 2*A*). Opacification seen at 2 h, however, showed a marked recovery after 24 h of continuous exposure even at higher osmolalities. After 6 days, lenses in media with osmolalities of 500 mmol/kg and below appeared similar to control lenses. At 600 mmol/kg, lens transparency did not recover, and there was a marked deterioration between 1 and 6 days. No significant opacity changes were observed in lenses

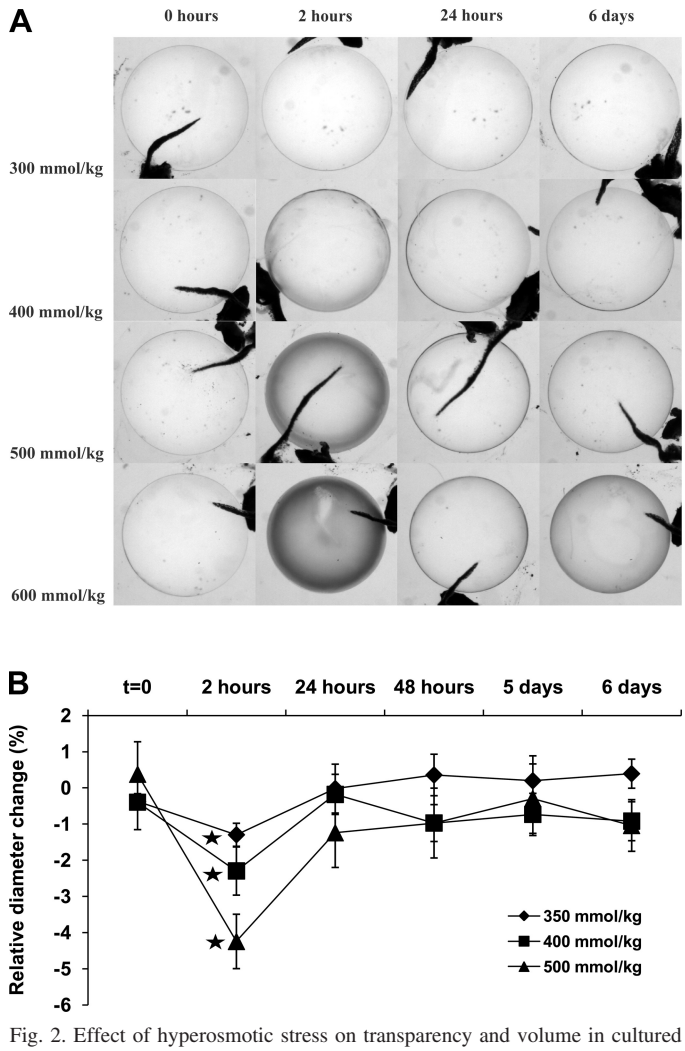

Fig. 2. Effect of hyperosmotic stress on transparency and volume in cultured salmon lenses. *A*: brightfield images of lenses exposed to hyperosmotic medium for 6 days. Note that attached to many of the lenses are black suspensory ligaments. *B*: relative diameter changes with time ($n = 5$). Changes in diameter are proportional to changes in volume. Error bars, means ± SE; $^*P \leq 0.05$ vs. control.

exposed to hypoosmotic conditions (data not shown). There was, however, a trend toward swelling in hypoosmotically exposed lenses, although, this was not significant (data not shown).

A similar pattern to the opacity changes were observed in the volume of lenses exposed to hyperosmotic conditions (Fig. 2*B*). During the first 2 h, the volume showed a significant decrease relative to control at all osmolalities, which was followed by a recovery during the next 24 h. Although there were some fluctuations, the lenses cultured in media of 350–500 mmol/kg showed a volume recovery after 24 h with no significant difference between control and treated lenses. The recovery was maintained for the remaining period of the experiment. Lenses exposed to 600 mmol/kg showed the largest decrease in diameter relative to the control lenses ($-12.5 \pm 1.5\%$ at 2 h) and failed to recover during the following 6 days (data not shown).

