Diminished social status affects ionoregulation at the gills and kidney in rainbow trout (*Oncorhynchus mykiss*)

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Abstract: Competition for social status can result in physiological differences between individuals, including differences in ionoregulatory ability. Subordinate rainbow trout (*Oncorhynchus mykiss*) had two-fold higher uptake rates of sodium across the gill and two-fold higher whole-body sodium efflux rates than the dominant fish with which they were paired. Sodium efflux was then divided into branchial and renal components, both of which were higher in subordinates. Branchial sodium efflux accounted for 95%–98% of sodium loss. Plasma sodium concentrations were more variable, although not significantly different, in subordinate fish, suggesting that the increased loss of sodium in these trout is compensated for by an increase in uptake rates. Urine flow rates and plasma cortisol concentrations were higher in subordinate fish, but there was no difference in glomerular filtration rate between dominants and subordinates. Renal sodium reabsorption was significantly reduced in subordinates. In summary, the ionoregulation of subordinate individuals was altered, most likely occurring as a result of stress-induced changes in gill permeability, resulting in a higher throughput of water and increased branchial sodium efflux. These changes in ionoregulatory ability have many physiological implications, including the increased susceptibility of subordinates to ionoregulatory challenges and an increased metabolic cost of ionoregulation.

Résumé : La compétition pour le statut social peut entraîner des différences physiologiques entre les individus, dont des différences dans la capacité d'ionorégulation. Les truites arc-en-ciel (*Oncorhynchus mykiss*) subordonnées ont une absorption deux fois plus grande de sodium par les branchies et des taux d'élimination du sodium au niveau du corps entier deux fois plus importants que les poissons dominants avec lesquelles elles sont appariées. Le sodium éliminé se divise en une composante branchiale et une composante rénale, qui sont toutes deux plus importantes chez les poissons subordonnées. L'élimination du sodium par les branchies représente 95 % à 98 % de la perte de sodium. Chez les poissons subordonnées, les concentrations plasmatiques de sodium sont plus variables, mais elles ne sont pas significativement différentes de celles des dominants, ce qui laisse croire que la perte accrue de sodium chez ces truites est compensée par des taux d'absorption plus élevées. Les taux d'écoulement de l'urine et les concentrations plasmatiques de cortisol sont plus élevés chez les poissons subordonnés, mais il n'y a pas de différences dans les taux de filtration glomérulaire entre les dominants et les subordonnés. La réabsorption de sodium par le rein est significativement réduite chez les subordonnés. En bref, l'ionorégulation des truites subordonnées est modifiée à cause très probablement de changements dans la perméabilité des branchies produits par le stress, ce qui entraîne un passage accru de l'eau et une élimination plus importante du sodium. Ces changements de la capacité ionorégulatrice ont plusieurs conséquences physiologiques, dont une susceptibilité accrue, chez les subordonnés, aux stress d'ionorégulation et un coût métabolique plus grand de l'ionorégulation.

[Traduit par la Rédaction]

Introduction

The stress encountered by a subordinate fish within a dominance hierarchy is sufficient to alter its physiology (reviewed by Sloman and Armstrong 2002). Recently, Sloman et al. (2002*a*) found that subordinate rainbow trout (*Oncorhynchus mykiss*) have higher branchial sodium uptake rates than their dominant counterparts and that this ionoregulatory disturbance can have implications for the accumulation of waterborne toxicants that enter via the sodium uptake path-

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way. However, the ionoregulatory differences that exist between dominant and subordinate animals are not clearly understood and it is not known whether this higher sodium uptake is driven by higher sodium efflux across the gills and (or) a difference in kidney function.

Salmonid fish form linear dominance hierarchies (Bachman 1984; Metcalfe et al. 1989) where position in the social hierarchy will denote both characteristic behaviours and physiological changes. These dominance hierarchies can be successfully replicated in the laboratory and when two fish are confined together, one fish will become dominant over the other. The present study aimed to examine sodium uptake and efflux and renal function in dominant and subordinate rainbow trout. Sodium crosses the apical membrane of the gill epithelia through a sodium channel driven by proton extrusion and is then transported across the basolateral membrane by Na^+/K^+-ATPase (Avella and Bornancin 1989; Lin and Randall 1995; Bury and Wood 1999). The stress response, i.e., to handling or confinement, is known to disturb sodium regulation (Mazeaud and Mazeaud 1981).

Loss of sodium is via two routes, branchial and renal. Sodium is lost by diffusion across the gills, accounting for approximately 90% of sodium loss at rest and even more during stress (see review by McDonald and Milligan 1997). Although usually a minor route of sodium efflux, sodium is also lost in the urine. In the majority of teleost fish, paired mesonephric ducts leave the kidney and unite in a structure known as the urinary bladder that serves as a functional extension of the kidney (Hickman and Trump 1969). Urine is stored in the urinary bladder for approximately 25–30 min before it is discharged via the urogenital papilla in periodic bursts (Curtis and Wood 1991). During this period of residence in the urinary bladder, ions can be reabsorbed to aid the kidney in salt conservation and this function of the urinary bladder may cut urinary losses of NaCl by at least 40% (Curtis and Wood 1991). Stress can also cause an increase in urine flow rate (diuresis) (Wood and Randall 1973; Vermette and Perry 1987), which along with decreased reabsorption of electrolytes in the kidney tubules and the urinary bladder can lead to an increased urinary sodium loss (Vermette and Perry 1987).

