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Chemical composition and growth indices in leptocephalus larvae

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Abstract Leptocephali grow at extremely high rates (>1 mm d^{-1}), but, unlike most fish larvae, 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 along with lipid reserves to fuel metamorphosis. Otolith growth rates were determined using scanning electron microscopy for four species of leptocephali common in the Gulf of Mexico, *Paraconger caudilimbatus* (Poey, 1867), *Ariosoma balearicum* (Delaroche, 1809), *Gymnothorax saxicola* (Jordan and Davis, 1891), and *Ophichthus gomesii* (Castelnou, 1855). Proximate composition, RNA:DNA ratios and protein growth rates were examined with respect to mass, length and age. The leptocephalus growth strategy was strongly reflected in the growth indices. Mass (Y) in all four species increased with increasing age (X) according to the equation $Y = aX^b$, where a is a species-specific constant and $1.05 < b < 2.40$. The accumulation of acellular mass was evident in protein growth rates and RNA:DNA ratios, and was observed as a shift in increasing size from rapid growth in length to a greater increase in mass with age. These results suggest that the proportion of actively metabolizing tissue declines with size and is replaced by the metabolically inert energy depot: the glycosaminoglycans. Leptocephali can thus grow to large size very rapidly with minimal metabolic penalty, an unusual and successful developmental strategy.

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Introduction

Body size is an important variable influencing the physiology, ecology, and behavior of living organisms (Brett and Groves 1979; Hunter 1981; Calder 1985; Miller et al. 1988). For example, the mortality rate of fish larvae decreases with increasing size (Cushing 1975). By increasing in size, a larva improves its ecological position; greater size increases it's 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 predators (Hunter 1981; Blaxter 1986; Webb and Weihs 1986).

Larval fish exhibit two distinctly different developmental strategies (Pfeiler 1986). In Type 1 larvae, representative of most larval fishes, increases in somatic mass are accomplished primarily by the accumulation of protein in the form of muscle (Balbontin et al. 1973; Ehrlich 1974a, b; Cetta and Capuzzo 1982). There is little or no energy storage; all energy exceeding metabolic requirements is devoted to growth (Brightman 1993). The larval period can range from 1 wk to ≥1 mo, and in some instances the duration of the Type 1 larval phase is controlled by the growth rate (Werner and Gilliam 1984). Overall, the strategy of the Type 1 larva is to reduce vulnerability to predation by maximizing growth and minimizing the duration of the larval phase, regardless of the potential for starvation.

Type 2 larvae, characterized by the leptocephalus, a unique larval form uniting eels, tarpon, bonefish, and ladyfish into the superorder Elopiformes, approach growth and survival differently. Larval growth is rapid, with substantial increases in mass and total length per day but growth in mass is accompanied by substantial

energy storage. Leptocephali typically reach a much larger maximum size than Type 1 larvae, commonly >50 mm (Bohlke 1989), and residence times in the plankton may vary from 30 d to as much as 1 yr (Schmidt 1925; Castonguay 1987; Crabtree et al. 1992). In fact, the onset of metamorphosis to the juvenile phase may be delayed until appropriate physical conditions are met (Tseng 1990).

The leptocephalus larval period is divided into two main phases, a premetamorphic period of growth in length and mass known as Phase I, and a period of metamorphosis and shrinkage called Phase II (Pfeiler 1986). Phase I, the leptocephalus premetamorphic larval period, is further divided into two subphases based upon metabolism (Bishop and Torres 1999) and proximate and nucleic acid composition (Donnelly et al. 1995). Phase Ia is characterized by growth via cellular proliferation, preferential synthesis of protein and carbohydrate relative to lipid, and growth manifested more as increased length than increased mass. During this period mass-specific metabolic rates decline precipitously with increasing mass (Bishop and Torres 1999). Phase Ib is distinguished by an accumulation of glycosaminoglycans, or GAGs, incorporated into the body matrix (Pfeiler 1999). GAGs are extracellular long-chain muccopolysaccharides. During this subphase, as the larvae increase in mass – in some cases more >1 order of magnitude – there is no significant change in mass-specific metabolism (Bishop and Torres 1999). Through the accumulation of GAGs, a unique mechanism for growth, the majority of the leptocephalus' increase in mass is in the form of nonmetabolizing tissue, minimizing the increased metabolic costs normally associated with increased size.

The objectives of this study were to address rates of growth in leptocephalus larvae using biochemical indicators of growth: changes in proximate and nucleic acid composition, RNA:DNA ratios, and percent increase in protein, and to compare these results with growth rates determined by otolith microstructural analysis. Four species of leptocephalus larvae were selected based on their high abundance in the Gulf of Mexico. The congrids, *Paraconger caudilimbatus* (Poey, 1867) and *Ariosoma balearicum* (Delaroche, 1809), a muraenid, *Gymnothorax saxicola*, (Jordan and Davis, 1891) and an ophichthid, *Ophichthus gomesii* (Castelnau, 1855) were selected for the determinations of daily growth and nucleic acid compositions. Proximate composition analyses were conducted on all species but *A. balearicum*, whose composition has been described previously (Donnelly et al. 1995).

Materials and methods

Collection

Premetamorphic leptocephalus larvae were collected at the edge of the continental shelf in the eastern Gulf of Mexico on six cruises from 1990 to 1996. Sampling was conducted from 26° to 27°N between 84° and 86°W. The collection gear 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 at between 1 and 2 knots 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 the collection of leptocephali.

Leptocephali were sorted from plankton samples and identified to species (Bohlke 1989). Larvae spanning the size ranges collected for each species were divided into two groups. Larvae collected for age determinations were measured using dial calipers to the nearest 0.1 mm total length, and were preserved in 95% ETOH. For the biochemical analyses, larvae were rinsed with deionized water, blotted, frozen in liquid nitrogen, and maintained at −80 °C.

