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ENERGY PARTITIONING IN FISH: THE ACTIVITY-RELATED COST OF OSMOREGULATION IN A EURYHALINE CICHLID

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SUMMARY

We have investigated how the maintenance, net cost of swimming and total (maintenance + net cost of swimming) metabolic rates of red, hybrid tilapia (*Oreochromis mossambicus* ♀ × *O. hornorum* ♂) responded to different acclimation salinities, and if these responses correlated with changes in ion-osmoregulation (= osmoregulation) costs.

Three groups of fish were acclimated to either fresh water (FW, 0‰), isosmotic sea water (ISW, 12‰) or full strength sea water (SW, 35‰) and oxygen consumption was measured while they swam at 10, 20, 30 and 40 cm s^{-1}. Maintenance oxygen consumption (estimated by extrapolation), for an average fish (63 g), increased among groups in the following order: FW < ISW < SW. The net cost of swimming increased in the order ISW < SW < FW, and total oxygen consumption (maintenance + net cost of swimming) increased in the order ISW < FW < SW.

We assumed that the contribution of cardiac, branchial and swimming muscles to the net cost of swimming was proportional to swimming speed only, and therefore, at similar speeds, differences in the net cost of swimming among salinities were due to changes in the activity-related cost of osmoregulation. Consequently, the order in which the net cost of swimming increases from one group to another is the same as the order in which the cost of osmoregulation increases. Since the sequences for maintenance and total metabolic rates differed from that for the net cost of swimming, salinity-related increases in these rates cannot be attributed exclusively to changes in osmoregulation cost.

We conclude, based on the differences in the net cost of swimming, that osmoregulation in FW is more expensive than in SW, and that it is cheapest in ISW. Although we were not able to estimate the total cost of osmoregulation in FW and SW, we estimated the activity-related cost, relative to the cost in ISW, at different swimming speeds (net cost of swimming in FW or SW minus net cost of swimming in ISW at each speed). For a 63-g fish in FW, this cost increased from zero at rest, to 41 mg O_2 kg^{-1} h^{-1} (16% of the total metabolic rate, 24% of the net cost swimming) at 40 cm s^{-1}. In SW the same cost increased only to 32 mg O_2 kg^{-1} h^{-1} (12% of the total metabolic rate, 20% of the net cost of swimming) at 40 cm s^{-1}. The net cost of swimming in FW or SW increased with swimming speed at a rate 3–4 times faster

Key words: bioenergetics, fish, oxygen consumption, exercise, osmoregulation, tilapia.

than the rate of increase in osmoregulation costs, suggesting that the latter did not limit the delivery of oxygen to the swimming, and other supporting, muscles.

INTRODUCTION

The use of oxygen consumption rates to study the energetics of ion-osmoregulation (= osmoregulation) in fish has led only to confusion (Potts & Parry, 1964; Parry, 1964; Nordlie, 1978). It is generally accepted that metabolic rates at rest, or during sustained activity, are lowest in isosmotic salinities because of negligible osmoregulation costs in such conditions, and the extra oxygen consumed in increasingly non-isosmotic media is attributed to proportional increases in osmoregulation requirements. However, this concept is challenged by the lack of consistent responses which have been observed (e.g. Hickman, 1959; Rao, 1968, 1971; Farmer & Beamish, 1969; Job, 1969*a*,*b*; Muir & Niimi, 1972; Nordlie & Leffler, 1975; Nordlie, 1978; Stuenkel & Hillyard, 1981; Fang, 1982; Furspan, Prange & Greenwald, 1984). That is, changes in the metabolism of fish due to altered salinities often do not correlate with changes expected in osmoregulatory work (Potts & Parry, 1964; Nordlie, 1978). Notwithstanding, specific estimates of osmoregulation cost, based on salinity-related differences in metabolism, have been made for rainbow trout, *Salmo gairdneri* (i.e. approx. 20 % of its metabolic rate in fresh water, 27 % in sea water; Rao, 1968), tilapia, *Oreochromis niloticus* (19 % in fresh water, 29 % in sea water; Farmer & Beamish, 1969), catfish, *Ictalurus nebulosus* (50 % in fresh water; Furspan *et al.* 1984), and striped mullet, *Mugil cephalus* ('negligible' in fresh water, 'high' in sea water; Nordlie & Leffler, 1975). Others may have refrained from making similar estimates of total osmoregulation costs because no differences in total metabolic rates among salinities were apparent (suggesting that no energy was required for osmoregulation, e.g. Nordlie, 1978; Fang, 1982) or because rates in salinities near isosmotic with the blood turned out to be higher than rates in FW or SW (suggesting that more energy may have been required in isosmotic conditions, contrary to the 'no cost' assumption; e.g. Hickman, 1959; Job, 1969*a*,*b*; Stuenkel & Hillyard, 1981).

Most of the problems of estimating accurately the cost of osmoregulation from differences in metabolic rate seem to originate from (i) having to rely on measurements of steady-state oxygen consumption taken from different groups of individuals, each representing a different acclimation salinity (and cost), and (ii) our inability to identify and isolate properly osmoregulatory costs from other metabolic activities in intact fish. These limitations make it impossible to attribute salinity-related differences in metabolism to changes in the cost of osmoregulation. Therefore, the use of changes in metabolic rate to estimate the magnitude and cost of osmoregulation at different salinities needs revision.

Our experiments demonstrate that differences in the slope relating metabolic rate to swimming speed, and in the net cost of swimming at a particular speed (total metabolic rate swimming minus maintenance metabolic rate), at different salinities provide a better estimate of how osmoregulation costs increase (or decrease) than d

differences in total metabolic rate. Unlike total metabolic rate, differences in the slope can be attributed almost exclusively to the effect of salinity on the activity-related cost of osmoregulation, a term proposed here for the extra cost of osmoregulation, above maintenance requirements, in steadily swimming fish (Webb, 1975; Jones & Randall, 1978; Stevens & Dizon, 1982). This cost is probably the result of increases with activity in the rate of active transport of ions at the gills, kidneys and/or intestine, in response to increments in passive ion and water fluxes (Randall, Baumgarten & Malyusz, 1972; Wood & Randall, 1973; Hofmann & Butler, 1979). Presumably, passive fluxes increase secondarily to activity-related increases in the functional surface area of the gills (Booth, 1978, 1979; Farrell, Sobin, Randall & Cosby, 1980; Randall & Daxboeck, 1984; Rankin & Bolis, 1984; Ungell, Kiessling & Nilsson, 1984) and in the glomerular filtration rate in kidneys (due to a rise in arterial blood pressure) (Hofmann & Butler, 1979). The fact that blood $[Na^+]$ and $[Cl^-]$ and osmolarity in fish exercised in different salinities differ only slightly from the values in resting fish (Rao, 1969; Farmer & Beamish, 1969; Byrne, Beamish & Saunders, 1972) further supports the idea of activity-related increases in osmoregulatory costs. Otherwise, fish would die following prolonged swimming if increases in passive ion and water losses (or gains) with activity went unchecked.