The present study aimed to identify and quantify any socially mediated differences in sodium balance at the gills and kidney as well as to assess the effects of social stress on kidney function using radioisotopic measurements of sodium turnover and renal processing. It was hypothesized that the chronic stress experienced by subordinate trout would severely impact upon ionoregulatory ability.

Materials and methods

Rainbow trout (weight 211.9 ± 6.8 g, fork length 26.7 ± 0.4 cm) were obtained from Humber Springs Trout Hatchery (Thamesford, Ont.) and held in 500-L stock tanks supplied with aerated, flowing, dechlorinated City of Hamilton tap water (hardness = 120 $mg \cdot L^{-1}$ as $CaCO_3$, Na^+ = 0.6 $mmol \cdot L^{-1}$, Cl^- = 0.7 $mmol \cdot L^{-1}$, Ca^{2+} = 1.0 $mmol \cdot L^{-1}$, 12 °C, pH = 8.0). Fish were fed to satiation once daily with commercial trout food (Martin Mills Inc., Elmira, Ont.). Feeding was stopped 24 h prior to experiments. Three experiments were carried out to investigate sodium turnover in relation to social status. The first documented sodium uptake in dominant and subordinate fish. The second measured whole-body sodium excretion (branchial and renal combined). Finally, kidney function of dominant and subordinates was examined by measuring urinary sodium excretion, urinary flow rate (UFR), and glomerular filtration rate (GFR) using [1,2-^{3}H]-polyethylene glycol ([^{3}H]PEG-4000) as a marker for urination events. All experiments were carried out according to the guidelines of the Canadian Council for Animal Care.

Behavioural observations

During each observation period, the fish were scored according to three variables: position, colour, and food acquisition. Fish swimming in the water column scored three points, fish resting on the bottom of the tank scored two points, and fish swimming at the water surface scored one point. Swimming at the water surface is characteristic of subordinate fish (Sloman et al. 2000*a*). Where there was a colour difference between the pair of fish, the lighter fish scored one point and the other zero points, as darkening in colouration is indicative of subordinance (Keenleyside and Yamamoto 1962; O'Connor et al. 1999). Finally, during each behavioural observation, a single food item was introduced into the tank. The fish that acquired the food item scored one point and the other fish zero points. With the exception of this one food item (given three times a day at each behavioural observation), fish were not fed during the experiment to minimize the effect of sodium uptake from the diet. At the end of the experiment, the fish within each pair with the higher behavioural score was identified as the dominant fish.

Branchial sodium uptake rates

Eighteen rainbow trout were removed from the stock tank, anaesthetized in benzocaine (0.05 $mg \cdot mL^{-1}$), and individually marked with alcian blue dye injected into their fins (Kelly 1967). Initial fork lengths and weights were recorded and fish were allocated to size-matched pairs (mean size difference = 0.6 ± 0.1 cm), as used in many other behavioural studies (Abbott and Dill 1989; Øverli et al. 1999; Sloman et al. 2000*a*). Each pair of fish was placed in a 26-L plastic tank and individuals were separated from each other by an opaque plastic partition. The stocking density was therefore approximately 15 $g \cdot L^{-1}$, which is similar to other studies using an equivalent size of trout to examine social interactions (Sloman et al. 2002*b*). Following 24 h of acclimation to the tank, the plastic partitions were removed and the fish allowed to socially interact. Behavioural observations were made three times daily (0900–0930, 1300–1330, and 1700–1730) to determine the dominant and subordinate fish of each pair (Behavioural observations section). Sodium uptake was measured in the two fish simultaneously while social interaction continued. In detail, after 48 h of social interaction, the water flow to the tanks was stopped and ^{22}Na (1 $\mu Ci \cdot L^{-1}$ (37 kBq)) (NEN-Dupont, Boston, Mass.) was added to the tank. Water samples were taken 15 min and 4 h after the addition of the isotope and analysed for both ^{22}Na radioactivity and total sodium concentration using an 8-cm-well NaI crystal gamma counter (Canberra Packard MINAXI Auto

Gamma, 5000) and flame atomic absorption spectrophotometer (Varian AA-220), respectively.

Four hours after the addition of isotope, a lethal dose of benzocaine was added to the tank (0.5 mg·mL^{-1}), which killed the two fish within 3 min. The fish were then removed and placed in a "cold" displacement rinse of sodium (100 mM) to remove any surface-bound ^{22}Na. The whole fish were cut up and then homogenized separately in a blender and 10 preweighed plastic scintillation vials filled with homogenate. The vials were reweighed and then counted for ^{22}Na activity with the same method as for water samples. Uptake rates of sodium were calculated as

$$\text{Uptake} = [(a/b)/c]/d$$

where a is total counts per minute in the fish, b is mean specific activity of the water (activity per unit concentration), c is weight of the fish (grams), and d is duration of exposure (hours) (Grosell et al. 1998).

