The euryhaline milkfish (Chanos chanos) is an excellent subject for studies of the physiological and behavioral processes involved in salinity adaptation. In this study, energy partitioning for metabolism, activity and growth, maximal activity performance and blood osmotic concentrations were assessed at two activity levels in juvenile milkfish fed equal rations and maintained at a relatively constant temperature (26±2 °C) and at salinities (15, 35 and 55 ‰) that represented a wide range of osmoregulatory challenges. Changes in the measured parameters were not consistently related to the magnitude of the trans-integumentary osmotic gradients. Routine oxygen consumption rates were high in 35 ‰ salinity (mean ±1 S.E.M. 167±8 mg O₂ kg⁻¹ h⁻¹) and comparably low in 15 and 55 ‰ salinity (133±6 and 127±3 mg O₂ kg⁻¹ h⁻¹, respectively). Routine activity levels (relative swimming velocity) were highest in 35 ‰ salinity (0.96±0.04 L.s⁻¹), where L is standard length, intermediate in 15 ‰ salinity (0.77±0.03 L.s⁻¹) and lowest in 55 ‰ salinity (0.67±0.03 L.s⁻¹). Growth was significantly higher in 55 ‰ salinity (3.4±0.2 % increase in wet body mass per day) than in 35 ‰ salinity (2.4±0.2 % increase per day) and intermediate in 15 ‰ salinity (2.9±0.5 % increase per day). Maximum swimming velocities decreased with increases in salinity, from 9.9±0.7 L.s⁻¹ in 15 ‰ salinity to 6.6±0.5 L.s⁻¹ in 55 ‰ salinity. Sustained swimming activity above routine levels for 2 h resulted in an increase in blood osmotic concentrations in milkfish in 55 ‰ salinity, but osmoregulation was re-established during the second 2 h of activity. Thus, patterns of variation in metabolic rate and growth were largely parallel to variations in routine activity although, comparing 15 and 55 ‰ salinity, elevated maintenance costs for osmoregulation at the high salinity were detectable. Reduced osmoregulatory abilities and reductions in maximal swimming performance suggest that high salinity may constrain activity. The results demonstrate that investigations of salinity adaptation in euryhaline fishes should take into account the interactive effects of salinity on physiology and behavior.

Key words: fish, salinity, energy partitioning, metabolism, growth, swimming, osmoregulation, milkfish, Chanos chanos.
study of salinity adaptation. It is widely distributed throughout the tropical and subtropical Indo-Pacific (Gordon and Hong, 1986; Bagarinao, 1994) and extremely euryhaline. Milkfish occur naturally and are commercially cultured in fresh, brackish and oceanic waters and also in hypersaline lagoons (up to 158‰ salinity; Crear, 1980). Milkfish are good osmoregulators (Ferraris et al. 1988) and are euryhaline throughout their life history (i.e. non-diadromous; Pannikar et al. 1953; Juliano and Rabanal, 1963; Gordon and Hong, 1986). In addition, they are active, cruising fish, so quantitative measurements of routine activity levels, measured in terms of swimming velocity, can be made with precision.

This paper reports the results of an integrated study of salinity adaptation in the milkfish. The objective was not to quantify the energetic costs of osmoregulation but rather to describe and compare the overall energy relationships, behavior and performance of milkfish acclimated to salinities which presented a wide range of osmoregulatory challenges. The experiments on metabolic rate, activity, growth, swimming performance and osmoregulatory capacity were designed specifically to examine the energetic responses of routinely active, minimally confined fish and to assess the possible interactive effects of salinity on activity, maximal performance and osmoregulatory ability.

Materials and methods

Experimental organisms

Experiments were conducted at laboratories in the United States (Hawaii) and the Philippines using locally collected and/or cultured juvenile milkfish Chanos chanos Forskål (Table 1). The Hawaiian milkfish were produced from spawnings by wild-caught adult fish (Lee et al. 1986) at the Oceanic Institute, Oahu, and reared there in hatchery tanks and ponds. In the Philippines, wild-caught juvenile milkfish from three localities were used. These fish were collected from coastal waters as fry (approximately 10–20 days post-hatch) and reared in ponds until used for the experiments. Morphometric, meristic and biochemical analyses of milkfish population structure indicate greater genetic variation within than among populations over the wide distribution of the species (Tamaru, 1986; Bagarinao, 1994), although some slight but statistically significant differences have been detected between milkfish from Hawaii and the Philippines (Smith, 1978; Winans, 1980, 1985).

Juvenile milkfish (less than 2 years old, less than 25 cm in standard length, L; milkfish begin maturation at >4–5 years old and at >0.5 m in length; Gordon and Hong, 1986) were used in all experiments to avoid the effects of sexual and seasonal differences in gonadal growth. The sizes of the fish used in the experiments (Table 1) differed because of technical constraints as well as biological considerations. For example, small fish were used for the growth experiments because of their higher relative growth rates (Ricker, 1979), while larger fish were required for blood collection in the blood osmotic concentration experiments.

Experimental salinities

Three salinities, 15, 35 and 55‰, were used in all experiments. Compared with the concentration of milkfish body fluids, 15‰ (437 mosmol kg⁻¹) is nearly iso-osmotic, 35‰ (1050 mosmol kg⁻¹) is moderately hyperosmotic and 55‰ (1730 mosmol kg⁻¹) is strongly hyperosmotic. Salinities were prepared using mixtures of sea water (32–35‰) and fresh water (untreated well water; 0–1‰) or sea water and evaporated sea salt (locally produced in either Hawaii or the Philippines) and measured using a salinity refractometer (Aquafauna Bio-Marine, Hawthorne, CA, USA) accurate to 1‰.

