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LEPTOCEPHALUS ENERGETICS: METABOLISM AND EXCRETION

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Summary

Leptocephali are the unusual transparent larvae that are typical of eels, bonefish, tarpon and ladyfish. Unlike the larvae of all other fishes, leptocephali may remain in the plankton as larvae for several months before metamorphosing into the juvenile form. During their planktonic phase, leptocephali accumulate energy reserves in the form of glycosaminoglycans, which are then expended to fuel metamorphosis. The leptocephalus developmental strategy is thus fundamentally different from that exhibited in all other fishes in two respects: it is far longer in duration and energy reserves are accumulated.

It was anticipated that the unusual character of leptocephalus development would be reflected in the energy budget of the larva. This study describes the allocation of energy to metabolism and excretion, two important elements of the energy budget. Metabolic rates were measured directly in four species of leptocephali, *Paraconger caudilimbatus*, *Ariosoma balearicum*, *Gymnothorax saxicola* and *Ophichthus gomesii*, using sealed-jar respirometry at sea. Direct measurements of metabolic rates were corroborated by measuring activities of lactate dehydrogenase and citrate synthase, two key enzymes of intermediary metabolism, in addition to that of Na^+/K^+-ATPase, a ubiquitous ion pump important in osmotic regulation. Excretion rates were determined by subsampling the sea water used in the respiratory incubations. The entire premetamorphic size range for each species was used in all assays.

Mass-specific oxygen consumption rate, excretion rate and all enzyme activities (y) declined precipitously with increasing mass (M) according to the equation $y=aM^b$, where a is a species-specific constant and $-1.74<b<-0.44$. In leptocephali, the highly negative slope of the familiar allometric equation describing the relationship between mass-specific metabolic rate and mass, normally between –0.33 and 0, showed that a massive decline in metabolic rate occurs with increasing size. The result suggests that the proportion of actively metabolizing tissue also declines with size, being replaced in large measure by the metabolically inert energy depot, the glycosaminoglycans. Leptocephali can thus grow to a large size with minimal metabolic penalty, which is an unusual and successful developmental strategy.

Key words: leptocephalus, energy budget, metabolism, excretion, glycosaminoglycan, larval development.

Introduction

Two fundamentally different strategies characterize the early development of marine teleosts (Pfeiler, 1986). The first and most common strategy (type 1) consists of a post-hatch period in which the yolk-sac is resorbed; exogenous feeding begins immediately thereafter and is continued throughout the larval period as the larva grows into a juvenile fish. In the second strategy (type 2), after a similar post-hatch period during which the yolk-sac is resorbed, the larval fish shows a dramatic increase in size. Two potential sources of nutrition have been proposed for type 2 larvae: dissolved organic carbon and particulate organic carbon in the form of zooplankton fecal pellets and larvacean houses (Mochioka and Iwamizu, 1996; Otake et al., 1993). Type 2 larvae may remain in the plankton for several months and are typical of five orders of bony fishes: the albuliformes (the bonefish), the anguilliformes (the eels), the elopiformes (the tarpon and ladyfish), the notacanthiformes (the spiny eels) and the saccopharyngiformes (the gulper eels). The type 2 larva has an unusual morphology. It is decidedly laterally compressed, almost leaf-like in appearance, with a perfectly clear body and a slender head that gives it its name: the leptocephalus.

Development proceeds in two main phases. In phase I, the larvae grow in size until they reach a maximum that is typical of the species. During phase I, unlike most (type 1) fish larvae, energy reserves are accumulated within the leptocephalus as lipid and an acellular mass within a mucinous pouch. The mucinous pouch consists of proteoglycans, compounds made up of a conjugated peptide and glycosaminoglycan carbohydrates, most familiar as mucus and cartilage. Phase II of leptocephalus development consists of a size shrinkage and a profound change in shape to the juvenile morph, fueled by combustion of most of

the accumulated energy reserves in the form of glycosaminoglycans and lipids (Pfeiler, 1996).

The two elements in the energy budget equation that influence the amount of energy available for growth in larval fishes are metabolic rate and excretion. Metabolic rate receives the greatest allocation of energy: 80–85 % of the total ingested (Brett and Groves, 1979). Excretion commands a much lower percentage of ingested energy, with values ranging from 4–40 % (Torres et al., 1996; Houde, 1989; Brett and Groves, 1979).

Enzyme activities can be used as proxies for metabolic rate. Citrate synthase, located in the mitochondria and positioned at the beginning of the Krebs citric acid cycle, catalyzes the formation of citrate from acetyl-CoA and oxaloacetate (Lehninger, 1982). Citrate synthase activity correlates directly with oxygen consumption rate in fishes and is a good index of maximum aerobic potential (Torres and Somero, 1988). Lactate dehydrogenase is the terminal enzyme in anaerobic glycolysis in vertebrates and is an indicator of anaerobic potential (Hochachka and Somero, 1984). Both enzymes are indicators of the maximum capacity of the organism for ATP production. Na^+/K^+-ATPase utilizes the ATP produced in these pathways to regulate monovalent ion concentrations in marine teleosts, and the activity of this enzyme is directly related to osmoregulatory requirements (Epstein et al., 1980). All three enzyme activities are indicative of maximum metabolic processes.