Laboratory analysis

Proximate and nucleic acid composition

Specimens were assayed for water, ash, protein, carbohydrate, lipid, RNA, and DNA following the methods described in Donnelly et al. (1995). Because of homogenate volume limitations, particularly in the very small larvae, each analysis was not conducted on every specimen. The entire suite of analyses for proximate and nucleic acid composition were conducted on specimens of *Paraconger caudilimbatus*, *Gymnothorax saxicola*, and *Ophichthus gomesii*. Only nucleic acid composition was determined for *Ariosoma balearicum*.

Proximate composition was expressed in three ways: as the total content per larva (μg $individual^{-1}$), as percent of wet mass (%WM), and as percent of ash-free dry mass (%AFDM). Content refers to the absolute quantity of a particular component measured in an individual; concentration is the component's proportion of the total mass. Components were examined in relation to total length (TL), wet mass (WM), and ash-free dry mass (AFDM).

Age determinations

For age determinations, the heads of larvae from the four species were removed, dehydrated, and infiltrated with increasing concentrations of low-viscosity embedding media (Spurr 1969). Only the otoliths from the largest *Ariosoma balearicum* larvae were removed under a dissecting microscope using polarized light Dehydration and infiltration times varied with the size of the larvae, ranging from hours to several days at each step. The heads were cured in blocks of resin, and the otoliths were prepared as described by Haake et al. (1982). Sagittal otoliths were used for age analysis because of their consistently larger size and superior clarity of increments.

After exposing the primordia of each otolith in the sagittal plane, otolith microincrements were examined using a compound microscope. A cursory examination of the otoliths revealed that increment widths approached or exceeded the resolving capabilities of light microscopy.

To prepare otoliths for analysis with the scanning electron microscope (SEM), an etching agent, 0.10 *N* HCl, was applied to erode the organic portions of each increment (Secor et al. 1992). Each otolith image was photographed or stored digitally. A minimum of three micrographs were taken of each otolith: the primordium, the entire otolith, and the best counting plane. For very large otoliths, a series of micrographs were taken using landmarks on the otolith to overlap each micrograph. Maximum and minimum primordium diameters were measured for each otolith. Any otoliths possessing a primordium diameter greater than the mean ± 2 SD were rejected from the age analyses on the basis that not all increments were properly exposed in the polishing process. Since pre-metamorphic leptocephalus otoliths were spherical, otolith radius measurements were calculated from the mean of the maximum

and minimum distances from the center of the primordium to the otolith margin.

Daily increment counts began with the first consistent increment distal to the primordium. Otolith increments were counted by two readers, each of whom did three independent readings. In each instance, the readings were made with the reader unaware of larval size or any previous age estimates. Any otolith reading varying by $>10\%$ of the mean in any of six separate readings was rejected. No significant differences were detected between the mean counts of each reader (paired t-test, $P < 0.01$, $df = 232$) . Increment counts were pooled for further analysis. The mean of the six increment counts represented the larval age in days post-hatch.

Protein growth rate(G_{pi}), expressed as the percent change in protein content per day, was calculated using the natural logarithm of the change in each individual's protein content (μg larvae^{-1}) and age in days (t) (Buckley 1984):

$$G_{pi} = \frac{\ln(\mu g\,\text{protein}) - \ln(\mu g\,\text{initial protein})}{t} \times 100 \ .$$

Initial protein values were not available for the four species examined. To obtain initial protein values, the mean protein content [94.60 μg ($\pm$19.236)] for 9 eggs and larvae from *Callechelys* sp., an ophichthid eel, were used. The eggs were collected 1 d prior to hatch, and the larvae were 1 d post-hatch.

Data analysis

Equations were obtained using least-squares regressions of ln transformed data. Graphs depict the raw data, and values in the tables have been back-transformed.

Results

Proximate and nucleic acid composition

Differences were observed in the scaling of compositional contents and concentrations with increasing size in *Paraconger caudilimbatus*, *Gymnothorax saxicola* and *Ophichthus gomesii*. The amount of a component would be expected to scale positively with increasing mass, since the total mass of any component will increase as the mass of the larva increases. In contrast, the concentration, the percent a component contributes to the total mass may scale positively, negatively, or not at all with increases in mass.

Larval total length and wet mass were measured on premetamorphic larvae ranging from 29.6 to 110.7 mm TL and 0.04 to 1.39 g WM. Mass, WM and AFDM increased allometrically with increasing length over the entire size range (Table 1). The relationships between water content and TL were similar to that between WM and TL (Table 1). Water content (g) increased multiplicatively with increasing WM, as did ash, protein, lipid, carbohydrate, RNA, and DNA contents (Table 1). Mean percent water (% WM) for the three species ranged from 91.8 to 93.9% (Table 2). Neither percent water nor percent ash showed significant trends with respect to TL or WM.

Relationships of the concentrations of the components to WM and AFDM were variable across the three species. Only *Paraconger caudilimbatus* showed a decrease in percent protein with increasing WM and AFDM; *Gymnothorax saxicola* and *Ophitchthus gomesii* showed a general increase, but the relationships were not significant (Table 1). Mean protein concentrations ranged from 1.56 to 2.84% of the WM and 34.95 to 67.01% of AFDM (Table 2). There were no significant relationships between lipid concentration and WM. Mean lipid values as percent WM ranged from 0.81 to 1.01%, and as percent AFDM from 17.44 to 29.14%. Carbohydrate concentrations varied with increasing mass, resulting in no significant relationships with WM except in *O. gomesii*. However, carbohydrate concentrations as a function of AFDM increased in both *G. saxicola* and *O. gomesii*. As a function of WM, mean carbohydrate concentrations ranged from 0.01 to 0.07%; as a function of AFDM, concentrations ranged from 0.36 to 2.95% (Table 2).