Acclimation and maintenance

After collection, all fish were maintained in tanks with flowing sea water (32–35‰) and allowed 4–14 days to recover from handling. Following this recovery period, fish were

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Location</th>
<th>Size</th>
<th>Rearing conditions</th>
<th>Temperature °C</th>
<th>Salinity ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolism/activity</td>
<td>Hawaii</td>
<td>33.5</td>
<td>All year round</td>
<td>22–30</td>
<td>30–37</td>
</tr>
<tr>
<td>Growth</td>
<td>Philippines</td>
<td>0.29</td>
<td>April–September</td>
<td>25–32</td>
<td>30–45</td>
</tr>
<tr>
<td>Maximal activity</td>
<td>Philippines</td>
<td>0.12</td>
<td>June–September</td>
<td>25–32</td>
<td>30–35</td>
</tr>
<tr>
<td>Blood osmotic concentration</td>
<td>Philippines</td>
<td>34.5</td>
<td>October–December</td>
<td>24–32</td>
<td>30–40</td>
</tr>
</tbody>
</table>

Table 1. Description of juvenile milkfish Chanos chanos used in the experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Size</th>
<th>Rearing conditions</th>
<th>Location</th>
<th>Temperature °C</th>
<th>Salinity ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolism/activity</td>
<td>33.5–108.0</td>
<td>All year round</td>
<td>Hawaii</td>
<td>22–30</td>
<td>30–37</td>
</tr>
<tr>
<td>Growth</td>
<td>0.29–0.97</td>
<td>April–September</td>
<td>Philippines</td>
<td>25–32</td>
<td>30–45</td>
</tr>
<tr>
<td>Maximal activity</td>
<td>0.12–0.68</td>
<td>June–September</td>
<td>Philippines</td>
<td>25–32</td>
<td>30–35</td>
</tr>
<tr>
<td>Blood osmotic concentration</td>
<td>34.5–120.0</td>
<td>October–December</td>
<td>Philippines</td>
<td>24–32</td>
<td>30–40</td>
</tr>
</tbody>
</table>

aRange.

bEstimated.

cEstimated range of environmental conditions prior to acclimation for use in experiments.

dThe Oceanic Institute, Honolulu, HI, USA.

eUniversity of the Philippines, Marine Science Institute, Bolinao, Pangasinan, Philippines, and Silliman University, Dumaguete, Philippines.

fSilliman University, Dumaguete, Philippines.

gSoutheast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines.
randomly assigned to one of the salinities and transferred to static experimental tanks (volumes: metabolism/activity, 350 l; growth, 25 l; maximal activity, 40 l; blood osmotic concentration, 500 l). In these tanks, the salinity was adjusted to the experimental levels by 5–10% per day. All fish were allowed to acclimate to the new salinities for a minimum of 14 days before any measurements were made, and all measurements were made on fish in their acclimation salinities.

All tanks were aerated and equipped with either a subsurface sand filter or a cartridge filter containing synthetic filter wool and activated charcoal. Approximately 50% of the water was replaced and the tank surfaces and filters were cleaned 4–8 times per month, depending on tank volume and stocking density (maximum stocking density 2 g fish L$^{-1}$). Throughout the recovery, acclimation and experimental periods, fish were maintained under natural light conditions and at relatively constant temperatures (26±2 °C). All fish were fed a uniform diet (4–6% body mass day$^{-1}$, depending on fish size) of a commercial diet (Purina Trout Chow in Hawaii, Kem Bang Fish Pellets in the Philippines). Fish used in the metabolism/activity experiments were individually marked using a subcutaneous injection of acrylic paint (Hill and Grossman, 1987) prior to transfer to the experimental salinities.

**Routine metabolism and activity**

Routine metabolic rates (oxygen consumption rates) and activity levels (swimming velocity) were measured concurrently in a non-rotating, annular respirometer (Fig. 1) equipped with three light–photocell activity monitors and a customized, computer data-acquisition system (ADC-1, Remote Measurement Systems, Inc., Seattle, WA, USA, and a TRS-80 model 100 portable computer). The respirometer consisted of a sealed annular swimming chamber (volume 122 l; outer diameter 90 cm; inner diameter 45 cm) and an attached sensor chamber (volume 8 l) which contained a subsmersible pump (output 10 l min$^{-1}$), an oxygen electrode (YSI self-stirring BOD oxygen probe connected to a YSI model 58 dissolved oxygen meter, Yellow Springs Instruments, Inc., Yellow Springs, OH, USA) and a temperature sensor (AD5900 temperature transducer, Remote Measurement Systems, Inc., Seattle, WA, USA). Water was continuously pumped from the sensor chamber into the swimming chamber, mixing the water in the apparatus and generating a 4 cm s$^{-1}$ unidirectional orientation current before returning to the sensor chamber. Temperature was maintained at 26±1 °C. The respirometer was shielded from outside activity behind a semi-opaque screen.

A single, post-absorptive (no food for 18 h prior to transfer to the respirometer) milkfish was used for each 36–48 h metabolism/activity experiment. After transfer to the respirometer, the fish was allowed a minimum of 16 h to recover from handling before measurements were begun. During the following 20–32 h, several metabolism/activity measurements (duration 1–3.5 h) were made. Between successive measurements, the respirometer was flushed with filtered, aerated water. Dissolved oxygen concentrations in the respirometer were 90–98% of air-saturation values at the start of a measurement and were never allowed to fall below 80% of air-saturation. Oxygen consumption rates (mg O$_2$ kg$^{-1}$ h$^{-1}$) were measured as oxygen depletion with time. Swimming direction (with or against the orientation current) and absolute velocity (cm s$^{-1}$, velocity through the water, corrected for direction) were measured between each pair of activity monitors for seven consecutive minutes every 10 min throughout every measurement. For each measurement period, mean swimming velocity and the number of passes (expressed as a percentage of total passes) between activity monitors swimming against the orientation current were calculated. After the final measurement, the fish was removed from the respirometer, anesthetized with 2-phenoxethanol (0.1 ml L$^{-1}$), identified, weighed (to ±0.01 g), measured (standard length, L, to ±0.1 cm) and returned to the experimental holding tanks. Relative swimming velocities (L s$^{-1}$), were calculated from absolute swimming velocity and standard length (cm s$^{-1}$/L in cm).