The energy lost in excretion can be divided into two subcomponents: feces, the portion of ingested energy that is indigestible, and nonfecal nitrogen, which in fishes is usually in the form of ammonia or urea. Teleosts are ammoniotelic, excreting the majority of their nitrogen in the form of ammonia (Brett and Groves, 1979). The present study examines the changes in metabolic processes with increasing mass in leptocephalus larvae. Four representatives of the most abundant leptocephalus species in the Gulf of Mexico, all belonging to the order anguilliformes, were selected for analysis: two congrids, *Paraconger caudilimbatus* (Poey) and *Ariosoma balearicum* (Delaroche), the muraenid *Gymnothorax saxicola* (Jordan and Davis), and an ophichthid, *Ophichthus gomesii* (Castelnau). The metabolic rates of the leptocephali were determined directly, using oxygen consumption rate, and indirectly, through the activities of enzymes in the pathways of intermediary metabolism.

Materials and methods

Collection

Premetamorphic leptocephalus larvae were collected at the edge of the continental shelf in the eastern Gulf of Mexico on three cruises from 1995–1996. Sampling was conducted from 26°N to 28°N between 84°W and 86°W. The collection apparatus consisted of a 2 m plankton net with 505 μm mesh and a 9 m^2 mouth area Tucker trawl constructed of 6.8 mm mesh. Both nets were equipped with quick-release blind cod ends to minimize damage to the larvae. Nets were towed between 1.5 and 3 $km\,h^{-1}$ in a double oblique from the surface to a depth of 100 m. Tow times varied from 10 to 60 min, depending upon plankton density. All sampling was conducted at night to maximize collection of leptocephali.

Immediately upon reaching the deck, the contents of the entire cod end were transferred to a larger (28 l) volume of clean sea water and all leptocephali were removed. Active and apparently undamaged larvae were placed in filtered (0.45 μm pore size) sea water for respiration and excretion analysis. The remaining larvae were measured to the nearest 0.1 mm total length, rinsed with deionized water, blotted and frozen in liquid nitrogen. Frozen larvae were maintained at −80 °C until mass determinations and enzyme analyses were conducted.

Shipboard respiration

Larvae were placed in water-jacketed Lucite chambers specifically designed to accommodate the long, thin, leptocephalus body form. Chamber volumes ranged from 80 to 375 ml, depending upon the size of the larvae. The chambers were filled with filtered sea water and maintained at an experimental temperature of 25±0.2 °C by a circulating, refrigerated water bath. Once larvae had been placed in the respirometers, 10 ml duplicate water samples were taken for determination of the initial ammonia concentrations. All water samples were taken with a syringe previously rinsed twice with sample water. Samples were subsequently dispensed into acid-washed, oven-dried polyethylene containers. A phenol-alcohol reagent, the initial step of the Solorzano technique for the determination of ammonia content, was added immediately to stabilize the ammonia concentration in the samples until return to the laboratory (Degobbis, 1973; Solorzano, 1969). Sample volumes were replaced with filtered sea water, the chambers were sealed and oxygen electrodes inserted into the chimneys. The system was covered with black plastic to reduce visual stress to the larvae. Oxygen partial pressure, P_{O_2}, was monitored continuously as larvae reduced the oxygen levels to low partial pressures using Clark-type, microcathode, polarographic oxygen electrodes (Clark, 1956). Electrodes were calibrated before and after each respiration determination using air- and nitrogen-saturated sea water at the experimental temperature.

Data were recorded continuously for the duration of the respiration analysis using a data-logging system that scanned each of ten channels for a period of 1 s every minute. Respiratory determinations were of the routine type: activity was monitored hourly but was not controlled. After an acclimation period of 30 min, routine respiratory rates were obtained by regressing the change in oxygen concentration in the respirometers on the time elapsed. Maximum and minimum oxygen consumption rates were taken from the maximum 30 min rate and the minimum 30 min rate, respectively.

When incubations were complete, larvae were removed, blotted and immediately frozen in liquid nitrogen. Final ammonia samples were taken in duplicate. To determine the effect of microorganisms upon oxygen consumption and ammonia excretion, larvae were removed after selected

incubation periods and their volume was replaced with fresh, filtered sea water. The chambers were resealed and microbial rates of oxygen consumption and changes in ammonia levels were measured for 2–10 h.

Oxygen consumption rates y were reported as absolute oxygen consumption ($\mu l\, O_2\, individual^{-1}\, h^{-1}$) *versus* mass M (g), with mass expressed in wet mass and dry mass, and as mass-specific rates ($\mu l\, O_2\, g^{-1}\, wet\, mass\, h^{-1}$ or $\mu l\, O_2\, g^{-1}\, dry\, mass\, h^{-1}$) *versus* mass as wet mass and dry mass (Table 1).

Mass determinations and enzyme activities

For dry mass determinations and enzyme analyses, individual larvae were introduced frozen into the homogenizing medium, ice-cold distilled water, at a dilution of 24:1 (v:v) and homogenized at 0–4 °C using a sonifier, and by hand using conical glass homogenizers with ground-glass contact surfaces. Duplicate 1 ml samples were dispensed into preweighed crucibles, dried for 72 h at 60 °C, allowed to cool, and reweighed. The remaining homogenates were centrifuged at 2500 ***g*** for 10 min and the supernatants saved for the enzyme analyses.

