The Journal of Experimental Biology 201, 3355–3366 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JEB1475

INTERACTIVE EFFECTS OF SALINITY ON METABOLIC RATE, ACTIVITY, GROWTH AND OSMOREGULATION IN THE EURYHALINE MILKFISH (*CHANOS CHANOS*)

CHRISTINA SWANSON*

Department of Biology, University of California, Los Angeles, CA 90095, USA

*Present address: Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, CA 95616, USA
(e-mail: cswanson@ucdavis.edu)

Accepted 5 October; published on WWW 17 November 1998

Summary

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 O_2 kg^{-1} h^{-1}) and comparably low in 15 and 55 ‰ salinity (133±6 and 127±3 mg O_2 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*.

Introduction

Salinity adaptation by euryhaline teleosts is a complex process involving a suite of physiological and behavioral responses to environments with differing osmoregulatory requirements. The mechanics of osmoregulation (i.e. total solute and water regulation) are reasonably well understood (for reviews, see Evans, 1984, 1993), and most researchers agree that salinities that differ from the internal osmotic concentration of the fish must impose energetic regulatory costs for active ion transport. There is less agreement concerning the magnitude of these costs (e.g. Farmer and Beamish, 1969; Rao, 1971; Potts *et al.* 1973; Furspan *et al.* 1984; Febry and Lutz, 1987; Morgan and Iwama, 1991; Nordlie *et al.* 1991) and very little information on the related energetic and physiological consequences of life in different salinities. One reason for the diverse and often contradictory results is that most research has focused on a single aspect of salinity adaptation, usually osmoregulation costs or growth, while ignoring other, probably related, physiological and/or behavioral responses, such as changes in routine activity. Additionally, the research has often been conducted using conditions in which the direction as well as the magnitude of the osmotic gradient between the fish and the environment are varied (e.g. fresh water compared with sea water; Muir and Niimi, 1972; Marais, 1978), on species in which euryhalinity is associated with specific life history stages (e.g. salmonid fishes; Morgan and Iwama, 1991) and/or using protocols that may be ecologically irrelevant to the fish (e.g. abrupt salinity change and/or stenohaline species; Furspan *et al.* 1984; Morgan and Iwama, 1996). This investigation of salinity adaptation in the milkfish *Chanos chanos* Forskål was designed to avoid some of these limitations.

The milkfish is an exceptionally well-suited subject for a

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

		Size[a]				Rearing conditions[a,b,c]	
Experiment	Location	Mass (g)	Standard length (cm)	Age[b] (years)	Season	Temperature (°C)	Salinity (‰)
Metabolism/activity	Hawaii[d]	33.5–108.0	13.8–19.0	0.5–1.5	All year round	22–30	30–37
Growth	Philippines[e]	0.29–0.97	2.8–4.0	0.2–0.4	April–September	25–32	30–45
Maximal activity	Philippines[f]	0.12–0.68	2.2–3.8	0.2–0.4	June–September	25–32	30–35
Blood osmotic concentration	Philippines[g]	34.5–120.0	13.7–20.4	0.5–0.8	October–December	24–32	30–40

[a]Range.
[b]Estimated.
[c]Estimated range of environmental conditions prior to acclimation for use in experiments.
[d]The Oceanic Institute, Honolulu, HI, USA.
[e]University of the Philippines, Marine Science Institute, Bolinao, Pangasinan, Philippines, and Silliman University, Dumaguete, Philippines.
[f]Silliman University, Dumaguete, Philippines.
[g]Southeast 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 ration (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 submersible 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 (AD590JH 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 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-phenoxyethanol (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

