Resting Metabolic Rate, Critical Swimming Speed, and Routine Activity of the Euryhaline Cyprinodontid, *Aphanius dispar*, Acclimated to a Wide Range of Salinities

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**ABSTRACT**

Specimens of the euryhaline cyprinodont fish, *Aphanius dispar*, collected in salt ponds, were acclimated to salinities of <1 (freshwater), 35 (seawater), 70, 105, and 140 ppt for 4 wk before measurement of oxygen consumption, critical swimming speed, and routine activity level. Oxygen consumption was similar in <1, 35, and 70 ppt (mean ± SD) but decreased in 105 and 140 ppt (mean ± SD) respectively. Critical swimming speed and routine activity levels showed the same trend. These results suggest a general decrease in physiological function of *A. dispar* at extreme salinities.

**Introduction**

Teleost fishes are osmoregulators, maintaining their body fluids’ osmotic concentration within a relatively narrow range, from ~260 to 400 mOsmol (Jobling 1995), usually different from the osmotic concentration of their environment. To osmoregulate, fish actively create a flow of ions and water in the opposite direction to the passive flow imposed by the osmotic gradient. In addition, they may reduce their gill membrane and/or integument permeability to passive flows. Most fishes are capable of tolerating only a narrow range of salinities in their natural environment (Haney and Nordlie 1997), and exposure to salinities substantially outside their range may exceed their osmoregulatory capabilities and reduce their performance (Beamish 1978; Webb 1994). By contrast, some fishes from habitats characterized by a wide range of salinity fluctuations (estuaries, salt marshes, intertidal pools, etc.) show physiological adaptations that enable them to tolerate and survive extreme salinity levels and fluctuations.

Most studies on the physiological and behavioral effects of euryhalinity and salinity have been done either with a narrow range of salinity, usually subranges between freshwater and full-strength seawater (Igram and Wares 1979; Nelson et al. 1996; Young and Cech 1996; Morgan et al. 1997; Kolok and Sharkey 1997; Morgan and Iwama 1998; Plaut 1998, 1999a, 1999b; Shikano and Fujiyo 1998) or under acute exposures to salinity fluctuations (Davenport and Vahl 1979; Moser and Miller 1994). Data on the effects of long-term acclimation to extreme salinities (above seawater) on fish are rare. Recent studies have included only two species, the milkfish, *Chanos chanos* (Swanson 1998), and the sheephead minnow, *Cyprinodon variegatus* (Haney and Nordlie 1997; Haney et al. 1999). Few fish species can tolerate extreme salinity, which may explain the small number of studies in this area (Nordlie and Haney 1998).

The cyprinodontid, *Aphanius dispar* (Teleostei, Cyprinodontidae) is a euryhaline fish species found in a wide range of salinities, from freshwater to >500% (~175 ppt) seawater in springs around the Dead Sea and in salt ponds in Atlit, Israel (Lotan 1969, 1971; Lotan and Skadhauge 1972; Skadhauge and Lotan 1974). Lotan (1971) showed that *A. dispar* is capable of maintaining its plasma osmotic pressure and ionic concentration when acclimated to salinities from 16 to 105 ppt. However, freshwater acclimation reduced *A. dispar* plasma osmotic pressure to 70% (compared with fish in seawater), and acclimation to 140 ppt increased its plasma osmotic pressure to about 135%. The ability of *A. dispar* to tolerate such a wide range of salinities makes it an excellent candidate for studies of salinity effects on other physiological, ecological, and behavioral traits.

In this study, I examine the effects of extremely high salinity on physiological and behavioral functions of *A. dispar*. I exposed *A. dispar* to a range of salinities, from freshwater to 175 ppt (500% seawater) for 4 wk, and then measured oxygen consumption rate, critical swimming speed, and routine activity level.