Since previous experiments have indicated that NAH may have a role in osmoregulation in salmon lens (7, 29), we measured the levels of NAH and histidine in lenses cultured in both hyper- and hypoosmotic media (250–400 mmol/kg) for 24 h (Fig. 3). Histidine levels remained relatively constant over the range of osmolalities investigated. In contrast to this, however, levels of NAH were found to have a linear relationship ($R^2 = 0.967$), decreasing in lenses exposed to hypoosmotic and increasing in lenses exposed to hyperosmotic conditions.

Hypoosmotic exposure stimulates the efflux of histidine. To discover how histidine and histidine metabolites behave when lenses are osmotically challenged, we carried out tracer experiments using [^{14}C]-histidine. The lenses were exposed to hypoosmotic medium (200 mmol/kg) and the efflux of [^{14}C]-histidine monitored.

Resting efflux of [^{14}C]-histidine was tightly regulated in both feed groups (Fig. 4, *A–C*), although, under control conditions, the rate constant for [^{14}C]-histidine efflux was greater in the HD lenses compared with the CD lenses. In both feed groups there was an increase in efflux on exposure to hypoosmotic medium. The efflux rate was greatest after 30-min exposure to hypoosmotic conditions, increasing by a factor of 9.2 (CD) and 2.6 (HD) above the resting level (Fig. 4*C*). The rate started to regulate downward during the hypoosmotic exposure and had returned to resting values by the end of the experiment.

From these experiments, it was also possible to calculate the relative amount of labeled histidine accumulated over the 24-h loading period. The specific activity ratios (internal/external) were 17.3 (CD) and 9.27 (HD), showing that there is a greater accumulation of histidine in the CD lenses.

Exposure to hypoosmotic conditions stimulates the efflux of NAH. Because we could not distinguish between the efflux of histidine and NAH in the [^{14}C]-histidine tracer experiments, we carried out a parallel experiment in which we directly measured by HPLC histidine and NAH efflux from cultured lenses following hypoosmotic challenge. Lenses were exposed to hypoosmotic conditions for 24 h. Resting histidine and NAH efflux from lenses in control conditions (300 mmol/kg) was greater from HD compared with CD lenses (Fig. 4*D*), confirming the [^{14}C]-histidine data. Exposure to hypoosmotic medium, however, showed an osmolality-dependent increase in the efflux of histidine and NAH in all lenses, although differences between the two feed groups were observed. When CD lenses were exposed to 250 mmol/kg, the efflux of histidine was greater than the efflux of NAH, while at 200 mmol/kg NAH efflux was greater. At both 250 and 200 mmol/kg NAH, efflux from HD lenses was greater than that of histidine and was considerably greater than the efflux from CD lenses.

Lens histidine and NAH levels respond to in vivo fluctuations in AH osmolality. If histidine and NAH behave as osmolytes in vivo, it would be anticipated that they would respond to fluctuations in AH osmolality. Lens samples were therefore taken from the same fish used to obtain the AH measurements in Fig. 1, and these were analyzed for histidine and NAH content. In both feed groups, NAH content was greater than histidine content (Fig. 5*A*). Additionally, there were differences in basal levels of histidine and NAH between the two feed groups with both being higher in HD compared with CD lenses. On exposure to seawater, both histidine and NAH changed with time. For histidine there was a small decrease in the first 2 h followed by an increase after 24 and 48 h exposure to seawater, and the pattern was similar in both lenses from CD and HD fish. In contrast, a pronounced difference was seen between the HD and the CD fish in the NAH response to the seawater challenge (Fig. 5*B*). A 24% increase of 1.8 μmol/g (~5 mM) in the NAH level was seen in lenses of HD fish in the first 2 h following exposure to seawater. This increased level was maintained over the following 48 h. This compares to a slight downward shift in the level of NAH that was seen in lenses of the CD group.