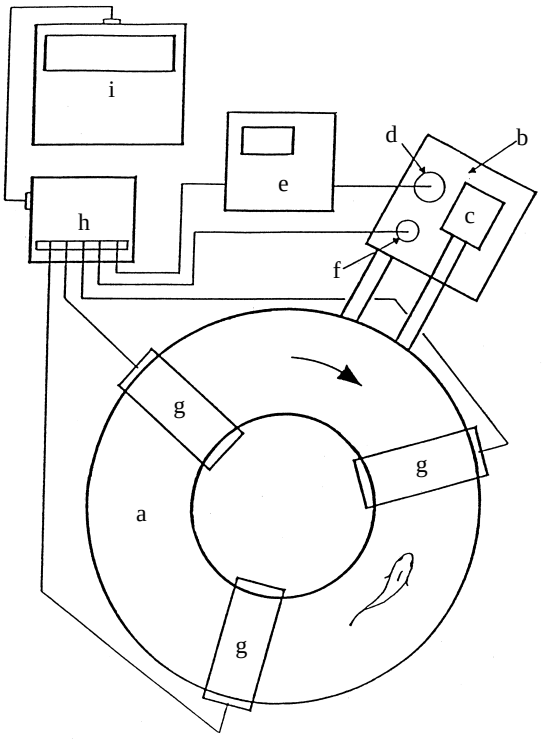

Fig. 1. Non-rotating annular respirometer used to measure routine metabolism and activity in milkfish *Chanos chanos*. a, annular swimming chamber; b, sensor chamber; c, submersible pump; d, oxygen electrode; e, dissolved oxygen meter; f, temperature sensor; g, activity monitors; h, analog-to-digital converter; i, portable computer. The arrow indicates the direction of the orientation current.

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

Experiment	N[a]	Size[b] Mass (g)	Size[b] Standard length (cm)	Duration (days) Acclimation	Duration (days) Growth	Stocking density[c] (fish per tank)	Ration (% body mass)	Daily increase in mass (%)
1	3	0.73±0.03 (21)	3.70±0.03 (21)	14	29	7–10	5	2.5
2	4	0.35±0.02 (6)	3.01±0.06 (6)	16	28	9	6	3

[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.

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, 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:

$$R = [(w_2 - w_1)/w_1(t_2 - t_1)] \times 100\,, \qquad (1)$$

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

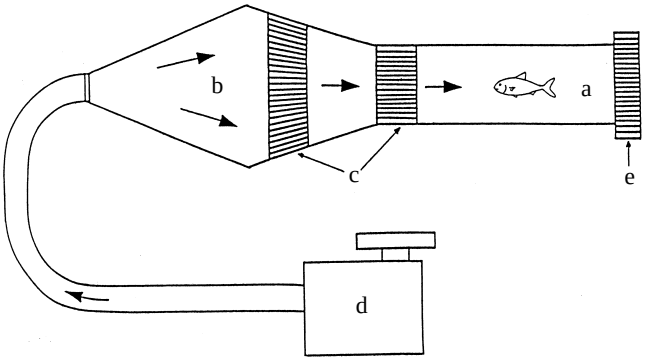

Fig. 2. Swimming flume used to measure critical swimming velocities in milkfish *Chanos chanos*. a, swimming chamber; b, expansion/contraction cone; c, flow straighteners; d, submersible pumps; e, downstream barrier. The arrows indicate the flow direction.

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, $L\,s^{-1}$ (cm s^{-1}/L in cm), were calculated using the formula from Brett (1964) as:

$$U_{crit} = U + (dU \times T_i/T), \quad (2)$$

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 Sigmastat software.

Results

Routine metabolism and activity

Routine metabolic rates (RMR) and swimming velocities ($U_{routine}$) 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 $U_{routine}$ (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 $U_{routine}$ 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 $U_{routine}$ did not change ($P>0.05$). Between successive experiments using the same fish, both RMR and $U_{routine}$ (overall and daily) generally decreased with time (a decrease in RMR of less than 1 % day^{-1}, $P<0.01$; a decrease in $U_{routine}$ 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 $U_{routine}$ 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 ±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 $U_{routine}$ 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 $U_{routine}$ (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 ($P>0.10$), 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 3. *Routine oxygen consumption rates and relative swimming velocities of milkfish* Chanos chanos *in three salinities*