**Material and Methods**

**Fish Collection, Holding, and Acclimation**

Adult *Aphanius dispar* (standard length [SL], 31.9 ± 0.5 mm [mean ± SE, range 24.6–46.3 mm], body mass [BM],
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0.72 ± 0.04 g [range 0.23–1.37]) were collected in the salt ponds of Atlit, near Haifa, Israel, by hand net. Salinity in the salt ponds at the time of collection was ~57 ppt (~160% seawater) and water temperature was 27°C. In the laboratory, fish were divided into five groups, each subdivided among three aquaria. These aquaria contained continuously aerated water from the salt ponds. Fish were treated prophylactically for 24 h with malachite green (0.15 ppm) and with methylene blue (1 ppm). Seven percent of the fish died within 3 d of collection; afterward mortality was very low. Two weeks after collection, salinity acclimation was begun. Salinity was changed by addition of artificial sea salt (Instant Ocean) or by dilution with distilled water. Each day, the salinity was increased or decreased by ~9 ppt. After 14 d, there were five groups in each of five different salinities: freshwater (<1 ppt salt), 35, 70, 105, and 140 ppt. Then, fish acclimated to each salinity were pooled to one larger tank for the rest of the acclimation period (five salinity groups in five tanks). Salinities were then monitored daily, and distilled water was added accordingly to compensate for evaporation. Fish remained in these salinities for 4 wk before experiments were begun. The aquaria were equipped with biological filters and temperature control (25°C ± 1°C), and fish were fed twice a day with Tetramin flakes and live *Daphnia magna* and *Artemia salina*.

**Oxygen Consumption Measurements**

The respirometer used in this study was described in detail in Plaut (1999a, 1999b). This is a semiclosed respirometer (Stefensen 1989) with eight measuring cells. Water was directed from an aerated (100% O₂ saturation), temperature-controlled reservoir containing water at the experimental salinity to the respirometer chamber and back to the reservoir by peristaltic pumps via c-flex tubing. Three-way stopcocks made it possible to disconnect a chamber from the reservoir and to direct the water flow from the respirometer chamber to a cell with an oxygen electrode and back to the chamber in closed circulation. Thus, oxygen depletion in the chamber could be measured without disturbance to the fish.

Fish were not fed for 24 h before measurement. Postabsorptive fish were placed in each of seven chambers (one chamber was left empty as a blank control) and left for 4–5 h to recover from handling. Preliminary tests showed that oxygen consumption was high immediately after placement of the fish in the chambers and then decreased to a stable value after approximately 2 h and remained stable for more than 24 h. At the beginning of the measurement, fish were completely quiescent in the chamber, so the oxygen consumption rates are regarded as resting metabolic rate (RMR). Following the recovery period, oxygen consumption was measured over about 15 min, long enough to detect depletion of oxygen concentration in the water (>5% decrease of saturation). Oxygen saturation was not allowed to decrease below 85%. Blank measurements were performed both before and after the measurements of oxygen consumption of the fish. Each fish was used only once, and after the measurement, the fish were weighed (±0.01 g) in a preweighed beaker with water and transferred to a new aquarium containing “used” fish.

Linear regression analysis of oxygen depletion over time was performed for each fish, and the regression coefficient was used to calculate oxygen consumption:

\[ \dot{V}_O_2 (\mu mol h^{-1}) = a \times 3,600 \times S_o_2 \times V \times P/760, \]

where \( a \) is the linear regression coefficient (percentage s⁻¹), the value 3,600 converts seconds to hours, \( S_o_2 \) is oxygen concentration at 100% saturation in the experimental salinity at 25°C at an atmospheric pressure of 760 mmHg (Green and Carritt 1967), \( V \) is the water volume in the closed respirometry system measured specifically for each chamber (L), and \( P \) is the actual atmospheric pressure in mmHg. Background (bacterial) oxygen consumption measured in the blank chamber was always below 4% of the fish’s oxygen consumption measurements. Corrections were made for blank oxygen consumption rates if those rates exceeded 1% of the experimental values per run.

**Critical Swimming Speed (U_{crit})**

Swimming performance was measured as critical swimming speed (\( U_{crit} \)) in a water tunnel described in Plaut (2000). Postabsorptive fish (24 h) were placed in the water tunnel containing the relevant water salinity at a temperature of 25°C ± 0.5°C. Water velocity was set to 4 cm s⁻¹ (about 1 SL s⁻¹), and the fish were left undisturbed for 2 h to recover from handling (Kolok 1991; Peake et al. 1997). Water velocity was then increased in increments of 4 cm s⁻¹ at 5-min intervals, until the fish fatigued. Fatigue was determined as the situation where the fish could no longer maintain position against the current and were swept downstream and held against the mesh screen at the downstream end of the water tunnel. \( U_{crit} \) was calculated according to the equation (Brett 1964)

\[ U_{crit} = U_i + [U_{ii}(T_i/T_o)], \]

where \( U_i \) is the highest velocity maintained for the whole 5 min (cm s⁻¹), \( U_{ii} \) is the velocity increment (i.e., 4 cm s⁻¹), \( T_i \) is the time elapsed at fatigue velocity (min), and \( T_o \) is the interval time (5 min). \( U_{crit} \)'s were calculated both as absolute (cm s⁻¹) and relative (SL s⁻¹) swimming speeds.

