ספרית אורות

המאמרים המשמשים במערכת הדפסים והמודיעoyo על-

הוקם ועדות ויזראים

dפסת מאמרים נוספים לצרכי לימוד והוראה בלבד

אין לעשות כל שימוע מסחרי במאמרים.
Effects of salinity acclimation on oxygen consumption in the freshwater blenny, *Salaria fluviatilis*, and the marine peacock blenny, *S. pavo*

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**Abstract.** The effect of salinity on standard metabolic rate was studied in a freshwater blenny, *Salaria fluviatilis*, and a marine congener, *S. pavo*. These blennies tolerate both fresh water (FW, <30 mOsm L\(^{-1}\)) and full-strength sea water (SW, 1000 mOsm L\(^{-1}\)). Fish of both species were acclimated to FW, isosmotic concentration (IOC, 375 mOsm L\(^{-1}\)) and SW at 21 ± 2°C for three months before oxygen consumption rates were measured. Slopes of the regressions relating oxygen consumption rates to body mass did not differ significantly between species and among salinity treatments. Oxygen consumption rates of *S. fluviatilis* in FW were significantly higher than in IOC and SW, whereas oxygen consumption rates of *S. pavo* were similar in IOC and SW salinity but were significantly lower in fish in FW. These results do not seem to be accounted for by different costs of osmoregulation or by different concentrations of oxygen in the water among different salinities, and they may demonstrate reduction in metabolic activities as a reaction to chronically unfavourable, sub-optimal environmental conditions.

**Extra keywords:** *Salaria pavo*, fish, resting metabolic rate.

**Introduction**

Freshwater and marine bony fishes are usually stenohaline (Jobling 1995), whereas euryhaline fishes are able to live in habitats of fluctuating salinities, such as estuaries (Evans 1993; Madsen *et al.* 1996), or pass through different phases of both marine and freshwater habitats during their life history (Hoar 1988; Boeuf 1993). There are, however, some fishes with euryhaline capabilities that rarely encounter wide salinity fluctuations in their natural habitat; one of these is the freshwater blenny, *Salaria fluviatilis* (Asso 1801), which inhabits freshwater habitats and is widely distributed throughout the northern and eastern parts of the Mediterranean basin (Steinitz 1954; Kosswig 1967). Although the population in Lake Kinneret (Sea of Galilee, Israel) has not encountered salinity fluctuations since the upper Pliocene, the fish may tolerate exposure to water of salinity up to that of full-strength sea water (Plaut 1998).

The marine peacock blenny, *Salaria pavo* (Risso, 1810), is closely related to *S. fluviatilis* and inhabits intertidal rocky coasts of the Mediterranean and European Atlantic coasts (Fishelson 1963; Zander 1973). Kosswig (1967) considered *S. fluviatilis* to be a polytopic derivative of *S. pavo*, and Zander (1973) postulated a eurythermal and euryhaline common ancestor for both species. As in *S. fluviatilis*, *S. pavo* also tolerates exposure to a wide range of salinities from full-strength sea water to fresh water (Plaut 1998).

The effect of environmental salinity on the metabolic rates of fishes has been well studied (see review by Kirschnier 1993, 1995; Peterson and Meador 1994). However, the physiological responses of fish to salinity change may be complex; salinity may affect a number of physiological systems, and salinity rarely changes in isolation from other environmental factors, such as temperature and concentrations of dissolved oxygen and CO\(_2\) (Wheatly 1988).

Studies of the effects of salinity on fishes often use oxygen consumption to estimate the metabolic cost of osmoregulation; this cost is expected to be lowest for fishes in an isosmotic environment, and is expected to increase as salinity increases or decreases and the medium becomes hyper- or hypo-osmotic to the body fluids (Febry and Lutz 1987). This holds if it is assumed that the permeability of gills remains constant (but see Gordon 1963; Shehadeh and Gordon 1969). However, results of experiments on the relationship between water salinity and metabolic rate vary from an absence of effect to a significant increase or decrease in metabolic rate at acclimation to low or high salinities (see review by Peterson and Meador 1994).

This study tested the effects of acclimation to different salinities on the standard metabolic rates of *S. fluviatilis* and *S. pavo*. The working hypothesis was that since both species are closely related, they would show similar responses. Metabolic rates were measured indirectly in terms of oxygen consumption rates.