RNA and DNA concentrations decreased rapidly in similar multiplicative relationships with WM (Table 1) and AFDM (Fig. 1 for DNA). RNA:DNA ratios ranged from 0.61 $\pm$ 0.034 for *Gymmothorax saxicola* to 1.11 $\pm$ 0.044 for *Ariosoma balearicum* (Table 3) and exhibited no significant trends with TL, WM, AFDM, or larval age.

Age determinations

The otoliths from 233 leptocephali were examined to determine age. Only sagittae and lapilli were present in the otic capsules. Asterisci were not present in any larvae examined. Deposition of the asterisci in leptocephalus larvae does not occur until the onset of metamorphosis (Bishop and Torres personal observations). Sagittae were consistently the largest otoliths. Both sagittae and lapilli exhibited daily increment microstructure as described by Dean et al. (1983). Sagittae of each species possessed a distinct primordium followed by a wide, diffuse band (Fig. 2). Mean core diameters were determined for each species and ranged from 12.7 $\pm$ 0.309 μm in *Ophitchthus gomesii* to 18.8 $\pm$ 0.762 μm for *Paraconger caudilimbatus*, with *Ariosoma balearicum* and *Gymnothorax saxicola* possessing intermediate core diameters. Increment widths ranged from 0.67 $\pm$ 0.035 μm for *A. balearicum* to 1.58 $\pm$ 0.120 μm in *G. saxicola*. Otolith increment number was linearly related to mean otolith radius in three of the four species, suggesting consistent otolith growth throughout the larval period. Otoliths examined for *G. saxicola* spanned a very narrow size range (19 to 43 μm radius), resulting in a non-significant relationship between increment number and otolith radius. Otolith ages ranged from 4 to 111 d for larvae of 8.0 mm to 240.0 mm TL. The intercepts, corresponding to length at hatch, ranged from 2.5 to 6.0 mm, again with *G. saxicola* being the exception. The regressions of age in days against TL, WM, and AFDM conformed to multiplicative relationships (Figs. 3, 4; Table 4). Growth in TL ranged from 0.63 $\pm$ 0.075 mm d^{-1} in *O. gomesii* to 1.42 $\pm$ 0.11 mm d^{-1} in *P. caudilimbatus*. Although all four species grew rapidly in both length and mass, the rate

Table 1 *Paraconger caudilimbatus* (*PC*), *Gymnothorax saxicola* (*GS*), *Ophichthus gomesii* (*OG*). Relationships of proximate and nucleic acid composition with total length (*TL*), wet mass (*WM*) and ash-free dry mass (*AFDM*). Data were ln-transformed. Equations are $Y = aX^b$, where Y = independent variable, X = dependent variable, and b = rate of change; a shown is inverse ln, and represents intercept (n number of larvae analyzed; $*p < 0.05$; $**P < 0.01$; $***P < 0.001$; *NS* relationships with $P > 0.05$)

Dependent variable	Independent variable	Species	a	b	(n)	P	r^2
WM (g)	TL (mm)	*PC*	1.162×10^{-5}	2.446	(60)	***	0.96
		GS	1.630×10^{-6}	2.957	(40)	***	0.64
		OG	5.525×10^{-6}	2.487	(27)	***	0.91
AFDM (g)	TL (mm)	*PC*	9.830×10^{-7}	2.290	(59)	***	0.60
		GS	1.287×10^{-10}	4.473	(39)	***	0.54
		OG	2.201×10^{-8}	3.030	(25)	***	0.85
Water content (g)	TL (mm)	*PC*	1.875×10^{-3}	1.161	(44)	**	0.21
		GS	1.863×10^{-6}	2.904	(38)	***	0.65
		OG	5.611×10^{-6}	2.463	(25)	***	0.90
	WM (g)	*PC*	0.377	0.476	(41)	*	0.28
		GS	0.892	0.968	(39)	***	0.99
		OG	0.909	0.994	(25)	***	0.99
Ash content (mg)	TL (mm)	*PC*	2.910×10^{-3}	1.877	(43)	**	0.22
		GS	1.913×10^{-5}	3.102	(36)	***	0.46
		OG	3.73×10^{-7}	2.334	(25)	***	0.66
	WM (g)	*PC*			(43)	NS	
		GS	22.822	1.029	(37)	***	0.64
		OG	0.028	0.852	(25)	***	0.60
Protein content (mg)	TL (mm)	*PC*	3.361×10^{-3}	1.856	(60)	***	0.71
		GS	2.581×10^{-7}	4.082	(40)	***	0.49
		OG	5.232×10^{-6}	3.133	(27)	***	0.73
	WM (g)	*PC*	18.981	0.775	(60)	***	0.78
		GS	25.822	1.398	(41)	***	0.78
		OG	22.206	1.266	(27)	***	0.80
	AFDM (mg)	*PC*	153.629	0.702	(59)	***	0.89
		GS	231.044	0.877	(39)	***	0.83
		OG	409.031	1.039	(25)	***	0.94
Protein (% WM)	WM (g)	*PC*	2.028	−0.193	(43)	*	0.12
		GS			(41)	NS	
		OG	2.221	0.266	(27)	*	0.16
Lipid content (mg)	TL (mm)	*PC*	7.122×10^{-7}	2.550	(60)	***	0.88
		GS	2.091×10^{-5}	2.854	(29)	***	0.35
		OG	1.306×10^{-6}	3.287	(17)	***	0.68
	WM (g)	*PC*	9.936	1.041	(60)	***	0.91
		GS	0.785	1.088	(30)	***	0.60
		OG	7.506	0.953	(17)	***	0.66
	AFDM (g)	*PC*	67.480	0.740	(59)	***	0.65
		GS	57.916	0.729	(28)	***	0.58
		OG	38.994	0.653	(16)	***	0.67
Carbohydrate content (mg)	TL (mm)	*PC*	4.736×10^{-5}	1.999	(60)	***	0.69
		GS	3.17×10^{-7}	3.190	(36)	***	0.33
		OG	5.311×10^{-12}	5.532	(19)	***	0.83
	WM (g)	*PC*	0.508	0.809	(60)	***	0.71
		GS	0.561	1.090	(37)	***	0.50
		OG	1.695	1.896	(19)	***	0.85
	AFDM (g)	*PC*	2.155	0.566	(59)	***	0.48
		GS	1.944	0.593	(35)	***	0.41
		OG	90.784	1.446	(17)	***	0.88
Carbohydrate (% WM)	WM (g)	*PC*			(26)	NS	
		GS			(37)	NS	
		OG	0.169	0.891	(19)	***	0.57
Carbohydrate (% AFDM)	AFDM (g)	*PC*			(26)	NS	
		GS	0.194	−0.407	(35)	**	0.25
		OG	9.078	0.446	(17)	**	0.41

