

## THE EFFECT OF INCREASED METABOLIC RATE ON RENAL FUNCTION IN THE RAINBOW TROUT, *SALMO GAIIRDNERI*

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### SUMMARY

1. A highly significant linear correlation was found between glomerular filtration rate and urine flow rate in both freshwater and seawater trout. The percentage tubular reabsorption of water was approximately 33% greater in seawater trout than in freshwater trout.

2. As oxygen consumption increased, the urine flow rate increased in freshwater and seawater rainbow trout. The proportional increase in urine flow with incremental changes in oxygen consumption was approximately the same in fresh water and sea water.

3. A significant positive linear relationship between oxygen consumption and glomerular filtration rate was found in both salinities. The proportional increase in GFR with incremental changes in the oxygen consumption rate was higher in the seawater trout than in the freshwater trout.

4. As oxygen consumption increased, the percentage tubular reabsorption of water decreased in the freshwater trout. No significant relationship between these two factors was found in the seawater trout.

### INTRODUCTION

The regulation of electrolytes and water is of the utmost importance to animals which maintain body fluids at concentrations different than that of the external environment. Two different patterns of physiological adaption are seen in euryhaline teleosts in fresh water and sea water. In the former the fish excretes excess water by forming a copious and dilute urine and counterbalances the loss of body salts by actively absorbing ions across the gills. In sea water the fish regains water which is lost osmotically through the gills and possibly through the skin by drinking sea water. Monovalent ions are transported across the intestine and water follows passively.  $\text{Na}^+$  and  $\text{Cl}^-$  ions are subsequently excreted through the gills. Although  $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$  ions, for the most part, are not absorbed across the intestine, any of these ions which do enter the blood are excreted by the kidney.

Little work has been done on the effect of swimming on urine flow rate and glomerular filtration rate. With the exception of Wood & Randall (1973*c*), previous studies of renal function in rainbow trout (Holmes & McBean, 1963; Holmes & Stainer, 1966; Fromm, 1963) have not considered that the metabolic rates of resting and con-

With activity or excitement the metabolic rate of the animal is increased. This necessitates increased uptake of oxygen, which is accomplished by increased ventilation and cardiac output (Stevens & Randall, 1967). Thus, the effective branchial exchange area – that is, the volume of blood which is brought into contact with the environment – increases per unit time. This in turn permits increased gaseous, ionic, and water exchange (Randall, Baumgarten & Malyusz, 1972). Following this argument it can be concluded that renal function, which is important for satisfactory water and electrolyte regulation in fish, would be integrated with the physiological processes linked to gas transport by the gills.

Wood & Randall (1973*c*) observed that increased metabolism in freshwater-acclimated rainbow trout was associated with increased urine flow rate. However, the effect of increased metabolic rate on GFR has not been investigated.

The purpose of this study was to assess the effect of different oxygen consumption levels on urine flow rates and glomerular filtration rates in freshwater-adapted and seawater-adapted *Salmo gairdneri*. We propose to examine first what changes in renal function occurred with changes in oxygen consumption and, secondly, to see if different strategies of physiological adaptation took place in the seawater trout than in the freshwater trout.

#### MATERIALS AND METHODS

##### *Animals*

Rainbow trout (*Salmo gairdneri*) were obtained from Humber Springs Hatchery, Orangeville, Ontario, and the Ontario Ministry of Natural Resources, Maple, Ontario. The average weight was 480 g, with a range of 297–865 g. The sex of the fish was not determined. Freshwater-trout tanks were supplied with running dechlorinated tap water, and seawater-trout tanks had continuous recirculation of artificial sea water (Instant Ocean, Aquarium Systems, Inc., Ohio) through gravel filters. Seawater trout were acclimated to 30‰ salinity by moving the fish from fresh water to sea water in increments of 7.5‰ salinity at 2-week intervals. Acclimation temperature ( $7 \pm 1$  °C) was reached by a change of 1 °C per day. Fish were acclimated to proper temperature and salinity for at least 2 weeks prior to experimentation. The photoperiod was controlled by an astronomical timer (Westinghouse, Tork Model no. 7120z, latitude 45 °N) and a dimmer switch which provided a half-hour transition period to approximate natural conditions. Fish were fed daily with a diet of commercial food in dry pellet form. However, fish were starved for 48 h prior to any oxygen consumption measurements (Beamish, 1964).