Whole-body sodium efflux

Nine pairs of size-matched fish (mean size difference = 0.7 ± 0.1 cm) were marked as outlined previously. Each pair was placed in a 26-L tank and the dominant and subordinate individual of each pair identified as in the previous experiment. However, it was not possible to measure sodium efflux separately in the two fish while social interaction continued. Instead, following 48 h of social interaction as before, the fish were removed from the tank and anaesthetized in a solution of benzocaine (0.05 mg·mL^{-1}). Each fish was then injected with a dose of 50 μCi of ^{22}Na in 0.5 mL of Cortland saline (Wolf 1963) into the caudal vein as a marker for the appearance of sodium in the water. Fish were then placed individually in 5-L black Perspex "flux" boxes (9 cm × 35 cm × 16 cm) (McDonald and Rogano 1986) and supplied with flowing, aerated City of Hamilton tap water for a 14-h recovery period.

Following the recovery period, the inflow to the flux boxes was stopped, although aeration was continued, and a 5-mL water sample was taken every hour for the following 8-h period. A regression analysis was carried out to calculate the rate of appearance of ^{22}Na in the water. At the end of the 8-h sampling period, a lethal dose of benzocaine was added to the flux boxes (0.5 mg·mL^{-1}) and the fish removed. A blood sample was immediately withdrawn by caudal venipuncture into a heparinized syringe and centrifuged at 13 000g for 2 min. Half of the plasma was placed in a vial for later analysis of ^{22}Na activity and the remainder frozen in liquid nitrogen for later analysis of plasma total sodium concentration.

^{22}Na activity was quantified in both water and plasma samples by counting in a gamma counter. Sodium concentrations in the water and plasma were determined by flame atomic absorption spectrophotometry. Whole-body sodium efflux rates were calculated as

$$\text{Efflux} = (a/b)/c$$

where a is the rate of appearance of sodium in the water (counts per minute per hour), b is the plasma specific activity (counts per minute per micromole), and c is the weight of the fish (kilograms).

Renal sodium excretion and kidney function

Twenty-two rainbow trout were removed from the stock tank, anaesthetized, and marked in the same way as before. Size-matched pairs of fish (mean size difference = 0.3 ± 0.1 cm) were then allocated to 26-L plastic tanks and the dominant and subordinate fish within each pair determined as in the previous experiment. The disturbance and time (at least 48 h needed for postsurgery recovery; Wood and Patrick 1994) involved in implantation of urinary catheters for direct collection of excreted urine (Curtis and Wood 1991) would likely have obliterated any effects of dominance or subordinance on renal function. Therefore, the indirect method of Curtis and Wood (1991) for assessing urinary excretion was employed.

In detail, after 48 h of social interaction, fish were drawn from the tank and rapidly anaesthetized in benzocaine (0.05 mg·mL^{-1}). Fish were then injected with a dose of 17 μCi of [^{3}H]PEG-4000 (New England Nuclear; half life ~12.3 years) in 0.66 mL of Cortland saline (Wolf 1963) into the caudal vein as a marker for GFR and for the appearance of urine in the water. Fish were then placed individually in 5-L black Perspex "flux" boxes and supplied with flowing, aerated City of Hamilton tap water for a 14-h recovery period.

Following the recovery period, the inflow to the flux boxes was stopped and a 5-mL water sample was taken every 5 min for the following 8-h period. At the end of the 8-h sampling period, a lethal dose of benzocaine was added to the tank (0.5 mg·mL^{-1}) and the fish removed and immediately sampled for blood and urine. The blood samples were taken by caudal venipuncture as before. Half of the plasma was placed in a scintillation vial for later analysis of [^{3}H]PEG-4000 radioactivity and the remainder was frozen in liquid nitrogen and stored at –80 °C for later analysis of plasma ions and cortisol. A urine spot sample was then taken using a 30-cm piece of Clay-Adams PE-60 tubing with one end heat-flared to 1.5 times its original diameter and the other attached to a 1-mL syringe. The flared end of the catheter was inserted through the urogenital papilla into the urinary bladder and a slight suction applied to drain the urinary bladder as completely as possible (Curtis and Wood 1991). The urine sample was divided in the same way as the plasma and stored for later analysis of ion concentrations and isotope activity.

[^{3}H]PEG-4000 activities were quantified in water, urine, and plasma samples by scintillation counting. Plasma and urine samples were diluted with nanopure water and 5 mL of sample added to 10 mL of ACS fluor (Amersham) and then counted in a scintillation counter (LKB Rackbeta 1217 counter). Tests demonstrated that quenching was constant. Plasma and urine ions were determined by flame atomic absorption spectrophotometry. Cortisol concentrations were analysed using a radioimmunoassay (ICN Pharmaceuticals).

[^{3}H]PEG-4000 activity in the water increased with intervening periods of relative consistency as seen in previous studies (Curtis and Wood 1991). These "steps" were interpreted as points of urination, which were considered to have occurred when there was an increase in at least 20 counts·min^{-1} in a 5 mL sample compared with the one taken 5 min earlier. Total [^{3}H]PEG-4000 activity in the water (A_w, in total counts per minute) was calculated at each time

point by multiplying the measured [^{3}H]PEG-4000 activity by the flux box volume. Individual urination burst volumes (V_{burst}) were calculated for each step increase in counts as

$$V_{burst} = ((A_{w_n} - A_{w_{n-1}})/A_u)/\text{mass}$$

where A_u is the measured [^{3}H]PEG-4000 activity in the urine in counts·min^{-1}·mL^{-1}. The average urination burst volume and the frequency of urination bursts were then calculated for each fish.