The subjects of our study were farm-reared (South Fisheries), hybrid tilapia developed from an original cross between *Oreochromis mossambicus* ♀ (red mutants) × *O. hornorum* ♂ (Sipe, 1979). Hybrids from this cross show close genetic similarity with pure strain *O. mossambicus* (Chen & Tsuyuki, 1970), a warm-water, euryhaline species often utilized in studies of teleost osmoregulation (Fukusho, 1969; Dharmamba, 1970; Dharmamba & Maetz, 1972, 1976; Fishelson, 1980; Nicoll, Wilson, Nishioka & Bern, 1981; Foskett *et al.* 1981; Mainoya, 1982; Assem & Hanke, 1983; Dange, 1985). The morphology and biology of *O. hornorum* and *O. mossambicus* are almost indistinguishable (Wohlfarth & Hulata, 1981) and both species, as well as their hybrid offspring, show high tolerance to SW (Talbot & Newell, 1957; Potts, Foster, Rudy & Howells, 1967; Lobel, 1980; Murray & Mitsui, 1982; Mitsui, Entenmann & Gill, 1983).

MATERIALS AND METHODS

Animals

Fish weighing between 20 and 170 g were used in the experiments. Three groups, each of about 20 fish, were acclimated to fresh water, isosmotic sea water or full strength sea water (Table 1). They were kept at 28° ± 2°C in a 320-l circular, aerated tank. During experiments with FW and ISW two-thirds of the water was replaced daily to maintain the level of metabolites in the water at low concentrations. For fish acclimated to SW, a continuous flow ($160\,l\,h^{-1}$) of sand-filtered Biscayne Bay water was directed through the tank. After 1 week of acclimation the fish were forced to swim continuously, for the next 3 weeks, at about $10\,cm\,s^{-1}$. A steady current was generated in the holding tank by a propeller connected to a variable-speed motor. Swimming was interrupted once a day, during feeding to satiation, throughout the

training period. This regime prompted the fish to swim readily in the respirometer at any imposed speed (Farmer & Beamish, 1969; Beamish, 1970), and increased their stamina and aerobic capacity at high sustainable speeds (Beamish, 1978; Johnston, 1982).

Respirometer

Oxygen consumption was measured using a modified Blazka-type respirometer (Blazka, Volf & Cepela, 1960; Smith & Newcomb, 1970; F. W. H. Beamish, personal communication) (Fig. 1), operated with intermittent flow by means of solenoid valves automatically actuated by high–low oxygen tension limit switches built into the O_2 meter. A 62-W submersible pump driving an impeller at the rear of the chamber was capable of generating a water current of up to $50\,cm\,s^{-1}$ in the swimming portion of the respirometer. A secondary circulation pump also drew water continuously from the respirometer through the O_2 probe (YSI 5331), temperature controller and heat exchanger and back to the respirometer. Aerated water was fed into the respirometer from a closed, vigorously aerated reservoir (75 l) to which water from the chamber was simultaneously returned during each reflush. This water was replaced regularly between tests to avoid accumulation of metabolites. The total effective volume of the respirometry system was 8·8 l. Oxygen saturation in the chamber during closed cycles never dropped below 75 %.

To facilitate heat dissipation during closed cycles, the chamber was submerged in a 250 l bath at 28 °C and appropriate salinity (0, 12, or 35 ‰). The temperature within the respirometer did not change more than ±0·3 °C even at the highest speed tested. With a bath of appropriate salinity it was also possible to open the chamber without removing it from the bath each time a new fish was introduced.

Table 1. *Chemical composition of the three media used in this study*

Parameter	Medium: Fresh water*	Medium: Isosmotic sea water	Medium: Sea water†	Blood‡ (±S.D., $N = 28$)
Na^+ ($mmol\,l^{-1}$)	0·9	160	468	158 ± 21·5
Cl^- ($mmol\,l^{-1}$)	1·1	188	546	130 ± 5·2
Ca^{2+} ($mmol\,l^{-1}$)	0·5	3	10	
K^+ ($mmol\,l^{-1}$)	0·05	3·3	10	
SO_4^{2-} ($mmol\,l^{-1}$)	0·25	9·4	28	
Mg^{2+} ($mmol\,l^{-1}$)	0·13	18	53	
Osmolality ($mosmol\,kg^{-1}$)	<20	355	1034	331 ± 11·9
pH	8·00–9·00	8·00–8·50	8·20	
Salinity (‰)	<3	12	35	

Mean blood concentrations of Na^+ and Cl^- and osmolarity are also included for comparative purposes.

* From the Alexander Orr Treatment Plant, Dade County, FL.

† Riley & Chester (1971).

‡ Mean concentrations derived from fish acclimated for 4 weeks to <3, 6, 12, 24, 35 ‰ salinities (R. Febry & P. Lutz, in preparation).

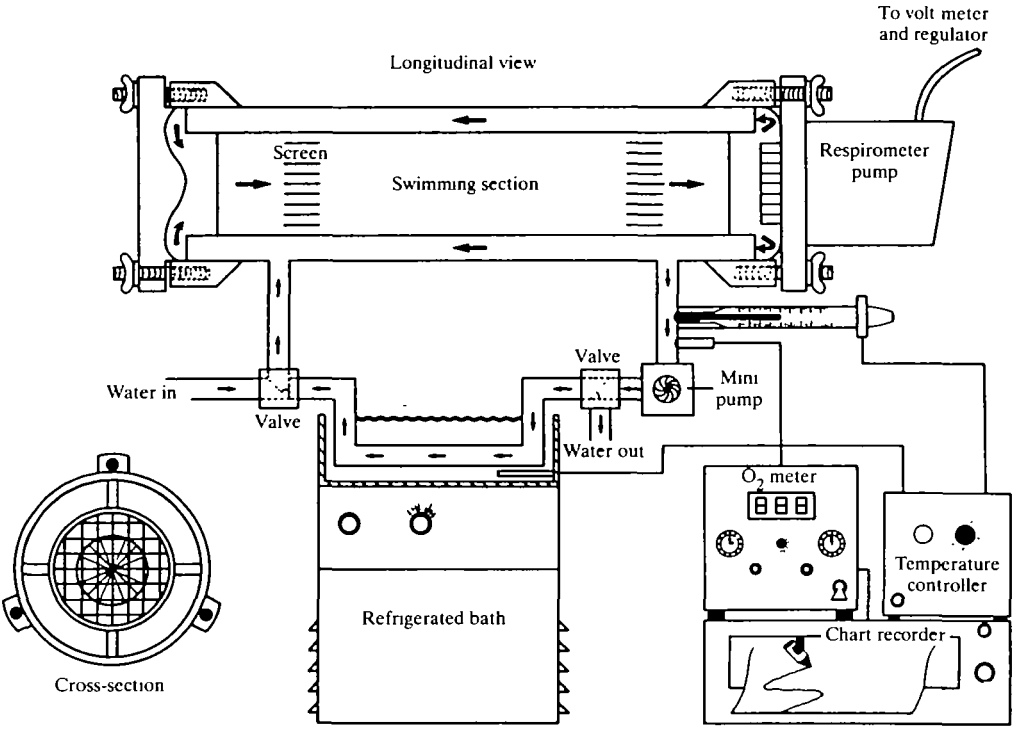

Fig. 1. Diagram of Blazka-type respirometer and support equipment. The respirometer functions in an open–closed cycle, with the oxygen meter and limit selector automatically controlling solenoid valves to allow aerated water in periodically.