##### *Equipment*

A Blazka respirometer (Blazka, Volf & Capela, 1960; Smith & Newcomb, 1970) was modified to allow continuous monitoring of the oxygen concentration of the water and collection of blood and urine in swimming fish (Hofmann, 1976). Two tubes were fitted through to the interior chamber so that there were two connections from the exterior of the respirometer to the fish chamber in the inner cylinder for the blood and urine cannulae. The tubes were fitted with nylotron plugs and O-rings to prevent any leakage of air. An oxygen electrode (Yellow Springs Instruments, Model no. 5)

was used to measure the dissolved oxygen concentration of the water. The accuracy of the electrode determinations was checked against those obtained by the modified Winkler method (Strickland & Parsons, 1965).

The current velocity in the respirometer was determined by the rate of rotation of the propeller which is controlled through a variable-speed drive on the motor. A black curtain was hung around the respirometer to prevent the fish from being visually disturbed by movement in the room.

### *Experimental procedure*

Each experiment lasted approximately 3 days. On the first day the fish was cannulated and catheterized. It was then placed in the respirometer to recover for 24 h. On subsequent days glomerular filtration rates, urine flow rates and oxygen rates were measured.

Fish were anaesthetized to anaesthetic stage II, plane 2 (MacFarland, 1959) with MS-222 (tricaine methanesulphonate), diluted 1:10000. The dorsal aorta was cannulated in the area of the first two gill arches, following the method of Smith & Bell (1964) with a few modifications. Polyethylene tubing (PE 50, Clay-Admas, 0.58 × 0.96 mm) with a 23 g Huber point hypodermic needle was used as the blood cannula. The cannula was filled with Cortland saline (Wolfe, 1963) containing 10 i.u./ml ammonium heparin. It was found that an extra suture, placed at the base of the dorsal fin, was necessary to prevent dislodgement of the cannula while the fish was swimming.

The urinary catheter was composed of a piece of PE 50 tubing which was inserted through the uro-genital papilla to the urinary bladder. A ligature of silk suture thread (size 5-0) was tied carefully around the papilla to prevent any leakage. The catheter was also attached to the base of the anal fin.

Immediately after the cannulations, while the fish was still under the effect of the anaesthetic, it was placed in the Blazka respirometer for a 24 h recovery period. Houston, Czerwinski & Woods (1973) found that cardiovascular-respiratory activities, such as oxygen consumption, cardiac rate and ventilation rate, stabilized within 24 h of anaesthesia and surgery in the brook trout, *Salvelinus fontinalis*. Hunn & Willford (1970) reported that urine flow rates returned to a normal condition within 24 h of anaesthetization and urinary bladder catheterization in rainbow trout.

The clearance of radioactive inulin by the kidney was used to estimate the glomerular filtration rate. One millilitre (5  $\mu$ Ci) of [ $^{14}$ C]carboxyl inulin (New England Nuclear, Boston, Lot No. 787-057, specific activity = 2.94  $\mu$ Ci/mg) in a 0.9% saline solution was injected into the fish through the dorsal aortic cannula within 4 h after the fish had been placed in the respirometer.

### *Sampling procedure*

Sampling began on the second day. Changing the water velocity in the respirometer and therefore the swimming speed of the fish was used to vary the level of oxygen consumption. At each determination, glomerular filtration rate and urine flow rate were measured in relation to a particular metabolic rate. Although the procedure varied for individual fish, the normal routine involved taking five samples per day: three in the morning and two in the afternoon. The first determination was at a slow swimming

speed (12 cm/s) to train the fish to swim in the chamber. The second and third samples were taken at a higher speed which the fish could sustain easily (18–36 cm/s). Then the fish was given a rest period and the remaining samples of that day were taken at intermediate swimming speeds.

Each sampling period lasted for 1 h. Oxygen consumption measurements were made during the first half hour. Urine samples were collected automatically into graded centrifuge tubes by a fraction collector for the entire sampling period. Sample volumes were recorded and the samples were frozen. Blood samples were taken at the midpoint of a urine collection and after the measurement of oxygen consumption. A 0.3 ml blood sample was taken and one drop of ammonium heparin was added to prevent clotting. An equivalent amount of Cortland saline was injected to replace the blood sample. Samples were spun in a clinical centrifuge and the plasma was removed and frozen.