Measured oxygen consumption rates were corrected for background (blank) oxygen consumption by the respirometer and for electronic drift by the dissolved oxygen meter. Two blank measurements were made for each experiment, the first before the fish was introduced into the respirometer and the

---

**Fig. 1.** Non-rotating annular respirometer used to measure routine metabolism and activity in milkfish Chanos chanos. a, annular swimming chamber; b, sensor chamber; c, submersible pump; d, oxygen electrode; e, dissolved oxygen meter; f, temperature sensor; g, activity monitors; h, analog-to-digital converter; i, portable computer. The arrow indicates the direction of the orientation current.
second after the fish had been removed. If oxygen consumption by the empty respirometer exceeded 50% of the oxygen consumption of the fish and/or the results from the two blank measurements differed by more than a factor of two, the results from that experiment were discarded and the respirometer was cleaned before further use. The dissolved oxygen meter was calibrated according to the manufacturer's specifications before and after every measurement. If drift during the measurement interval was greater than 2%, the results from that measurement were discarded. The oxygen probe was serviced and the membrane replaced and the respirometer was cleaned after every two or three experiments or as necessary. Measured swimming velocities were not corrected for solid blocking (Brett, 1964) because the maximal cross-sectional areas of fish used were less than 5% of the cross-sectional area of the swimming chamber.

Experiments were conducted over a period of 1 year and, for each salinity, during both the summer (April–September) and winter (October–March). Twenty-five fish were used in the experiments (15 ‰, \(N=7\); 35 ‰, \(N=9\); and 55 ‰, \(N=9\)) and each fish was used in the respirometer 1–4 times (each salinity; mean, two experiments per fish). The mean elapsed time between successive experiments using the same individual was 15 days (range 4–52 days). Three to eight (mean six) measurements were made during each experiment.

### Growth

Two growth experiments were conducted using replicate groups of milkfish in each salinity. Fish size, stocking density, ration size and the durations of the acclimation and growth periods differed between the two experiments but not between salinities within each experiment (Table 2). In each experiment, fish mass (to ±0.01 g) and standard length \(L_s\), to ±0.1 cm) were measured at the beginning of the acclimation period and at the beginning and end of the growth period. Relative growth rates \(R\) (percentage increase in mass per day; Ricker, 1979) were calculated as:

\[
R = \left[\frac{(w_2 - w_1)/w_1(t_2 - t_1)}\right] \times 100, \quad (1)
\]

where \(w_1\) is mass (g) at the beginning of the growth period, \(w_2\) is mass (g) at the end of the growth period and \(t_2 - t_1\) is the duration of the growth period (days).

In experiment 1, fish from each tank were subsampled for these measurements. In experiment 2, fish were anesthetized, measured and then returned to their experimental tanks. The daily ration, fed in two meals per day, was calculated for fish in each tank using mean fish mass from that tank at the beginning of the growth period and increased daily to compensate for increases in fish size. The size of the incremental increase was based on the mean growth rate measured during the acclimation period.

### Maximal activity

Maximal activity levels were measured in terms of critical swimming velocity \((U_{crit})\), the maximum swimming velocity that a fish can sustain for a prescribed period (Brett, 1964). Measurements for milkfish in each salinity were made using a flow-through, cylindrical swimming flume (swimming chamber volume 620 ml; diameter 6.3 cm; length 20 cm; Fig. 2) submerged in a 100 l glass aquarium equipped with vigorous aeration and a cartridge filter containing synthetic filter wool and activated charcoal. Flow through the flume was generated by five submersible pumps (output range 6–12 l min\(^{-1}\)), arranged in parallel, and stabilized and straightened through an expansion/contraction cone, and two

---

**Growth**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>(N^a)</th>
<th>Mass (\text{g}) ((\text{g}))</th>
<th>Standard length (\text{cm}) ((\text{cm}))</th>
<th>Duration (days)</th>
<th>Stocking density(^c)</th>
<th>Ration (%) body mass</th>
<th>Daily increase in mass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>0.73±0.03 (21)</td>
<td>3.70±0.03 (21)</td>
<td>14</td>
<td>29</td>
<td>7–10</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.35±0.02 (6)</td>
<td>3.01±0.06 (6)</td>
<td>16</td>
<td>28</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

\(^a\)Number of replicate tanks per salinity.

\(^b\)Size (mean ± 1 s.e.m.) \((N)\) at the beginning of the acclimation period. The number of fish measured is given in parentheses.

\(^c\)Stocking density during the growth period.

---

**Table 2. Description of two growth experiments using milkfish Chanos chanos**

---

![Fig. 2. Swimming flume used to measure critical swimming velocities in milkfish Chanos chanos. a, swimming chamber; b, expansion/contraction cone; c, flow straighteners; d, submersible pumps; e, downstream barrier. The arrows indicate the flow direction.](image-url)
sets of flow straighteners upstream of the swimming chamber. Flow velocities were calibrated using pump output rates and the cross-sectional area of the swimming chamber. Flow characteristics were studied using both neutrally buoyant particles and test fish in the chamber, and flow through the swimming chamber was visually laminar at all speeds. Temperature in the aquarium was maintained at 26±1 °C during all experiments.