Protocol

For each trial, a fish was netted from the training tank (selected at random from the rest of the batch for each particular salinity) and isolated in an aquarium for 48 h without food. This allowed the gut to empty of any food residues so that fish could be tested in a post-absorptive state; furthermore, it allowed the fish enough time to rest from the training regime prior to being exercised in the chamber. Fish were then lightly anaesthetized with Tricaine methanesulphonate (MS 222, $0{\cdot}4\,g\,l^{-1}$) and measured (total length, depth and breadth) prior to being transferred to the swimming portion of the respirometer. The measurements taken at this point were utilized to correct for the solid blocking effect of the fish on the speed of the water flowing over its body (Webb, 1975), ensuring that every fish was exercised at exactly the same speeds regardless of differences in size.

To enable the fish to orientate on recovery from anaesthesia, a water current of $5\,cm\,s^{-1}$ was applied immediately and maintained until the following morning, when the exercise experiments began; fish were placed in the respirometer the evening before an experiment to allow them enough time (approx. 16 h) to recover from handling. Measurements of O_2 consumption were always performed at the same time each day (Fry, 1971).

During each test, fish were exercised progressively at speeds of 10, 20, 30 and 40 cm s^{-1} for 45–60 min (prolonged swimming); their oxygen consumptions were measured during the last 30 min of swimming at each speed (Webb, 1971). For a few fish in the freshwater-acclimated group, oxygen consumption rates were measured for an additional 3 h following exercise to determine if any significant oxygen debts had been incurred during the 4-h periods of sustained activity (Webb, 1971). At the end of each trial, fish were removed from the respirometer and weighed. A blank run was then performed, and the oxygen consumed by the respirometry system was subtracted from that consumed by the fish and the system combined.

Calculations

Oxygen consumption rates ($\dot{M}_{O_2}$) for each fish and swimming speed were computed using the equation:

$$\dot{M}_{O_2} = \left(\frac{\Delta Pw_{O_2}}{\Delta T}\right) \times V \times \alpha,$$

where ΔPw_{O_2} is the change in partial pressure of oxygen in the water (in mmHg, 1 mmHg = 133·3 Pa), ΔT is time (in min), V is respirometer volume (in l) and α is the O_2 solubility coefficient at 28 °C for each salinity tested. Data for mass, length, salinity and O_2 consumption rates at each activity level were entered into a computer (UNIVAC 1181/80), and analysed statistically using appropriate subroutines for analysis of covariance (Nie *et al.* 1975).

RESULTS

Oxygen consumption rate versus *swimming speed*

Steady-state consumption of oxygen increased with swimming speed in a typically exponential fashion (Fig. 2). At a maximum speed of 40 cm s^{-1} the smallest fish tested (mass 23 g, length 11 cm) swam at 3·6 body lengths s^{-1} (L s^{-1}). Perciforms of similar size have been reported to be capable of cruising at approximately 6·3 L s^{-1} (Webb, 1975; p. 52). Therefore, it seemed fair to assume that in our samples most, if not all, of the energy required for swimming was supplied aerobically (Webb, 1971). Indeed, oxygen consumption immediately after exercise was similar to pre-exercise rates in the few animals examined (e.g. Fig. 2). The main morphological characteristics of each group of fish tested are summarized in Table 2.

The mean rates of oxygen consumption (uncorrected for possible weight-related biases) *versus* swimming speed at each salinity are shown on semi-logarithmic coordinates (Brett, 1964) in Fig. 3. Mean resting (= maintenance) metabolic rates were estimated by extrapolating to zero swimming speed the curves obtained for each individual fish. At any given swimming speed the mean rate of oxygen consumption was lowest in fish acclimated to ISW. At rest and at 10 cm s^{-1}, the rates were slightly higher in SW than in FW, the situation reversing itself at 20, 30 and 40 cm s^{-1}. The rate of increase in metabolic rate (i.e. the slope) was smallest in fish swimming in

ISW. The rates of oxygen consumption increased more rapidly (steeper slope) in FW-adapted than in SW-adapted fish.

Effect of mass and swimming speed interactions on oxygen consumption rates

Before testing the statistical significance of the effect of salinity on metabolism, it was necessary to determine if, at each salinity, size and swimming speed had a supplementary, interactive effect on oxygen consumption, in addition to their independent effects. The individual rates of oxygen consumption at each swimming

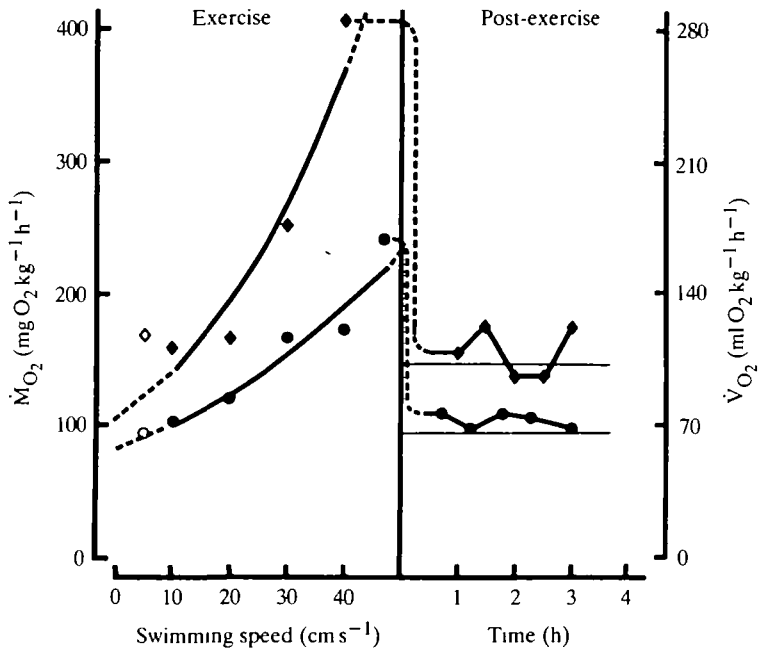

Fig. 2. Oxygen consumption rates *versus* swimming speed and time following exercise for two representative fish (◆, ●) in fresh water; open symbols on left and horizontal lines on right of figure represent pre-exercise metabolic rates (see text for details).

Table 2. *Mean morphometric characteristics of each group of fish tested*

	Medium			
Parameter	Fresh water	Isosmotic sea water	Sea water	Overall
Mass (g)	57·65	72·68	61·94	63·02
range	22·8–170·0	31·4–132·5	24·5–121·8	22·8–170·0
Length (cm)	15·5	17·2	16·4	16·2
range	10·7–22·7	13·0–22·4	11·3–21·3	10·7–22·7
Condition factor*	1·35	1·35	1·35	1·35
range	1·14–1·86	1·15–1·67	1·25–1·70	1·14–1·86
N	16	10	10	36

* Condition factor = (mass/length^3)×100.