**Materials and methods**

*Fish collection, maintenance and acclimation*

*S. pavo* (62 males and 52 females with body mass range 0.88–6.78 g) were collected from tide-pools on the rocky shore of Habonim, Israel (32°35'N,34°54'E), in May 1996. Quinaldine solution (10% Quinaldine in 90% ethanol) was poured into the pools in amounts sufficient to anaesthetize the fishes in it (Summerfelt and Smith, 1990) within 10–30 min. The fish
were transferred to a sea-water container, where they recovered within 5 min, and were then transferred to a 10 L container with continuously aerated sea water, and the other fish were released. Sea-water temperature in the tide pools during collection ranged between 23° and 28°C. S. fluviatilis (77 males and 89 females with body mass range 0.52–6.52 g) were collected from shallow water at the northern end of Lake Kinneret, Israel (32°52’N,35°32’E), in May 1996. The fish were captured by a diver using a hand-net. Water temperature at the collection site was 21.5°C. The fish were transported to the laboratory, within 2 h of collection, in 10 L cans filled with continuously aerated water from the collection site.

The blennies were held indoors in 20 L glass aquaria, 10–20 fish per aquarium, at a temperature of 21 ± 1°C, and were exposed to a 12:12 LD photoperiod under continuous aeration and biological filtration. In the holding tanks, concentration of ammonia was always <4 mg L⁻¹, concentration CaCO₃ was 150–240 mg L⁻¹ (within the range of the habitat water of S. fluviatilis) and chlorine in dechlorinated fresh water was undetectable. S. pavo was held in natural Mediterranean sea water, and S. fluviatilis in dechlorinated tap water. The fish were fed twice a day with dry Tetramin flax, live Daphnia spp. and Tubifex sp. Small stones and PVC tubes were placed in the aquaria as shelters. The fish were held under these conditions for 3 weeks to recover from handling.

Three weeks after the fish were brought into the laboratory, acclimation to different salinities began. Fish of each species were divided into three treatment groups, and each group was acclimated to a different salinity: fresh water, FW, <30 mOsm L⁻¹; iso-osmotic concentration, (IOC) 375 mOsm L⁻¹ (Davenport and Vahl 1979); and sea water (SW), 1000 mOsm L⁻¹. Acclimation was made in a stepwise manner, by decreasing (for S. pavo) or increasing (for S. fluviatilis) the salinity by ~100 mOsm L⁻¹ each day, either by adding sea salt (Instant Ocean) or by diluting the sea water in the aquaria with dechlorinated tap water. Thus, all groups reached their designated salinity within 0–10 days, and they were then held at these salinities for an additional 3 months before measurements of oxygen consumption rates began.

Apparatus

Oxygen consumption measurements were undertaken in a semi-closed respirometry system containing four cylindrical measurement chambers. The chambers, 11 cm long and 3.5 cm internal diameter, were submerged in a water bath and were connected by c-flax tubing (MasterFlex 6424-16) to a reservoir containing aerated water at the experimental salinity and temperature. Total volume of each chamber (including tubing and electrode cell) was ~135 mL (exact volumes were used for each cell for the calculation of oxygen consumption). A peristaltic pump (MasterFlex, Cole-Parmer Instruments Co.) maintained water flow from the reservoir to the respirometer chambers and back to the reservoir. Water flow from a chamber could be directed to a small container with a polarographic oxygen electrode (YSI 5331 connected to Biological Oxygen Monitor, YSI Model 5300) via 3-way valves, and depletion in oxygen concentration in the water could be measured. The oxygen electrode was calibrated in aerated water (100% saturation) and N₂ bubbled water (0% saturation) before and after each run. Recordings were stored every 10 s for 15 min on a IBM PC compatible computer equipped with A/D hardware and software (Logal Explorer, MBL). Experimental temperature was 20 ± 0.2°C and oxygen saturation was always kept at >80% of air-saturation levels.

Experiments

One fish, unfed for 24 h, was placed in each of three chambers and left for 4–5 h to recover from handling. Preliminary tests showed that oxygen consumption was high immediately after placement in the chambers, and then decreased to a stable value after ~2 h and remained stable for >24 h. At this time the fish were completely quiescent in the chamber, so the oxygen consumption rates are regarded as standard metabolic rates. The fourth chamber was left empty of fish (blank) to measure electrode drift and bacterial oxygen consumption. Following the recovery period, oxygen consumption was measured over ~15 min, long enough to obtain a depletion of oxygen concentration in the water (>5% of saturation). Blank measurements were performed both before and after the measurements of oxygen consumption by the fish. Each fish was used only once, and after the measurement the fish was weighed (± 0.01 g) in a pre-weighted can with water and transferred to a new aquarium.