Citrate synthase [EC 4.1.3.7; citrate: oxaloacetate-lyase (CoA-acetylating)] activity was assayed using the methods of Torres and Somero (1988). L-Lactate dehydrogenase (EC 1.1.1.27; lactate: NAD^+ oxidoreductase) activity was assayed in the pyruvate reductase direction (Torres and Somero, 1988). Because of the lack of specimens in all size ranges, only *P. caudilimbatus* and *A. balearicum* were selected for the determination of Na^+/K^+-ATPase enzyme activity. Na^+/K^+-ATPase activity was measured using the coupled pyruvate kinase/lactate dehydrogenase assay system described by Gibbs and Somero (1989).

All enzyme activities were assayed on a Cary spectrophotometer at 20±0.1 °C in triplicate and means are reported as μmol substrate converted to product per minute. To compare the citrate synthase enzyme activity with oxygen consumption, a series of determinations was made at both 20 °C and 25 °C, and the remaining enzyme activities were temperature-corrected using the resulting Q_{10} value of 2.35.

Ammonia determination

Because the primary excretory product of fishes is ammonia, excretion rates were determined as the change in ammonia concentration over the duration of the incubation period. Ammonia content of the samples was analyzed within 1 week of collection using the technique developed by Solorzano (1969). Ammonia excretion rates y were expressed as absolute ($\mu mol\, NH_3\, individual^{-1}\, h^{-1}$) and mass-specific ($\mu mol\, NH_3\, g^{-1}\, wet\, mass\, h^{-1}$ or $\mu mol\, NH_3\, g^{-1}\, dry\, mass\, h^{-1}$) values.

Data were analyzed using Statgraphics statistical graphics system 6.1. Regressions were generated using the least-squares method with significance at $P<0.05$.

Results

Virtually all organisms experience an increase in whole-animal respiration rates as they increase in mass. This was not the case with leptocephalus larvae. There was no significant correlation between any of the absolute metabolic processes examined and mass, indicating that individual metabolic rate

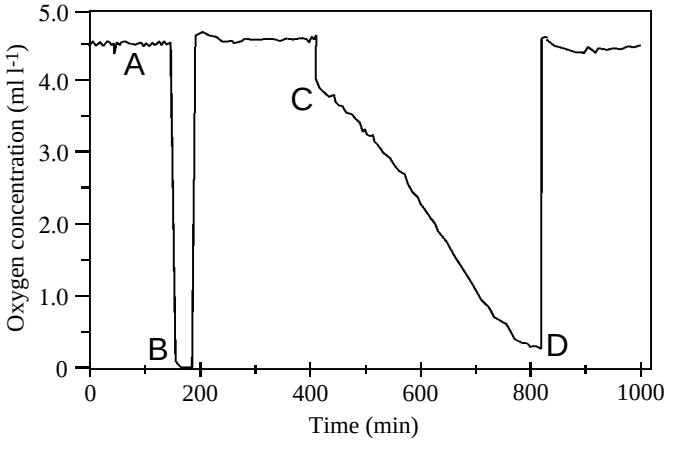

Fig. 1. Recording from a *Paraconger caudilimbatus* respiration incubation with oxygen concentration on the *y*-axis and time on the *x*-axis. (A) Maximum calibration; (B) minimum calibration; (C) time the larvae is sealed in the chamber; (D) time when the incubation is terminated.

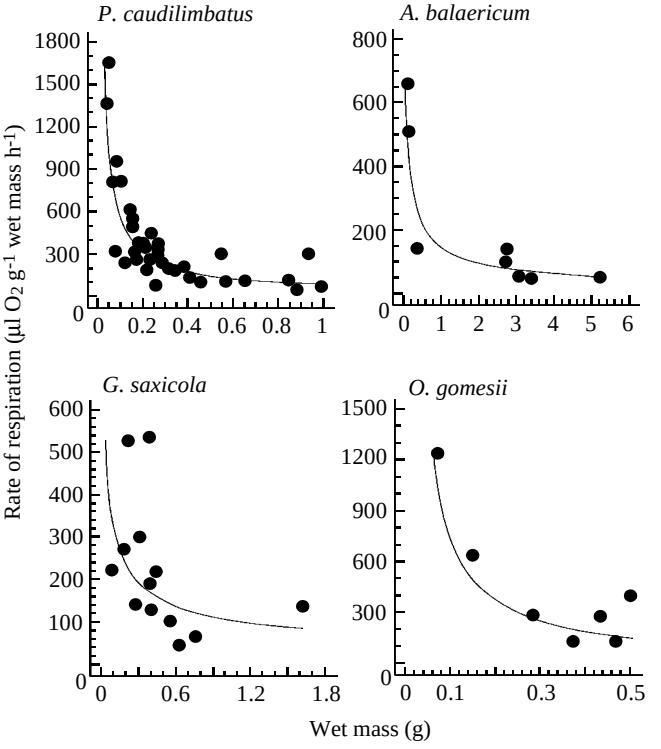

Fig. 2. Wet-mass-specific rate of respiration against wet mass in different larvae. Regression equations and r^2 values are reported in Table 1.