	Overall[a]		Daily[b]		Fish[c]	
Salinity (‰)	RMR ($mg\,O_2\,kg^{-1}\,h^{-1}$)	$U_{routine}$ ($L\,s^{-1}$)	RMR ($mg\,O_2\,kg^{-1}\,h^{-1}$)	$U_{routine}$ ($L\,s^{-1}$)	RMR ($mg\,O_2\,kg^{-1}\,h^{-1}$)	$U_{routine}$ ($L\,s^{-1}$)
15	132.3±3.2 (93)	0.79±0.01 (93)	132.7±5.6 (16)	0.77±0.03 (16)	136.0±6.8 (7)	0.77±0.04 (7)
35	170.4±4.1 (93)	0.97±0.02 (93)	167.2±7.8 (15)	0.96±0.04 (15)	171.7±10.3 (9)	1.02±0.05 (9)
55	126.0±2.4 (119)	0.67±0.02 (119)	126.5±3.4 (21)	0.67±0.03 (21)	127.7±4.2 (9)	0.70±0.05 (9)
Effect						
RMR	35 ‰>15 ‰=55 ‰ $P<0.001$		35 ‰>15 ‰=55 ‰ $P<0.001$		35 ‰>15 ‰=55 ‰ $P<0.01$	
$U_{routine}$	35 ‰>15 ‰>55 ‰ $P<0.001$		35 ‰>15 ‰>55 ‰ $P<0.05$		35 ‰>15 ‰=55 ‰ $P<0.01$	
Regressions[d]						
15 ‰	$\log y=1.89+0.29x$	$N=42$, $r^2=0.093$				
35 ‰	$\log y=1.93+0.28x$	$N=71$, $r^2=0.238$				
55 ‰	$\log y=1.85+0.31x$	$N=41$, $r^2=0.180$				

[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; $U_{routine}$, 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‰ salinity had higher relative growth rates (increase 3.35±0.17 % day^{-1}) than milkfish in 35‰ salinity (increase 2.36±0.19 % day^{-1}) (P<0.05). Growth was intermediate and more variable in 15‰ salinity (increase 2.86±0.49 % 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‰, 2.7±0.1 cm; 35‰, 2.9±0.1 cm; and 55‰, 3.2±0.1 cm; 15<35<55‰, P<0.05). Therefore, results were analyzed as U_{crit} and as maximum swimming velocity (U_{max}, which included U_{crit} values from fish that fatigued and the maximum velocity of the flume for fish that did not fatigue) with velocity expressed as cm s^{-1} and L s^{-1} (Table 4). U_{max} underestimated the true value of U_{crit}.

Salinity did not significantly affect absolute or relative U_{crit} (both tests, P>0.1), but the numbers of fish that fatigued differed between the salinities. In 15‰ salinity, only three of the ten fish (30 %) fatigued, in 35‰ salinity, nine of the 14 fish (64 %) fatigued and in 55‰ salinity, all but three of the 13 fish (77 %) fatigued. Among fish that swam to fatigue and provided a U_{crit} measurement, some post-fatigue mortality was observed in fish from sea water (one fish) and 55‰ salinity (three fish) but not in fish in 15‰ salinity. Salinity significantly affected both absolute and relative U_{max}: milkfish in 15‰ salinity, despite their smaller size, achieved higher swimming velocities than fish in 55‰ salinity (both tests, P<0.05).

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

	U_{crit}		U_{max}[a]	
Salinity (‰)	Absolute (cm s^{-1})	Relative (L s^{-1})	Absolute (cm s^{-1})	Relative (L s^{-1})
15	19.4±1.2 (3)	7.4±0.5 (3)	26.3±1.8 (10)	9.9±0.7 (10)
35	19.0±1.3 (9)	6.2±0.4 (9)	21.9±1.4 (14)	7.6±0.6 (14)
55	19.4±1.4 (10)	6.0±0.4 (10)	21.4±1.5 (13)	6.6±0.5 (13)
Effect				
Absolute (cm s^{-1})	NS[b]		15‰>55‰ P<0.05	
Relative (L s^{-1})	NS		15‰>55‰ P<0.05	

[a]U_{max} includes U_{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_{crit}, critical swimming velocity; U_{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*

	Osmotic concentration (mosmol kg^{-1})			
		Sustained activity		
Salinity (‰)	Routine	2 h	4 h	Activity effect[a]
15	361±6 (4)	363±7 (4)	366±5 (4)	NS[c]
35	372±14 (4)	372±6 (5)	361±7 (5)	NS
55	401±12 (4)	430±14 (4)	374±8 (4)	2 h>4 h P<0.01
Salinity effect[b]	55‰>15‰ P<0.05	55‰>15‰=35‰ P<0.01	NS	

[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).

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‰ salinity were the highest, those of fish in sea water were intermediate and those of fish in 15‰ salinity were the lowest (55>15‰, P<0.05; all other contrasts not significant). After 2 h of swimming, the plasma osmotic concentrations of fish in 55‰ 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‰ 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 $0.1\,L\,s^{-1}$, 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 $1\,L\,s^{-1}$) were low, this analytical approach and interpretation are less convincing.