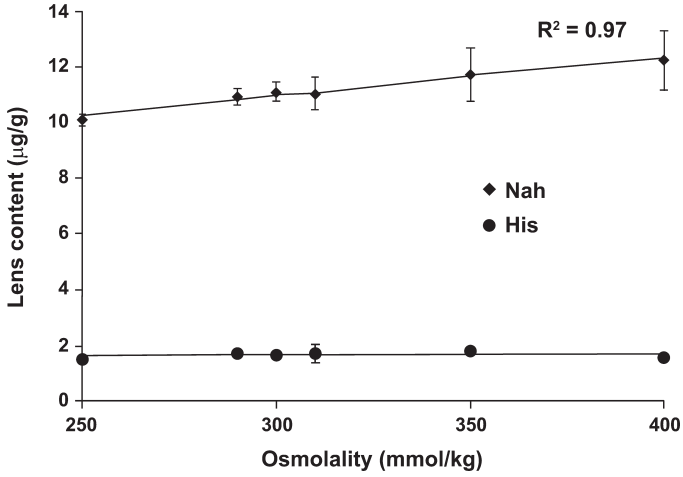

Fig. 3. The effect of osmolality on lens histidine (His) and *N*-acetylhistidine (NAH) in cultured salmon lenses. Cultured lenses were exposed to media of varying osmolality for 24 h, and histidine and NAH content was measured by HPLC ($n = 4$).

DISCUSSION

It has been shown that supplementing dietary histidine reduces cataract in farmed Atlantic salmon and this may be linked to an increase in lens NAH, which is observed when salmon are transferred from freshwater to seawater (4, 6, 7). Here we show that NAH is a lens osmolyte and suggest that reduced histidine availability compromises lens osmoregulation and increases cataract development.