Table 1. *Mass-specific respiration, enzyme activities (citrate synthase, lactate dehydrogenase, Na^+/K^+-ATPase) and NH_3 excretion with respect to wet mass and dry mass in different larvae*

	Wet mass specific			Dry mass specific		
Species	Regression equation‡	r^2	N	Regression equation‡	r^2	N
Paraconger caudilimbatus						
Rate of respiration	$y=45.970M_W^{-0.932***}$	0.604	35	$y=43.337M_D^{-0.986***}$	0.689	23
CS activity	$y=0.206M_W^{-1.091***}$	0.649	39	$y=0.257M_D^{-1.359***}$	0.688	36
LDH activity	NS		38	NS		38
Na^+/K^+-ATPase activity	$y=0.029M_W^{-0.982***}$	0.325	29	$y=0.572M_D^{-0.734***}$	0.317	29
Rate of NH_3 excretion	$y=0.426M_W^{-0.598***}$	0.344	28	$y=1.597M_D^{-0.573***}$	0.293	28
Ariosoma balearicum						
Rate of respiration	$y=146.510M_W^{-0.573**}$	0.845	8	$y=446.930M_D^{-0.565**}$	0.872	6
CS activity	$y=0.116M_W^{-1.243***}$	0.799	31	$y=0.065M_D^{-1.219***}$	0.799	31
LDH activity	$y=8.109M_W^{-0.645*}$	0.255	27	$y=19.273M_D^{-0.668*}$	0.276	27
Na^+/K^+-ATPase activity	$y=0.007M_W^{-1.123***}$	0.791	23	$y=0.0267M_D^{-1.045***}$	0.681	23
Rate of NH_3 excretion	$y=1.974M_W^{-0.814*}$	0.706	5	$y=0.718M_D^{-0.897*}$	0.901	4
Gymnothorax saxicola						
Rate of respiration	$y=104.654M_W^{-0.529***}$	0.225	13	$y=1.431M_D^{-0.909***}$	0.395	10
CS activity	$y=0.204M_W^{-1.207***}$	0.795	33	$y=0.155M_D^{-1.131***}$	0.828	29
LDH activity	$y=4.629M_W^{-1.739**}$	0.554	12	$y=0.838M_D^{-1.675**}$	0.408	10
Rate of NH_3 excretion	NS		17	NS		14
Ophichthus gomesii						
Rate of respiration	$y=0.067M_W^{-1.070*}$	0.483	7	$y=62.780M_D^{-1.039*}$	0.504	7
CS activity	$y=0.279M_W^{-0.869***}$	0.695	21	$y=0.4223M_D^{-0.848***}$	0.721	21
LDH activity	$y=249.184M_W^{-0.436***}$	0.406	19	$y=1084.039M_D^{-0.424**}$	0.398	19
Rate of NH_3 excretion	NS		4	NS		4

Regression equations and statistics are given for mass-specific respiration rate ($\mu l\, O_2\, g^{-1}$ wet mass h^{-1}), enzyme activities (CS, citrate synthase; LDH, lactate dehydrogenase; Na^+,K^+-ATPase, Na^+,K^+-adenosine triphosphatase; all in μmol substrate converted $min^{-1}\, g^{-1}$ wet mass) and NH_3 excretion (μmol g^{-1} wet mass h^{-1}) with respect to wet mass (M_W) and dry mass (M_D).

‡Regression equation is $y=aM^b$, where y is the mass-specific metabolic process. r^2, coefficient of determination; N, is the number of larvae assayed.

NS, non-significant relationships. *P<0.05; **P<0.01 and ***P<0.001.

did not correlate with increasing wet mass or total length. This is a significant result that has not been observed previously in any organism.

Oxygen consumption

Respiratory incubations ranged from 2 to 18 h. Microbial oxygen consumption, obtained during the controls, was negligible and required no correction of the data. Oxygen consumption rates of the 44 premetamorphic leptocephali showed little variability during the course of an incubation (e.g. Fig. 1); maximum and minimum oxygen consumption rates were not significantly different from the slope for the mean (Student's t-test, P>0.05). No obvious critical oxygen partial pressures ($P_{O_2,crit}$), were observed in leptocephalus respiration. A $P_{O_2,crit}$ is normally observed as a decline in oxygen consumption rate below a critical P_{O_2} and indicates an inability by the gas-exchange system to extract oxygen below that P_{O_2} (Prosser, 1973).

Mass-specific oxygen consumption ($\dot{V}_{O_2}$) decreased precipitously with increasing wet and dry mass in all four species according to the power function:

$$\dot{V}_{O_2} = aM^b, \quad (1)$$

where $\dot{V}_{O_2}$ is the mass-specific oxygen consumption rate, M is mass in g, a is a scaling factor, and b is the species-specific slope (Table 1). The greatest change in $\dot{V}_{O_2}$ with increasing mass occurred in larvae weighing less than 0.20 g wet mass (Fig. 2). To illustrate the rapid changes occurring in $\dot{V}_{O_2}$ with mass in the smallest larvae, raw data rather than conventional double logarithmic plots were used in Fig. 2. Mass-specific oxygen consumption rates for larvae weighing less than 0.20 g wet mass ranged from 130 to 1650 $\mu l\, O_2\, g^{-1}$ wet mass h^{-1} (*P. caudilimbatus*). For larvae larger than 0.20 g wet mass, $\dot{V}_{O_2}$ ranged from 50 $\mu l\, O_2\, g^{-1}$ wet mass h^{-1} (*G. saxicola*) to 450 $\mu l\, O_2\, g^{-1}$ wet mass h^{-1} (*O. gomesii*).