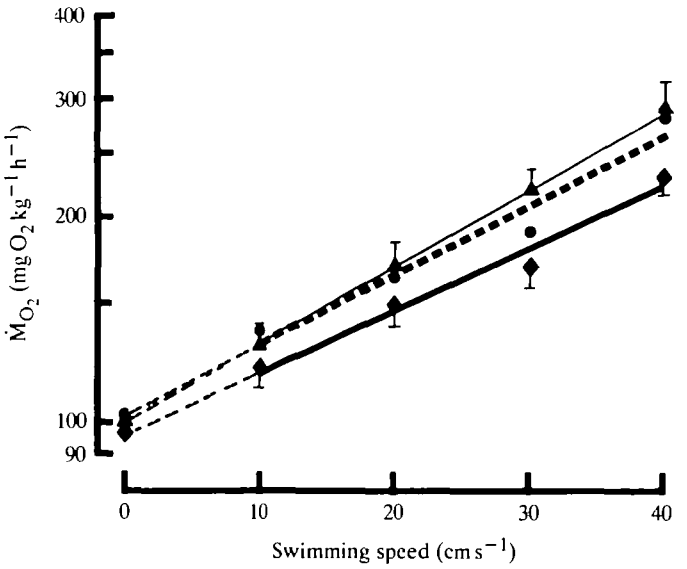

Fig. 3. Means and lines of best fit for oxygen consumption rates *versus* swimming speed at each salinity. Bars indicate ±S.E., $N = 16$ in fresh water, $N = 10$ in isosmotic sea water and $N = 10$ in sea water. ▲, fresh water; ●, sea water; ◆, isosmotic sea water.

speed and salinity are plotted against mass on logarithmic coordinates (Fig. 4). At each salinity, oxygen consumption increased considerably with mass at any given swimming speed, the effect always being highly significant ($P < 0{\cdot}001$). Although at each salinity the slopes of the regression lines at different swimming speeds were not significantly different ($P > 0{\cdot}25$, two-tailed test), the y-intercepts were different in all three media ($P < 0{\cdot}001$, one-tailed test). The lack of change in slope means that, within the range examined, swimming speed did not alter the relationship between the rate of oxygen consumption and mass (Fig. 4). Rather, the influence of swimming speed on metabolic rate was independent of the size of the fish, as only the y-intercepts were affected significantly. This is not to say, however, that over wider ranges of size and/or swimming speeds a significant interaction between size and speed might not occur (Brett, 1965), only that none was found within the limits of our tests.

Effect of salinity on the relationship between rate of oxygen consumption, size and swimming speed

Since, at each salinity, the relationship between metabolic rate and size did not change significantly with varying swimming speeds, a single regression model can be used to describe the relationship within the range of speeds tested. In addition, the effect of swimming speed on metabolic rate can also be incorporated into the existing metabolic rate/size equations as a second, independent variable. The result is a single, multiple regression model for each salinity describing simultaneously the relationship of metabolic rate to mass and swimming speed (Table 3). Note that as salinity increases, so does the partial regression coefficient for the log mass variable

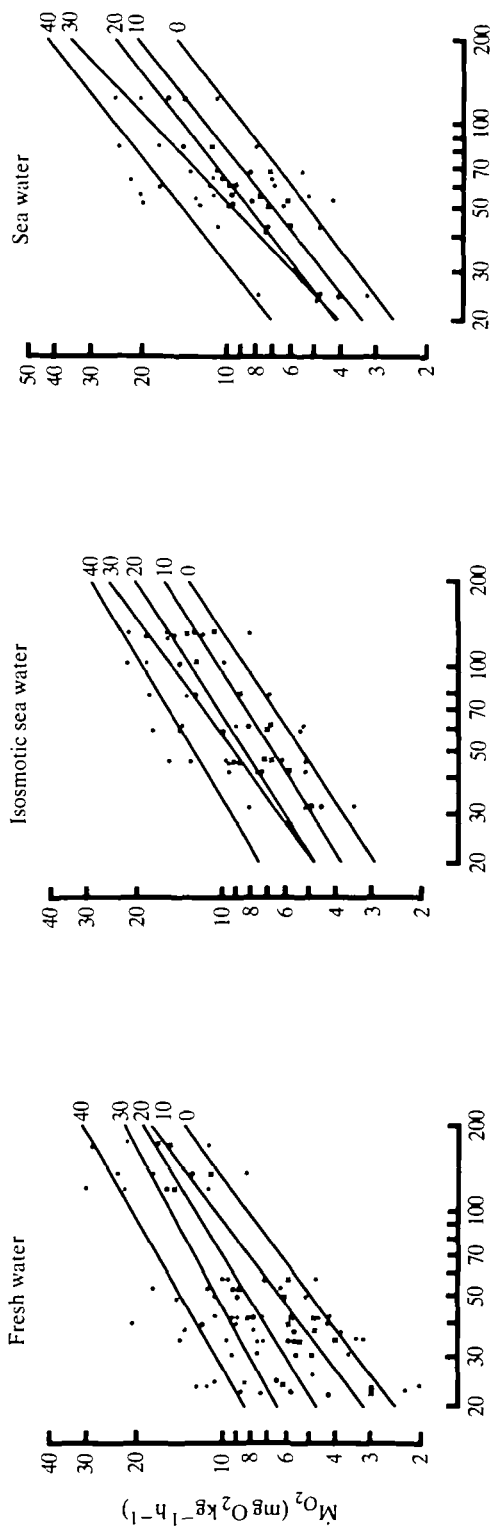

Fig. 4. Individual oxygen consumption rates *versus* mass, and lines of best fit, at each swimming speed (given in $cm\,s^{-1}$) for fish in fresh water, isosmotic sea water and sea water; resting rates were estimated by extrapolating to zero swimming speed the individual metabolic rate *versus* speed functions.

