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Proximate composition and nucleic acid content of premetamorphic leptocephalus larvae of the congrid eel *Ariosoma balearicum*

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Abstract Water, ash, proximate composition (protein, lipid, carbohydrate, hexosamine), and nucleic acid (DNA, RNA) content were measured in premetamorphic larvae of the congrid eel *Ariosoma balearicum* (Delaroche) collected from the eastern Gulf of Mexico. Specimens ranged from 15.0 to 202.3 mm total length (TL) and 0.0116 to 4.3860 g wet mass (WM). Water content increased linearly with increasing specimen mass over the entire size range; consequently, percent water was uniform and had a mean value of 92.9 ± 1.09% WM. Ash content also increased linearly with specimen mass, but only up to a mass of ≃ 2.5 g WM (165 mm TL). Ash content in specimens >165 mm TL showed only a small increase with mass, suggesting an improved osmoregulatory capability in larger individuals. The absolute amount of all proximate components increased with increasing specimen size, but rates of deposition among the components varied, resulting in different patterns in the relative concentrations of each with growth. Protein dominated the ash-free dry mass (AFDM) throughout development (29 to 59% AFDM); carbohydrate and hexosamine occurred in similar proportions (8 to 24% AFDM). Lipid was a significant proportion of the AFDM in only the smallest individuals. Lipid concentrations decreased initially as mass increased in individuals smaller than ≃ 0.4 g WM (90 mm TL), indicating a low rate of lipid deposition in small individuals. In specimens >90 mm TL, lipid concentrations were uniform and had a mean of 12% AFDM. Trends for biochemical components and nucleic acids suggest that growth of Phase I leptocephali occurs in two subphases (Ia and Ib). Phase Ia is characterized by cellular proliferation, preferential synthesis of protein and carbohydrate relative to lipid, and growth manifested more as increased length rather than increased mass. For *A. balearicum*, Phase Ia extends from yolk-sac absorption to ≃ 90 mm TL. In Phase Ib, nucleic acid content levels off, lipid deposition increases, and mass increases exponentially.

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Introduction

A distinguishing characteristic of elopomorph fishes (orders Elopiformes, Albuliformes, Notacanthiformes, Anguilliformes, and Saccopharyngiformes) is that they have a leptocephalus larval stage (Robins 1989; Nelson 1994). In addition to a distinctive morphology (a laterally compressed body composed mainly of acellular gelatinous material), premetamorphic leptocephali (Phase I) are unique among teleost larvae because although a considerable increase in size may take place during this period, ingestion of prey or other macroscopic particles has not been demonstrated. As noted by Smith (1989), there is little data on the trophic relationships of leptocephali. Direct absorption of dissolved organic matter (DOM) has been suggested (Pfeiler 1986; Hulet and Robins 1989) and, more recently, Otake et al. (1993) showed that particulate organic matter (POM) may also be an important food source.

Hydromineral studies on premetamorphic leptocephali indicate a high water and salt content (Callamand 1943; Hulet et al. 1972; Hulet and Robins 1989), conditions that may be associated with synthesis of the gelatinous matrix (Pfeiler 1986). Data on chemical composition, however, are scant and deal principally with metamorphosing individuals (Pfeiler 1984a, b, 1988; Pfeiler and Luna 1984). Early work indicated that the gelatinous material in the congrid eel *Ariosoma*

balearicum tested positive for proteins and polysaccharides (Hulet 1978). More recently, specific glycosaminoglycan (GAG) components of the gelatinous matrix have been identified for several species (Pfeiler 1988, 1991, 1993; Pfeiler et al. 1991). The only information available on overall chemical composition in leptocephali indicates that lipid and carbohydrate stores generated during Phase I are utilized during metamorphosis in bonefish (Pfeiler and Luna 1984).

This study presents data on the proximate composition and nucleic acid content of premetamorphic leptocephali of the congrid eel *Ariosoma balearicum* and describes changes in these components during Phase I development. This species was used primarily because it is common in the Gulf of Mexico and the Caribbean and attains a relatively large premetamorphic size (220 mm standard length; Hulet 1978). Secondarily, within the lineage of eels having fused frontal bones (after Regan 1912, *in* Hulet 1978), *A. balearicum* is a fairly generalized eel taxonomically and thus may be considered representative.