Enzyme activities

Mass-specific enzyme activities decreased with increasing wet mass (Figs 3–5) and dry mass (Table 1), again following a power function. The only exception occurred in *P. caudilimbatus* lactate dehydrogenase mass-specific enzyme activities, which showed no significant relationship with increasing mass (Fig. 4). From the wet-mass-specific regressions for citrate synthase, b values ranged from –0.869 to –1.243; b values for lactate dehydrogenase ranged from

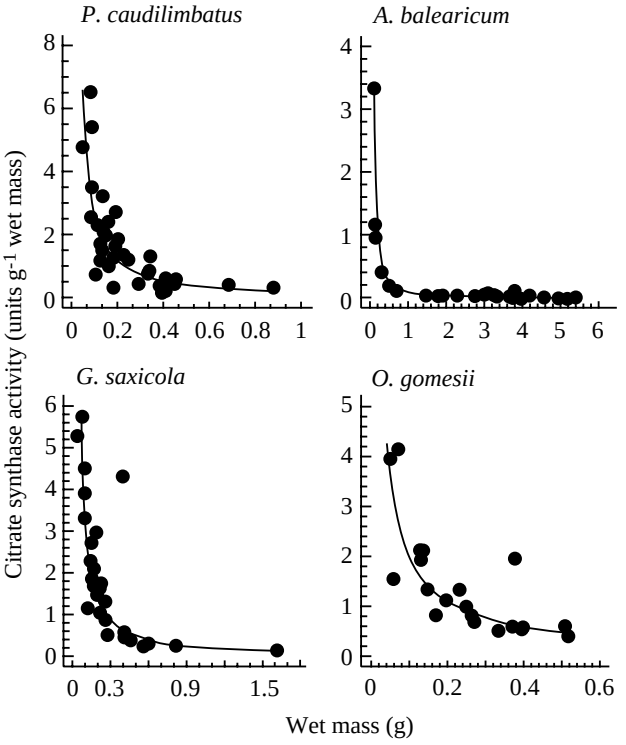

Fig. 3. Wet-mass-specific citrate synthase activity (1 unit is 1 μmol substrate converted min^{-1}) *versus* wet mass in different larvae. Regression equations and r^2 values are reported in Table 1.

–0.436 to –1.739 and Na^+/K^+-ATPase slopes were –0.982 and –1.123 (Table 1). For all three enzymes, the greatest decrease in activity occurred for larvae weighing less than 0.20 g wet mass (*P. caudilimbatus*, *G. saxicola* and *O. gomesii*) and 0.50 g wet mass (*A. balearicum*), after which all activities leveled off.

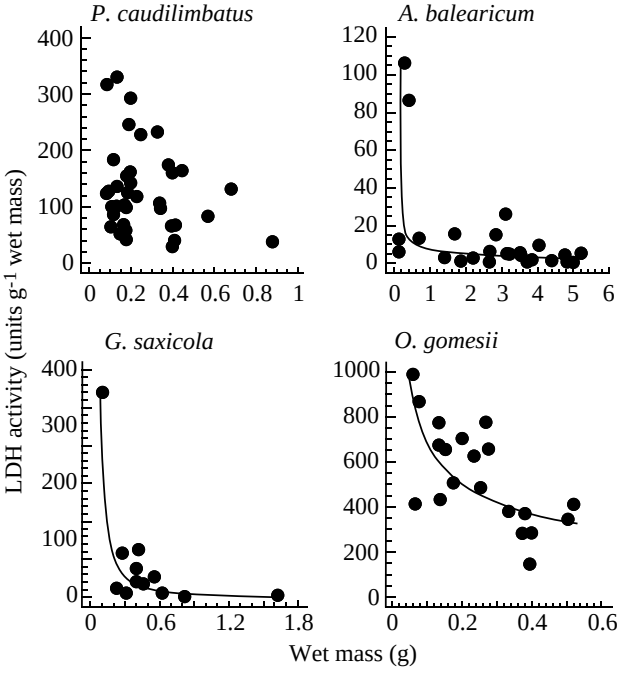

Fig. 4. Wet-mass-specific lactate dehydrogenase activity (1 unit is 1 μmol substrate converted min^{-1}) *versus* wet mass in different larvae. Regression equations and r^2 values are reported in Table 1.

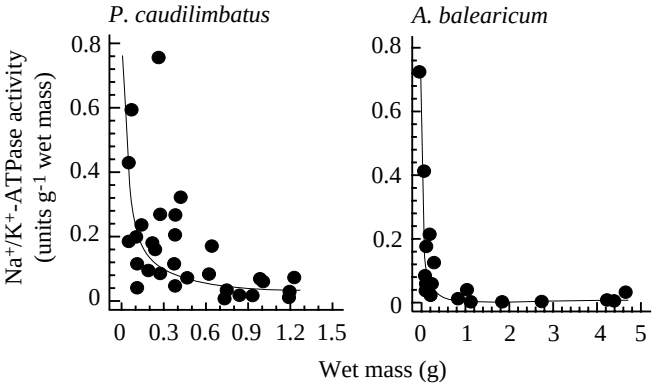

Fig. 5. Wet-mass-specific Na^+/K^+-ATPase activity (1 unit is 1 μmol substrate converted min^{-1}) *versus* wet mass in different larvae. Regression equations and r^2 values are reported in Table 1.

There was a significant linear relationship between the temperature-corrected wet-mass-specific citrate synthase activities and rates of oxygen consumption in all four species (Table 2; Fig. 6).

Excretion rates

Ammonia excretion rates were analyzed for 51 larvae. Changes in ammonia levels resulting from the presence of microorganisms were not significant and no correction of the data was required. Mass-specific ammonia excretion rate declined with increasing mass, for both wet mass (Fig. 7; Table 1) and dry mass (Table 1). The maximum decline in mass-specific excretion with increasing mass occurred in larvae weighing 0.50 g wet mass or less. Larvae weighing more than 0.50 g wet mass exhibited wet-mass-specific excretion rates that remained relatively constant with increasing mass.