Table 1 (continued)

Dependent variable	Independent variable	Species	*a*	*b*	(*n*)	*P*	r^2
DNA content (μg)	AFDM (mg)	*PC*	58.958	0.534	(35)	***	0.34
		GS	70.107	0.457	(24)	***	0.77
		OG	133.798	0.672	(18)	***	0.96
DNA conc. (μg DNA mg^{-1})	WM (g)	*PC*	11.478	−0.484	(35)	***	0.33
		GS	21.810	−0.282	(29)	***	0.57
		OG	19.442	−0.169	(19)	***	0.50
	AFDM (mg)	*PC*	58.958	−0.466	(35)	***	0.28
		GS	70.107	−0.543	(24)	***	0.83
		OG	133.798	−0.328	(18)	***	0.85
RNA content (μg)	TL (mm)	*PC*	2.668×10^{-5}	2.957	(33)	***	0.73
		GS			(28)	NS	
		OG	3.498×10^{-5}	2.751	(19)	***	0.91
	WM (g)	*PC*	16.159	0.874	(33)	***	0.86
		GS	16.996	0.912	(29)	***	0.95
		OG	18.335	0.968	(19)	***	0.96
	AFDM (mg)	*PC*	288.932	0.931	(33)	***	0.85
		GS	63.446	0.546	(24)	***	0.85
		OG	144.207	0.748	(18)	***	0.91
RNA conc. (μg RNA mg^{-1})	WM (g)	*PC*	10.097	−0.395	(35)	**	0.24
		GS	16.996	−0.087	(29)	*	0.15
		OG			(19)	NS	
	AFDM (mg)	*PC*	69.329	−0.371	(35)	**	0.20
		GS	63.446	−0.454	(24)	***	0.80
		OG	144.207	−0.250	(18)	***	0.54

of growth in total length decreased with age, while rate of growth in mass increased. Inflections in six of the eight growth curves occurred at approximately 20 d (Figs. 3, 4).

Protein growth scaled significantly with larval age, TL, WM (Table 4; Fig. 5), and AFDM, fitting a multiplicative relationship. A rapid decline in protein growth rate occurred in larvae <0.2 to 0.5 g WM, and <20 d old, indicating that the rate of protein accumulation per gram decreased with increasing size and larval age. Larvae >0.5 g WM showed relatively little change in protein growth. Mean protein growth rates (% protein d^{-1}) for each species ranged from 16.12 ± 0.984% (*Paraconger caudilimbatus*) to 37.36 ± 0.521% (*Gymnothorax saxicola*) (Table 3).

Table 2 *Paraconger caudilimbatus*, *Gymnothorax saxicola*, *Ophichthus gomesii*. Proximate composition as a function of wet mass (*% WM*) and ash-free dry mass (*% AFDM*). Data are means ± SE (*n* number of larvae analyzed; *na* not applicable)

Species	% WM ± SE	% AFDM ± SE	(*n*)
P. caudilimbatus			
Water	92.23 ± 0.249	na	(41)
Protein	2.84 ± 0.113	67.01 ± 5.689	(60)
Lipid	1.01 ± 0.036	29.14 ± 3.602	(59)
Carbohydrate	0.07 ± 0.003	2.95 ± 0.476	(59)
Ash	2.84 ± 0.194	na	(41)
G. saxicola			
Water	93.88 ± 0.405	na	(39)
Protein	1.56 ± 0.149	42.61 ± 2.291	(39)
Lipid	0.87 ± 0.059	23.56 ± 2.395	(28)
Carbohydrate	0.06 ± 0.005	1.93 ± 0.238	(35)
Ash	2.41 ± 0.152	na	(39)
O. gomesii			
Water	91.75 ± 0.184	na	(25)
Protein	1.67 ± 0.179	34.95 ± 1.747	(25)
Lipid	0.81 ± 0.037	17.44 ± 0.878	(16)
Carbohydrate	0.01 ± 0.091	0.36 ± 2.411	(17)
Ash	3.88 ± 0.295	na	(25)

Discussion

The unique developmental strategy utilized by the leptocephalus is apparent in metabolic rate, enzyme activities (Bishop and Torres 1999) and proximate composition (Donnelly et al. 1995), and, as this study demonstrates, in growth. The division of the leptocephalus larval phase into two subphases can be observed in each of the growth indices examined, including protein growth rate, nucleic acids both as RNA:DNA ratios and as changes in concentration with size, and in the relationships between otolith age and size. The proximate composition components and each of the growth indices examined are addressed individually below.