Materials and methods

Premetamorphic *Ariosoma balearicum* (Delaroche) larvae were captured off the west coast of Florida (near 27°N; 84°W) in oblique trawls within the upper 60 m, using either a 2 m hoop-net or a 1 m × 2 m plankton net, during the spring and summer of 1992 and 1993. Immediately after capture, specimens were measured to the nearest 0.1 mm total length (TL), rinsed with deionized water, blotted, and frozen in liquid nitrogen. Samples were stored at −80 °C until analysis. In the laboratory, individual specimens were weighed to the nearest 0.1 mg wet mass (WM) and homogenized in deionized water with both a sonifier and a hand-held glass tissue-grinder. Water content was determined either directly by weighing intact specimens after drying or indirectly from dried homogenate aliquots. Ash content was measured either from dried specimens or from dried homogenate aliquots combusted at 500 °C for 3 h. Separate homogenate aliquots (in duplicate or triplicate) were dispensed for analysis of proximate components (protein, lipid carbohydrate, hexosamine) and nucleic acids (DNA, RNA). Due to homogenate volume limitations, we were not able to conduct analyses on every specimen. Protein was determined using the method of Lowry et al. (1951) with human albumin and globulin (Sigma, 540-10) as the standard. Lipids were extracted according to the method of Reisenbichler and Bailey (1991); extracts were then dried under a flow of nitrogen at 35 °C and total lipid was determined using the charring method of Marsh and Weinstein (1966), with stearic acid (Kodak, 402) as the standard. Carbohydrate was determined by the method of DuBois et al. (1956), with glucuronic acid (Sigma, G-5269) as the standard. Hexosamine was determined using the method of Tsuji et al. (1969), with D-glucosamine hydrochloride (Sigma, G-4875) as the standard. Nucleic acids were measured fluorimetrically following procedures adopted from Bentle et al. (1981); calf thymus DNA (Sigma, D-3664) and bakers' yeast RNA (Sigma, Type XI, R-6750) were used as standards.

Data are expressed quantitatively in both absolute and relative terms. "Content" refers to the absolute amount of a particular component measured in an individual, "concentration" to the relative proportion a component constitutes of the total mass. Changes in measured components were examined in relation to total length, wet mass, and ash-free dry mass (AFDM). Regressions were generated using the least-squares method with significance at $p < 0.05$. Multiple-parameter nonlinear functions were selected by trial and error, based on their goodness of fit for the variables in question, and were considered only after simple linear ($y = a + bx$), multiplicative ($y = ax^b$), or exponential ($y = \exp[a + bx]$) functions had been examined and failed to adequately fit the data.

Results

Proximate composition

Total length and wet mass were measured on 84 premetamorphic specimens of *Ariosoma balearicum* ranging from 15.0 to 202.3 mm TL (0.0116 to 4.3860 g WM). Wet mass increased with increasing length over the entire size range (Fig. 1a). The regressions for dry mass (DM) and AFDM against TL were similar to that for WM (Table 1).

The relationship between water content (g) and TL (Fig. 1b) was similar to that between WM and TL; consequently, water content increased linearly with WM (Table 1). Percent water (%WM) ranged from 90.2 to 95.7% (mean ± SD = 92.9 ± 1.09% WM) and showed no significant trends with respect to TL or WM.