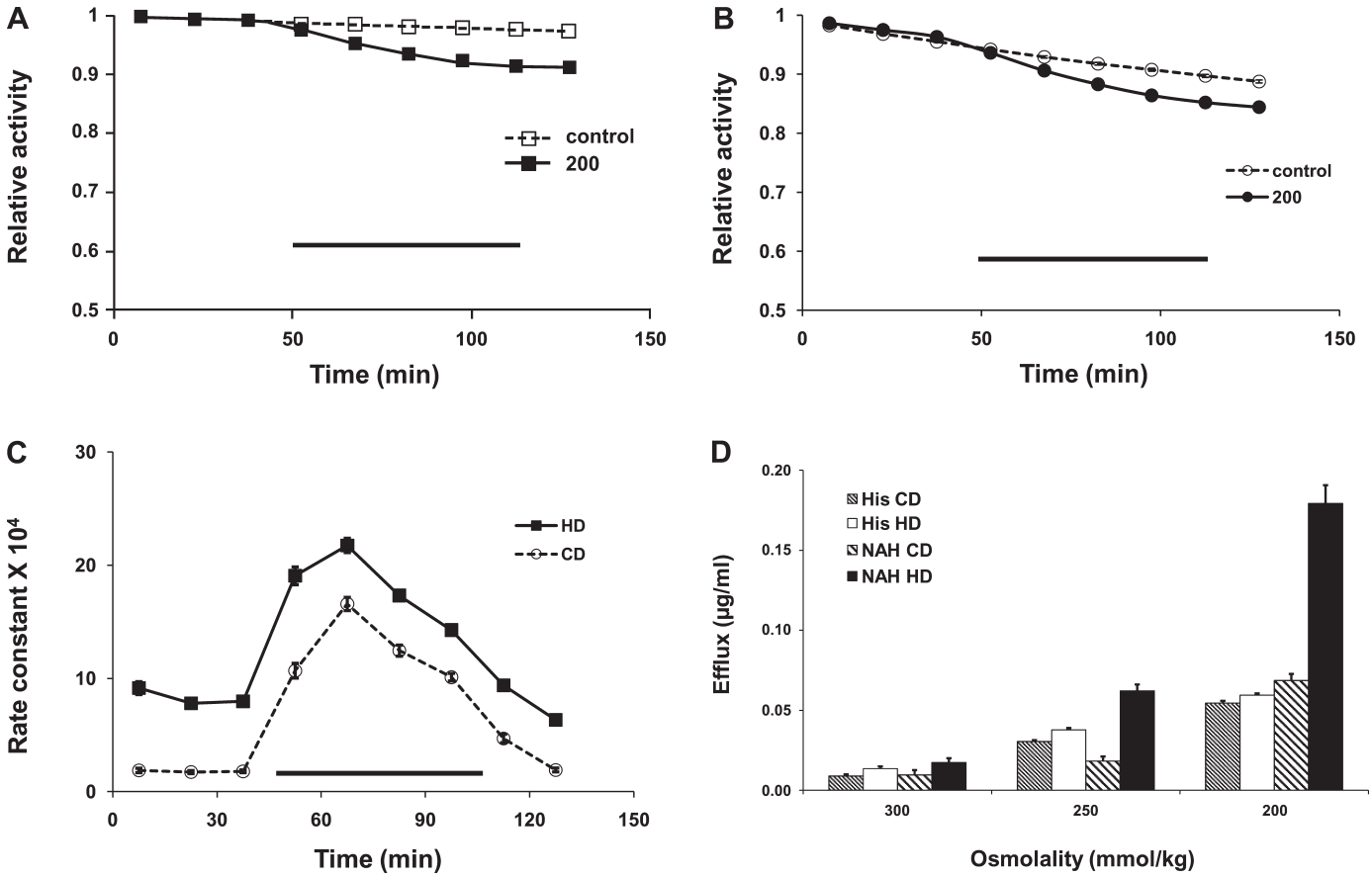

Fig. 4. Efflux of histidine and NAH from cultured lenses of fish fed CD or HD diets, exposed to hypoosmotic conditions. *A–C*: [^{14}C]-histidine efflux from lenses exposed to hypoosmotic (200 mmol/kg) conditions (1 h) denoted by black bar. *A* and *B*: change in relative activity of [^{14}C]-histidine (content in lens) in CD (*A*) and HD (*B*) lenses. *C*: stimulation of [^{14}C]-histidine efflux following exposure to hypoosmotic conditions. *D*: efflux of histidine and NAH measured by HPLC from lenses exposed to hypoosmotic medium for 24 h. Error bars, means ± SE; $n = 6$.

The rapid change in AH osmolality (~20 mmol/kg) when fish were transferred from freshwater to seawater (Fig. 1) shows that the lens is acutely exposed to osmotic stresses during the life cycle of farmed salmon. Using in vitro culture techniques, we also show that exposure to hyperosmotic media causes an immediate decrease in transparency that is comparable to the osmotic cataracts observed in vivo (9, 22). The cultured lenses also had a remarkable ability to regulate transparency and volume after exposure to osmotic stress (Fig. 2), enabling us to investigate the mechanisms of osmoregulation in vitro. In an earlier study (29), no significant changes in lens opacity or volume were observed after 24 h in hyperosmotically challenged lenses. From the present study, however, we now know that within this period the lenses do experience loss of transparency and decrease in volume, but that they show a remarkably rapid recovery despite continued exposure to hyperosmotic stress. In the mammalian lens, it has been shown that permanent damage to cortical fiber cell structure occurs when the mechanisms of lens volume regulation are inhibited (15). In this study, the fact that transparency was recovered and the highly organized architecture of the lens was maintained is evidence that the lenses regulated volume by active cellular mechanisms and remained viable despite long-term exposure to osmotic stress. Significantly, such regulation of volume and transparency did not occur in lenses exposed to hyperosmotic solutions of 600 mmol/kg, indicating that if the volume regulation processes are overwhelmed, recovery does not occur.

We did not observe a significant change in volume in lenses exposed to hypoosmotic conditions, although, there was a trend toward volume increase (data not shown) and this is in agreement with a previous study (29). It has recently been shown that in hypoosmotic conditions rabbit and cow lenses changed shape primarily in the posterior-to-anterior axis with little or no change in the equatorial axis (25). It is important to note that nonrodent mammalian lenses are often ellipsoid in shape and relatively soft compared with the nearly spherical and relatively rigid teleost lens in which volume changes would be manifest as an even change in diameter along all axes. Also in contrast to mammalian lenses, in which accommodation is achieved by shape change, in the teleost eye accommodation is achieved by movement of the lens toward or away from the retina (28). The lens is held firmly in place by a number of suspensory ligaments attached to the lens capsule (24) and is pulled backward against the resistance of the vitreous humor by the lens muscle. It can be argued that the relatively rigid nature of the teleost lens is a consequence of this mechanism, as shape distortion when the lens is pulled by the lens muscle would cause unwanted errors in accommodation. Nevertheless increased osmotic pressure within the fish lens as a consequence of exposure to hypotonic solution would be damaging,