For each measurement, a single milkfish was introduced into the swimming chamber and allowed at least 10 min to recover from handling. After recovery, flow through the flume was initiated using the slowest velocity. Fish that failed to orient to the current and swim steadily at this velocity were considered unsuitable for the experiments and removed from the apparatus. Current velocity was increased incrementally every 45 min until the fish fatigued. The incremental increase in velocity ranged from 1 to 6 cm s\(^{-1}\), but the velocity sequence used in the experiments was the same for all fish. Fatigue was indicated by failure to hold position in the swimming chamber and at least three contacts of the caudal fin and posterior trunk with the downstream barrier within a 2 min period. Critical swimming velocity, expressed as absolute velocity, cm s\(^{-1}\), and relative velocity, \(L_s\) (cm s\(^{-1}\)/L in cm), were calculated using the formula from Brett (1964) as:

\[
U_{crit} = U + (dU \times T/T_i)
\]

where \(U\) is the highest velocity maintained for the entire interval, \(dU\) is the velocity increment, \(T_i\) is the time elapsed at fatigue velocity and \(T\) is the prescribed swimming period. For fish that did not fatigue within the 45 min interval at the flume’s highest velocity, the maximum swimming velocity of the fish, equal to the flow velocity, was recorded. For all experiments, fish size was small relative to the swimming chamber; velocities were therefore not corrected for solid blocking (Brett, 1964).

At the end of the measurement, the fish was removed from the swimming chamber, anesthetized, weighed (to ±0.01 g), measured (\(L\), to ±0.1 cm) and released into a tank. Thirty-seven fish were used in the experiments (15 ‰, \(N=10\); 35 ‰, \(N=14\); and 55 ‰, \(N=13\)).

**Blood osmotic concentration**

Blood osmotic concentrations were measured in routinely active milkfish and in milkfish that had been forced to swim steadily at 40 cm s\(^{-1}\) (2–3 \(L_s\)\(^{-1}\)) for 2 and 4 h. The apparatus consisted of a round tank (volume 450 l) partitioned into an annular swimming chamber (outer diameter 150 cm; inner diameter 70 cm; depth 25 cm) and an inner pumping chamber, a design similar to that used by Postlethwaite and McDonald (1995). The unidirectional current in the swimming chamber was generated by directing the outputs of five submersible pumps located in the inner chamber into the swimming chamber. The swimming chamber was divided into two equal-sized, hemi-annular sections to allow sampling of the fish from one section (2 h sample) without disturbing the fish in the other section (4 h sample).

After transfer to the swimming chamber, the fish were allowed 16 h to recover from handling before the current was established. Blood was collected from the caudal vein by caudal transection from fish removed from the holding tank (routine) or swimming tank (active), immediately anesthetized with 2-phenoxyethanol (0.3 ml l\(^{-1}\)), weighed (to ±0.1 g) and measured (\(L\), to ±0.1 cm). Blood samples were centrifuged (Autocrit II, Becton, Dickinson and Co., Parsippany, NJ, USA), and the osmotic concentration (mosmol kg\(^{-1}\)) of the plasma was measured using a vapor pressure osmometer (Wescor 5100 C, Wescor, Inc. Logan, UT, USA). Blood osmotic concentration of each fish was calculated as the mean of the replicate samples taken from that fish (variation, s.d., among replicate samples <8 mosmol kg\(^{-1}\)). Thirty-nine fish were used in the experiments and, for each salinity/activity treatment, four or five fish for were sampled.

**Statistical methods**

Oxygen consumption rates and routine swimming velocities were analyzed at three levels: (1) overall, all measurements made during all experiments; (2) daily, weighted means of the measurements made during each experiment (weight duration of experiment) and; (3) fish, means of the daily oxygen consumption rates and swimming velocities for each individual. Analysis of variance (ANOVA) and multiple contrasts were used to test for differences in oxygen consumption rates and swimming velocities between salinities, between experiments within each salinity and between successive experiments using the same fish. Regression analysis was used to describe the effects of activity on oxygen consumption rates and to test for effects of fish size and time (time of day, time spent in the respirometer, time between successive trials with the same fish and duration of the acclimation period) on oxygen consumption rates and swimming velocities. A second, smaller data set, constructed using only results from those measurements in which the fish swam steadily against the orientation current at least 90% of the time, was subjected to regression analysis to describe the effects of swimming velocity on oxygen consumption rates and to analysis of covariance (ANCOVA) to test for salinity effects on the slopes and intercepts of the regressions.

Growth rates were analyzed by analysis of variance using a two-way layout to test for the effects of salinity (main effect) and experiment (block). After the removal of non-significant variables (e.g. block), one-way analysis of variance and multiple contrasts were used to test for the effects of salinity on relative growth rates from the pooled results of experiments 1 and 2.

Swimming performance results were analyzed using analysis of variance and regression analysis to test for the effects of salinity and fish size.

Blood osmotic concentrations were analyzed using analysis of variance and multiple contrasts to test for differences associated with salinity for each group of milkfish (routinely active and 2 and 4 h active milkfish) and within salinities for the effects of activity. Values are expressed as mean ±1 S.E.M. unless otherwise
noted. Statistical procedures were conducted according to the methods of Afifi and Azen (1979) using Systat and Sigmasstat software.