Proximate and nucleic acid composition

The unique developmental strategy utilized by leptocephali is reflected most prominently in the nucleic acid composition of the larvae. Nucleic acid concentrations declined with increasing AFDM, illustrating a transition in the character of growth from increases in cell number

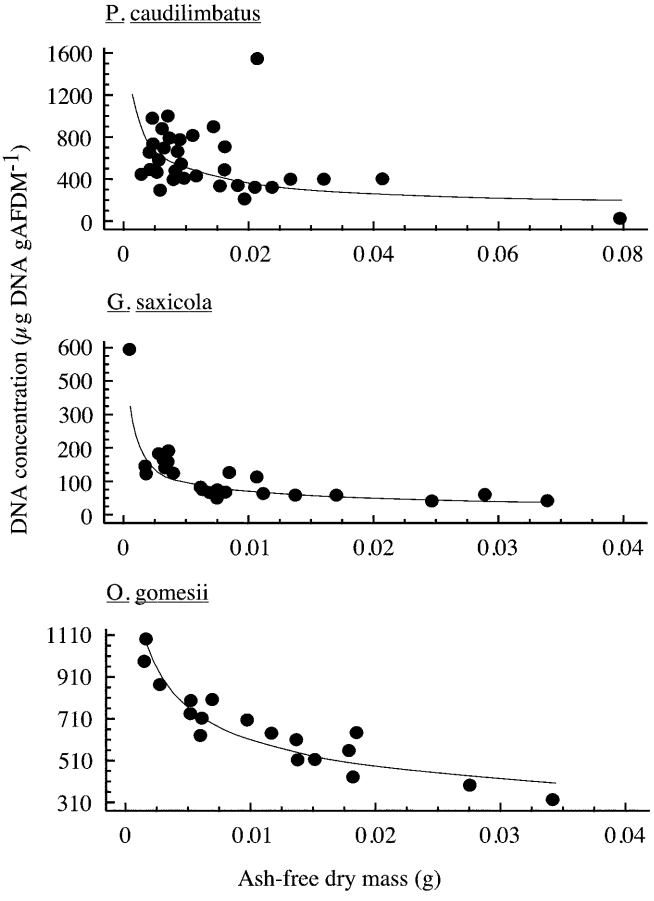

Fig. 1 *Paraconger caudilimbatus*, *Gymnothorax saxicola*, *Ophitchthus gomesii*. Least-squares regression of DNA concentration against ash-free dry mass. Raw data shown; equations and r^2 values in Table 1 (*AFDM* ash-free dry mass)

to growth by accumulation of acellular mass. The protein, carbohydrates, and lipid contents increased in the leptocephali with increasing mass in a manner similar to that in other larval fish (Balbontin et al. 1973).

The conclusion of the present study is that cellular proliferation continues throughout the size range of the larvae analyzed, albeit at a reduced rate. This is in contrast to the results of Donnelly et al. (1995), who observed an asymptote in relation to RNA and DNA content and increasing mass in *Ariosoma balearicum*. An asymptotic relationship was not observed in the larvae

Table 3 *Paraconger caudilimbatus*, *Ariosoma balearicum*, *Gymnothorax saxicola*, *Ophichthus gomesii*. Protein growth rates and RNA:DNA ratios (means ± SE). Protein growth G_{pi} (% d^{-1}) was calculated using natural logrithm of the change in a larvae's protein content (μg $larvae^{-1}$) divided by age in days, and converted to a percent (*n* number of larve)

Species	G_{pi}	RNA:DNA	(*n*)
P. caudilimbatus	16.12 ± 0.984	0.86 ± 0.115	(49)
A. balearicum	23.75 ± 3.779	1.11 ± 0.044	(83)
G. saxicola	37.36 ± 0.521	0.61 ± 0.034	(36)
O. gomesii	31.56 ± 0.568	0.78 ± 0.039	(81)

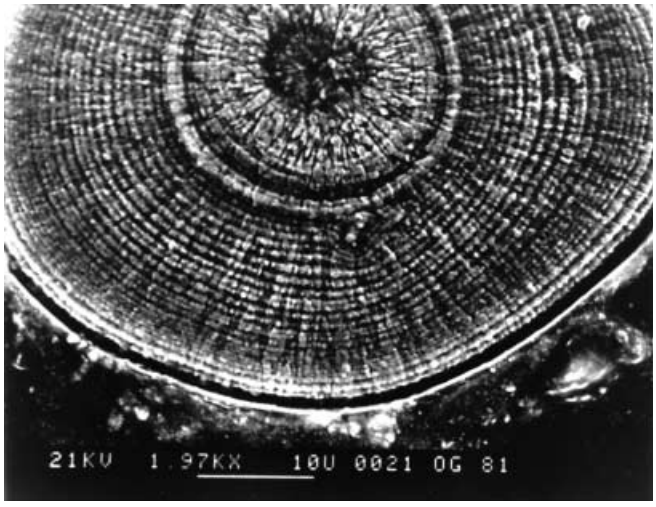

Fig. 2 *Ophitchthus gomesii*. Scanning electron micrograph of sagittal otolith of 32 d-old larva (43 mm) (Scale bar = 10 μm)

examined in this study. This is most likely not an artifact of the size range of the larvae examined. The largest larva analyzed by Donnelly et al. was 85% of maximum mass prior to metamorphosis. The largest *Paraconger caudilimbatus* and *Ophitchthus gomesii* had >85% maximum premetamorphic mass. An explanation for the differences in nucleic acid content with increasing mass between two species in the same family is not available at this time.