UFR was calculated as

$$\text{UFR} = \frac{\sum V_{burst}}{\text{time}}$$

and GFR was calculated as

$$\text{GFR} = \frac{\text{UFR} \times A_u}{A_p}$$

where A_p is the measured [^{3}H]PEG-4000 activity in the plasma in counts·min^{-1}·mL^{-1}. Extrarenal clearance rate (ECR) could then be estimated as

$$\text{ECR} = \frac{A_w - \left(\sum V_{burst} \times A_u\right)}{A_p \times \text{mass} \times \text{time}}$$

where A_w (total counts·min^{-1}) is the total [^{3}H]PEG-4000 activity appearing in the water over the 8-h period. Calculation of ECR assumes that any appearance of [^{3}H]PEG-4000 that did not occur in the periodic "steps" represented extrarenal excretion.

Urinary excretion rates (U) of sodium and potassium (X) were then calculated according to McDonald and Wood (1998) using the following equation:

$$U_X = [X]_u \times \text{UFR}$$

where $[X]_u$ is the concentration in the urine.

Clearance rates (C) were calculated as

$$C_X = \frac{[X]_u \times \text{UFR}}{[X]_p}$$

where $[X]_p$ was the measured plasma concentration. Finally, clearance ratios (CR_X) for both sodium and potassium were calculated, relating the clearance of these ions to the GFR (i.e., to the clearance of [^{3}H]PEG-4000) (McDonald and Wood 1998):

$$CR_X = \frac{C_X}{\text{GFR}}$$

The lower the clearance ratio, the higher the efficiency of reabsorption. For example, a CR_X of 0.1 would represent 90% reabsorption of filtered X by the renal system. The clearance ratio of water was calculated as

$$CR_{H_20} = \frac{\text{UFR}}{\text{GFR}}$$

Statistical analysis

Data are given as means ± SE of the mean. Physiological measurements were compared between pairs of fish using Wilcoxon signed ranks test. SPSS® software was used for statistical analyses and the limit of significance in all analyses was $p < 0.05$.

Results

Subordinate fish had a twofold higher branchial sodium uptake rate of 730 μequiv.·kg^{-1}·h^{-1} when compared with 390 μequiv.·kg^{-1}·h^{-1} in dominant fish ($p = 0.038$) (Fig. 1).

When total sodium loss was measured using injected ^{22}Na as a radiotracer, subordinate fish had a higher overall loss of sodium when compared with dominants, the values being –744 and –324 μequiv.·kg^{-1}·h^{-1}, respectively ($p = 0.008$) (Fig. 2*a*), which were very comparable with the difference in uptake rates (Fig. 1).

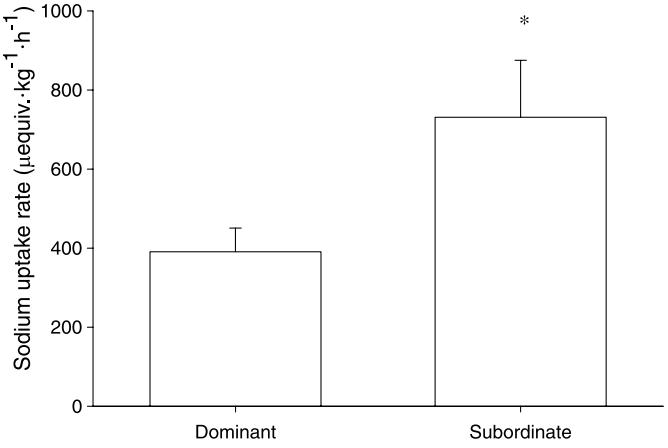
Subordinate fish in the experiments using injected [^{3}H]PEG-4000 to measure renal clearance had significantly higher plasma cortisol concentrations ($p = 0.012$) (Table 1) and significantly higher concentrations of sodium in their urine ($p = 0.021$) (Table 1). However, urine potassium levels and plasma ion levels were not significantly affected by social status (urine K^+, $p = 0.123$; plasma Na^+, $p = 0.081$; plasma K^+, $p = 0.177$; plasma Ca^{2+}, $p = 0.161$) (Table 1). There was a trend towards lower and more variable plasma sodium concentrations in subordinates, the values being 137.5 ± 5.3 μequiv.·L^{-1} in dominants and 111.7 ± 15.1 μequiv.·L^{-1} in subordinates (Table 1).