**Routine Activity Rate**

Routine activity rates of *A. dispar* were measured in five isolated identical aquaria, 28 × 14 × 18 cm (length, width, and height, respectively), containing water with the relevant salinity of the
tested group at 25° ± 1°C under photoperiods of 12L:12D. Ten (two for each aquarium) infrared beam projectors and recording photocells were placed along the long wall of the aquarium opposite a reflector. Photocells were placed in one-third and two-thirds of the aquaria length (9 cm from each other), 6 cm above the bottom. A counter connected to a recorder attached to a PC recorded every time a fish crossed a beam. The computer recorded the accumulated crosses for each photocell every 15 min.

Because A. dispar is a gregarious species, activity levels were measured in groups of six fish for each salinity. A group of six fish, acclimated to a certain salinity, was placed in one aquarium for 72 h before measurement. After about 60 h, aquaria were inspected to ensure that all fish were in good condition and behaving normally. Measuring began 12 h after the experimenter left the room and continued for 24 h. Five sets of measurements were taken, each with different fish. Data for each group (salinity) for the five sets were assembled for each hour of the day (24 h) for analysis.

Statistical Analyses

Resting oxygen consumption was compared among salinities using ANOVA and the Tukey’s post hoc test. Relative critical swimming speed (U\text{crit}, SL s^{-1}) values were plotted against standard lengths of the fish. Regression lines were tested for homogeneity of slopes, and since all were found homogeneous, ANCOVA was performed. Because ANCOVA detected significant differences between salinities, a Tukey’s HSD multiple comparison was conducted to test for differences between each pair of salinity (Wilkinson 1990).

Activity data were compared among the different salinities for each hour of the day using ANOVA and Tukey’s post hoc test. Differences were considered significant at \( P < 0.05 \).

Results

Oxygen Consumption

Oxygen consumption rate is expected to be strongly influenced by body size (Haney and Nordlie 1997). However, no size effects were detected, which probably reflects the small size range of the fish examined. Resting oxygen consumption of Aphanius dispar did not differ among fish held in freshwater, 35 ppt, and 70 ppt (0.18 ± 0.07 \([n = 22]\), 0.17 ± 0.06 \([n = 14]\), and 0.16 ± 0.04 \([n = 24]\) mL h^{-1} g^{-1}, respectively). However, oxygen consumption was lower for fish held in 105 and 140 ppt (0.12 ± 0.02 \([n = 20]\) and 0.09 ± 0.02 \([n = 20]\) mL h^{-1} g^{-1}, respectively; Fig. 1).

Critical Swimming Speed

Absolute (cm s^{-1}) critical swimming speed of fish in freshwater increased with body size (SL; \( R^2 = 0.35, F_{1,19} = 10.4, P = 0.004 \)), but no body size effect was detected at other salinities. Relative swimming speeds (U\text{crit} measured as SL s^{-1}) decreased significantly as body size increased for fish in all salinities (Table 1; Fig. 2). The slopes were homogeneous among groups (\( F_{1,78} = 0.272, P = 0.895 \)). U\text{crit} of fish acclimated to freshwater, 35 ppt, and 70 ppt did not differ (ANCOVA). However, U\text{crit} was lower in fish acclimated to 105 ppt. It decreased further in fish acclimated to 140 ppt. Several fish acclimated to 140 ppt did not survive the tests, so the experiments of this group were limited to a sample size of seven fish only.

Routine Activity Rate

Aphanius dispar showed a striking circadian rhythm, being active during the light period and relatively quiescent during the dark period (Fig. 3). Fish acclimated to freshwater, 35 ppt, and 70 ppt showed similar rates of activity in light and dark periods (data compared for each hour of the day by ANOVA and Tukey’s post hoc test). In some hours, one of these groups was significantly more, or less, active than the others, but no clear pattern could be detected. However, activity was lower in fish acclimated to 105 and 140 ppt, and during some hours, fish acclimated to 140 ppt were significantly less active than those acclimated to 105 ppt.