Discussion

Body size has a profound influence on the physiology, ecology and behavior of fishes, particularly during

Table 2. *Linear regressions of corrected wet-mass-specific citrate synthase activities against wet-mass-specific oxygen consumptions in various larvae*

Species	Regression equation	r^2	*N*
Paraconger caudilimbatus	$y=0.005x+0.919$***	0.517	20
Ariosoma balearicum	$y=0.006x-0.468$**	0.859	6
Gymnothorax saxicola	$y=0.002x+0.263$*	0.626	9
Ophichthus gomesii	$y=0.002x+0.298$***	0.795	5

y, corrected citrate synthase (CS) activity (μmol substrate converted g^{-1} wet mass min^{-1}); *x*, wet-mass-specific oxygen consumption (μl O_2 g^{-1} wet mass h^{-1}). r^2, coefficient of determination; *N*, number of larvae assayed.

*P<0.05; **P<0.01; ***P<0.001.

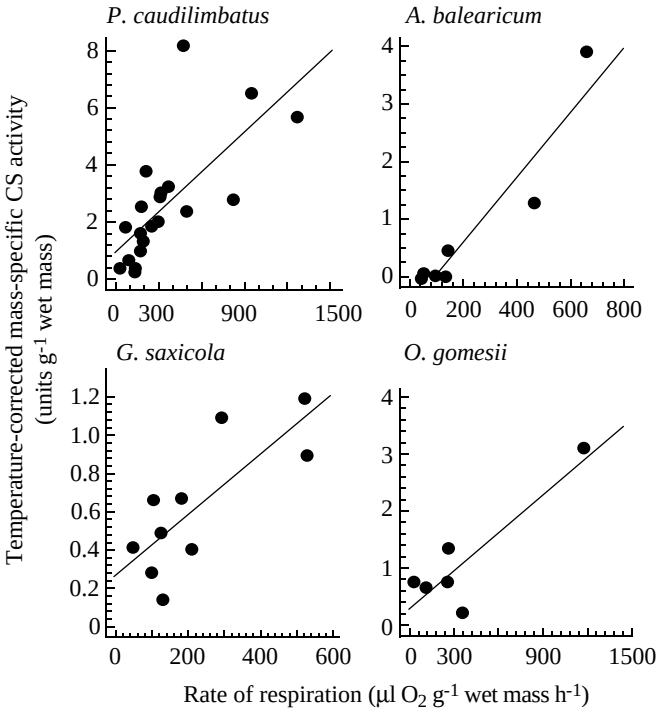

Fig. 6. Assay-temperature-corrected wet-mass-specific citrate synthase activity (1 unit is 1 µmol substrate converted min^{-1}) against wet-mass-specific respiration rate in different larvae. Regression equations and r^2 values are reported in Table 2.

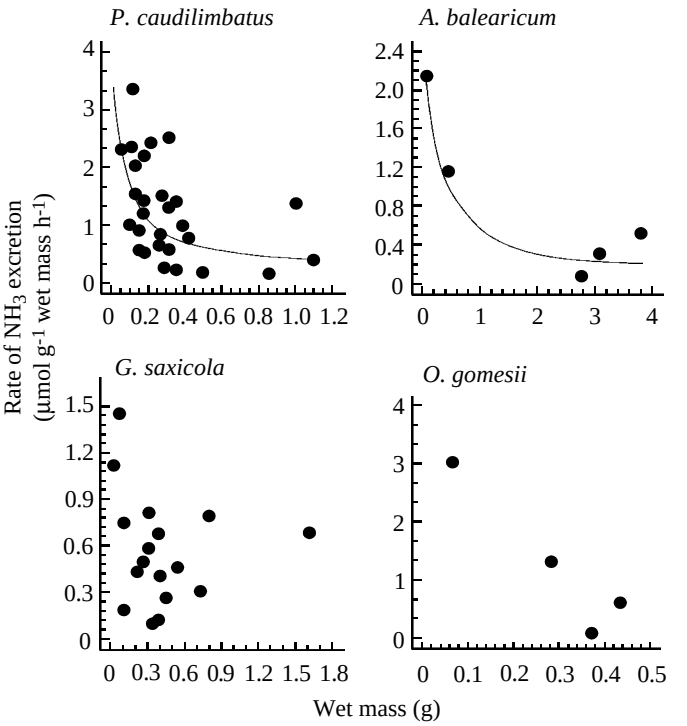

Fig. 7. Wet-mass-specific rates of NH_3 excretion against wet mass in different larvae. Regression equations and r^2 values are reported in Table 1.

development (Brett and Groves, 1979; Miller et al., 1988; Hunter, 1981). For example, mortality in fish larvae decreases with increasing size (Cushing, 1974). With increasing size, a larva improves its ecological position; greater size increases its prey spectrum by improving locomotory capabilities and enlarging the gape, in turn reducing the predator spectrum by enhancing escape potential and exceeding the gape of a predator (Blaxter, 1986; Webb and Weihs, 1986; Hunter, 1981).