#### *Measurement of glomerular filtration rate*

The rate of glomerular filtration was estimated by measuring the clearance of inulin from the blood by the kidney. Inulin is an inert polyfructose molecule, with a molecular weight of 5200, which can pass across the glomerulus. There is considerable evidence from mammalian experiments that it is neither absorbed nor secreted by the tubules (Pitts, 1968). Hickman (1972) found that [<sup>14</sup>C]carboxy inulin did not bind to plasma proteins in any significant amount. Some recent investigations (Beyenbach & Kirschner, 1976; Hickman, Newcombe & Kinter, 1972; Schmidt-Nielsen, Renfro & Benos, 1972) have questioned the reliability of various mammalian glomerular markers to measure GFR in teleosts. The clearance of inulin may be an underestimation of the actual rate of filtration. However, even if this were the case, information on the changes in GFR relative to various oxygen consumption levels would still be consistent. Samples of the respirometer water, taken after each experiment, revealed that no leakage of inulin was occurring either across the gills or at the urogenital papilla.

The following formula was used to calculate inulin clearance:

$$\text{inulin clearance} = \frac{U_{\text{in}} V}{P_{\text{in}}},$$

where  $U_{\text{in}}$  = urinary inulin concentration (cpm/100  $\mu$ l),  $P_{\text{in}}$  = plasma inulin concentration (cpm/100  $\mu$ l) and  $V$  = urine volume (ml/kg.h).

The concentration of inulin in blood and urine was determined by counting the amount of radioactivity in prepared samples with a Packard Tri-Carb Liquid Scintillation Spectrometer (Packard Instrument Co.). Samples were checked for counting efficiency and quenching.

#### *Statistical analysis*

Analysis of covariance, following the method of Snedecor & Cochran (1967), was used to analyze the data. This analysis regressed the data from each fish to find the common slope for all fish. A two-tailed  $t$  test was used to check whether the common slope was significantly different from 0.

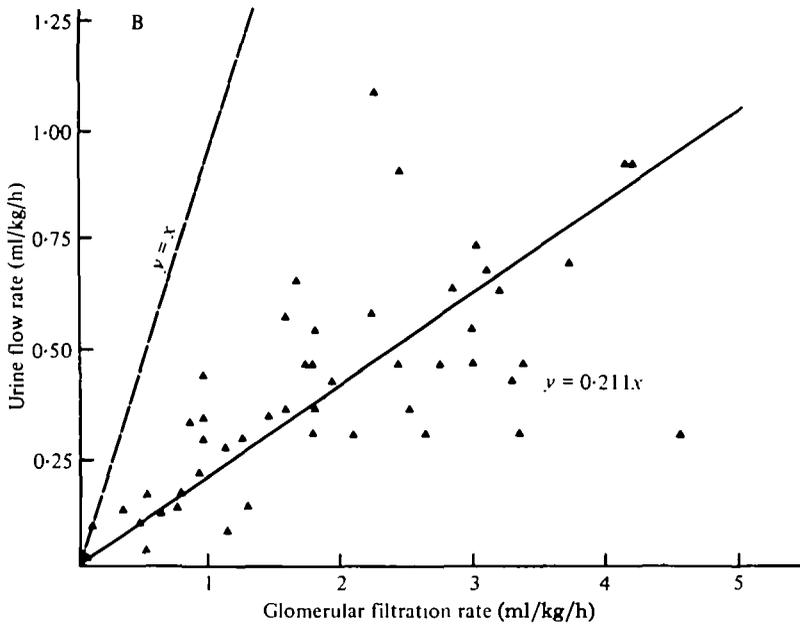
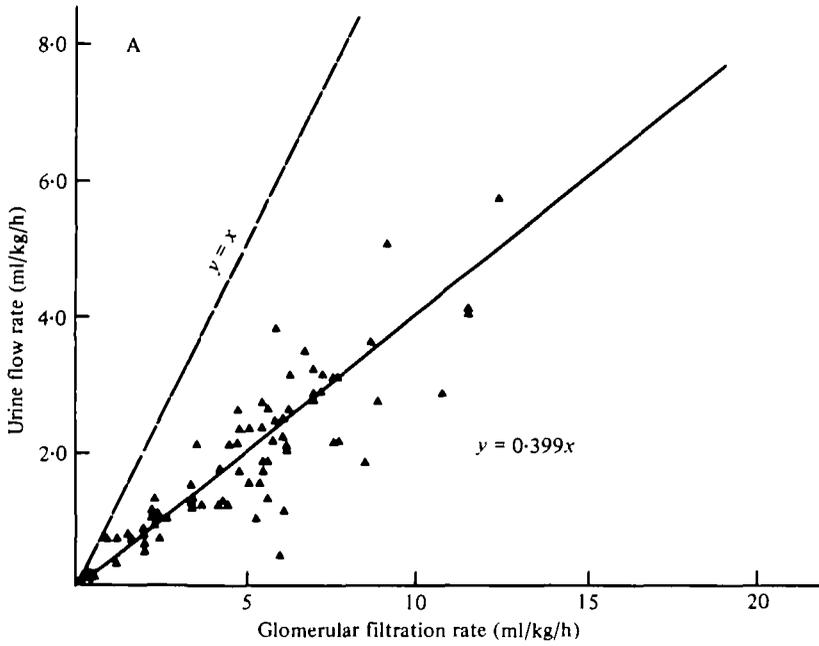