Linear regression analysis was performed on each data set of oxygen depletion over time, and the regression coefficient was used to calculate oxygen consumption, \( \dot{V}_O_2 \) (μmol h⁻¹):

\[
\dot{V}_O_2 = (a - a_o)3600S_O_2 WP/760
\]

where \( a \) is the linear regression coefficient (%), \( a_o \) is the regression coefficient of the relevant blank measurement, \( S_O_2 \) is oxygen concentration at 100% saturation in the experimental salinity at 20°C at an atmospheric pressure of 760 mmHg (285, 262 and 230 μmol L⁻¹ at FW, IOC and SW, respectively; Green and Carritt 1967), \( W \) is the water volume (L) in the closed respirometry system, and \( P \) is the atmospheric pressure (mmHg). Background (bacterial) oxygen consumption, the ‘blank’ measurement, was always <3% of the fish oxygen consumption measurements. Correction were made for blank oxygen consumption rates if those rates exceeded 1% of the experimental values per run.

Statistical analysis

Data for oxygen consumption and body mass were log transformed, and linear regressions were plotted for each data set (i.e. species and salinity): log \( \dot{V}_O_2 = \log a + \log b M \) where \( M \) is body mass (g). Within each species, data sets were tested for homogeneity of the slope.

Log transformations of the data do not allow analysis of covariance, so oxygen consumption values were converted to values for an ‘average’ fish with 2.9 g body mass. The conversions used the following equation (Carefoot 1989):

\[
\dot{V}_O_2 (\text{avg}) = (x/M)^b \dot{V}_O_2 ,
\]

where \( x = 2.9 \), \( M \) is the actual body mass and \( b \) is the regression coefficient of each data set. Values of \( \dot{V}_O_2 \) for an ‘average’ 2.9 g fish were tested with one-way ANOVA, and Tukey HSD tests were used to determine differences between salinity for each species. All data analyses were performed with Systat 5.04 for Windows (Wilkinson 1990). Values are presented as mean ± s.e., and \( P \leq 0.05 \) was taken as the level of significance.

Results

The slopes of the regression equations relating log oxygen consumption to log body mass were not significantly different among species and salinities and ranged between 0.57 and 0.65 (test for homogeneity of slopes, \( F_{5,156} = 0.172, P = 0.97 \)) (Fig. 1). These values are at the lower end of the range known for fish, 0.65–0.9 (Jobling 1994).

Oxygen consumption rate of S. fluviatilis decreased significantly as salinity increased (Table 1), with fish in FW consuming 47% more oxygen than fish in IOC and 70% more than fish in SW. Oxygen consumption rate of S. fluviatilis held in IOC was not significantly different from that of fish in SW. For S. pavo the trend was the opposite (Table 1): oxygen consumption of S. pavo held in SW was not significantly different from that of fish in IOC, but S. pavo held in FW consumed significantly less (~30%) oxygen than the fish held in IOC and SW.

Fish of both species consumed similar amounts of oxygen when acclimated to IOC and SW (Table 1), but when acclimated to FW, S. pavo consumed about 2.3 times less oxygen
Salinity effects on oxygen consumption in blennies

than *S. fluviatilis*. When oxygen consumption rates of the two species were compared at the salinities of their respective natural habitats (*S. fluviatilis* in FW and *S. pavo* in SW), *S. fluviatilis* was found to consume about 1.6 times more oxygen than *S. pavo*.

**Discussion**

Salinity is expected to affect metabolism through changes in energy expenditure for osmoregulation. Fish in iso-osmotic environments (330–380 mOsm L\(^{-1}\)) are expected to expend minimum energy for osmoregulation, whereas energy costs are expected to increase in water that deviate from the iso-osmotic level. Such a trend was reported for *Oreochromis mossambicus* (Febry and Lutz 1987; Kültz et al. 1992) after long-term acclimation (but see the opposite in Morgan et al. 1997 after acute exposure to different salinity). The energy costs of osmoregulation remain unclear and have been reported to vary widely, from an empiric value of >45% (Maceina et al. 1980; Furspan et al. 1984) to a theoretical value of 1–2% (Jobling 1994) of metabolic rate.