Ash content increased multiplicatively with increasing specimen length (Table 1). As a function of mass,

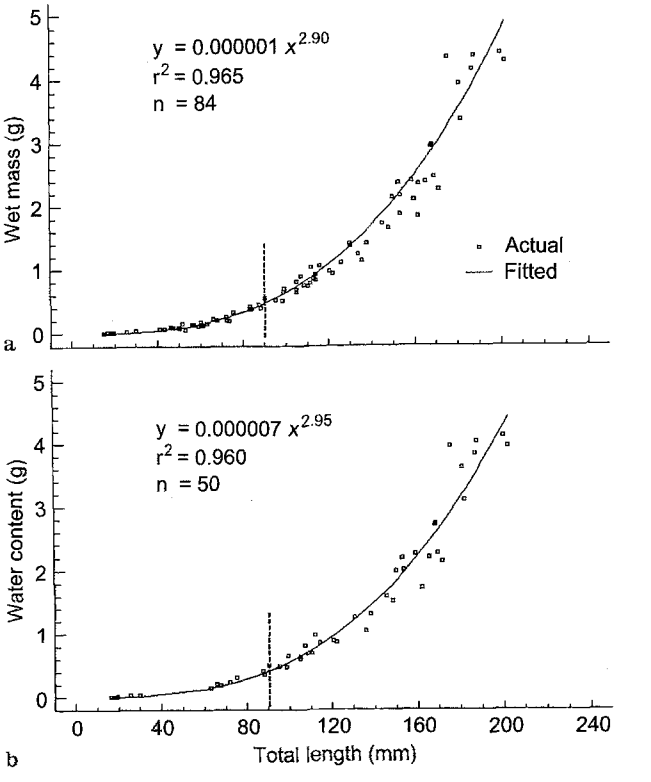

Fig. 1 *Ariosoma balearicum*. Regression of wet mass (**a**) and water content (**b**) against total length. Here and in Figs. 2 to 9, vertical dotted line marks approximate transition point from Phase Ia to Phase Ib

Table 1 *Ariosoma balearicum.* Regressions of mass versus length and various biochemical components versus mass and length (*DM* dry mass; *AFDM* ash-free dry mass; *TL* total length; *WM* wet mass). All regressions significant at $p < 0.05$

Dependent variable	Independent variable	Regression	r^2	(n)
DM (mg)	TL (mm)	$y = 0.00009x^{2.850}$	0.905	(50)
AFDM (mg)	TL (mm)	$y = 0.000005x^{3.360}$	0.856	(43)
Water content (g)	WM (g)	$y = 0.0007 + 0.927x$	0.999	(49)
Ash content (mg)	TL (mm)	$y = 0.0002x^{2.460}$	0.902	(43)
Protein content (mg)	TL (mm)	$y = 0.00005x^{2.700}$	0.942	(38)
	WM (g)	$y = 19.887x^{0.921}$	0.990	(37)
	AFDM (mg)	$y = 1.008x^{0.797}$	0.956	(29)
Lipid content (mg)	TL (mm)	$y = e(-0.843 + 0.021x)$	0.937	(30)
	WM (g)	$y = 5.060x^{1.142}$	0.953	(30)
	AFDM (mg)	$y = 0.566 + 0.110x$	0.968	(29)
Carbohydrate content (mg)	TL (mm)	$y = 0.00004x^{2.520}$	0.958	(29)
	WM (g)	$y = 6.538x^{0.903}$	0.958	(29)
	AFDM (mg)	$y = 0.250x^{0.850}$	0.949	(29)
Hexosamine content (mg)	TL (mm)	$y = e(-1.014 + 0.025x)$	0.952	(28)
	WM (g)	$y = -1.248 + 9.235x$	0.856	(28)
	AFDM (mg)	$y = 0.310 + 0.167x$	0.855	(28)
DNA content (μg)	TL (mm)	$y = 0.296x^{1.347}$	0.919	(47)
	AFDM (mg)	$y = 1/(0.003 + 0.060/x)$	0.736	(23)
DNA concentration (μg mg^{-1} AFDM)	AFDM (mg)	$y = 39.631x^{-0.599}$	0.818	(23)
RNA content (μg)	TL (mm)	$y = 0.039x^{1.731}$	0.796	(48)
	AFDM (mg)	$y = 1/(0.003 + 0.064/x)$	0.404	(24)
RNA concentration (μg mg^{-1} AFDM)	AFDM (mg)	$y = 35.705x^{-0.634}$	0.707	(23)

ash content increased in specimens up to a mass of $\simeq 2.5$ g WM (190 mg DM), after which values leveled off. The relationship between ash content (AC, mg) and mass (M) was best described by a Gompertz function (Ratkowsky 1990) as