Fig. 1. The relationship between glomerular filtration rate (ml/kg/h) and urine flow rate (ml/kg/h) in freshwater (A) and seawater (B) rainbow trout. Equation of the line was derived by linear regression. Number of fish in A, 9. Number of fish in B, 6.

The analysis of glomerular filtration rate and urine flow rate was calculated according to the method of Snedecor & Cochran (1967) to find a straight line through the origin. A  $t$  test was used to test the null hypothesis that the line goes through the origin.

## RESULTS

### *GFR and urine flow rate*

The relationship between inulin clearance, as an estimate of GFR, and urine flow rate is shown in Fig. 1. All samples with these measurements were plotted, regardless of metabolic rate or swimming speed. When the data for the freshwater trout were regressed as a straight line through the origin, the equation was  $Y = 0.399X$ , where  $X$  is the inulin clearance (ml/kg.h) and  $Y$  is the urine flow rate (ml/kg.h). The equation for the seawater trout was  $Y = 0.211X$ . Both regressions were significant ( $P = 0.001$ ). For both sets of data a  $t$  test was performed to test that the regression does pass through the origin. Since the error in estimating GFR is so much greater than the error in measuring urine flow, these equations were derived by regressing the values for glomerular filtration rates on the values for urine flow rates. However, the equations were then rewritten to express urine flow as a function of GFR.

The data for both freshwater and seawater trout show that there is a direct linear correlation between glomerular filtration rate and urine flow rate. As the GFR increased there was a proportional rise in urine flow. This rise was twice as great in the freshwater trout as it was in the seawater trout.

In Fig. 1 the line  $Y = X$  has been plotted. This line represents the theoretical condition when all the filtrate is excreted, without any reabsorption and secretion. Lines which fall to the right of this represent cases where there is a net reabsorption of water from the filtrate by the kidney tubules. In the freshwater trout there was approximately 60% tubular reabsorption of water whereas in the seawater trout it was approximately 79%.

### *The effect of increased oxygen consumption*

Analysis of the data for oxygen consumption and urine flow rate in both freshwater and seawater experiments shows that the data are best described as a linear relationship. Fig. 2 shows the graphs of the data for these experiments. Analysis of a covariance showed that the relationship between these two factors could be described by the equation  $Y = 1.0673 + 0.0077X$  (s.d. of  $b = 0.0021$ ) for the freshwater fish ( $P = 0.001$ ) and by the equation  $Y = 0.1798 + 0.0016X$  (s.d. of  $b = 0.0008$ ) for the seawater fish ( $P = 0.1$ ), where  $X$  is the oxygen consumption (mg  $O_2$ /kg.h) and  $Y$  is the urine flow rate (ml/kg.h).