In general, urinary parameters were also more variable in subordinate fish. There were nonsignificant trends towards a higher average frequency and a higher average volume of urination bursts in subordinates (Table 1), which, in combination, resulted in significantly higher UFR in subordinate animals ($p = 0.012$) (Fig. 3*a*). Social rank did not affect GFR (Fig. 3*b*) or ECR (Table 1). Subordinate fish had significantly higher sodium (Fig. 2*b*) and potassium urinary excretion rates (Table 1) when compared with dominant fish (Na^+, $p = 0.018$; K^+, $p = 0.043$). Urinary sodium excretion rates were 31.7 μequiv.·kg^{-1}·h^{-1} in subordinates compared with 8.0 μequiv.·kg^{-1}·h^{-1} in dominant fish (Fig. 2*b*). Branchial sodium efflux was estimated (by subtraction of urinary sodium excretion rates from total sodium loss in the previous experiment) at 712 μequiv.·kg^{-1}·h^{-1} in subordinates and 316 μequiv.·kg^{-1}·h^{-1} in dominants (Fig. 2*c*). Subordinate trout also had significantly higher sodium clearance ratios when measured against the glomerular filtration marker [^{3}H]PEG-4000 (Na^+, $p = 0.018$) (Fig. 4), but potassium and water clearance ratios were not significantly different (Fig. 4). Sodium reabsorption efficiency by the renal system therefore fell from 99% in dominants to 87% in subordinates.

Discussion

The present study clearly illustrates that the branchial influx and efflux and renal clearance of sodium differ dramatically between subordinate and dominant animals. Firstly, subordinate animals demonstrate an elevation in sodium influx. Sodium influx rates for unstressed adult trout in Hamilton tap water are usually around 320 μequiv.·kg^{-1}·h^{-1} in fish of similar size (Goss and Wood 1990), which was approximately the same as seen in dominant fish (390 μequiv.·kg^{-1}·h^{-1}) of the present study. Higher sodium influx rates as a result of subordination have been illustrated previously in smaller fish (~5 cm)

Fig. 1. Sodium uptake rates for dominant and subordinate fish measured using the radiotracer ^{22}Na. The asterisk denotes a significant difference (Z = –2.073, p = 0.038; n = 9 pairs of fish).

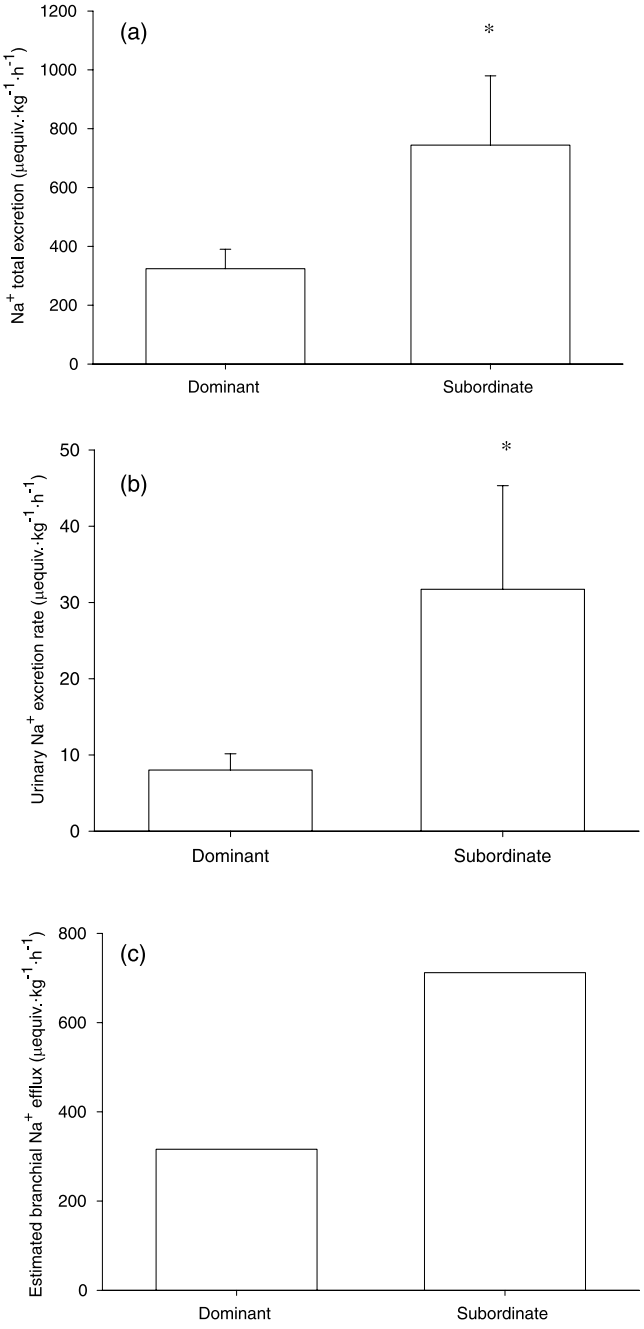


and have implications for the accumulation of toxicants that cross the gill via apical sodium channels (Sloman et al. 2002*a*). Total sodium efflux was comparably higher in subordinates when compared with dominants. The sodium influx and efflux calculations of the present study are based on different pairs of fish, but the effect of social status on sodium turnover appears relatively constant under these conditions with values for influx and efflux being comparable from both experiments.