$$AC = a\exp[-\exp(b - cM)],$$

where a is the asymptote, with an inflection point at $M = b/c$ (Fig. 2). Ash concentration ranged from 17.3 to 50.0% DM, and showed a curvilinear trend with increasing length (Fig. 3). Values decreased rapidly in specimens from 60 to $\simeq 90$ mm TL; from 90 to $\simeq 150$ mm TL, values gradually increased, followed by a rapid decrease again in specimens >150 mm TL. Specimens >180 mm TL had the lowest ash concentrations. The curvilinear pattern was also seen when ash concentration was plotted against either WM or DM; however, the initial decreasing trend was less obvious due to the meager mass change in smaller specimens.

Protein content increased multiplicatively with both increasing specimen length and mass (Table 1). Regression slopes for protein content as a function of either WM or AFDM were significantly less than one (Student's t-test, $p < 0.05$), indicating a higher rate of protein synthesis in small specimens. Protein concentrations ranged from 1.4 to 4.1% WM and from 29.0 to 58.0% AFDM. As a function of WM, protein concentrations decreased rapidly with increasing mass up to

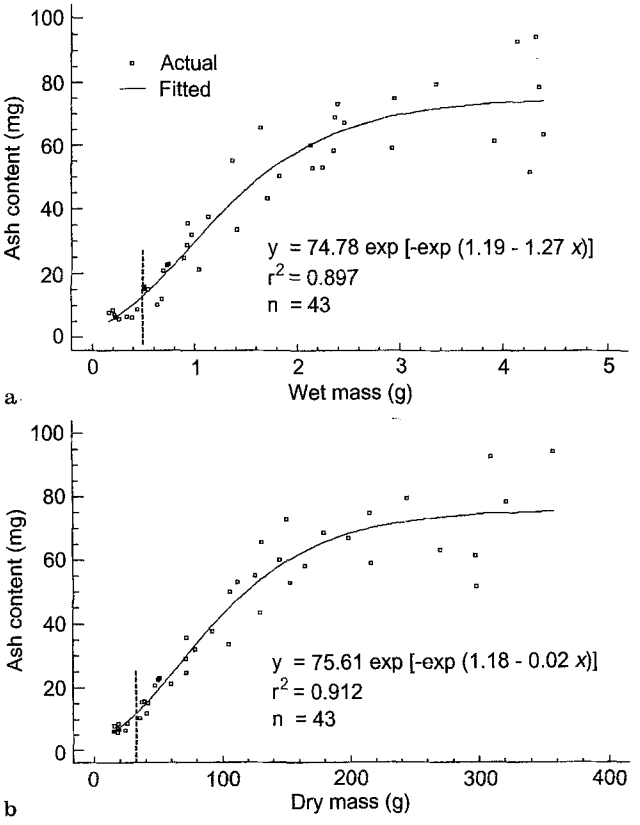

Fig. 2 *Ariosoma balearicum.* Regression of ash content against wet mass (**a**) and dry mass (**b**)

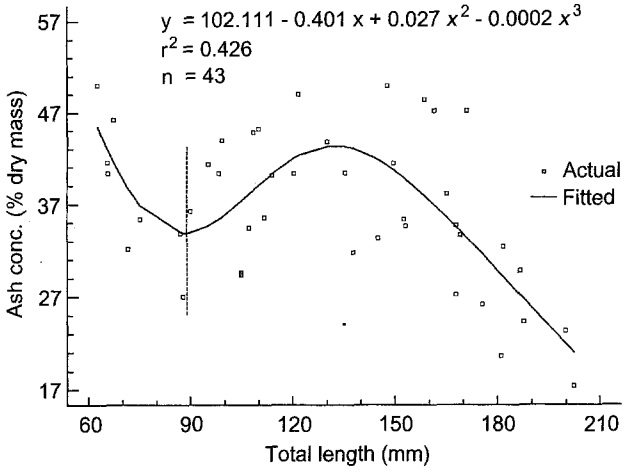

Fig. 3 *Ariosoma balearicum.* Regression of ash concentration against total length