At the same oxygen consumption level, 40 mg  $O_2$ /kg.h, the urine flow rate was 1.4 ml/kg.h in fresh water and 0.24 ml/kg.h in sea water. When the metabolic rate was doubled to 80 mg  $O_2$ /kg.h, the urine flow rate increased 22% to 1.7 ml/kg.h in the freshwater trout whereas in the seawater trout there was an increase of 26% to 0.3 ml/kg.h. Although the slopes of the equations are different the proportional change in the urine flow values is very similar.

As in oxygen consumption and urine flow, the relationship between oxygen con-

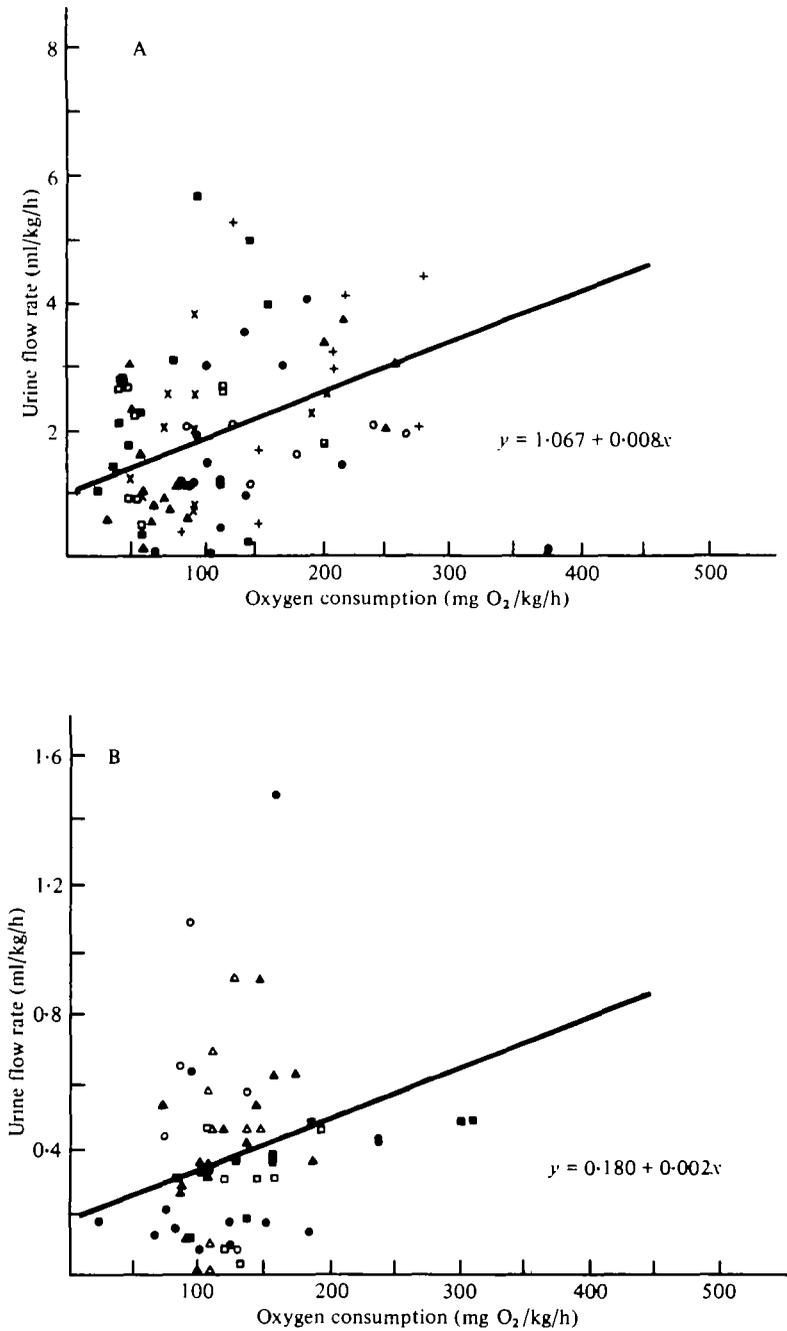


Fig. 2. The relationship between oxygen consumption (mg O<sub>2</sub>/kg/h) and urine flow rate (ml/kg/h) in freshwater (A) and seawater (B) rainbow trout. The equation of the line was derived by analysis of covariance. Each symbol represents an individual fish.