Table 3. *Multiple regression models derived for each salinity describing the effects of the log transformations of mass (g) and of swimming speed ($cm\,s^{-1}$) on log transformations of metabolic rates ($mg\,O_2\,h^{-1}$)*

	Multiple regression models					
	$\log \dot{M}O_2 = \log A +$		$B_1(\log \text{mass}) +$		$B_2(\text{swimming speed})$	
Acclimation medium	N		(B_1, S.E.) r^2		(B_2, S.E.) r^2	(y−x, S.E.) r^2
Fresh water	16	−0·3559	0·6143 (0·0436) 0·76		0·0115 (0·0010) 0·69	0·089 0·84
Isosmotic sea water*	10	−0·3705	0·6460 (0·0534) 0·79		0·0090 (0·0010) 0·71	0·069 0·86
Sea water	10	−0·6823	0·8282 (0·0631) 0·83		0·0102 (0·0010) 0·76	0·068 0·88

* Reference regression model against which coefficients in fresh water (FW) and sea water (SW) were compared (see text for details); ISW, isosmotic sea water.
$\Delta B_{1,\text{ISW}\leftrightarrow\text{FW}}$, $0{\cdot}20 > P > 0{\cdot}10$, not significant.
$\Delta B_{1,\text{ISW}\leftrightarrow\text{SW}}$, $0{\cdot}20 > P > 0{\cdot}10$, not significant.
$\Delta B_{2,\text{ISW}\leftrightarrow\text{FW}}$, $0{\cdot}10 > P > 0{\cdot}05$, significant.
$\Delta B_{2,\text{ISW}\leftrightarrow\text{SW}}$, $P > 0{\cdot}25$, not significant.
$\Delta \log A_{\text{ISW}\leftrightarrow\text{SW}}$, $0{\cdot}20 > P > 0{\cdot}10$, not significant.

(B_1). However, the partial regression coefficient for swimming speed (B_2) decreases from FW to ISW, then increases again in SW (Table 3). This means that the effect of salinity on the total metabolic rate of a standard-size fish can be different from the effect of salinity on its cost of swimming alone.

Because water, Na^+ and Cl^- concentration gradients between the blood and the external medium were virtually absent in ISW (Table 1) we anticipated that osmoregulatory work in these conditions would be minimal (see Discussion). Therefore, the multiple regression model for ISW was chosen as a convenient reference model against which to compare, statistically, the FW and SW models. Either of the latter two, however, would have been equally acceptable as the reference model. An analysis of covariance was performed, using dummy variable regression methods (Kleinbaum & Kupper, 1978), to determine if significant deviations occurred in FW or SW from the reference model in ISW. Deviations in the log mass regression coefficient (ΔB_1), or the y-intercept ($\Delta \log A$), were tested based on a two-tailed hypothesis. The null hypothesis was that no deviations occurred in FW (or SW) (null hypothesis: ΔB_1, $\Delta \log A = 0$). Since the effect of salinity on the slope of the relationship between metabolic rate and size, as well as on the y-intercept, is not related to differences in osmoregulation costs, we were only concerned with whether or not changes due to salinity were significant, regardless of the direction of change. These preconditions called for a two-tailed test (Zar, 1974). Deviations in the regression coefficient for swimming speed (ΔB_2), however, were tested for significance based on a one-tailed test. In this case we were interested only in increases in

the slope relating metabolic rate to swimming speed from its reference value in ISW. The null hypothesis was that changes in the swimming speed coefficient in FW or SW did not occur, or were negative changes (i.e. slope decreased) (null hypothesis: $\Delta B_2 \leq 0$). Since we were only interested in showing whether swimming costs would increase in FW and SW compared to ISW, as a result of increases in osmoregulation costs, a one-tailed test was required (Zar, 1974).

The deviations in log mass regression coefficient (ΔB_1), in either the freshwater or seawater treatments, were not significantly different from zero ($0{\cdot}20 > P > 0{\cdot}10$). In sea water, the lack of statistical significance of what was a large ΔB_1 can be attributed to the relatively small size range of fish tested (less than one order of magnitude between the smallest and largest fish in each treatment). This often leads to large standard errors in regression coefficients resulting from scaling effects. In FW, the deviation in the swimming speed coefficient (ΔB_2) from the value in ISW was significant ($0{\cdot}10 > P > 0{\cdot}05$); in SW the deviation was not significant, however ($P > 0{\cdot}25$). Since the ISW and SW models were statistically equivalent, the difference in y-intercept ($\Delta \log A$) was also tested and found to be not significant ($0{\cdot}20 > P > 0{\cdot}10$).

DISCUSSION

Estimates of relative costs of osmoregulation based on differences in total metabolic rate

Previous estimates of costs in non-isosmotic salinities have been based on the assumption that in an isosmotic salinity no energy is expended on osmoregulation. Consequently, the differences obtained by subtracting total metabolic rates measured in fish held in isosmotic conditions from rates measured in non-isosmotic conditions have customarily been attributed to the extra cost of osmoregulation (see reviews by Potts & Parry, 1964; Nordlie, 1978; Eddy, 1982; and reports by Rao, 1968; Farmer & Beamish, 1969; Furspan *et al.* 1984). Because it is likely that some energy is still utilized in isosmotic conditions, we prefer to consider these as estimates of *relative*, not *absolute*, costs of osmoregulation. This allows for the cost in ISW to be different from zero without violating any assumptions.

We have estimated the relative costs of osmoregulation in tilapia hybrids acclimated to FW (0‰), ISW (12‰) and SW (35‰) using the approach outlined above. At rest, and when swimming at $10\,cm\,s^{-1}$, the corrected total metabolic rate of a standard fish (63 g; from the last column of Table 2) increased with salinity (Table 4; Fig. 5). This suggests that when resting, or during slow swimming, more energy is spent on osmoregulation in SW and in ISW than in FW. However, at swimming speeds of 20, 30 and $40\,cm\,s^{-1}$, total metabolic rates were higher in FW than in ISW (Fig. 5). It appears from this that, in comparison with isosmotic conditions, the cost of osmoregulation in FW rises as swimming speed increases: at rest it is lower, at some intermediate speed identical, and at greater speeds higher in FW than in ISW (Fig. 5). Since the osmotic and electrochemical gradients against which regulatory work occurs in FW compared with ISW should not be significantly

affected by the level of activity of the fish (Rao, 1969; Farmer & Beamish, 1969; Byrne *et al.* 1972), such a shift in the salinity at which the cost of osmoregulation is lowest would not be expected. In other studies, as well as in ours, total metabolic rates measured in acclimated fish have either increased, remained unchanged or decreased as salinity increased (Hickman, 1959; Job, 1969*a*,*b*; Muir & Niimi, 1972; Nordlie & Leffler, 1975; Nordlie, 1978; Stuenkel & Hillyard, 1981; Fang, 1982). Thus, comparisons between published reports also provide conflicting estimates of the relative order in which the cost of osmoregulation increases with salinity.