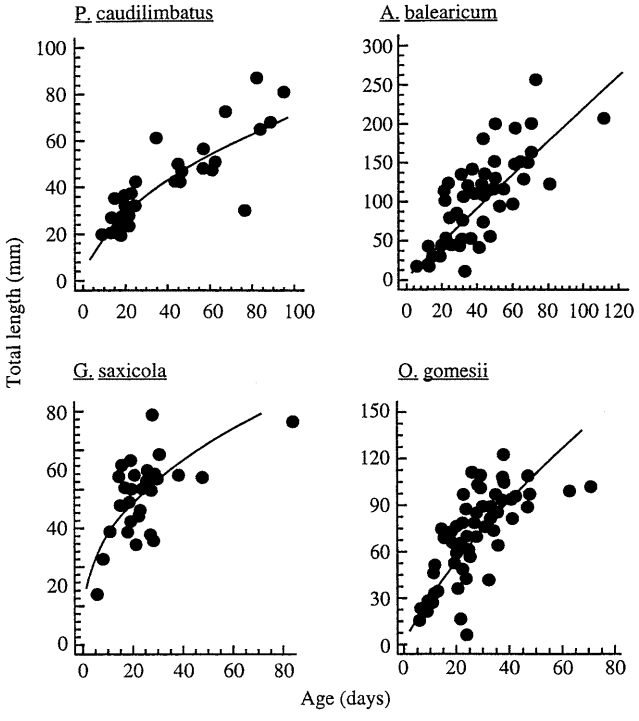

Fig. 3 *Paraconger caudilimbatus*, *Gymnothorax saxicola*, *Ophitchthus gomesii*. Least-squares regression of age (d) determined from sagittal otolith against total length (mm). Raw data shown; equations and r^2 values in Table 4

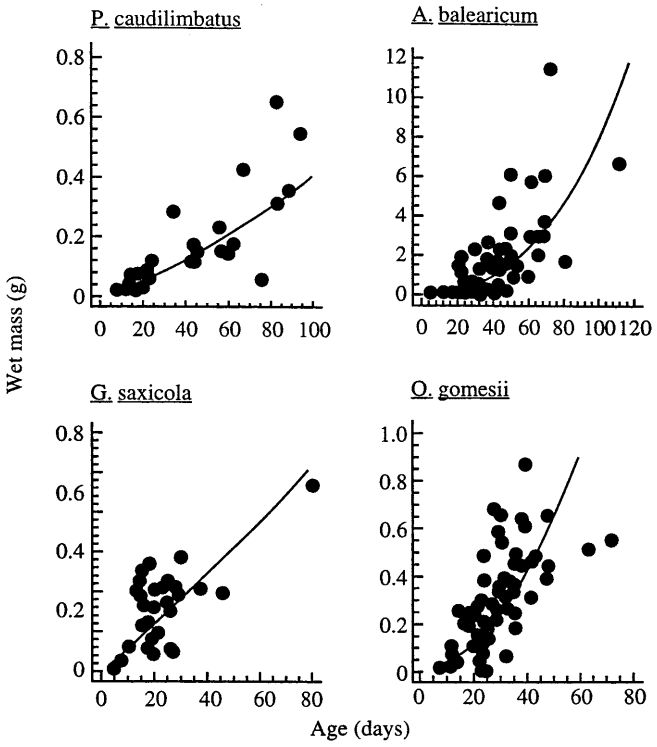

Fig. 4 *Paraconger caudilimbatus*, *Gymnothorax saxicola*, *Ophitchthus gomesii*. Least-squares regression of age (d) determined from sagittal otolith against wet mass. Raw data shown; equations and r^2 values in Table 4

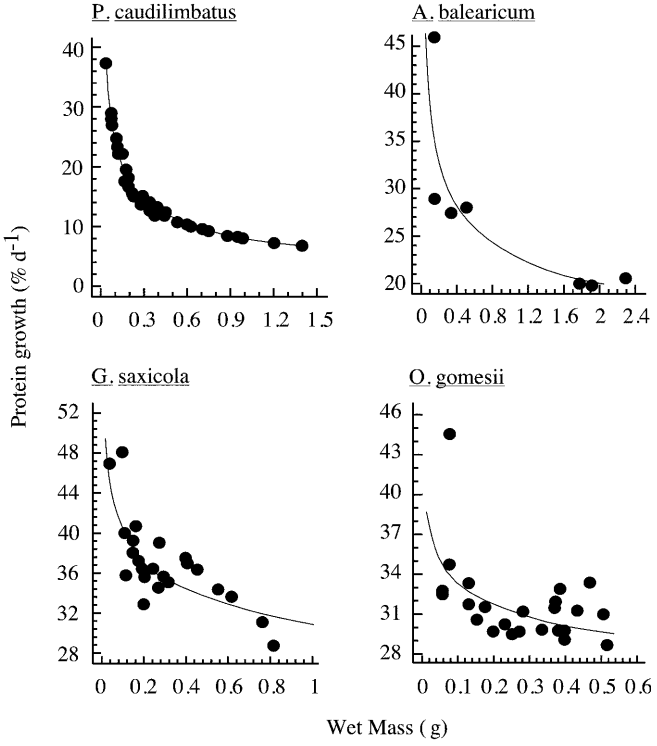

Fig. 5 *Paraconger caudilimbatus*, *Gymnothorax saxicola*, *Ophitchthus gomesii*. Least-squares regression of protein growth rate (% d^{-1}) against wet mass. Protein growth calculated as ln of change in protein content over time. Raw data shown; equations and r^2 values in Table 4