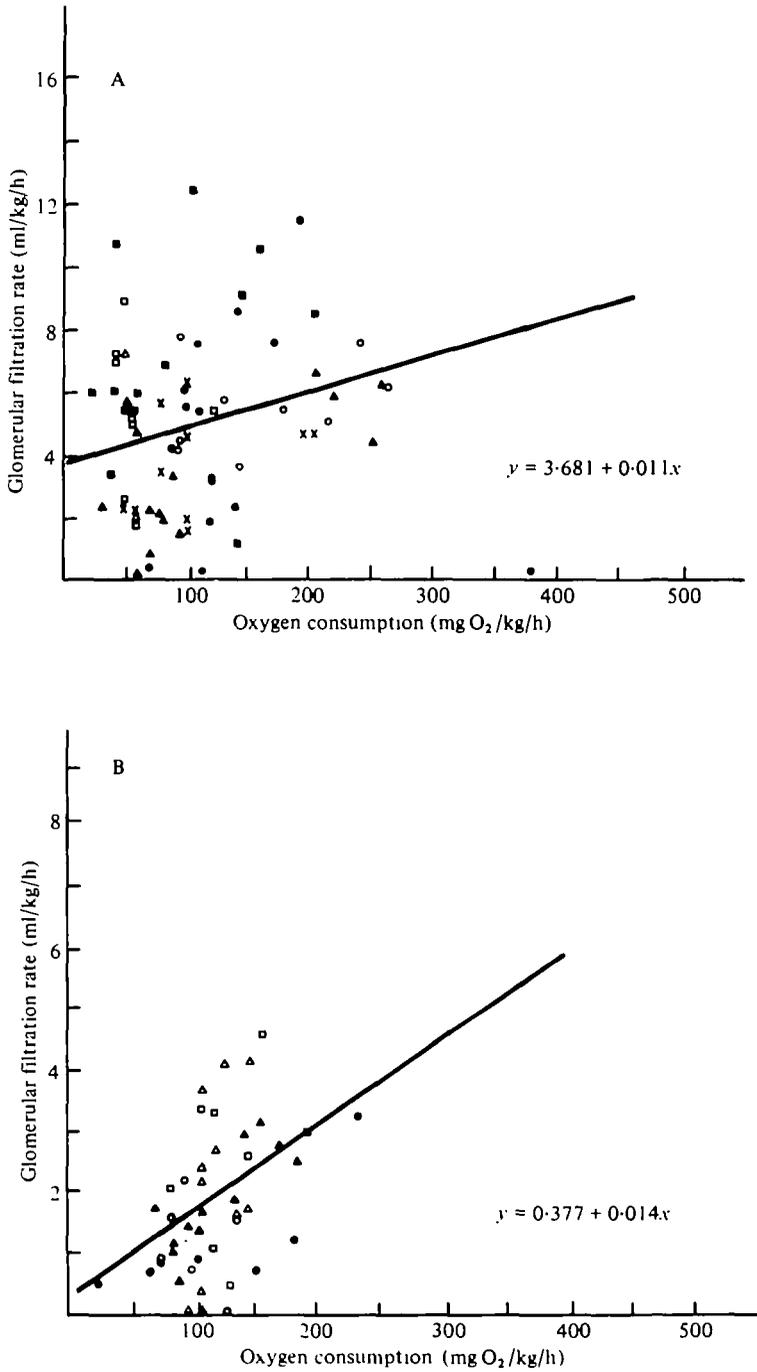


Fig. 3. The relationship between oxygen consumption (mg O<sub>2</sub>/kg/h) and glomerular filtration rate (ml/kg/h) as estimated by the chance of [<sup>14</sup>C]inulin in freshwater (A) and seawater (B) rainbow trout. The equation of the line was derived by analysis of covariance. Each symbol represents an individual fish.

Table 1. *The relationship between oxygen consumption (X, mg O<sub>2</sub>/kg.h) and tubular reabsorption of water (Y, %) in freshwater- and seawater-acclimated rainbow trout*

Equation†	S.D. of slope	No. of fish	No. of samples	Significance
Fresh water				
$Y = 55.244 - 0.053X$	0.0172	9	71	$P = 0.001$
Sea water				
$Y = 73.338 - 0.027X$	0.0352	6	44	N.S.*

† Equations were derived by analysis of covariance.