Low routine metabolic rates in nearly iso-osmotic and

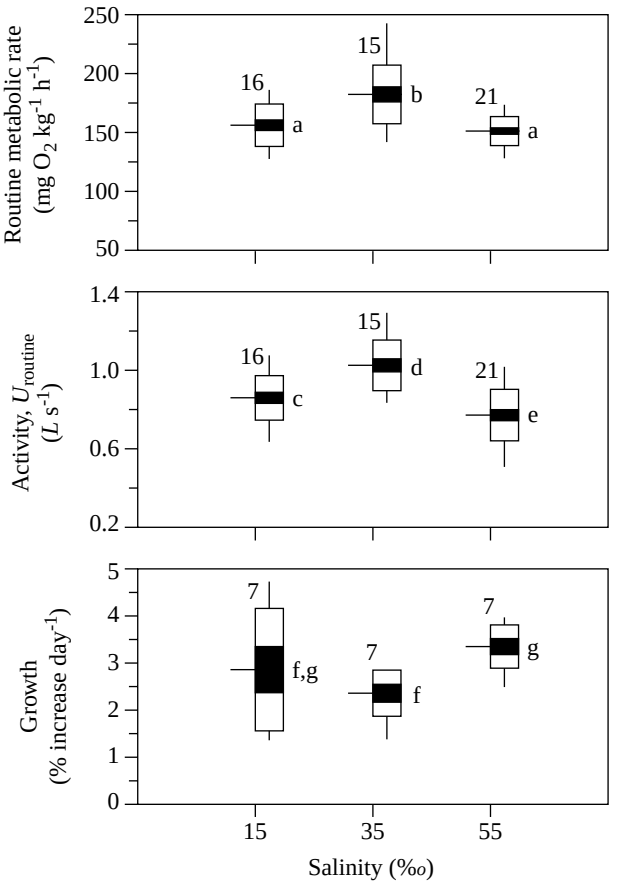

Fig. 3. Routine metabolic rates (daily RMR), routine activity levels (daily $U_{routine}$) and relative growth rates of milkfish *Chanos chanos* in three salinities. Each Dice-gram shows the mean (horizontal bar), ±1 S.E.M. (filled column), ±1 S.D. (open column) and the range (vertical bar). Sample sizes are given. Values with different letters are significantly different ($P<0.05$). *L*, standard length.

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

(>35‰) 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, 1973*a*,*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*, Pérez-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, 1973*a*,*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 $L\,s^{-1}$ *versus* <1 $L\,s^{-1}$) 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, 1973*b*; 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,

1971; Farmer and Beamish, 1969; Muir and Niimi, 1972; Skadhauge and Lotan, 1974; Nordlie and Leffler, 1975; Courtois, 1976; Marais, 1978; Nordlie, 1978; Maceina *et al.* 1980; Barton and Barton, 1987; Febry and Lutz, 1987; Moser and Hettler, 1989; Morgan and Iwama, 1991; Nordlie *et al.* 1991; Pérez-Pinzón and Lutz, 1991; Morgan *et al.* 1996; Nelson *et al.* 1996) and growth rates (Kinne, 1960; Vallet *et al.* 1970; Otwell and Merriner, 1975; Brocksen and Cole, 1972; Peters and Boyd, 1972; DeSilva and Perera, 1976; Hettler, 1976; Hu and Liao, 1976; MacLeod, 1977; Martinez-Palacios *et al.* 1990) measured in different salinities for the purpose of estimating or comparing the costs of osmoregulation have been reported by many investigators using a wide variety of species. The results are distinguished by their lack of consistency and a general lack of evidence for either minimal metabolic rates or enhanced growth in nearly iso-osmotic salinities expected to impose minimal maintenance costs. Collectively, these disparate results suggest that the relationships between osmoregulatory costs, metabolic rates, growth rates and the osmotic gradient between the fish and the environment vary among species and, within species, are probably not linear.