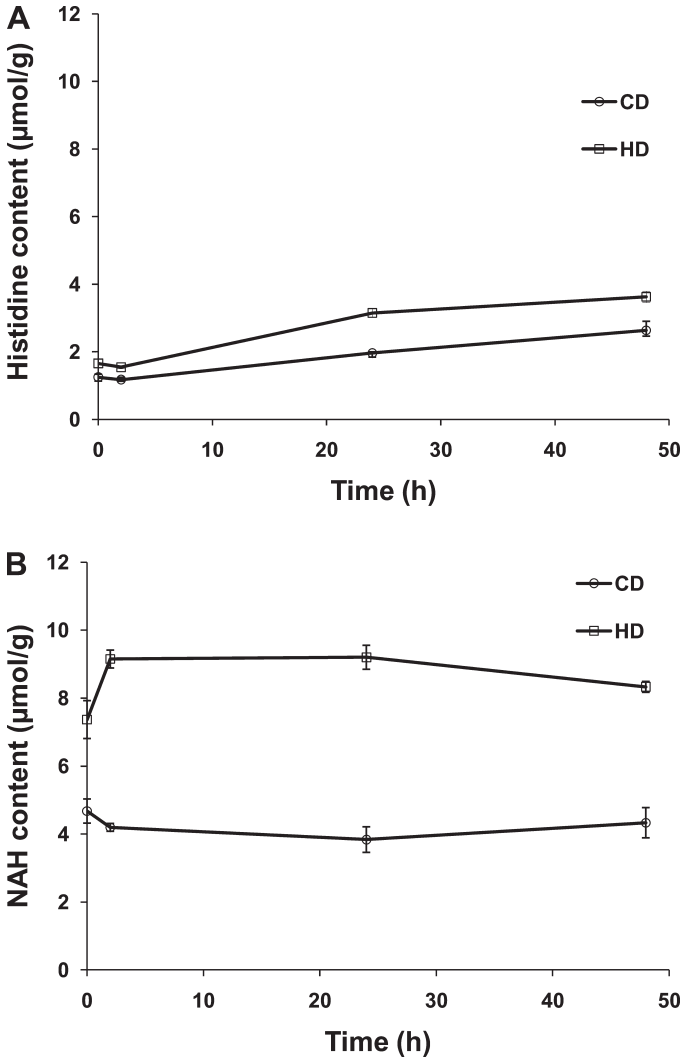

Fig. 5. Effect of environmental osmolality on lens histidine (*A*) and NAH (*B*) in smolts transferred from FW to SW. Histidine and NAH were measured by HPLC. Error bars, means ± SE; $n = 15$.

and mechanisms to equilibrate the osmolality of the internal environment must, therefore, exist.

In common with most animal cells, the lens is highly permeable to water via aquaporin channels (18), and cell volume is determined by the extracellular osmolality and the cell's content of osmotically active molecules. The immediate response of cells exposed to an osmotic change in their environment is to allow ions, osmolytes, and water to flow across the plasma membrane. To avoid having to maintain a high concentration of ions in the intracellular environment, during long-term exposure to hyperosmotic conditions, cells utilize organic molecules, termed compatible osmolytes, which do not interfere with normal cellular function (21). Taurine, for example, has been shown to be taken up and released by mammalian lens cells in response to osmotic changes in the external medium (10), but, interestingly, has been shown not to change in the lenses of salmon in seawater compared with freshwater (7). Other osmolytes, which have roles in volume regulation in mammalian lenses, include sorbitol and myoinositol (11, 12). In fish, both taurine and myoinositol have been shown to play an important role in the response of tilapia (*Oreochromis mossambicus*) to fluctuations in environmental salinity (17). In different tissues, different compatible osmolytes were important, with myoinositol responding in brain and kidney, and taurine and glycine in muscle and liver. Tissue differences are further highlighted in studies investigating enzymes associated with myoinositol synthesis following acclimation to seawater in the European eel (*Anguilla anguilla*) (23). Myoinositol monophosphatase is upregulated in gills and kidney, but there was no change in expression in intestinal tissue.