Calculation of the urinary sodium excretion rate allowed for the division of total sodium loss into branchial and renal components. Urinary sodium excretion rates were higher in subordinates when compared with dominants, as was branchial sodium efflux. Urinary sodium excretion accounted for approximately 5% of sodium loss in subordinates and only 3% in dominant fish. Total sodium efflux rates of dominant fish were around 324 μequiv.·kg^{-1}·h^{-1}, which is comparable with sodium efflux rates of nonstressed fish of comparable size from previous studies (220 μequiv.·kg^{-1}·h^{-1}; Goss and Wood 1990). Urinary sodium excretion rates of nonstressed fish are approximately 5–8 μequiv.·kg^{-1}·h^{-1} (Curtis and Wood 1991), which is similar to the urinary excretion rate of dominant fish in the present study (8.0 μequiv.·kg^{-1}·h^{-1}). Plasma ions were not significantly different between dominant and subordinate fish, although there was a trend towards lower and more variable plasma sodium in subordinates. The greater loss of sodium from subordinates is counteracted to a variable degree by the higher sodium uptake rates.

Branchial sodium efflux was estimated to be around 712 μequiv.·kg^{-1}·h^{-1} in subordinates compared with 316 μequiv.·kg^{-1}·h^{-1} in dominants. Subordinate fish also had a higher frequency and average volume of urination bursts, resulting in a significantly higher UFR, which is symptomatic of stress-induced diuresis (Wood and Randall 1973; Vermette and Perry 1987). Subordinate animals demonstrate many physiological changes indicative of a stress response (Pottinger and Pickering 1992; Øverli et al. 1999; Sloman et al. 2000*a*). The major hormones released during the stress response are catecholamines and cortisol. Significantly elevated concentrations of plasma cortisol have been

Fig. 2. (*a*) Whole-body sodium loss, (*b*) sodium urinary excretion rates, and (*c*) estimated branchial sodium efflux (whole-body minus urinary excretion) for dominant and subordinate fish measured using ^{22}Na. The asterisks denote a significant difference (whole-body sodium loss, Z = –2.666, p = 0.008; sodium urinary excretion rates, Z = –2.521 p = 0.012; n = 9–11 pairs of fish).



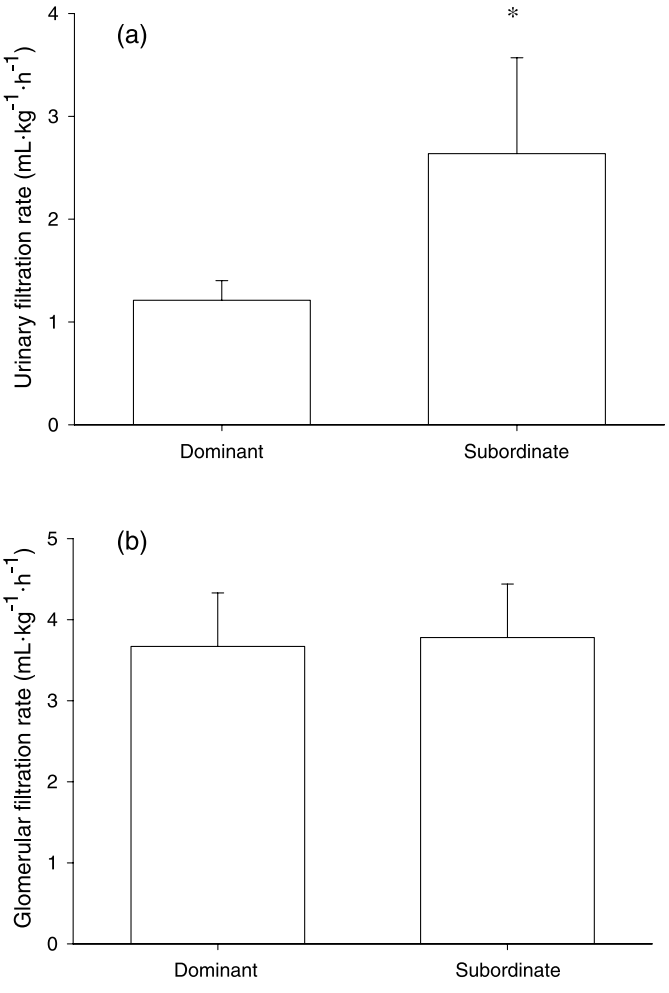
widely documented in subordinate animals (reviewed by Sloman and Armstrong 2002), and in the present study, subordinate fish had higher plasma cortisol concentrations than their dominant counterparts. Plasma cortisol concentrations measured here are slightly higher than would be expected in unstressed fish (0–5 ng·mL^{-1}; Pickering and Pottinger 1989) but this is not surprising, as confinement in a flux box would act as an additional stressor. However,

Table 1. Plasma cortisol, urine and plasma ions, extrarenal [^{3}H]PEG-4000 clearance rates (ECR), and water clearance ratios for dominant and subordinate fish (n = 11 pairs of fish).