Variations among the reported results probably reflect both methodological and physiological factors. First, the species studied differ in their natural history, degree of euryhalinity and life history stage, all factors that Morgan and Iwama (1991) suggested define the type of metabolic response to salinity. Second, in measurements of routine metabolism, routine activity levels were never measured and were assumed to be similar among all salinities. On the basis of the present results with milkfish, this may be a serious source of error for both metabolic rate and growth studies. The importance of the routine activity component in the overall energy budget, demonstrated in these experiments and in well-developed bioenergetic models for other species (Boisclair and Leggett, 1989), cannot be overlooked. The use of measurements of active metabolic rate to estimate osmoregulatory costs benefit from controlled and known levels of activity, but the relevance of the results may be limited if the experiments subject the fish to unnatural levels of activity. In the present study, routinely active milkfish never swam faster than 1–1.5 $L\,s^{-1}$, lower velocities than those used in most forced activity experiments and below those at which Febry and Lutz (1987) detected activity-related costs for osmoregulation. In addition, while activity may indeed affect osmoregulatory costs, salinity may also affect activity in ways that compensate for elevated maintenance costs and/or control or minimize activity-related costs of osmoregulation. Finally, metabolism and energy partitioning in different salinities may be influenced by other interactive physiological processes unrelated or only indirectly related to the energetics of osmoregulation, including changes in hormone levels (e.g. cortisol, Morgan and Iwama, 1996), tissue permeabilities to water and ions (Isaia, 1984; Rankin and Bolis, 1984), gill ventilation, perfusion, functional surface area and permeabilities (Jones and Randall, 1978; Rankin and Bolis, 1984), types of metabolic substrates catabolized (Smith and Thorpe, 1977; Chan and Woo, 1978) and ingestion rates 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, Pangasinan, 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

AFIFI, A. A. AND AZEN, S. P. (1979). *Statistical Analysis. A Computer Oriented Approach*. New York: John Wiley & Sons.

BAGARINAO, T. (1994). Systematics, distribution, genetics and life history of milkfish, *Chanos chanos*. *Env. Biol. Fishes* **39**, 23–41.

BARTON, M. AND BARTON, A. C. (1987). Effects of salinity on oxygen consumption of *Cyprinodon variegatus*. *Copeia* **1987**, 230–232.

BOISCLAIR, D. AND LEGGETT, W. C. (1989). The importance of activity in bioenergetics models applied to actively foraging fishes. *Can. J. Fish. aquat. Sci*. **46**, 1859–1867.

BRETT, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd Can*. **21**, 1183–1226.

BROCKSEN, R. W. AND COLE, R. E. (1972). Physiological responses of three species of fishes to various salinities. *J. Fish. Res. Bd Can.* **29**, 399–405.

BULLIVANT, J. S. (1961). The influence of salinity on the rate of oxygen consumption of young quinnat salmon (*Oncorhynchus tschawytscha*). *N.Z. J. Sci*. **4**, 381–391.

CHAN, D. K. O. AND WOO, N. Y. S. (1978). Cortisol in eel metabolism. *Gen. comp. Endocr*. **35**, 205–215.

COURTOIS, L. A. (1976). Respiratory responses of *Gillichthys mirabilis* to changes in temperature, dissolved oxygen and salinity. *Comp. Biochem. Physiol*. **53**A, 7–10.

CREAR, D. (1980). Observations on the reproductive state of milkfish populations (*Chanos chanos*) from hypersaline ponds on Christmas Island (Pacific Ocean). *Proc. World Maricult. Soc*. **11**, 548–556.

DESILVA, S. S. AND PERERA, P. A. B. (1976). Studies on the young grey mullet, *Mugil cephalus* L. I. Effects of salinity on food intake, growth and food conversion. *Aquaculture* **7**, 327–338.

DESILVA, S. S. AND PERERA, M. K. (1984). Digestibility in *Sarotherodon niloticus* fry: effect of dietary protein level and salinity with further observations on variability of daily digestibility. *Aquaculture* **38**, 293–306.

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

mechanisms in euryhalinity. In *Fish Physiology*, vol. 10B (ed. W. S. Hoar and D. J. Randall), pp. 239–283. New York: Academic Press.

EVANS, D. H. (1993). Osmotic and ionic regulation. In *The Physiology of Fishes* (ed. D. H. Evans), pp. 315–341. Boca Raton, FL: CRC Press.

FARMER, G. J. AND BEAMISH, F. W. H. (1969). Oxygen consumption of *Tilapia nilotica* in relation to swimming speed and salinity. *J. Fish. Res. Bd Can.* **26**, 2807–2821.

FEBRY, R. AND LUTZ, P. (1987). Energy partitioning in fish: the activity-related cost of osmoregulation in a euryhaline cichlid. *J. exp. Biol.* **128**, 63–85.