Results

Routine metabolism and activity

Routine metabolic rates (RMR) and swimming velocities (\(U_{\text{routine}}\)) differed significantly between salinities (Table 3), between experiments and between fish within each salinity (\(P<0.05\)) and, for some fish, between successive experiments (overall and daily rates only, \(P<0.05\)). Time of day, fish mass and fish length did not significantly affect RMR or \(U_{\text{routine}}\) (all tests; \(P>0.05\)). Overall RMR increased slightly with the duration of the acclimation period (an increase in RMR of less than 1 \% day\(^{-1}\), \(P<0.001\)), but \(U_{\text{routine}}\) did not change (\(P>0.05\)). During an experiment, overall RMR generally decreased slightly with time (a decrease of less than 1 \% h\(^{-1}\), \(P<0.05\)), but \(U_{\text{routine}}\) did not change (\(P>0.05\)). Between successive experiments using the same fish, both RMR and \(U_{\text{routine}}\) (overall and daily) generally decreased with time (a decrease in RMR of less than 1 \% day\(^{-1}\), \(P<0.01\); a decrease in \(U_{\text{routine}}\) of less than 1 \% day\(^{-1}\), \(P<0.01\)).

In all salinities, overall RMR was higher at higher swimming velocities (all regressions, \(P<0.05\), although \(r^2\) values were low (Table 3). Analysis of covariance showed that neither the slopes nor the intercepts of the regressions differed between the salinities (\(P>0.5\)). The ranges of \(U_{\text{routine}}\) were small (0.38–1.12 \(L\) s\(^{-1}\), 0.54–1.61 \(L\) s\(^{-1}\) and 0.30–1.09 \(L\) s\(^{-1}\) in 15, 35 and 55 \%, respectively), and variation in velocities reflected differences between experiments rather than within experiments. During an experiment, milkfish swam at relatively constant velocities, usually within \(\pm 0.1 \, L\) s\(^{-1}\) of their mean velocity during that experiment.

Given the length of time between most experiments using the same fish and the concurrent changes in fish size, as well as the smaller variances in both RMR and \(U_{\text{routine}}\) during experiments than between experiments, the daily rate (weighted mean of measurements made during one experiment) was considered to be a better estimate of routine metabolism and activity than that calculated for individual fish.

Salinity significantly affected daily RMR and \(U_{\text{routine}}\) (Table 3). Milkfish in 35 \% salinity swam at higher velocities (both tests, \(P<0.05\)) and consumed more oxygen (both tests, \(P<0.001\)) than milkfish from either 15 or 55 \% salinity. Fish in 15 and 55 \% salinity consumed equal amounts of oxygen (\(P>0.1\)), but fish in 55 \% salinity swam significantly more slowly (\(P<0.05\)).

Growth

In both experiments and at all salinities, milkfish regularly consumed all their daily ration, and fish mass increased almost

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>RMR (mg O(_2) kg(^{-1}) h(^{-1}))</th>
<th>(U_{\text{routine}}) (L s(^{-1}))</th>
<th>RMR (mg O(_2) kg(^{-1}) h(^{-1}))</th>
<th>(U_{\text{routine}}) (L s(^{-1}))</th>
<th>RMR (mg O(_2) kg(^{-1}) h(^{-1}))</th>
<th>(U_{\text{routine}}) (L s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>132.3±3.2 (93)</td>
<td>0.79±0.01 (93)</td>
<td>132.7±5.6 (16)</td>
<td>0.77±0.03 (16)</td>
<td>136.0±6.8 (7)</td>
<td>0.77±0.04 (7)</td>
</tr>
<tr>
<td>35</td>
<td>170.4±4.1 (93)</td>
<td>0.97±0.02 (93)</td>
<td>167.2±7.8 (15)</td>
<td>0.96±0.04 (15)</td>
<td>171.7±10.3 (9)</td>
<td>1.02±0.05 (9)</td>
</tr>
<tr>
<td>55</td>
<td>126.0±2.4 (119)</td>
<td>0.67±0.02 (119)</td>
<td>126.5±3.4 (21)</td>
<td>0.67±0.03 (21)</td>
<td>127.7±4.2 (9)</td>
<td>0.70±0.05 (9)</td>
</tr>
</tbody>
</table>

**Effect**

<table>
<thead>
<tr>
<th>RMR</th>
<th>(P&lt;0.001)</th>
<th>(35%&gt;15%&lt;55%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(U_{\text{routine}})</td>
<td>(P&lt;0.001)</td>
<td>(35%&gt;15%&lt;55%)</td>
</tr>
</tbody>
</table>

**Regressions**

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>Regression Equation</th>
<th>(N)</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 %</td>
<td>(y=1.89x+0.29)</td>
<td>42</td>
<td>0.093</td>
</tr>
<tr>
<td>35 %</td>
<td>(y=1.93x+0.28)</td>
<td>71</td>
<td>0.238</td>
</tr>
<tr>
<td>55 %</td>
<td>(y=1.85x+0.31)</td>
<td>41</td>
<td>0.180</td>
</tr>
</tbody>
</table>

---

Table 3. Routine oxygen consumption rates and relative swimming velocities of milkfish *Chanos chanos* in three salinities

---

\(a\) All measurements made during all experiments using all fish.

\(b\) Weighted means of rates measured for each experiment.

\(c\) Means of daily rates calculated for each fish.

\(d\) \(y\) is overall RMR, \(x\) is swimming velocity (L s\(^{-1}\)), where \(L\) is standard length. Regressions were generated using data from fish that swam against orientation current at least 90 \% of the time.

RMR, routine oxygen consumption rate; \(U_{\text{routine}}\), relative swimming velocity.

Statistical comparisons of rates in the different salinities and regressions of oxygen consumption on swimming velocity are shown.

Values are means ± 1 S. E. M. (\(N\)).
twofold during the 4 week growth period. Relative growth rates did not differ between the two experiments \( (P>0.05) \), so the results were pooled for analyses.