Studies with euryhaline fish (acclimated to either FW or SW) indicate that electrochemical gradients and gill permeabilities are similar among species (Potts, 1984). Furthermore, the mechanisms of osmoregulation show no significant interspecific differences (Evans, 1980*a*,*b*, 1984). Therefore, it can be assumed that similar osmoregulatory mechanisms, operating at the same rate against similar electrochemical gradients, would require equivalent amounts of energy. Thus, if the minimum thermodynamic energy required for osmoregulation, in FW for example, is more than that required in ISW or SW, we would expect this to be the case consistently for most euryhaline species (assuming no radical differences in body

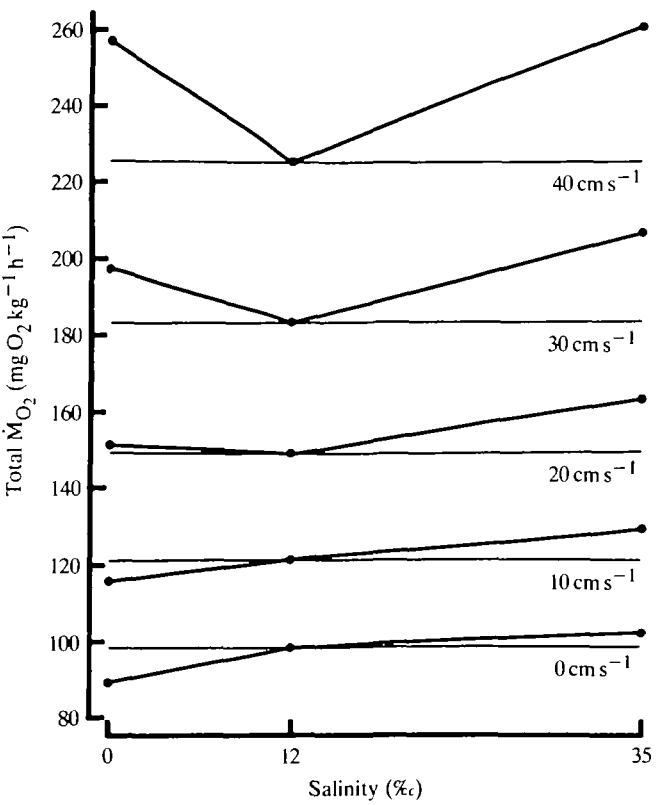

Fig. 5. Total oxygen consumption rates *versus* salinity for an average fish (63 g) at rest, and swimming at 10, 20, 30 and 40 cm s^{-1} (calculated from Table 3). Horizontal lines represent the reference rate in isosmotic sea water at the swimming speeds indicated.

Table 4. *Total oxygen consumption rates, and net costs of swimming for an average fish (63 g) acclimated to fresh water (0 ‰), isosmotic sea water (12 ‰) or full-strength sea water (35 ‰) (calculated from Table 3)*

Swimming speed		Acclimation medium					
		Fresh water		Isosmotic sea water		Sea water	
($L\,s^{-1}$)	($cm\,s^{-1}$)	Total metabolic rate ($mg\,O_2\,kg^{-1}\,h^{-1}$)	Net cost* of swimming ($mg\,O_2\,kg^{-1}\,h^{-1}$)	Total metaboic rate ($mg\,O_2\,kg^{-1}\,h^{-1}$)	Net cost* of swimming ($mg\,O_2\,kg^{-1}\,h^{-1}$)	Total metabolic rate ($mg\,O_2\,kg^{-1}\,h^{-1}$)	Net cost* of swimming ($mg\,O_2\,kg^{-1}\,h^{-1}$)
0	0	89	0	98	0	102	0
0·6	10	116	27	121	23	129	27
1·2	20	151	62	149	51	163	61
1·8	30	197	108	183	85	206	104
2·4	40	257	168	225	127	261	159

L, body length.
* Total swimming metabolic rate minus total resting metabolic rate.

permeability). It follows, then, that total metabolic rates for each species should always be higher in FW than in ISW, if indeed differences in metabolism accurately represent changes in the cost of osmoregulation. However, as mentioned in the Introduction, no such correlation can be established from experiments with whole fish. Therefore, we must conclude that differences in total metabolic rate at different salinities are not always exclusively related to changes in osmoregulatory cost. Under these circumstances, the accuracy of previous estimates (e.g. Rao, 1968; Farmer & Beamish, 1969; Nordlie & Leffler, 1975; Furspan *et al.* 1984) remains uncertain, and the estimates themselves may be of limited relevance. In fact, the procedure of comparing total metabolic rates between salinities has previously been considered unreliable, and its use discouraged, when studying osmoregulation energetics in fish (Potts & Parry, 1964; Nordlie, 1978).

Compensating for the interference by non-osmoregulatory events using swimming performance data

The variety of responses of total metabolic rate to different salinities suggests that these are being influenced by undetermined physiological processes not necessarily associated with the energetics of osmoregulation. Upon acclimation to different salinities, permanent hormonal changes occur in fish which, in addition to inducing the necessary osmoregulatory adjustments, may also have secondary effects on other, non-related variables affecting total metabolic rates (Smith & Thorpe, 1976, 1977; Baker & Wigham, 1979; Foskett, Bern, Machen & Conner, 1983). Cortisol, for example, is known to enhance nitrogen excretion (protein catabolism), and thus metabolic rates, in fish (Smith & Thorpe, 1977; Chan & Woo, 1978), while prolactin has been associated with an increase in lipid metabolism (Baker & Wigham, 1979). Since the production of these two hormones is generally affected in opposite directions by changes in salinity, their independent or interactive effects on the overall metabolism could be confounding the specific effect of salinity on osmoregulation costs. The lack of consistent responses in metabolic rate to otherwise similar experimental or physiological conditions, as obtained in independent studies using different species, supports this hypothesis. Therefore, somehow these unrelated influences of salinity on total metabolic rates need to be compensated for during, or following, measurements of oxygen consumption, before proper estimates of the cost of osmoregulation can be made.

Compensation for non-osmoregulatory effects can be obtained from curves relating oxygen consumption to swimming speed at different salinities. The rationale is as follows: a fish swimming at progressive, sub-maximal speeds, can be expected to have a total rate of oxygen consumption which will be approximately proportional to, or increase in proportion to, the increase in the mechanical work performed by the swimming muscles. To a smaller extent, the extra cost of operating the branchial and cardiac muscles (Jones, 1971; Webb, 1975; Beamish, 1978) and the additional osmoregulatory work associated with swimming (Webb, 1975) will also contribute proportionately to the increase in metabolism. Since exercise rates are kept constant among salinities, the output from swimming, cardiac and branchial muscles should

also be constant. However, osmoregulatory work is not constant between salinities, as changes in body permeability and in ion and osmotic gradients take place. Thus, at similar swimming speeds only the differences in osmoregulatory requirements among salinities will exist. Therefore, differences in the amount by which metabolic rates increase with swimming speed, for similar rates of mechanical work, must be due to differences in osmoregulation cost. Differences in the density of the medium can be ignored (Webb, 1975), and any salinity can be chosen as the reference, or control, against which the others can be compared.

The above arguments allow us to examine more accurately differences in osmoregulatory cost in terms of the relationship between metabolic rate and swimming speed. The multiple regression models derived above indicate that the rate of increase in total metabolic rate with speed (i.e. the slope) is lowest in ISW (Table 3). This would be expected given the absence of significant Na^+ and Cl^- and osmolarity gradients between the fish and the acclimation medium (Table 1). However, the partial regression coefficient in FW was higher than in SW, suggesting higher osmoregulatory costs for fish acclimated to the former. These results contradict the conclusions of Rao (1968), Farmer & Beamish (1969) and Nordlie & Leffler (1975), who proposed higher total osmoregulatory costs in SW than in FW. Instead, our results agree with the predictions of Eddy (1982), based on thermodynamic principles, which estimated that the minimum cost of osmoregulation in *S. gairdneri* in FW was higher than in SW. Comparisons of the slopes relating metabolic rate to swimming speed are relevant for, in this case, they agree with thermodynamic considerations. This agreement is encouragement for standardizing measurements of metabolic rate prior to making comparisons among salinities. It should be noted, though, that the relationship between metabolic rate and size (Rao, 1968, 1971; Farmer & Beamish, 1969; Job, 1969*a*,*b*; Muir & Niimi, 1972) does not serve this purpose well, as size, contrary to swimming speed, cannot be modified as required during short tests in the respirometer.