Table 4 *Paraconger caudilimbatus*, *Ariosoma balearicum*, *Gymnothorax saxicola*, *Ophichthus gomesii*. Larval growth equations ($Y = aX^b$) obtained by regressing otolith age (X, d) against growth parameters [Y, total length (TL), wet mass (WM, g), and ash-free dry mass ($AFDM$). Protein growth rates (G_{pi}) are shown as percent increase in protein per day (X, % protein d^{-1}) regressed against wet mass (Y, WM) (n number of larvae; ** $P < 0.01$; *** $P < 0.001$)

Species	Growth equation		(n)	r^2	P
	a	b			
P. caudilimbatus					
TL	5.95	0.54	(49)	0.84	***
WM	9.1×10^{-4}	1.33	(49)	0.84	***
AFDM	5.8×10^{-5}	1.24	(49)	0.86	***
G_{pi}	8.01	−0.49	(46)	0.99	***
A. balearicum					
TL	2.58	0.97	(83)	0.69	***
WM	1.2×10^{-4}	2.42	(83)	0.69	***
AFDM	1.1×10^{-6}	2.92	(83)	0.73	***
G_{pi}	23.35	−0.22	(6)	0.84	**
G. saxicola					
TL	16.19	0.37	(36)	0.42	***
WM	6.9×10^{-3}	1.05	(36)	0.4	***
AFDM	2.7×10^{-5}	1.74	(36)	0.42	***
G_{pi}	30.92	−0.12	(39)	0.61	***
O. gomesii					
TL	6.03	0.75	(81)	0.47	***
WM	5.0×10^{-4}	1.87	(81)	0.48	***
AFDM	5.1×10^{-6}	2.27	(81)	0.5	***
G_{pi}	28.26	−0.07	(27)	0.31	**

The concentrations of the components of proximate composition also illustrated slightly different trends from those observed by Donnelly et al. (1995). Protein concentrations were comparable to those found in *Ariosoma balearicum* (Donnelly et al. 1995: 1.4 to 4.1% WM and 29 to 58% AFDM), but the decline in protein concentration as a function of WM in very small larvae (< 0.4 g WM) was evident only in *Paraconger caudilimbatus*. Protein concentrations in *P. caudilimbatus* decreased slightly up to 0.2 g WM, but leveled off in the larger larvae. *Gymnothorax saxicola* and *Ophitchthus gomesii* showed highly variable protein concentrations with increasing mass. The trends toward declining lipid and carbohydrate concentration with increasing WM and AFDM observed by Donnelly et al. were not evident in this study. Lipid and carbohydrate concentrations remained variable with increasing mass.

The division of Phase I development in leptocephali into the Subphases Ia and Ib was initially based upon the shift from growth in length via cellular proliferation to an increase in acellular mass (Donnelly et al. 1995). Evidence confirming the developmental strategy utilized by the leptocephalus came from an examination of metabolism of the four species of larvae used in the present study. The transition between Phases Ia and Ib occurs at ≃10% of the total premetamorphic wet mass; thus, in *Ariosoma balearicum*, one of the largest leptocephali found in the Gulf of Mexico, the division occurs at 0.5 g

WM, or ≃90 mm TL. However, the three species examined here reach a substantially smaller maximum larval size; *Paraconger caudilimbatus* and *Ophitchthus gomesii* both metamorphose at 120 mm and *Gymnothorax saxicola* at 90 mm (Bohlke 1989). Using 10% of the maximum premetamorphic mass as the predicted transition from Phase Ia to Phase Ib, the division for *P. caudilimbatus*, *O. gomesii* and *G. saxicola* would occur at 0.1 to 0.2 g WM. This point coincides with the decrease in protein concentration observed in *P. caudilimbatus*. Only 2 and 3 data points are present for *O. gomesii* and *G. saxicola* larvae of <0.1 g WM, respectively. The inflection points indicating the transition from Phase Ia to Ib are probably not clear for *O. gomesii* and *G. saxicola* because of the low number of very small larvae. The trends of decreasing concentrations observed by Donnelly et al. are not evident here, most likely as a result of a paucity of small larvae with respect to the species-specific maximum premetamorphic size.

Growth indices

The leptocephalus growth strategy is strongly reflected in the growth indices. Growth by the accumulation of acellular mass is evident in protein growth rates and RNA:DNA ratios, and as a shift in increasing size from increases in length to a greater increase in mass with age.

The protein growth rates obtained here (16 to 33%) are comparable to those obtained by Torres et al. (1996) for red drum larvae 6 to 14 d old (10.40 to 50.20% d^{-1}) at temperatures equivalent to summer Gulf of Mexico surface-water temperatures, but the age range for the leptocephali is substantially greater (4 to 111 d). Values for RNA:DNA were extremely low (<1.3) compared to Type 1 larvae. Wright and Martin (1985) reported that RNA:DNA values <2 were indicative of starvation. The accumulation of glycosaminoglycans, characteristic of Type 2 larval development, most likely violates the assumption that both somatic growth and gain in mass in teleosts are a result of protein synthesis. Therefore, the low protein growth rates and the depressed RNA:DNA ratios are a result of the Type 2 developmental strategy, reflecting the greater proportion of acellular tissue (Donnelly et al. 1995) as the larvae increase in mass.

Although the protein growth rates of the leptocephali were low, the relationship of protein growth to increasing mass clearly illustrates the transition from Phase Ia to Ib. In Phase Ia there is a rapid decline in the percent protein accumulating in the larvae per day, and in Phase Ib there is relatively little change in protein accumulation rate as the larvae rapidly increase in size.