\* N.S. = *t* value was not significant at  $P = 0.1$ .

sumption and glomerular filtration rate can also be described as linear (Fig. 3). Analysis of covariance was performed on the data to derive the equations. For the freshwater trout the equation was  $Y = 3.681 + 0.0114X$  (S.D. of  $b = 0.0048$ ) ( $P = 0.05$ ), where  $X$  is the oxygen consumption (mg O<sub>2</sub>/kg.h) and  $Y$  is the inulin clearance (ml/kg.h). For the seawater trout the relationship was described by  $Y = 0.377 + 0.014X$  (S.D. of  $b = 0.0075$ ) ( $P = 0.1$ ).

An interesting situation is present with oxygen consumption and GFR. The slopes of the equations for freshwater and seawater fish are almost equal, although the  $Y$ -intercept is much lower in the second case. A rise in oxygen consumption will increase the absolute amount of filtrate by approximately the same amount in both cases. Therefore, because the GFR is much lower in the seawater fish, the proportional increase in the filtration rate is greater in the seawater trout.

#### *Tubular reabsorption of water*

The tubular reabsorption of water represents the net percentage of water that is reabsorbed from the filtrate by the kidney tubules. The equations expressing the relationship between oxygen consumption (mg O<sub>2</sub>/kg.h) and tubular reabsorption of water (%) are shown in Table 1, along with the number of fish and samples. Equations were derived by analysis of covariance.

The equation for the freshwater trout shows that there is a significant reduction in the percentage tubular reabsorption of water with increasing oxygen consumption. No predictable pattern of tubular reabsorption of water with increasing metabolic rate was found in the seawater trout.

## DISCUSSION

### *Glomerular filtration rate and urine flow rate*

A highly significant relationship has been found between GFR and urine flow rate in both freshwater and seawater rainbow trout (Fig. 1). This correlation has been previously demonstrated in *Salmo gairdneri* (Holmes & Stainer, 1966), in *Anguilla rostrata* (Butler, 1969; 1973) and in *Paralichthys lethostigma* (Hickman, 1968).

Both GFR and urine flow rates were greatly reduced in the seawater trout to conserve water. The glomerular filtration rates were approximately 50% lower in the

seawater trout. In addition the percentage reabsorption of water by the kidney tubules in the seawater trout was approximately 33% greater than in the freshwater trout. Therefore our results indicate that upon adaptation to sea water, the most important change is in the filtration rate, although the increased tubular water reabsorption is significant. This conclusion was also reached by Holmes & McBean (1963) in seawater-adapted rainbow trout, Holmes & Stainer (1966) in smolting *Salmo gairdneri*, and Miles (1971) in seawater-adapted coho salmon.

Interestingly, this pattern of adaptation contrasts with that found in *Anguilla japonica* by Oide & Utida (1968). They found that 10 days after transfer to sea water the GFR was reduced by only 12% whereas the tubular reabsorption of water rose to 80%

#### *Kidney function and metabolic rate*

The main purpose of this work was to study the relationship between kidney function and metabolic rate. The increase in urine flow rate and GFR with increasing metabolic rate was expected for several reasons. First, Stevens & Randall (1967) observed a 500% increase in the amplitude of breathing and a 30% increase in the rate of breathing in swimming freshwater rainbow trout. Secondly, they observed a 450% increase in cardiac output, which increases the effective branchial exchange area. In freshwater-adapted fish, these two factors will cause a rise in the outflux of ions and the influx of water across the gills which must be excreted by the kidney. However, in sea water these cardiovascular changes would have the opposite effect. Therefore a different response by the kidney might be expected in the seawater trout.

Another factor which would contribute to a rise in GFR and urine flow rate is increased blood pressure during activity. Stevens & Randall (1967) found that during moderate swimming activity the systolic blood pressure in the dorsal aorta increased by 16%, whereas the diastolic pressure increased by 21%. Glomerular ultrafiltration is determined by a number of factors, such as arterial pressure, intracapsular pressure, and colloidal osmotic pressure (Pitts, 1968). However, there is no information on the changes in blood pressure in the renal arterioles with changes in exercise in teleosts. In lake trout, Hammond (1969, as quoted in Hickman & Trump, 1969) found a positive correlation between changes in dorsal aortic blood pressure during swimming activity with changes in GFR. Therefore a rise in the arterial pressure would probably be followed by an increase in GFR.