Discussion

Although Aphanius disparate inhabits water bodies of wide salinity range (Lotan and Skadahage 1972; Skadahage and Lotan 1974), it performs best at salinities between freshwater and 70 ppt, and its performance significantly decreases when salinity increases above 70 ppt. This decrease is demonstrated both at the physiological level (RMR) and the functional-ecological level (swimming performance and routine activity level). The
pattern observed for *A. dispar* is in agreement with the study of Haney et al. (1999) on the sheepshead minnow. Extreme salinities may be stressful for salt marsh resident fishes, resulting in reduced energy expediencies.

RMR of *A. dispar* was not affected by salinity acclimation of freshwater, 35 ppt, and 70 ppt but decreased when acclimated to salinities above 70 ppt. Most studies on effects of salinity on metabolic rate of fishes have measured either the effects of acute exposure to different salinities (Davenport and Vahl 1979; von Oertzen 1984; Moser and Miller 1994) or salinity effects on fish that can tolerate only a narrow range of salinity with no obvious euryhaline adaptation (Stuenkel and Hillyard 1981; Meador and Kelso 1989, 1990). Moreover, most of these studies tested salinities within the ranges between freshwater and seawater only (see also Plaut 1999). Thus, data on the effects of long-term acclimation to a wide range of salinity on fish’s metabolic rate are few. Several studies have demonstrated an increase in metabolic rate in fish with an increase in the difference between ambient salinity and isosmotic concentration, which may reflect an elevation in the cost of osmoregulation (Febry and Lutz 1987; Kültz et al. 1992; Morgan et al. 1997). However, other studies have reported a decrease in metabolic rate of euryhaline fishes as salinity increased. This trend was shown in the salt marsh fish, *Cyprinodon variegatus*, acclimated to salinity above 40 ppt (Nordlie et al. 1991; Haney and Nordlie 1997; Haney et al. 1999), in the freshwater blenny, *Salaria fluviatilis*, acclimated to 14 and 35 ppt (Plaut 1999a), and in the milkfish, *Chanos chanos*, acclimated to 55 ppt (Swanson 1998).

Nordlie et al. (1991) and Haney and Nordlie (1997) suggested that the decrease in oxygen consumption observed at extremely high salinity is related to permeability changes of the gill membrane and/or the integument. Haney and Nordlie (1997) compared the salinity effects on oxygen consumption and critical oxygen tension with data from Nordlie (1985) and showed that the point at which oxygen consumption is reduced corresponds well to a diminished ability of *C. variegatus* to osmoregulate efficiently. Lotan (1971) reported that in *A. dispar*, Na⁺ concentration in the plasma was stable between 14 and 70 ppt, but increased in 105 ppt, while other osmoregulatory factors, such as plasma osmolarity and Cl⁻ concentration, progressively increased as ambient salinity increased. Considering these data, it is not clear to what extent *A. dispar* is an osmoregulator or osmoconformer, but in the case of Na⁺ concentration it seems to be comparable to *C. variegatus*. Thus, these data support the suggestion that reduction in gill membrane and/or integument permeability helps to decrease ionic influx but reduces the ability of the fish to obtain oxygen from the water (Kristensen and Skadhauge 1974; Davenport and Sayer 1993; Haney and Nordlie 1997). Reduction in gill and opercular membrane permeability as a result of extreme osmotic conditions has been shown for several fish species (Karnaky et al. 1976; Kültz and Onken 1993; Bindon et al. 1994a, 1994b). Swanson (1998) reported that the milkfish, *C. chanos*, acclimated to 35 ppt, consumed more oxygen than it did under acclimation to both 15 ppt (close to isosmotic concentration) and 55 ppt. However, acclimation to 55 ppt resulted

<table>
<thead>
<tr>
<th>Salinity (ppt)</th>
<th>n</th>
<th>Linear Regression Equation</th>
<th>R²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>21</td>
<td>$U_{crit} = 16.03(±.96) - .19(±.03)SL $</td>
<td>.709</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>35</td>
<td>20</td>
<td>$U_{crit} = 15.87(±1.11) - .21(±.03)SL $</td>
<td>.691</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>70</td>
<td>22</td>
<td>$U_{crit} = 16.15(±1.38) - .21(±.04)SL $</td>
<td>.548</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>105</td>
<td>18</td>
<td>$U_{crit} = 14.15(±.80) - .21(±.02)SL $</td>
<td>.830</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>140</td>
<td>7</td>
<td>$U_{crit} = 12.28(±1.05) - .18(±.03)SL $</td>
<td>.864</td>
<td>.002</td>
</tr>
</tbody>
</table>

Note. Intercept values with different superscript letters are significantly different.
in a significant decrease in metabolic rate. In this case, the increase of metabolic rate at 35 ppt acclimation in comparison with 15 ppt may be explained as an increase of osmoregulatory cost, but the decrease at 55 ppt acclimation in comparison with 35 ppt may be a result of decreased permeability of the gill membrane and/or integument.