However, there may be a 'catch-22' associated with rapid growth in larval fishes. Instead of showing the decline in mass-specific metabolic rate with increasing mass observed in juvenile and adult fish, metabolic rate in most larval fishes correlates isometrically with increasing mass (Giguere et al., 1988; Manahan, 1990; Torres et al., 1996). As a larva grows, the whole-individual (absolute) rate of oxygen consumption increases more quickly with size than it does in juveniles and adults, requiring more combustible energy. A larger larva must ingest more to meet the higher metabolic energy demands that result from increased size. As a consequence, in most larval fish, little energy is stored. Most of the ingested energy that is not allocated to metabolism is devoted to increasing muscle mass (Brightman et al., 1997; Miller et al., 1988; Hunter, 1981). Thus, during periods of starvation, larger larvae are nearly as vulnerable as smaller larvae.

In the allometric equation $y=aM^b$, the slope value, b, provides a quantitative expression of the change in rates of metabolic processes with increasing individual size, and this slope can be used to estimate the influence of body size on metabolic processes. A slope of 0.67 indicates that the metabolic process correlates with surface area, and a slope of 1.00 indicates that it correlates directly with mass (Schmidt-Nielsen, 1990). In leptocephali, there was no significant relationship between increasing whole larval mass and any of the metabolic processes examined: rate of oxygen consumption, the activities of three intermediary metabolic enzymes and rate of ammonia excretion.

Mass-specific metabolic processes, however, conformed strongly to the power function $y/M=aM^b$, where y is the metabolic process examined and M is the mass. For oxygen consumption in a wide variety of organisms, b ranges between –0.33 (indicating that respiration rate correlates with surface area) and 0 (indicating that respiration rate correlates with mass) (Schmidt-Nielsen, 1990). For most larval fish, the value of b is close to zero, indicating that mass has the greatest impact on respiration rates (see Torres et al., 1996; Giguere et al., 1988). The slope values obtained for leptocephali (b=–0.53 to –1.05), however, indicated a precipitous decline in mass-specific respiration rate with increasing mass, much greater than any described previously for a larval fish. This result demonstrates that a lower proportion of the mass of the leptocephalus is invested in metabolizing tissue than in other larval fish. This is a very unusual situation for a larval fish, or for any organism. The wet-mass-specific respiration rates obtained (50–1650 µl O_2 g^{-1} wet mass h^{-1}) were low and not comparable with any reported wet-mass-specific metabolic rates obtained for type 1 larval fishes at similar temperatures (Table 3). The

Table 3. *Oxygen consumption rates ($\dot{V}_{O_2}$) for various teleost larvae*

Species	$\dot{V}_{O_2}$ (ml O_2 mg^{-1} h^{-1})*	*N*	*T* (°C)	Source
Achirus lineatus (lined sole)	6.2–19.7	15	28	Houde and Schekter (1983)
Anchoa mitchelli (bay anchovy)	5.7–8.2	11	26	Houde and Schekter (1983)
Archosargus rhomboidalis (sea bream)	8.7–11.0	9	26	Houde and Schekter (1983)
Albula vulpes (bonefish) (early metamorphosis)	67.9–281.1	4	24–25	Pfeiler and Govoni (1993)
Pseudopleuronectes americanus (winter flounder) (feeding larvae)	5.8–6.8	17	5	Cetta and Capuzzo (1982)
Sciaenops ocellatus (red drum) (10- to 17-day-old)	3.1–5.5	19	25	Torres et al. (1996)

*All respiration measurements are based upon larval dry mass (mg) except for those obtained for *Albula vulpes*, which are expressed as μl O_2 g^{-1} wet mass h^{-1} and those for *Pseudopleuronectes americanus*, which are expressed as μl O_2 mg^{-1} protein h^{-1}. *N*, number of individuals analysed; *T*, experimental temperature.

respiration rates for advanced premetamorphic leptocephali (>0.5 g wet mass) were comparable with rates obtained for early metamorphosing bonefish leptocephali (67.9 μl O_2 g^{-1} wet mass h^{-1}) (Pfeiler and Govoni, 1993) (Table 3). A comparison of the dry-mass-specific leptocephalus respiration rates (0.52–20.39 μl O_2 mg^{-1} dry mass h^{-1} for 5–180 μg individuals) to values for bay anchovy *Anchoa mitchelli* (5.7–8.2 μl O_2 mg^{-1} dry mass h^{-1} for 9–424 μg individuals) (Houde and Schekter, 1983) and red drum *Sciaenops ocellatus*, larvae (3.1–5.5 μl O_2 mg^{-1} dry mass h^{-1} for 21–68 μg individuals) (Torres et al., 1996) reveals that leptocephalus respiration, as a function of dry mass, encompasses and surpasses rates of other teleost larvae at a similar experimental temperature (Table 3).

Metabolic enzyme activities

As observed in the mass-specific respiration rates, there was a rapid decrease in mass-specific citrate synthase, lactate dehydrogenase and Na^+/K^+-ATPase activities with increasing mass. The wet-mass-specific citrate synthase activity decreased at a substantially greater rate (b=–0.87 to –1.24) than was observed in larval menhaden *Brevoortia tyrannus* (b=–0.8) (Power and Walsh, 1992).