As reported in the present study, increased metabolic rate, with accompanying changes in ventilation, cardiac output and blood pressure, coincided with a rise in GFR and urine flow rate. The changes in renal function with increasing oxygen consumption were more consistent in the freshwater trout than in the seawater trout, especially with respect to glomerular filtration rates (Figs. 2, 3). Because the volumes were smaller and the concentration of inulin higher in the seawater samples, even small pipetting errors would cause an increase in the variability of the results. Therefore the proportional error involved in measuring GFR was greater in the seawater fish.

The rise in GFR as a function of increased oxygen consumption was approximately the same for both the freshwater and seawater trout. Consequently the seawater trout

are faced with a greater proportional increase in the amount of filtrate with a rise in metabolic rate. This result is surprising because one might expect that in sea water where trout are faced with an increased outflux of water the GFR would not rise as rapidly as it does in fresh water in an effort to conserve water. Possibly, the same factor, such as arterial blood pressure, is responsible for the increased GFR in both experiments. The increased blood pressure and cardiac output, associated with a rise in oxygen consumption, is similar in both freshwater and seawater trout.

This situation makes the results from oxygen consumption and urine flow rates more interesting. In this case the slopes of the regressions for the freshwater- and seawater-acclimated trout were not the same, indicating that the rates of change in urine flow rate with increased oxygen consumption were different. However, the proportional change over a fixed increment in metabolic rate was similar. The results from the tubular reabsorption of water (Table 1) indicate that in the freshwater trout, as oxygen consumption increases, the percentage of filtrate which is reabsorbed significantly decreased to produce a higher urine flow rate. In this manner the freshwater trout were able to counterbalance the increased water influx across the gills. In the seawater trout there was no significant increase in the percentage tubular reabsorption of water. The fact that there was not greater compensation in the seawater trout may indicate that a limit in the efficiency of water reabsorption by the kidney tubules may have been reached. The greater osmoregulatory load of the seawater trout may explain the higher metabolic rates observed in the seawater trout at all swimming speeds than in the freshwater trout (Hofmann, 1976; Rao, 1968).

Wood & Randall (1973*c*) also observed that increased metabolism in rainbow trout acclimated to fresh water was associated with increased urine flow rates. They reported two other interesting findings which also were observed in our experiments. First, they found a much higher urine flow rate at the onset of exercise than after prolonged exercise, even though there were not any marked changes in oxygen consumption. Secondly, they observed that when swimming activity was interrupted the slope of the regression between oxygen consumption and urine flow decreased. Both of these tendencies suggest that there was a drop in the water influx in the freshwater trout per unit oxygen uptake and that the fish were able to compensate to relieve the osmotic load with prolonged swimming activity. Swift & Lloyd (1974) reported that the urine flow rates of freshwater trout exposed to hypoxic conditions decreased after 5 h of exposure. This change in urine flow rates during long-term exposure to hypoxia may indicate a mechanism which compensates for water influx, similar to that indicated during prolonged swimming activity.

The increased urine flow rate with a rise in oxygen consumption in both freshwater and seawater trout could alter the water balance within the fish. Changes in hematocrit and weight indices with either increased swimming activity or decreased ambient oxygen levels have been reported in several studies (Holeton & Randall, 1967; Swift & Lloyd, 1974; Stevens, 1968; Wood & Randall, 1973*c*). There is also evidence of increasing plasma osmotic concentrations with exercise (Farmer & Beamish, 1969; Byrne, Beamish & Saunders, 1972; Rao, 1969; Wood & Randall, 1973*a, b*).

In conclusion, increased oxygen consumption brings about a number of physiological changes. The need for additional oxygen uptake and waste removal alters the

respiratory and circulatory systems to increase the effective branchial area. This leads to concomitant increases in ion and water fluxes at the gills and increased excretion of water and electrolytes by the kidney. These conditions establish a dynamic situation in which gas exchange, ionoregulation, and waste excretion are weighed against each other to create a tolerable physiological compromise for the animal.

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