Salinity significantly affected growth rates: milkfish acclimated to 55% \( \text{salinity} \) had higher relative growth rates (increase 3.35±0.17 \( \text{day}^{-1} \)) than milkfish in 35% \( \text{salinity} \) (increase 2.36±0.19 \( \text{day}^{-1} \)) \( (P<0.05) \). Growth was intermediate and more variable in 15% \( \text{salinity} \) (increase 2.86±0.49 \( \text{day}^{-1} \)), and significant differences between growth in this salinity and the other salinities were not detected \( (P>0.10) \). This trend of higher growth in the low and high salinities was noticeable (although not statistically significant) even after the 2 week acclimation period.

**Maximal activity**

Critical swimming velocities were measured for only 22 of the 37 fish used in the experiments because, at the highest current velocity possible in the swimming flume, some fish did not fatigue. Analysis was further complicated by small but significant differences in fish size \( (L) \) among the salinities (15% \( \text{salinity} \), 2.7±0.1 cm; 35% \( \text{salinity} \), 2.9±0.1 cm; and 55% \( \text{salinity} \), 3.2±0.1 cm; \( 15<35<55 \% \), \( P<0.05 \)). Therefore, results were analyzed as \( U_{\text{crit}} \) and as maximum swimming velocity \( (U_{\text{max}}, \text{which included } U_{\text{crit}} \text{ values from fish that fatigued and the maximum swimming velocity of the flume for fish that did not fatigue}) \) with velocity expressed as \( \text{cm s}^{-1} \) and \( L\text{s}^{-1} \) (Table 4). \( U_{\text{max}} \) underestimated the true value of \( U_{\text{crit}} \).

Salinity did not significantly affect absolute or relative \( U_{\text{crit}} \) (both tests, \( P>0.1 \)), but the numbers of fish that fatigued differed

### Table 4. Critical and maximum swimming velocities of milkfish *Chanos chanos* in different salinities and statistical comparisons

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>( U_{\text{crit}} ) Absolute (( \text{cm s}^{-1} ))</th>
<th>( U_{\text{crit}} ) Relative ( (L\text{s}^{-1}) )</th>
<th>( U_{\text{max}}^a ) Absolute (( \text{cm s}^{-1} ))</th>
<th>( U_{\text{max}}^a ) Relative ( (L\text{s}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>19.4±1.2</td>
<td>7.4±0.5</td>
<td>26.3±1.8</td>
<td>9.9±0.7</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(3)</td>
<td>(10)</td>
<td>(10)</td>
</tr>
<tr>
<td>35</td>
<td>19.0±1.3</td>
<td>6.2±0.4</td>
<td>21.9±1.4</td>
<td>7.6±0.6</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(14)</td>
<td>(14)</td>
</tr>
<tr>
<td>55</td>
<td>19.4±1.4</td>
<td>6.0±0.4</td>
<td>21.4±1.5</td>
<td>6.6±0.5</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td>(13)</td>
<td>(13)</td>
</tr>
</tbody>
</table>

**Effect**

- **Absolute (\( \text{cm s}^{-1} \))**
  - NS\(^b\)
  - 15%\(>55\% \) \( P<0.05 \)
- **Relative (\( L\text{s}^{-1} \))**
  - NS
  - 15%\(>55\% \) \( P<0.05 \)

\(^a\)\( U_{\text{max}} \) includes \( U_{\text{crit}} \) values for fish that fatigued and the maximum flow velocity of the flume for fish that did not fatigue.

\(^b\)No significant effect.

\( U_{\text{crit}} \), critical swimming velocity; \( U_{\text{max}} \), maximum swimming velocity.

Values are means ± 1 s.e.m. \( (N) \).

\( L \), standard length.

---

### Table 5. Blood osmotic concentrations of milkfish *Chanos chanos* during routine and sustained activity and statistical comparisons of blood osmotic concentrations at different salinity and activity levels

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>Routine</th>
<th>Sustained activity</th>
<th>Activity effect(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>361±6</td>
<td>363±7</td>
<td>366±5</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>35</td>
<td>372±14</td>
<td>372±6</td>
<td>361±7</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>55</td>
<td>401±12</td>
<td>430±14</td>
<td>374±8</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

\(^a\)Effects of activity on blood osmotic concentration within each salinity.

\(^b\)Effects of salinity on blood osmotic concentration within each activity level.

\(^c\)No significant effect.

All contrasts not shown are not significant.

Values are means ± 1 s.e.m. \( (N) \).

---

between the salinities. In 15% \( \text{salinity} \), only three of the ten fish (30%) fatigued, in 35% \( \text{salinity} \), nine of the 14 fish (64%) fatigued and in 55% \( \text{salinity} \), all but three of the 13 fish (77%) fatigued. Among fish that swam to fatigue and provided a \( U_{\text{crit}} \) measurement, some post-fatigue mortality was observed in fish from sea water (one fish) and 55% \( \text{salinity} \) (three fish) but not in fish in 15% \( \text{salinity} \). Salinity significantly affected both absolute and relative \( U_{\text{max}} \): milkfish in 15% \( \text{salinity} \), despite their smaller size, achieved higher swimming velocities than fish in 55% \( \text{salinity} \) (both tests, \( P<0.05 \)).

### Blood osmotic concentration

Salinity and activity significantly affected blood osmotic concentrations of milkfish (Table 5). Among routinely active fish, the blood osmotic concentrations of fish acclimated to 55% \( \text{salinity} \) were the highest, those of fish in sea water were intermediate and those of fish in 15% \( \text{salinity} \) were the lowest (55>15% \( \text{salinity} \), \( P<0.05 \); all other contrasts not significant). After 2 h of swimming, the plasma osmotic concentrations of fish in 55% \( \text{salinity} \) increased to levels significantly higher than those of fish in the other two salinities \( (P<0.01) \). After 4 h of activity, the blood osmotic concentrations of fish in 55% \( \text{salinity} \) decreased significantly \( (P<0.01) \) to levels indistinguishable from those of fish in the other two salinities. Blood osmotic concentrations of fish in the other salinities did not change after activity \( (P>0.05) \).