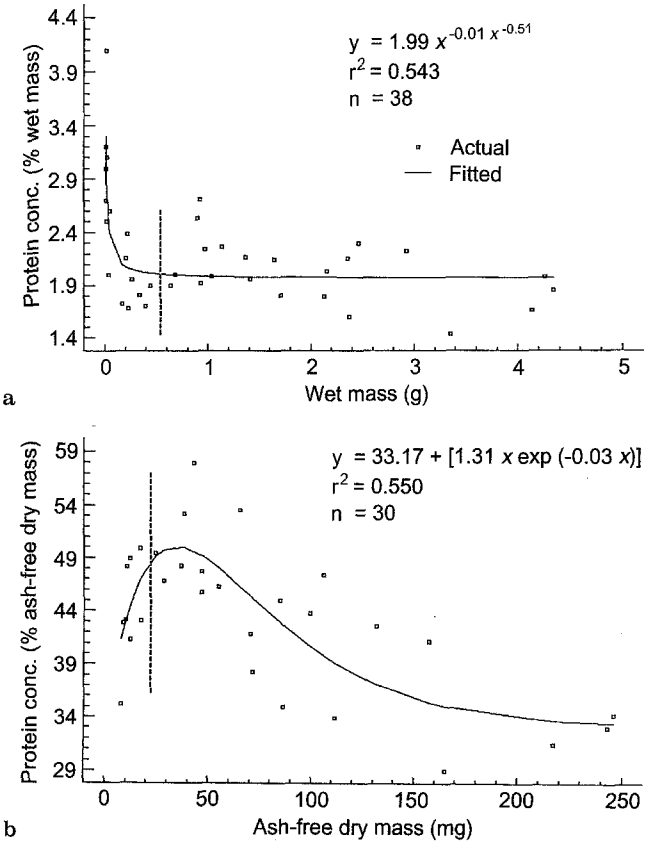

Fig. 4 *Ariosoma balearicum.* Regression of protein concentration against wet mass (**a**) and ash-free dry mass (**b**)

a mass of $\simeq 0.4$ g WM. In specimens > 0.4 g WM, values were uniform and had a mean of $2.0 \pm 0.28\%$WM (Fig. 4a). The relationship between protein concentration (PC) and WM was best described by an extended multiplicative function (Ratkowsky 1990) as

$$PC = aWM^{bWM^{-c}},$$

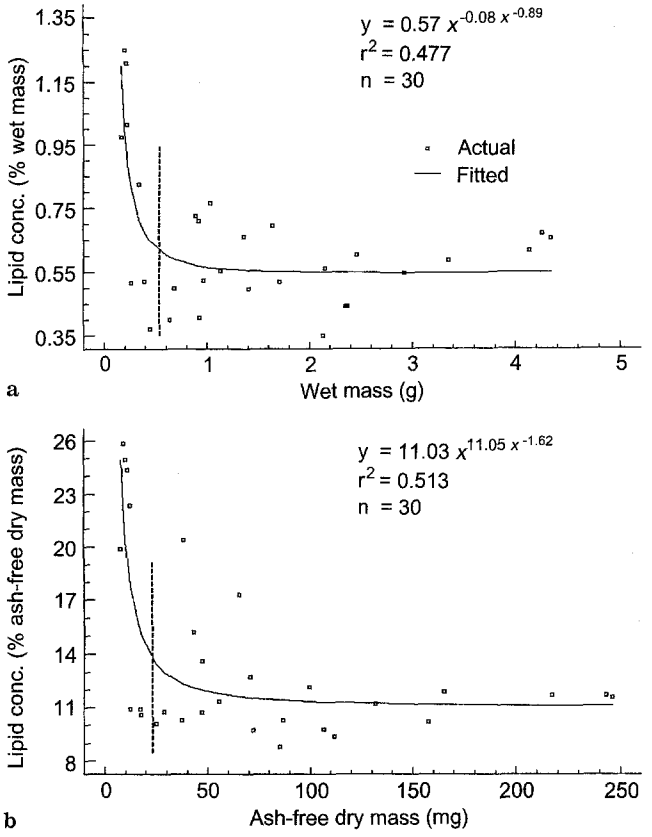

Fig. 5 *Ariosoma balearicum.* Regression of lipid concentration against wet mass (**a**) and ash-free dry mass (**b**)