FERRARIS, R. P., ALMENDRAS, J. M. AND JAZUL, A. P. (1988). Changes in plasma osmolality and chloride concentration during abrupt transfer of milkfish (*Chanos chanos*) from seawater to different test salinities. *Aquaculture* **70**, 145–157.

FERRARIS, R. P., CATACUTAN, M. R., MABELIN, R. L. AND JAZUL, A. P. (1986). Digestibility in milkfish, *Chanos chanos* (Forsskal): effects of protein source, fish size and salinity. *Aquaculture* **59**, 93–105.

FRY, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In *Fish Physiology*, vol. 6 (ed. W. S. Hoar and D. J. Randall), pp. 1–98. New York: Academic Press.

FURSPAN, P., PRANGE, H. D. AND GREENWALD, L. (1984). Energetics and osmoregulation in the catfish, *Ictalurus nebulosus* and *I. punctatus*. *Comp. Biochem. Physiol*. **77**A, 773–778.

GLOVA, G. J. AND MCINERNEY, J. E. (1977). Critical swimming speeds of coho salmon (*Oncorhynchus kisutch*) fry to smolt stages in relation to salinity and temperature. *J. Fish. Res. Bd Can*. **34**, 151–154.

GORDON, M. S. AND HONG, L.-Q. (1986). Biology. In *Aquaculture of Milkfish (*Chanos chanos*): State of the Art* (ed. C.-S. Lee, M. S. Gordon and W. O. Watanabe), pp. 1–35. Hawaii: The Oceanic Institute.

HANEY, D. C. AND NORDLIE, F. G. (1997). Influence of environmental salinity on routine metabolic rate and critical oxygen tension of *Cyprinodon variegatus*. *Physiol. Zool*. **70**, 511–518.

HETTLER, W. F. (1976). Influence of temperature and salinity on routine metabolic rate and growth of young Atlantic menhaden. *J. Fish Biol*. **8**, 55–65.

HILL, J. AND GROSSMAN, G. D. (1987). Effects of subcutaneous marking on stream fishes. *Copeia* **1987**, 492–495.

HU, F. AND LIAO, I.-C. (1976). Effects of salinity on growth of young milkfish, *Chanos chanos*. *Proceedings of the International Milkfish Workshop-Conference*, May 19–22, 1976, Tigbauan, Iloilo, Philippines. Working paper no. **2**, 33–42.

ISAIA, J. (1984). Water and nonelectrolyte permeation. In *Fish Physiology*, vol. 10B (ed. W. S. Hoar and D. J. Randall), pp. 1–38. New York: Academic Press.

JONES, D. R. AND RANDALL, D. J. (1978). The respiratory and circulatory systems during exercise. In *Fish Physiology*, vol. 7 (ed. W. S. Hoar and D. J. Randall), pp. 425–501. New York: Academic Press.

JORDAN, F., HANEY, D. C. AND NORDLIE, F. G. (1993). Plasma osmotic regulation and routine metabolism in the Eustis pupfish, *Cyprinodon variegatus hubbsi* (Teleostei: Cyprinodontidae). *Copeia* **1993**, 784–789.

JULIANO, R. O. AND RABANAL, H. R. (1963). The tolerance of milkfish fingerlings and fry, *Chanos chanos* (Forskal), to decreases in salinity. *Copeia* **1963**, 180–181.

KINNE, O. (1960). Growth, food intake and food conversion in a euryplastic fish exposed to different temperatures and salinities. *Physiol. Zool*. **33**, 288–317.

KOCH, F. AND WIESER, W. (1983). Partitioning of energy in fish: can reduction of swimming activity compensate for the cost of production? *J. exp. Biol.* **107**, 141–146.

LALL, S. P. AND BISHOP, F. J. (1976). Studies on nutrient requirements of rainbow trout (*Salmo gairdneri*) grown in seawater and freshwater. E24, *FAO Technical Conference on Aquaculture*, Kyoto, Japan, 26 May to 2 June 1976, 10pp.

LEE, C.-S., TAMARU, C. S., BANNO, J. E. AND KELLY, C. D. (1986). Induced spawning of the milkfish (*Chanos chanos*) by a single application of LHRH-analogue. *Aquaculture* **18**, 75–93.

MACEINA, M. J., NORDLIE, F. G. AND SHIREMAN, J. V. (1980). The influence of salinity on oxygen consumption and plasma electrolytes in grass carp, *Ctenopharygodon idella* Val. *J. Fish Biol*. **16**, 613–619.