Age determinations

Direct validation of daily increment deposition has been extolled as a requirement for a reliable age and growth study (Beamish and McFarlane 1983). A survey of papers published on the validation of larval otolith growth rates since their initial application in 1976 revealed that in every case discrepancies that arise between otolith age and known-age larvae could be attributed to inadequate otolith preparation, misinterpretation of subdaily increments (Geffen 1982, 1983; Campana and Neilson 1985; Campana et al. 1987) and exposure to extreme physical conditions, or arise as a result of starvation (Jones 1985; Lagardere and Troadec 1997). Discrepancies that arise in otolith preparations and increment interpretation can be eliminated by examining the otoliths using scanning electron microscopy. Exposure to extreme physical conditions is not relevant to this study, and the effects of possible starvation can never be removed from a natural population.

Of major concern is the age at which the first increment is formed. Although these data do not establish the age corresponding to the deposition of the first increment, age at yolk-sac absorption (4 to 6 d) is similar to that of hatchery-reared *Anguilla japonica* (yolk-sac absorption 5 to 7 d) (Umezawa et al. 1989), indicating that increment deposition occurred most likely at hatching. Establishing an average core-diameter criteria for the acceptance of otoliths reduces the possibility of underestimating increment numbers. The core diameters for all four species equate favorably with the results of Lecomte-Finiger (1992) (7.5 μm) and Castonguay (1987) for *Anguilla* sp. (16 ± 2 μm), supporting the assumption that ages were not underestimated through incomplete otolith preparation.

The intercepts of total length on age, corresponding to the size at hatch for *Ariosoma balearicum*, *Paraconger caudilimbatus*, and *Ophitchthus gomesii*, are 2.66 ± 0.34, 5.91 ± 0.21, and 6.05 ± 0.73 mm, respectively. These values are quite similar to the sizes at hatch reported for each species (Bohlke 1989) as well as for *Anguilla japonica* (2 to 2.9 mm) (Yamamoto et al. 1975; Umezawa et al. 1989). The intercept for *Gymnothorax saxicola* (16 mm) is erroneous as a result of the narrow size range of larvae collected for that species, and is not indicative of size at hatch.

A comparison of the growth rates of the leptocephali to other pelagic larvae in the Gulf of Mexico reveals that leptocephali grow at a substantially greater rate. Larval growth rates for yellow-fin tuna (*Thunnus albacares*) (Lang et al. 1994) and Atlantic bumper (*Chloroscombrus chrysurus*) (Leffler and Shaw 1992) were 0.47 and 0.26 to 0.40 mm d^{-1}, respectively. The maximum rate for the bay anchovy (*Anchoa mitchelli*) (Fives et al. 1986) was 1.1 mm d^{-1}, approaching the mean growth rate observed in the leptocephali.

The TL growth rates of the four leptocephali reported here are comparable and higher than rates for other leptocephalus larvae. Growth rates obtained by both length-frequency analysis and otolith analysis for other members of the Elopiformes are shown in Table 5. Crabtree et al. (1992) prepared otoliths using similar techniques and obtained growth rates of 0.92 mm d^{-1}

Table 5 Elopomorph larvae. Growth rates and duration of larval phase

Species	Growth rate (mm total length d^{-1})	Duration of larval phase	Source
Anguilla anguilla (European eel)	0.19–0.24	11–18 mo	Boetius and Harding (1985)
	0.38	5 mo	Castonguay (1987)
	0.26–0.30	1 yr	Lecomte-Finiger (1992)
Anguilla rostrata (American eel)		8–12 mo	Kleckner and McCleave (1985)
Anguillla japonica (Japanese eel)		155–169 d	Tabeta et al. (1987)
		151–276 d	Tsukamoto (1989)
		112–156 d	Tseng (1990)
	0.48–0.90		Tsukamoto et al. (1992)
Megalops atlanticus (tarpon)	0.92		Crabtree et al. (1992)

for *Megalops atlanticus*. Although growth was not linear in the present study, average growth rates for the larvae reported here are all >1 mm d^{-1}. The maximum size for *M. atlanticus* (23 mm) is substantially smaller than that for *Ariosoma balearicum* (240 mm), which may account for higher growth rates. The substantially lower growth rates for *Anguilla* sp. leptocephali may reflect the specialized life histories of these larvae. *Anguilla* spp. make extensive offshore spawning-migrations, with the larvae spending a year or more as leptocephali before beginning metamorphosis (Smith 1989). A prolonged larval period and accompanying slow growth rate may be a necessity for *Anguilla* spp. larvae.

The estimated length of Phase I development, determined using the largest reported total lengths and the growth equations for each species, ranged from 36 d for *Ophitchthus gomesii* to 111 d for *Paraconger caudilimbatus*. The largest larvae, *Ariosoma balearicum*, would require 77 d to attain maximum length. *Gymnothorax saxicola*, the smallest larva, was predicted to reach metamorphosis within 40 d. Winter flounder (*Pseudopleuronectes americanus*), a pelagic larva with Type 1 development that metamorphoses to a demersal juvenile, required 50 to 70 d until the onset of metamorphosis (Chambers and Leggett 1987), well within the range reported for the leptocephali. Total residence times in the plankton cannot be determined until the length of the metamorphic period for each species is determined.

Leptocephalus' growth rates, in both length and wet mass, are higher than those of Type 1 larvae. In contrast, leptocephalus growth rates in dry mass and percent protein are similar to values for other larval fish. The rapid growth in wet mass is accomplished primarily through the accumulation of glycosaminoglycans during Phase Ib of premetamorphic development. Because the glycosaminoglycans are metabolically inert compounds, the larvae are able to rapidly increase in size with minimal energy expenditure. Since energy is allocated to growth only after metabolic requirements are met, the leptocephalus' unique developmental strategy circumvents the necessary tradeoff between energy required for metabolism and the need to rapidly increase in size to reduce predation.

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