### Discussion

Salinity exerted substantial and interactive effects on the energy relationships and performance capacity of the euryhaline milkfish. The integrated approach used in this
study, the first to combine and quantify the behavioral component with physiological and energetic responses, demonstrated the interdependence of osmoregulation costs, behavioral compensation and physiological constraints in defining the responses of milkfish to different salinities. For milkfish, energetic costs were determined by both activity behavior and osmoregulatory requirements, but salinity also influenced activity, and activity, in turn, affected osmoregulatory ability. These results provide further evidence that fishes use flexible strategies for the allocation of energy for maintenance, activity and growth in response to changes in both environmental conditions and physiological status (Koch and Wieser, 1983; Wieser and Medgyesy, 1991).

**Effects of salinity on energy partitioning**

Salinity-related differences in energy expenditures for metabolism and energy storage for growth (Fig. 3), while consistent with each other, were not directly or proportionally related to the osmotic gradient between the fish and its environment and predicted osmoregulatory costs. Interpretation of these results was possible only because of concurrent metabolism and activity measurements. These results illustrate the hazards in attributing differences in routine metabolic rates or growth to differential maintenance costs alone.

The relatively high metabolic rates measured for fish in 35‰ salinity were related to and almost entirely accounted for by higher activity levels. Therefore, between this salinity and the others, differential osmoregulatory costs could not be easily detected. In contrast, between 15 and 55‰ salinity, elevated osmoregulatory costs in the high salinity were detectable but were offset by compensatory reductions in routine activity by the fish. Milkfish in 55‰ salinity consumed oxygen at rates indistinguishable from those of fish in the nearly isosmotic 15‰ salinity, but compensated for the increased osmoregulation overheads by reducing activity. The small magnitude of the reduction, approximately 0.1 Ls⁻¹, suggests that, even over this wide salinity range, osmoregulatory costs were small, probably approximately 7% of routine metabolic rate (range 6.7–7.4%; calculated using the regression analyses of oxygen consumption/swimming velocity for each salinity in Table 3). This value is consistent with thermodynamic calculations of Potts et al. (1973), who suggested that osmoregulatory costs in sea water, compared with those at an iso-osmotic salinity, were approximately 4% of maintenance costs for the flounder, *Pleuronectes platessa*.

Analyses of metabolic rates in the context of activity indicated that salinity neither affected standard metabolic rates (i.e. similar intercepts) nor imposed activity-related costs for osmoregulation (i.e. similar slopes) in milkfish. However, because the ranges of swimming velocities exhibited by the fish were small and differed among the salinities, and the highest swimming velocities measured in all salinities (approximately 1 Ls⁻¹) were low, this analytical approach and interpretation are less convincing.

Low routine metabolic rates in nearly iso-osmotic and hypersaline salinities similar to those measured in milkfish have been reported for other extremely euryhaline fish (*Cyprinodon variegatus*, Nordlie et al. 1991; Jordan et al. 1993; Haney and Nordlie, 1997; *Gillichthys mirabilis*, Courtois, 1976). The pattern of metabolic rates of *C. variegatus* over the salinity range 15–60‰ reported by Haney and Nordlie (1997) was remarkably similar to that reported here for milkfish. Activity was measured in none of these studies, although Nordlie et al. (1991) observed, but did not quantify, reduced activity levels in the high salinities.

The effects of salinity on milkfish growth mirrored its effects on routine metabolic rate. Milkfish consumed equal amounts of food in all salinities (within each experiment), but in 35‰ salinity, where the fish expended relatively larger proportions of energy, correspondingly less energy was available for storage as somatic growth. Higher growth rates in 55‰ and (possibly) 15‰ salinity reflected the activity-mediated, lower routine metabolic rates of these fish rather than differential osmoregulatory maintenance costs.

In the Philippines, milkfish ‘fingerlings’ (<10 g) are traditionally cultured under conditions of hypersalinity
perfusion (Randall et al. 1986) and high stocking density to stunt growth and to provide seed stock to culture ponds during times of diminished supply. Ferraris et al. (1986) suggested that reduced growth under these conditions was related to high energetic costs for osmoregulation. On the basis of the results presented in the present paper, this is probably not the case. It is more likely that the effects of salinity on activity and ingestion rates (held constant at levels below satiation in this laboratory study, but which probably vary in the wild and in culture ponds), as well as salinity-related variations in the availability and quality of the food organisms that grow naturally in milkfish culture ponds, have more influence on milkfish growth rates than possible differences in osmoregulatory costs. Milkfish are omnivorous planktivores (Gordon and Hong, 1986; Bagarinao, 1994), and foraging success is probably related to activity (O'Brien, 1979). Reduced activity in low and high salinities could translate into reduced feeding in situations where food is widely distributed throughout the environment. In another planktivore, Brevoortia tryannus, Hettler (1976) associated salinity-related differences in growth to variations in activity levels and feeding rates. Salinity may also affect a range of other properties related to food processing. Ferraris et al. (1986) suggested that reduced protein digestibility in milkfish in hyperosmotic conditions was related to higher rates of food movement through the intestine and increased drinking rates required for osmoregulation. This pattern would be consistent with increased food intake and/or protein requirements of fish in sea water (DeSilva and Perera, 1976; Lall and Bishop, 1977) and, possibly, with the relatively low growth rates of milkfish in sea water measured in this study. However, the high growth rates of milkfish in 55‰ salinity, a salinity at which drinking rates would be expected to be at least as high as those in sea water, suggest that other factors are also involved.