If the reduction in oxygen consumption occurs as a result of gill membrane and/or integument permeability, such a decrease should be observed whenever a fish experiences salinity beyond its osmoregulatory capability. When a marine fish is exposed to salinities below its body fluid osmotic concentration, to maintain its osmotic balance it should reverse the direction of its active osmotic flow. Instead of excreting ions, it should absorb them from the seawater, and instead of keeping water in its body, it should get rid of excess water. Since most marine fishes are limited in their ability to reverse the direction of osmotic flows, they may drastically reduce their gill membrane and integument permeability to ions and water, an action that will result in reducing the permeability to oxygen as well.

Such a phenomenon was observed in two species of marine blennies acclimated to freshwater. In the marine peacock blenny, *Salaria pavo*, long-term acclimation to 14 ppt did not affect its oxygen consumption, but long-term acclimation to freshwater reduced its metabolic rate (Plaut 1999a). A similar phenomenon was reported for the intertidal blenny, *Parablennius sanguinolentus* (Plaut 1999b). These results support the low-permeability hypothesis, which may be true also in the case of fishes in an extreme hypsaline environment.

Studies on the effects of salinity acclimation on swimming performance of fish are few. Beamish (1978), in his extensive review of swimming capacity, mentioned only three studies. Two dealt with ontogenetic salinity adaptation in salmonids (Alderdice 1963; Glova and McInnerney 1977), and one reported no significant effects on skipjack and yellowfin tuna exposed to a narrow salinity range of 29–34 ppt (Dizon 1977). Nelson et al. (1996) exposed *Gadus morhua* acclimated to 31–20 ppt and vice versa and found no effects on $U_{\text{crit}}$, but this is a very narrow range compared with the salinity range tested in this study. Kolok and Sharkey (1997) measured $U_{\text{crit}}$ of the euryhaline gulf killifish, *Fundulus grandis*, acclimated to freshwater (~1 ppt) and brackish water (~12 ppt) and showed that $U_{\text{crit}}$ of fish acclimated to brackish water was faster than that of fish acclimated to freshwater. Kolok and Sharkey (1997) attributed the decrease of $U_{\text{crit}}$ in fish acclimated to freshwater to the increase of metabolic cost of osmoregulation compared with fish in brackish, close to isosmotic water; thus, the additional cost of osmoregulation is met at the expense of oxygen available for aerobic swimming. However, this may also be a result of reduced gill membrane and integument permeability because of the increasing difference in osmotic pressure between body fluids and the environment. Swanson (1998) found that $U_{\text{crit}}$ of milkfish decreased as salinity increased. In light of Swanson’s (1998) metabolic rate data mentioned above, these results may be an expression of the effects of both osmoregulation cost (at 35 ppt) and decrease of permeability of the gill membrane/integument (at 55 ppt).

In this study, $U_{\text{crit}}$ show the same trend as RMR. It seems that the decrease in the ability to obtain oxygen from the water limited the aerobic ability of the fish and thus decreased $U_{\text{crit}}$ at high salinities.
Activity levels of *A. dispar* were high when fish were acclimated to freshwater, 35 ppt, and 70 ppt and progressively decreased in fish acclimated to 105 and 140 ppt. This pattern was observed in both light and dark phases. This trend is consistent with the findings in RMR and *U*<sub>crit</sub> measurement and with Plaut (2000).

The overall picture that emerges in this study is that although *A. dispar* is found in an extremely wide range of salinities, when the fish are exposed to salinities above 70 ppt their overall performance significantly decreases. This phenomenon is likely to have substantial ecological effects on *A. dispar* populations in extremely saline habitats. Decreases in metabolic rate, swimming capacity, and activity rate are expected to reduce the ability of the fish to prey, to avoid predation, and to perform other functions that are critical to ensure survival. The fact that *A. dispar* regularly inhabits such habitats is probably because its competitors and predators cannot tolerate such physical conditions of extreme salinity that may compensate for decreased performance.

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