	Dominant	Subordinate	Statistical values
Plasma cortisol (ng·mL^{-1})	16.60±5.23	46.63±8.17	Z = –2.521, p = 0.012*
Urine Na^+ (mequiv.·L^{-1})	6.5±1.2	22.1±8.1	Z = –2.310, p = 0.021*
Urine K^+ (mequiv.·L^{-1})	0.16±0.01	0.4±0.1	Z = –1.540, p = 0.123
Plasma Na^+ (mequiv.·L^{-1})	137.5±5.3	111.7±15.1	Z = –1.376, p = 0.169
Plasma K^+ (mequiv.·L^{-1})	3.2±0.2	4.0±0.1	Z = –1.459, p = 0.646
Plasma Ca^{2+} (mequiv.·L^{-1})	2.6±0.1	2.3±0.2	Z = –1.580, p = 0.114
Average V_{burst} volume (mL·kg^{-1})	1.05±0.15	1.90±0.95	Z = –0.840, p = 0.401
V_{burst} frequency (h^{-1})	1.17±0.12	1.63±0.28	Z = –1.825, p = 0.068
ECR (mL·kg^{-1}·h^{-1})	2.7±1.3	1.5±0.4	Z = –0.700, p = 0.484
K^+ urinary excretion rate (μequiv.·kg^{-1}·h^{-1})	2.0±0.3	3.0±1.0	Z = –2.023, p = 0.043*

Note: Statistical values are for Wilcoxon signed ranks tests and data are given as means ± SE of the mean. Asterisks denote statistically significant differences.

Fig. 3. (*a*) Urinary filtration rate and (*b*) glomerular filtration rate (measured using [^{3}H]PEG-4000) of dominant and subordinate fish. Asterisks denote significant differences (urinary filtration rate, Z = –2.521, p = 0.012; glomerular filtration rate, Z = –0.980, p = 0.327; n = 11 pairs of fish).



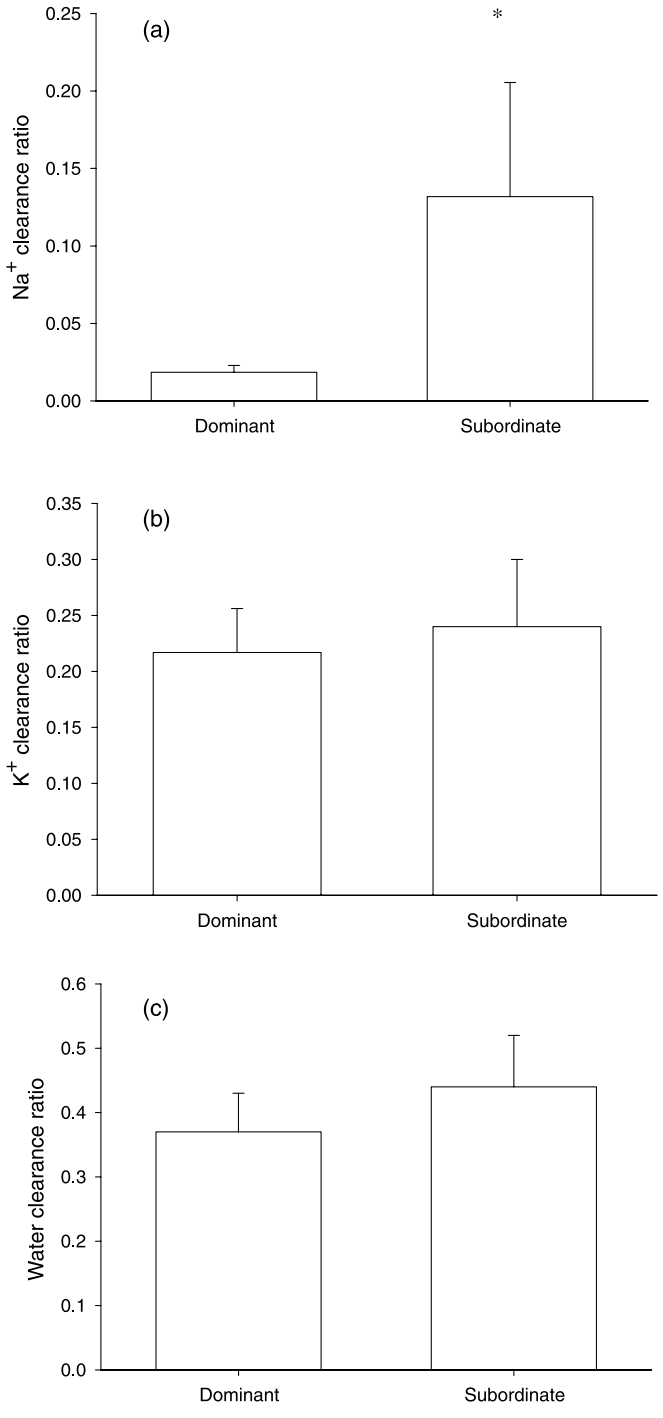
both dominant and subordinate fish would receive the same level of stress and the fact that values of sodium influx and efflux rates are comparable from the two experiments, one using flux boxes and the other not, suggests that this additional stressor did not greatly affect the results. While no significant effect of social status on catecholamine secretion rates upon stimulation of the chromaffin cells in situ with acetylcholine has been demonstrated (Sloman et al. 2002*b*), catecholamine secretion in vivo during social interactions in fish has not been examined. However, social interactions are known to affect catecholamine levels in mammals (Von Holst 1998), and therefore, it is likely that social status will influence catecholamine concentrations in fish as well.

During stress, increased concentrations of catecholamines alter blood flow through the gills, increasing the area available for ion and gas transfer (Randall et al. 1972). This increases gill permeability, in particular by increasing intralamellar pressure (Gonzalez and McDonald 1992), and results in an increase in diffusional water influx and in passive branchial efflux of ions (McDonald and Milligan 1997). During stressful events, this increase in diffusional surface area aids in increased oxygen uptake but leads to an osmorespiratory compromise, as the surface area for passive ion loss is also increased (Randall et al. 1972). Cortisol may also play a role in stress-induced ionoregulatory changes. McDonald and Wood (2004) found that blocking glucocorticoid receptors in rainbow trout reduced UFR but that further increases in cortisol using implants of cortisol had no effect on UFR.