Interactive effects of salinity and activity

For milkfish, salinity was a directive factor that influenced routine swimming behavior, a limiting factor that constrained maximal activity and a masking factor that imposed energetic costs for osmoregulation (Fry, 1971). Variations in routine activity, as with overall energy allocation, were not directly related to the osmotic gradient and presumed differences in maintenance costs, suggesting that milkfish do not consistently regulate either activity or metabolic expenditures at constant levels. In contrast, the magnitude of the osmotic gradient appeared to limit maximal activity and, at extreme levels (55‰ salinity), possibly restricted activity at moderate, submaximal levels by impairing osmoregulatory ability, at least in the short term. Therefore, depressed activity in the high salinity, in addition to compensating for higher osmoregulatory maintenance costs, may function to minimize activity-related costs for osmoregulation (the increase in osmoregulatory costs related to both the intensity of activity and the magnitude of the osmotic gradient; Febry and Lutz, 1987) and/or osmotic imbalance associated with the increased ventilation and gill perfusion (Randall et al. 1972; Wood and Randall, 1973a,b).

While the $U_{crit}$ measurements were problematic and biased by the velocity limitations of the flume, other results ($U_{max}$ and the proportions of fish that fatigued and died following fatigue) were consistent with a direct effect of salinity on maximal performance capacity. Despite their smaller size, milkfish in 15‰ salinity achieved higher swimming velocities, sustained swimming longer and suffered less post-fatigue mortality than those in 35 and 55‰ salinity. Since, for most fishes, absolute maximum sustained swimming velocities increase with fish size (Webb, 1977; Videler and Wardle, 1991), the size differences among the milkfish used in these experiments were small (<10 % of $L$), and $U_{max}$ underestimated $U_{crit}$, these results strongly suggest that milkfish in the nearly iso-osmotic salinity had a greater swimming capacity, or scope for activity, than fish in the higher salinities.

The effects of salinity on swimming performance have been investigated in few species (Oncorhynchus kisutch, Glova and McInerney, 1977; Centropomus undecimalis, Perez-Pinzón and Lutz, 1991; Gadus morhua, Nelson et al. 1996). The most commonly proposed explanation for reduced performance in high (and low) salinities is reduced scope for activity resulting from greater osmoregulatory maintenance costs and/or increased activity-related costs for osmoregulation (Febry and Lutz, 1987), an interpretation also proposed by Nelson (1989), who studied the swimming performance of Perca flavescens in relation to pH and water hardness. Nordlie et al. (1991) also suggested that salinity-related reductions in gill permeabilities may have limited oxygen uptake and curtailed the scope for activity. However, exercise and adrenaline are known to increase gill permeabilities to water and oxygen (Randall et al. 1967; Wood and Randall, 1973a,b; Isaia, 1984). Swimming failure by milkfish at relatively lower velocities in the high salinities could also have resulted from osmoregulatory failure and osmotic imbalance, a hypothesis supported both by the results of the experiments measuring activity effects on blood osmotic concentrations and by the salinity-dependent rates of post-fatigue mortality. Catecholamines, produced during exercise and in response to stress, not only increase gill permeabilities to water and oxygen but also inhibit salt extrusion in marine fishes (Zadunaisky, 1984). In 55‰ salinity, milkfish minimally confined but forced to swim at velocities above routine levels ($2–3 \text{Ls}^{-1}$ versus $<1 \text{Ls}^{-1}$) showed moderately diminished osmoregulatory abilities during the first 2 h of activity but re-established osmoregulation shortly thereafter, a pattern similar to that observed in rainbow trout (Oncorhynchus mykiss) subjected to steady exercise (Wood and Randall, 1973b; Postlethwaite and McDonald, 1995). In the $U_{crit}$ experiments, the continuously increased levels of forced activity leading to exhaustion and concomitant confinement stress may have overwhelmed the milkfish’s regulatory abilities. Postlethwaite and McDonald (1995) showed that confinement stress, as compared with steady swimming exercise, increased both the magnitude and duration of the resultant osmotic imbalance.

Use of metabolic rate and growth to estimate osmoregulatory costs

Comparisons of metabolic rates (Bullivant, 1961; Rao, 1968,
and digestive efficiencies (DeSilva and Perera, 1976, 1984; Lall and Bishop, 1976; MacLeod, 1977; Ferraris et al. 1986). Salinity adaptation by euryhaline fishes involves the coordination of all of these (and other) behavioral and physiological adjustments. Investigations of any one aspect of salinity adaptation, such as osmoregulatory costs, should take these other components into account.

This research was supported at the Oceanic Institute, Oahu, HI, by a grant from the United States Agency for International Development (DAN-4161-A-004055-00) and in the Philippines by a Fulbright Grant for graduate study abroad. I am grateful to the faculties and staffs of the Oceanic Institute, University of the Philippines, Marine Science Institute (Bolinao, Pangasianan, Philippines), Silliman University (Dumaguete, Philippines), the Southeast Asian Fisheries Development Center (Tigbauan, Iloilo, Philippines) and the Philippine–American Education Foundation (Manila, Philippines) for their invaluable assistance and support. I thank Drs M. S. Gordon, D. G. Buth, K. A. Nagy, J. J. Cech Jr, W. A. Walsh and C.-S. Lee for their support and useful comments.

References

EVANS, D. H. (1984). The roles of gill permeability and transport...


the teleost *Cyprinodon variegatus* Lacepède. *J. Fish Biol.* 38, 115–122.