We have previously suggested that in the salmon lens, NAH could act as an osmolyte (7, 8). Here we investigate this role in detail to provide definitive evidence of the osmoregulatory role of NAH. We observed a correlation with osmolality and NAH content in cultured lenses (Fig. 3) in line with the increase already observed in vivo (7) and our previous observations (29). For NAH to have an osmolyte role, it must be able to respond dynamically to osmotic stress. On exposure to hypoosmotic stress, there was a rapid, regulated efflux of [^{14}C]-histidine from the lens (Fig. 4*C*), and by HPLC we specifically identified a greatly increased NAH efflux. Remarkably, a 10-fold increase in NAH efflux in response to a −100 mmol/kg change was observed from HD lenses compared with only threefold from CD lenses. A major indication that NAH has a role in osmoregulation in the lens came from the seawater challenge test (Fig. 5) in which we show that in vivo, lens NAH responded rapidly to changes in AH osmolality (Fig. 5*B*) in the HD fish. Taken together, we believe that these data present compelling evidence that NAH is a primary osmolyte in the salmon lens, directly balancing extracellular osmolality. Furthermore, given the high water permeability of the lens and the NAH levels involved, there is no evidence to support its role as a molecular water pump (2).

The role of NAH as an osmolyte in other tissues has not been specifically investigated, but interestingly, there is no detectable NAH in the muscle of salmon (7), although, it has been detected in skeletal muscle of a number of freshwater species (32). However, another histidine metabolite, anserine (β-alanyl-*N*-methyl-histidine) is found in muscle and increases in seawater compared with freshwater in fish with a histidine-supplemented diet (7). Taurine, on the other hand, is decreased in seawater compared with freshwater in fish from both dietary backgrounds. This indicates species differences (17) and further highlights the importance of histidine in osmoregulation in Atlantic salmon.

Differences in responses to osmotic stress in lenses of salmon fed different levels of dietary histidine add further support to the argument for NAH as an osmolyte. The seawater challenge test shows that the lenses of both dietary groups were exposed to similar osmotic stress, and therefore any differences that may predispose lenses to cataract were at the level of the lens and were not due to perturbations in gill function, for example. However, lenses of CD fish contained less NAH at the outset, and this did not increase even after 48 h in seawater. In contrast, lens NAH in HD fish increased in response to the increase in AH osmolality. The in vitro data also highlighted differences between the two feed groups, with efflux of NAH on hypoosmotic challenge being much greater from HD lenses. Such a clear difference between the two dietary groups in the cultured lenses is surprising since lenses from both feed groups were maintained under the same conditions for 1 wk prior to the experiments. CD lenses accumulated histidine at a greater rate and had a resting efflux that was 80% lower than the HD

lenses (Fig. 4*C*). This increased demand revealed a histidine deficit that had not been redressed even after a protracted period of culture.

Interestingly, the osmolality of AH and the level of lens NAH recorded in smolts prior to seawater transfer was comparable to that measured in fish that had been in seawater for an extended period (Fig. 1*A*). This suggests that changes in AH and lens osmolality are part of a preadaptive developmental process and are not an acute response to seawater exposure. We note, however, that the initial exposure to seawater resulted in a transitory increase in AH osmolality and a parallel increase in lens NAH (Fig. 5*B*). Developmental preconditioning may, therefore, serve to limit the osmotic stress to which the lens is unavoidably exposed. One can speculate that once in the sea, wild fish may seek out an environment of stable salinity (i.e., deeper water) and thus avoid exposure to large fluctuations in osmotic stress. The environment of farmed salmon, in cages at the sea surface, may represent an osmotically variable environment to which wild fish would only rarely be exposed.

Perspectives and Significance

In experiments reported here, we show that NAH acts as an osmolyte in the salmon lens. More than this, it appears to be the major osmolyte that balances increases in AH osmolality when fish move from freshwater to seawater. This is a critical role in an anadromous fish. A deficiency in NAH would lead to a dysfunction of the normal osmoregulatory processes in the lens, and we propose that this would contribute to cataract formation in fish deficient in histidine.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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