An increase in gill permeability of subordinate trout caused by stress-related hormonal changes would explain the large increase in branchial sodium efflux. Branchial sodium efflux accounted for 95%–98% of overall sodium loss in the present study. It is likely that increased branchial sodium loss is the major driving force behind the changes in ionoregulation seen in subordinate fish, resulting in a compensatory increase in sodium uptake. However, in addition, the sodium clearance ratio of the renal system was significantly higher in subordinates (0.132 compared with 0.018 in dominants). Therefore, subordinate fish have reduced reabsorption efficiency (87% versus 99%) in the renal system, reflecting a change in tubular and (or) urinary bladder reabsorption.

Increased levels of stress hormones would also cause an elevation of diffusional branchial water influx which, coupled with an increase in UFR, suggest a stress-related, increased throughput of water (Wood and Randall 1973; McDonald and Milligan 1997). The elevation of UFR in

Fig. 4. (*a*) Sodium, (*b*) potassium, and (*c*) water clearance ratios of dominant and subordinate fish. Asterisks denote significant differences (Na^+ clearance ratio, $Z = -2.366$, $p = 0.018$; K^+ clearance ratio, $Z = -1.753$, $p = 0.080$; water clearance ratio, $Z = -0.314$, $p = 0.753$; $n = 11$ pairs of fish).



subordinate fish despite no change in GFR also suggests that the reduced reabsorption (i.e., increased excretion) of sodium and possibly other ions such as Cl^- and K^+ increases water loss and hence elevates UFR. The trend towards a higher water clearance ratio in subordinate trout also supports the concept of increased throughput of water as seen in response to other stressors (McDonald and Milligan 1997).

The stress associated with subordination results in an increased branchial efflux of sodium. Urine flow rates are increased and there is a decreased reabsorption of sodium during renal processing. In combination, this increased loss of sodium drives the need for higher uptake of sodium across the gills and possibly the potential for increased sodium uptake from the diet (not measured in the present study). However, subordinate trout acquire a lower share of food among dominance hierarchies (Abbott and Dill 1989), and so, compensation by sodium uptake from the diet seems unlikely. Subordinate fish will also encounter an increased metabolic cost of ionoregulation associated with the increased active uptake of sodium (and potentially other ions such as Cl^-) from their freshwater environment. This is likely to be a contributing factor to the increased metabolic rate associated with subordinate trout following a social encounter (Sloman et al. 2000*b*), which would in turn affect the osmorespiratory compromise (Randall et al. 1972).

Many physiological consequences of social status have been documented in trout and in other animals (reviewed by Sloman and Armstrong 2002), and the present study increases our knowledge of the physiological differences that can exist between fish of different social status. While the social interactions occurring between pairs of fish may be structurally and socially simple compared with those occurring in the natural environment, it is well known that trout exhibit linear social hierarchies in field situations (Bachman 1984; Nakano 1995). Fish held in groups in larger laboratory stream tanks are generally found to exhibit socially mediated variations in physiology, qualitatively similar to those from studies of dyads (Sloman and Armstrong 2002). There are surprisingly few studies on the physiology of fish in relation to dominance hierarchies in natural habitats, but it is likely that many socially mediated differences in physiology found in the laboratory environment, such as those documented in the present study, will play a role in the natural environment.

The ionoregulatory stress, particularly with regard to sodium, seen in the subordinate animals of the present study can therefore have several important implications. Recently, Sloman et al. (2002*a*, 2003) demonstrated that higher sodium uptake rates in subordinate fish leads to greater uptake and accumulation of trace metal contaminants that cross the gill epithelia via the apical sodium channel (i.e., copper and silver). Sloman et al. (2002*a*) found socially mediated differences in copper uptake and accumulation both between pairs of fish and among groups of fish held in a stream tank environment. Animals whose ionoregulatory abilities are already compromised, such as subordinates, would likely have lower thresholds of effect for ionoregulatory toxicants, such as copper and silver (Laurén and McDonald 1985; Morgan et al. 1997). Certainly, under the conditions routinely used for toxicological assays, i.e., a small group of fish confined together in a lentic aquarium, individual differences in ionoregulation caused by the formation of dominance hierarchies have the potential to affect toxicity thresholds. Subordinate fish may also be at a disadvantage when encountering other ionoregulatory challenges, e.g., acclimation to ion-poor or acidic water (McDonald and Rogano 1986; Perry and Laurent 1989; Wood 2001).

In conclusion, subordinate fish display differences in sodium uptake and excretion from dominant animals, suggest-

ing that the ionoregulatory ability of subordinate trout is compromised. There are significant implications for the fish in terms of physiological health, particularly during an ionoregulatory challenge such as acclimation to ion-deficient water or exposure to an ionoregulatory toxicant. Within populations of fish where strong dominance hierarchies exist, there is scope for the ionoregulatory abilities of individuals to vary markedly depending on their position in the social hierarchy.

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