where a is the intercept and bWM^{-c} is a slope term. As a function of AFDM, protein concentrations increased rapidly up to a specimen mass of $\simeq 40$ mg AFDM, followed by a gradual decrease as mass increased, described by a Ricker function (Prager et al. 1989) as

$$PC = a + [bAFDM \exp(-cAFDM)],$$

where a is an adjusted origin, b is the slope at the origin, and c is equal to $1/AFDM$ at maximum PC (Fig. 4b).

Lipid content increased exponentially as a function of TL, multiplicatively as a function of WM, and linearly as a function of AFDM (Table 1). The slope of the regression of lipid content against WM was significantly greater than 1 (Student's t-test, $p < 0.05$). Lipid concentrations ranged from 0.3 to 1.2% WM and from 8.8 to 25.9% AFDM. As a function of WM, lipid concentrations showed a pattern similar to that displayed by protein (Fig. 5a). Mean lipid concentration in specimens > 80 mm TL was $0.6 \pm 0.12\%$ WM. Unlike the relationship between protein and AFDM, the relationship between lipid concentration and AFDM was also explained by an extended multiplicative function. Lipid concentrations decreased rapidly with increasing mass up to a mass of $\simeq 15$ mg AFDM, after which values were uniform (mean $\pm$ SD = 11.8 ± 2.62, Fig. 5b).

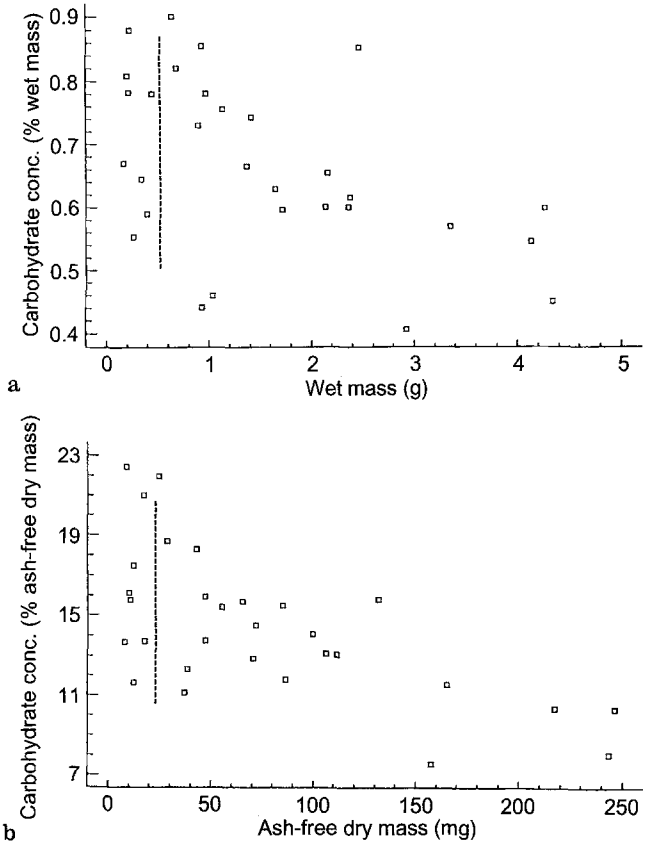

Fig. 6 *Ariosoma balearicum.* Carbohydrate concentration vs wet mass (**a**) and ash-free dry mass (**b**)