Citrate synthase activity correlated directly with oxygen consumption rate in the leptocephali, allowing the use of citrate synthase activity as a predictive tool for future analyses to determine respiration rates from frozen specimens. Lactate dehydrogenase wet-mass-specific activity did not correlate as tightly with increasing mass as did citrate synthase wet-mass-specific activity. This was particularly evident in *P. caudilimbatus*, in which less than 20 % of the change in lactate dehydrogenase mass-specific activity could be explained by changes in mass. The rapid decline in mass-specific Na^+/K^+-ATPase activity indicated that transport costs were much greater per unit of body mass in small larvae (<0.5 g wet mass) than in larger larvae (>0.5 g wet mass). Hulet and Robins (1989) determined the osmolality of 75–220 mm *A. balearicum* premetamorphic larvae to be 792±100 mosmol kg^{-1} H_2O (mean ± S.D.). Unfortunately, the authors pooled the larvae, so any distinction between larvae of less then 0.5 g wet mass and larger ones was not investigated. If smaller larvae are iso-osmotic, as Hulet and Robins (1989) speculated, it is possible that high mass-specific Na^+/K^+-ATPase activities are a result of transport processes other than osmoregulation. In any event, this is another area of leptocephalus biology that requires further investigation.

Ammonia excretion

These data are the first reported on ammonia excretion for leptocephalus larvae and contribute significantly to our limited knowledge of excretion in larval fishes. Absolute ammonia excretion values for leptocephali (0.04–1.37 μmol NH_3^{-1} individual h^{-1}) were higher than those found by Torres et al. (1996) for 10- to 14-day-old *Sciaenops ocellatus* (0.002 μmol NH_3^{-1} individual h^{-1} at 25 °C). Dry-mass-specific excretion rates in the leptocephali were similar to or higher than the values for red drum larvae (30.58 μmol NH_3 g^{-1} dry mass h^{-1}) at a mass of 0.10 mg (Torres et al., 1996).

Excretion values obtained in the present study may slightly underestimate total nitrogen excretion. Jobling (1981) found that 15–25 % of nitrogenous waste in plaice *Pleuronectes platessa* larvae occurred in the form of urea. Torres et al. (1996) determined that urea made up 18–30 % of the nitrogenous waste in red drum larvae. As a result, total nitrogen excretion values for leptocephali may be underestimated by 15–30 %.

For all the metabolic processes examined, the greatest change in slope occurred in larvae of less than 0.5 g wet mass in all four species (Figs 2–5, 7). Donnelly et al. (1995), based upon proximate and nucleic acid composition, reported that *A. balearicum* phase I development could be divided into two subphases (Ia and Ib). Phase Ia was characterized by a high rate of cellular proliferation, with growth occurring in length rather than in mass. In phase Ib, there was a leveling off of RNA and DNA levels at an asymptotic concentration, indicating that a greater proportion of each larva was composed of acellular mass: glycosaminoglycans. The size at which the

transition from phase Ia to phase Ib occurred was approximately 0.50 g wet mass. The changes observed by Donnelly et al. (1995) in the proximate composition of the larvae were also apparent in the rates of oxygen consumption and ammonia excretion and in the enzyme activities for the four larval species examined here. The greatest decrease in mass-specific metabolic processes occurred between 0.20 g wet mass for *P. caudilimbatus*, *G. saxicola* and *O. gomesii* and 0.50 g wet mass for *A. balearicum*, a much larger larva. Respiration rates declined by 51.6 % (*G. saxicola*) to 84.9 % (*A. balearicum*) from phase Ia to phase Ib.

The most significant results of the present study on the metabolic processes of leptocephalus larvae are the scaling coefficients relating mass-specific metabolic processes to mass through direct determinations of metabolic rate, excretion rate and intermediary metabolic enzyme activities. The mechanism responsible for the unusual relationship between mass and metabolic rate in these larvae is the formation of an energy depot in the form of glycosaminoglycans (Donnelly et al., 1995; Pfeiler, 1984). The larvae increase rapidly in mass but accumulate little metabolizing tissue, thereby maintaining low overall metabolic costs in very large larvae.

Glycosaminoglycans not only act as an energy depot but also serve a skeletal function. Most leptocephali are very large for fish larvae. Type 1 larvae are rarely greater than 20 mm; in contrast, *Ariosoma balearicum* exceeds 200 mm before it begins metamorphosis. Our personal observations suggest that leptocephali are also highly competent swimmers for a larval form. They exhibit well-developed backward and forward anguilliform locomotion, making them difficult to capture. It required extreme care to prevent them escaping from the respirometers before they could be sealed, yet, except for their teeth and otoliths, they are completely unossified. The glycosaminoglycans act as a firm, gelatinous skeleton for the musculature to work against, conferring an exceptional swimming ability without a bony skeleton, and without appreciable metabolic costs other than that needed for acquiring and depositing the glycosaminoglycans. Glycosaminoglycan deposition allows for a rapid low-cost increase in size, provides a skeleton in support of locomotion and, in conjunction with lipid reserves (Pfeiler, 1996), serves as an energy depot to fuel metamorphosis.

In conclusion, increasing in size has important implications for an individual's ecology and physiology. On the one hand, the rapid growth of a larva decreases the number of predators capable of ingesting it, thereby providing the larva with the ecological refuge of increased size. On the other hand, increased size may be accompanied by increased visibility and a greater vulnerability to larger visual predators. From a physiological perspective, in larvae exhibiting conventional teleost developmental strategies, where energy storage is virtually non-existent (Brightman, 1993; Hunter, 1981), the absolute energetic costs of being larger may also result in a higher susceptibility to death by starvation (see Torres et al., 1996; Miller et al., 1988). Leptocephali are highly transparent, ameliorating some of the ecological risks associated with larger size and, because individual energy depots in the form of glycosaminoglycans increase with increasing size, being larger is ecologically and physiologically beneficial. Thus, for the leptocephalus larvae, bigger is better.

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