Do we know how much energy is spent on osmoregulation?

The minimum cost of osmoregulation in FW, according to Eddy's (1982) estimates, was only 2 % of the resting metabolism of trout; far lower than previous total cost estimates based on differences in rates of oxygen consumption among salinities (Rao, 1968). Using the same principles, Potts, Fletcher & Eddy (1973) and Eddy (1975) had also predicted minimum osmoregulation costs as being no higher than 4 % of the metabolism of flounders (in SW) and goldfish (in FW). These minimal thermodynamic estimates are considerably lower than the more frequently quoted estimates of 20–30 % based on respirometry methods (Rao, 1968; Farmer & Beamish, 1969). Furspan *et al.* (1984) recently suggested that 50 % of oxygen consumption was used for osmoregulation in resting catfish. Such a large difference indicates that the osmoregulatory mechanisms in fish are, thermodynamically, relatively inefficient (apparent osmoregulatory efficiency in trout = $0{\cdot}02/0{\cdot}20 \times 100 = 10\,\%$). However, as discussed above, estimates based on differences in total oxygen consumption are not consistent in magnitude, nor in the order in which they

increase (or decrease) with salinity. The discrepancy *within* such respirometry data impedes any serious analysis of the efficiency with which osmoregulatory mechanisms truly operate.

There is little doubt that a direct correlation exists between rates of active solute transport and oxygen uptake, as has been demonstrated with isolated transporting tissues and organs (Zerahn, 1956; Leaf & Renshaw, 1956; Hess Thaysen, Lassen & Munck, 1961; Martin & Diamond, 1966; Sargent, Bell & Kelly, 1980). Yet, similar increases in consumption of oxygen during similar rates of active ion transport, in separate preparations of the same tissue, still amount to different percentages of the total rate of oxygen consumption (Martin & Diamond, 1966). These differences have been attributed to unrelated variations in maintenance uptake between preparations (Martin & Diamond, 1966). Similarly, differences in maintenance metabolism not related to the cost of osmoregulation (between tilapia acclimated to FW and ISW) may explain the shift in the order in which total rates of metabolism increased with salinity as swimming speed increased (Fig. 5). That is, regardless of the order indicated at rest, more energy for osmoregulation was always being spent in FW, but this only became apparent when the slopes of the relationship between metabolic rate and swimming speed were compared.

In post-absorptive fish, differences in maintenance (= resting) metabolic rate at different salinities may occur that are unrelated to changes in osmoregulatory work. However, although maintenance metabolism can be estimated fairly accurately (by extrapolating to zero activity the relationship between metabolic rate and swimming speed), we are not yet able to distinguish changes in the maintenance-related cost of osmoregulation, with salinity, from changes in other maintenance requirements (see Fig. 6). In resting fish this presents a fundamental problem, when (by assuming that changes occur only in the osmoregulatory compartment) we attempt to estimate the cost of osmoregulation from differences in maintenance rate without properly partitioning those differences. Not being able to make the necessary distinction between different maintenance requirements, accurate estimates of the total cost of osmoregulation in actively swimming fish are also precluded. Although the maintenance component becomes a smaller fraction of the total metabolic rate with increasing swimming speed, it will contribute at least 20–30% of the total at maximum sustainable speeds (Jones & Randall, 1978), still allowing considerable room for error in estimating osmoregulation costs when active metabolic rates are compared indiscriminately.

The remaining alternative is to subtract the maintenance expenditure from the total metabolic rate of active fish to obtain the net cost of swimming (Beamish, 1978; Stevens & Dizon, 1982) (Fig. 6). This quantity is more useful for comparison among salinities because its magnitude is always a function of swimming speed, a variable which is controlled by the investigator. Although, like maintenance uptake, the net cost of swimming is the sum total of various energy-requiring parameters, these can be separately identified as the swimming, branchial and heart muscles, and the activity-related cost of osmoregulation (Webb, 1975; Jones & Randall, 1978; Stevens & Dizon, 1982). As discussed earlier, the first three are assumed to be constant

among salinities, since their net, activity-related output is proportional to swimming speed, which is the same among salinities. Therefore, differences in the net cost of swimming, when fish are swimming at similar speeds, can be attributed to the effect of salinity on the activity-related cost of osmoregulation (Fig. 6).

Although the total rate of oxygen consumption of a 63-g fish, swimming at $10\,cm\,s^{-1}$, is higher in ISW than in FW, the net cost of swimming is lower, probably because the latter is not subject to unexplained, or uncontrolled, variation in the non-swimming component (Table 4; Figs 5, 7). The implications of comparing total with net costs of swimming are even more evident between the FW and SW treatments. Although in SW total metabolic rates at any given swimming speed are consistently higher than in FW, the net costs of swimming are either similar, or lower, for the 63-g fish (Table 4). Table 5 shows that in FW the activity-related cost of osmoregulation increases from 0 at rest, to $41\,mg\,O_2\,kg^{-1}\,h^{-1}$ (16·0 % of the total metabolic rate, 24 % of the net cost of swimming) at $40\,cm\,s^{-1}$. In SW the rate of increase is less marked, from 0 at rest, to $32\,mg\,O_2\,kg^{-1}\,h^{-1}$ (12·3 % of the total metabolic rate, 20 % of the net cost of swimming) at $40\,cm\,s^{-1}$. These are not insignificant costs in active