MACLEOD, M. G. (1977). Effects of salinity on food intake, absorption and conversion in the rainbow trout *Salmo gairdneri*. *Mar. Biol*. **43**, 93–102.

MARAIS, J. F. K. (1978). Routine oxygen consumption of *Mugil cephalus*, *Liza dumerili* and *L. richardsoni* at different temperatures and salinities. *Mar. Biol*. **50**, 9–16.

MARTINEZ-PALACIOS, C. A., ROSS, L. G. AND ROSADO-VALLADO, M. (1990). The effects of salinity on survival and growth of juvenile *Cichlasoma urophthalmus*. *Aquaculture* **91**, 65–75.

MORGAN, J. D., BALFRY, S. K., VIJAYAN, M. M. AND IWAMA, G. K. (1996). Physiological responses to hyposaline exposure and handling and confinement stress in juvenile dolphin (mahimahi: *Coryphaena hippurus*). *Can. J. Fish. aquat. Sci*. **53**, 1736–1740.

MORGAN, J. D. AND IWAMA, G. K. (1991). Effects of salinity on growth, metabolism and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon *Oncorhynchus tshawytscha*). *Can. J. Fish. aquat. Sci*. **48**, 2083–2094.

MORGAN, J. D. AND IWAMA, G. K. (1996). Cortisol induced changes in oxygen consumption and ionic regulation in coastal cutthroat trout (*Oncorhynchus clarki clarki*) parr. *Fish Physiol. Biochem*. **15**, 385–394.

MOSER, M. L. AND HETTLER, W. F. (1989). Routine metabolism of juvenile spot, *Leiostomus xanthurus* (Lacépède), as a function of temperature, salinity and weight. *J. Fish Biol*. **35**, 703–707.

MUIR, B. S. AND NIIMI, A. J. (1972). Oxygen consumption of the euryhaline fish aholehole (*Kuhlia sandvicensis*) with reference to salinity, swimming and food consumption. *J. Fish. Res. Bd Can.* **29**, 67–77.

NELSON, J. A. (1989). Critical swimming speeds of yellow perch *Perca flavescens*: comparison of populations from a naturally acidic lake and a circumneutral lake in acid and neutral water. *J. exp. Biol*. **145**, 239–254.

NELSON, J. A., TANG, Y. AND BOUTILIER, R. G. (1996). The effects of salinity change on the exercise performance of two Atlantic cod (*Gadus morhua*) populations inhabiting different environments. *J. exp. Biol*. **199**, 1295–1309.

NORDLIE, F. G. (1978). The influence of environmental salinity on respiratory oxygen demands in the euryhaline teleost, *Ambassis interrupta* Bleeker. *Comp. Biochem. Physiol*. **59**A, 271–274.

NORDLIE, F. G. AND LEFFLER, C. W. (1975). Ionic regulation and the energetics of osmoregulation in *Mugil cephalus* Lin. *Comp. Biochem. Physiol*. **51**A, 125–131.

NORDLIE, F. G., WALSH, S. J., HANEY, D. C. AND NORDLIE, T. F. (1991). The influence of ambient salinity on routine metabolism in

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

O'BRIEN, W. J. (1979). The predator–prey interaction of planktivorous fish and zooplankton. *Am. Sci.* **67**, 572–581.

OTWELL, W. S. AND MERRINER, J. V. (1975). Survival and growth of juvenile striped bass, *Morone saxitilis*, in a factorial experiment with temperature, salinity and age. *Trans. Am. Fish. Soc*. **104**, 560–566.

PANNIKAR, N. K., TAMPI, P. R. S. AND VISWANATHAN, R. (1953). Some aspects of adaptation in *Chanos chanos* (Forskal). *Proc. Indian Acad. Sci*. **37**, 203–213.

PÉREZ-PINZÓN, M. A. AND LUTZ, P. L. (1991). Activity-related cost of osmoregulation in the juvenile snook (*Centropomus undecimalis*). *Bull. mar. Sci*. **48**, 58–66.

PETERS, D. S. AND BOYD, M. T. (1972). The effect of temperature, salinity and the availability of food on the feeding and growth of the hogchoker, *Trinectes maculatus* (Bloch & Schneider). *J. exp. mar. Biol. Ecol*. **7**, 201–207.