Most of the data on the effects of environmental salinity on metabolic rate in fishes concern species that are exposed to salinity changes during their life cycle (Job 1969; Skadhauge and Lotan 1974; Hettler 1976; Davenport and Vahl 1979; Furspan et al. 1984; Langdon 1985; McClanahan et al. 1986; Barton and Barton 1987; McCormick and Saunders 1987; Moser and Hettler 1989; Morgan and Iwama 1991; Nordlie et al. 1991; Moser and Miller 1994). Although *S. pavo* and *S. fluviatilis* may be found in brackish water (Zander 1973), the usual habitats of the populations in this study are strictly marine (*S. pavo*) or fresh water (*S. fluviatilis*). For such species the salinity tolerance is unexpected and probably developed during previous geological ages (Kosswig 1967; Zander 1973). Thus, it is hard to compare them with other fishes studied previously. Moreover, in most of the previous studies fish were exposed to experimental salinity for shorter duration.

The decrease in oxygen consumption of *S. fluviatilis* as salinity increased above FW was expected as a sign of a decrease in osmoregulation cost when comparing FW with IOC; however, the further decrease in oxygen consumption when acclimated to SW cannot be explained in terms of a decrease in osmoregulation cost, since the osmotic gradient between the body fluids and the environment increases. The differences in oxygen consumption rates cannot be attributed to different activity levels, since the fish in all experiments, as in Swanson (1998), were quiescent and the data represent standard metabolic rates.

**Table 1.** Influence of salinity on oxygen consumption (mean ± s.e.; μmol h\(^{-1}\)) of *Salaria fluviatilis* and *S. pavo* at three salinities

<table>
<thead>
<tr>
<th>Species</th>
<th>Salinity (mOsm L(^{-1}))</th>
<th>Body mass, (M) (g)</th>
<th>(n)</th>
<th>(\frac{\log \dot{Y}_{\text{O}_2} = \log(a) + b \log(M)}{\log(a)})</th>
<th>(b)</th>
<th>(r^2)</th>
<th>Oxygen consumption of 2.9 g 'average' fish</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. fluviatilis</em></td>
<td>&lt;30</td>
<td>0.52–6.52</td>
<td>24</td>
<td>0.86</td>
<td>0.57</td>
<td>0.791</td>
<td>13.44 ± 0.60(^a)</td>
</tr>
<tr>
<td></td>
<td>375</td>
<td>0.56–5.72</td>
<td>27</td>
<td>0.69</td>
<td>0.63</td>
<td>0.755</td>
<td>9.71 ± 0.42(^b)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.70–5.88</td>
<td>35</td>
<td>0.63</td>
<td>0.65</td>
<td>0.787</td>
<td>8.67 ± 0.31(^c)</td>
</tr>
<tr>
<td><em>S. pavo</em></td>
<td>&lt;30</td>
<td>1.29–5.34</td>
<td>18</td>
<td>0.49</td>
<td>0.57</td>
<td>0.303</td>
<td>5.94 ± 0.47(^c)</td>
</tr>
<tr>
<td></td>
<td>375</td>
<td>0.95–6.78</td>
<td>32</td>
<td>0.64</td>
<td>0.63</td>
<td>0.571</td>
<td>8.71 ± 0.40(^b)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.88–6.57</td>
<td>32</td>
<td>0.66</td>
<td>0.57</td>
<td>0.388</td>
<td>8.80 ± 0.56(^b)</td>
</tr>
</tbody>
</table>
The decrease in oxygen consumption by *S. pavo* in FW can, similarly, not be attributed to changes in osmoregulation energy cost. There are only two reports on the effect of salinity on metabolic rate in blennies: exposure of the intertidal blenny *Parablennius sanguinolentus* to IOC and FW salinities (Plaut 1999) had an effect similar to that shown here for *S. pavo*, and exposure of *Blennius pholis* to acute salinity fluctuations did not significantly affect metabolic rate (Davenport and Vahl 1979). However, Davenport and Vahl (1979) measured the effect of acute exposure whereas the present study measured the effect of chronic exposure.

*S. pavo* and *S. fluviatilis* showed opposite metabolic responses to the salinities tested, but both decreased their metabolic rates when exposed to a salinity that differed from that of their natural habitat. The changes in metabolic rates do not seem to be related to differences in costs of osmoregulation. The decrease in metabolic rate may indicate a reaction to chronic exposure to unfavourable conditions, termed 'tertiary stress response' by Jobling (1994), which may lead to reduction in reproductive success, depression of growth rate and decrease in disease resistance.

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**References**


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