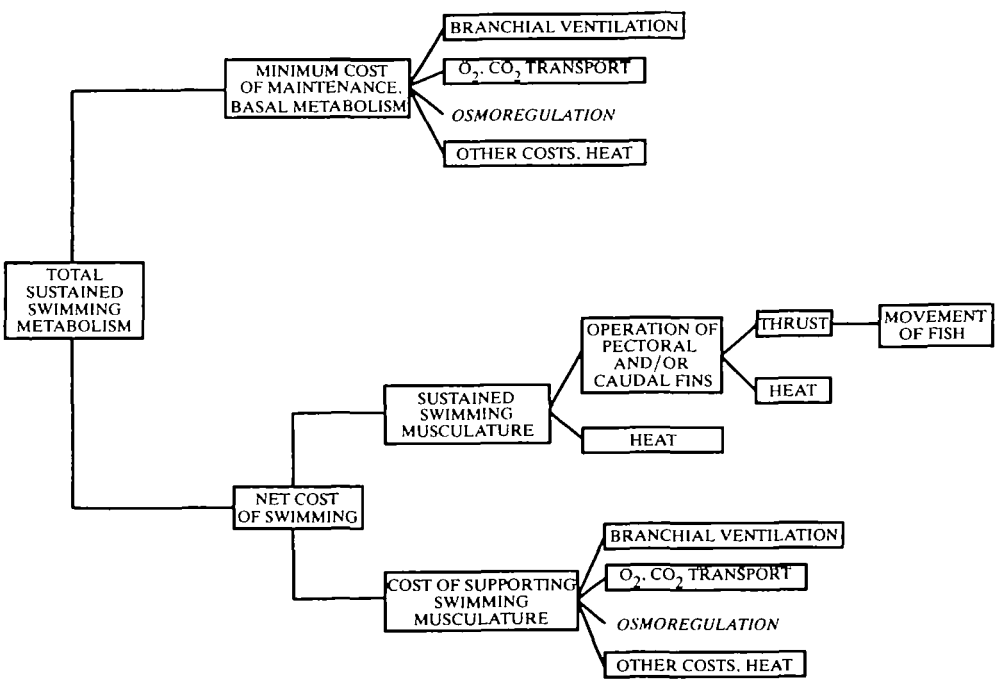

Fig. 6. Breakdown of total metabolic rate into the maintenance and activity-related compartments. The maintenance component, minus maintenance osmoregulation costs, varies among salinities to an unknown extent, whereas the net cost of swimming is always a function of activity-related work. Since the rate of mechanical work can be adjusted by the investigator, so that it remains constant among salinities, differences in the net cost of swimming at constant swimming speeds must be due to the activity-related cost of osmoregulation.

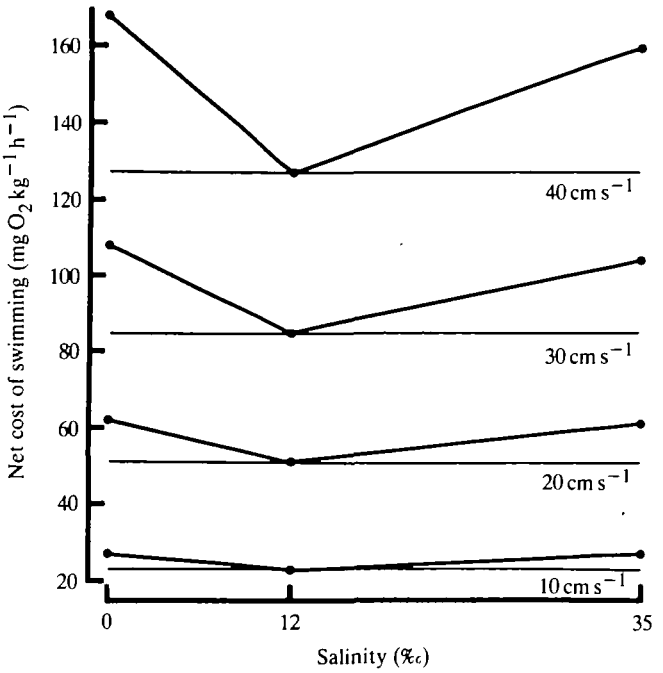

Fig. 7. Oxygen uptake above resting (maintenance) level *versus* salinity at different swimming speeds. Values are for a 63-g fish.

fish, contrary to thermodynamic estimates of the *minimum* cost in resting fish (Eddy, 1982).

The net cost of swimming in ISW increases linearly within the range of speeds examined here, the slope having a value equal to 3·46 ($r^2 = 0{\cdot}99$; derived from data in Table 4). In FW the activity-related cost of osmoregulation increases linearly with a slope of 1·23 ($r^2 = 0{\cdot}96$; derived from data in Table 5), while in SW the slope was 0·93 ($r^2 = 0{\cdot}97$). Thus, in non-isosmotic waters net oxygen uptake by tissues exclusively associated with swimming increases at a rate 3–4 times greater than the rate of uptake necessary to cover increases in osmoregulation cost. This means that oxygen supplied to the swimming muscles is not restricted by increases in osmoregulation cost (Jones & Randall, 1978). It is important to notice that although at 10 cm s^{-1} the net cost of swimming and activity-related cost of osmoregulation are exactly the same in FW and SW, the percentages of the total metabolic rate due to the activity-related cost of osmoregulation are slightly different (Tables 4, 5). This supports our contention that changes in maintenance metabolism due to non-osmoregulatory processes can bias estimates of osmoregulation cost, although in this case the effect would not have been particularly significant.

Why is the cost of osmoregulation lowest in isosmotic sea water?

In tilapia acclimated to ISW, the osmolarity and Na^+ concentrations of the external medium are about the same as in the blood plasma, while the external Cl^- concentration is about 60 mmol l^{-1} higher (Table 1). In isosmotic conditions the

Table 5. *Activity-related cost of osmoregulation (mg O_2 kg^{-1} h^{-1}) for an average fish (63 g) acclimated to fresh water (0 ‰) or sea water (35 ‰), with respect to an isosmotic sea water (12 ‰) reference*

Swimming speed		Acclimation medium					
		Fresh water			Sea water		
($L\,s^{-1}$)	($cm\,s^{-1}$)	Activity-related cost of regulation* (mg O_2 kg^{-1} h^{-1})	Percentage of total metabolic rate	Percentage of net cost of swimming	Activity-related cost of regulation* (mg O_2 kg^{-1} h^{-1})	Percentage of total metabolic rate	Percentage of net cost of swimming
0	0	0	0	0	0	0	0
0·6	10	4	3·4	15	4	3·1	15
1·2	20	11	7·3	18	10	6·1	16
1·8	30	23	11·7	21	19	9·2	18
2·4	40	41	16·0	24	32	12·3	20

L, body length.
* Net cost of swimming in fresh or sea water minus net cost in isosmotic sea water.

large influx of water from the environment, characteristic of fish living in FW, would be reduced to a minimum. Not surprisingly, in comparison with FW-acclimated individuals, compensatory production of large volumes of urine decreases to a marginal level in those acclimated to an isosmotic medium (Hickman & Trump, 1969; McVicar & Rankin, 1983; Furspan *et al.* 1984). This reduction implies a saving of energy spent reabsorbing important solutes from the ultrafiltrate (Hess Thaysen *et al.* 1961). A reduction in the osmotic-related work performed by the intestine and the gills, in SW-acclimated fish, would also be expected on transfer to ISW. In intact fish, transepithelial potentials measured in ISW (or in Ringer solutions) generally approach the Nernst equilibrium potential for Na^+ (Pic, 1978; Eddy & Bath, 1979), implying that little energy is required for its regulation in this condition (Eddy & Bath, 1979). However, this also means that the transepithelial potential in ISW is further away from the Nernst equilibrium potential for Cl^-, necessitating the expenditure of more energy for Cl^- than for Na^+ regulation (Eddy & Bath, 1979). Relative costs of ion regulation in non-isosmotic salinities are more difficult to predict because of differences in electrical, as well as concentration, gradients, and in body permeability. Nevertheless, the oxygen consumption data indicate that the sum of ion and water costs of regulation in ISW is still smaller than in FW or SW.

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