POSTLETHWAITE, E. K. AND MCDONALD, D. G. (1995). Mechanisms of Na^+ and Cl^- regulation in freshwater-adapted rainbow trout (*Oncorhynchus mykiss*) during exercise and stress. *J. exp. Biol*. **198**, 295–304.

POTTS, W. T. W., FLETCHER, C. R. AND EDDY, F. B. (1973). Analysis of the sodium and chloride fluxes in the flounder *Platichthys flesus*. *J. comp. Physiol*. **82**, 21–28.

RANDALL, D. J., BAUMGARTEN, D. AND MALYUSZ, M. (1972). The relationship between gas and ion transfer across the gills of fishes. *Comp. Biochem. Physiol*. **41**A, 629–637.

RANDALL, D. J., HOLETON, G. F. AND STEVENS, E. D. (1967). The exchange of oxygen and carbon dioxide across the gills of rainbow trout. *J. exp. Biol*. **46**, 339–348.

RANKIN, J. C. AND BOLIS, L. (1984). Hormonal control of water movement across the gills. In *Fish Physiology*, vol. 10B (ed. W. S. Hoar and D. J. Randall), pp. 177–201. New York: Academic Press.

RAO, G. M. M. (1968). Oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. *Can. J. Zool*. **46**, 781–786.

RAO, G. M. M. (1971). Influence of activity and salinity on the weight-dependent oxygen consumption of the rainbow trout *Salmo gairdneri*. *Mar. Biol*. **8**, 205–212.

RICKER, W. E. (1979). Growth rates and models. In *Fish Physiology*, vol. 8 (ed. W. S. Hoar, D. J. Randall and J. R. Brett), pp. 677–743. New York: Academic Press.

SKADHAUGE, E. AND LOTAN, R. (1974). Drinking rate and oxygen consumption in the euryhaline teleost *Aphanius dispar* in waters of high salinity. *J. exp. Biol.* **60**, 547–556.

SMITH, A. C. (1978). Pathology and biochemical variation in the milkfish, *Chanos chanos*. *J. Fish Biol*. **13**, 173–177.

SMITH, M. A. K. AND THORPE, A. (1977). Endocrine effects on nitrogen excretion in the euryhaline teleost, *Salmo gairdneri*. *Gen. comp. Endocr*. **32**, 400–406.

TAMARU, C. S. (1986). Population structure. In *Aquaculture of Milkfish (*Chanos chanos*): State of the Art* (ed. C.-S. Lee, M. S. Gordon and W. O. Watanabe), pp. 37–55. Waimanalo, HI: The Oceanic Institute.

VALLET, F., BERHAUT, J., LERAY, C., BONNET, B. AND PIC, P. (1970). Preliminary experiments on the artificial feeding of Mugilidae. *Helgoländer wiss. Meeresunters*. **20**, 610–619.

VIDELER, J. J. AND WARDLE, C. S. (1991). Fish swimming stride by stride: speed limits and endurance. *Rev. Fish Biol. Fisheries* **1**, 23–40.

WEBB, P. W. (1977). Effects of size on performance and energetics of fish. In *Scale Effects in Animal Locomotion* (ed. T. J. Pedley), pp. 315–331. New York: Academic Press.

WIESER, W. AND MEDGYESY, N. (1991). Metabolic rate and cost of growth in juvenile pike (*Esox lucius* L.) and perch (*Perca fluviatilis* L.): the use of energy budgets as indicators of environmental change. *Oecologia* **87**, 500–505.

WINANS, G. A. (1980). Geographic variation in the milkfish, *Chanos chanos*. I. Biochemical evidence. *Evolution* **34**, 558–574.

WINANS, G. A. (1985). Geographic variation in the milkfish, *Chanos chanos*. II. Multivariate morphological evidence. *Copeia* **1985**, 890–898.

WOOD, C. M. AND RANDALL, D. J. (1973*a*). Influence of swimming activity on sodium balance in the rainbow trout (*Salmo gairdneri*). *J. comp. Physiol*. **82**, 207–233.

WOOD, C. M. AND RANDALL, D. J. (1973*b*). Sodium balance in the rainbow trout (*Salmo gairdneri*) during extended exercise. *J. comp. Physiol*. **82**, 235–256.

ZADUNAISKY, J. A. (1984). The chloride cell: the active transport of chloride and the paracellular pathways. In *Fish Physiology*, vol. 10B (ed. W. S. Hoar and D. J. Randall), pp. 129–176. New York: Academic Press.