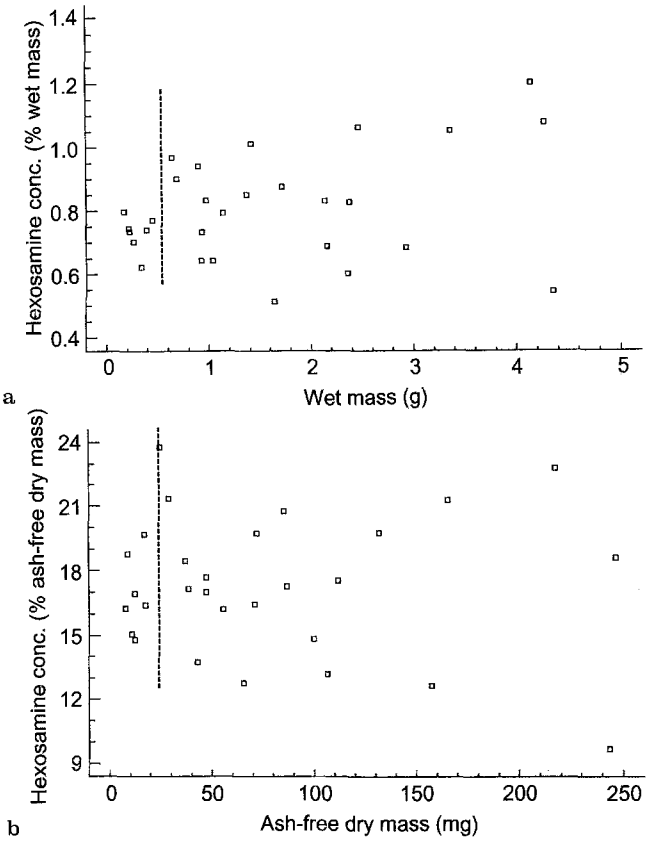

Fig. 7 *Ariosoma balearicum.* Hexosamine concentration vs wet mass (**a**) and ash-free dry mass (**b**)

Carbohydrate content increased multiplicatively with increasing TL, WM, and AFDM (Table 1). As for protein, regression slopes for carbohydrate content as a function of either WM or AFDM were significantly less than 1 (Student's t-test, $p < 0.05$). As a function of WM, carbohydrate concentrations ranged from 0.4 to 0.9% (mean $\pm$ SD $= 0.7 \pm 0.14\%$); as a function of AFDM, carbohydrate concentrations ranged from 7.6 to 22.4%. Both relations appeared to show a trend similar to that shown by protein concentration (%AFDM), but the regressions were not significant (Fig. 6). Carbohydrate concentrations were, however, significantly lower in specimens >150 mm TL (Student's t-test, $p < 0.04$ for %WM, $p < 0.004$ for %AFDM). As a function of WM, mean values were $0.7 \pm 0.13\%$ ($n = 20$) for specimens <150 mm compared to $0.6 \pm 0.13\%$ ($n = 9$) for specimens >150 mm. As a function of AFDM, mean values for each group were $15.7 \pm 3.39\%$ ($n = 20$) and $11.5 \pm 2.75\%$ ($n = 9$).

Hexosamine content increased exponentially with increasing TL and increased linearly with both increasing WM and AFDM (Table 1). Hexosamine concentrations ranged from 0.5 to 1.2% WM (mean $\pm$ SD $= 0.8 \pm 0.17\%$) and from 9.7 to 23.8% AFDM (mean $\pm$ SD $= 17.3 \pm 3.21\%$) and, except for an increased variability in larger specimens, showed no trends as a function of either WM or AFDM (Fig. 7).

Nucleic acid composition

Nucleic acid content was measured in 48 specimens ranging from 15.0 to 202.3 mm TL (0.0116 to 4.3428 g WM). DNA content increased multiplicatively with increased TL (Table 1). As a function of mass, DNA content increased asymptotically with both WM (Fig. 8a) and AFDM (Table 1). The relationship was best described by a Beverton-Holt recruitment function (Prager et al. 1989) as

$$DNA = 1/(a + b/M),$$

where $a = DNA^{-1}$ at $M = \infty$, and b is the reciprocal of the slope at the origin. In contrast, DNA concentration (μg DNA g^{-1} WM) decreased exponentially with increasing TL (Table 1) and decreased multiplicatively with increasing WM (Fig. 8b). The mass-specific pattern observed as a function of AFDM was similar to that shown for WM.

Changes in RNA content as specimen size increased were similar to those in DNA content; however, RNA

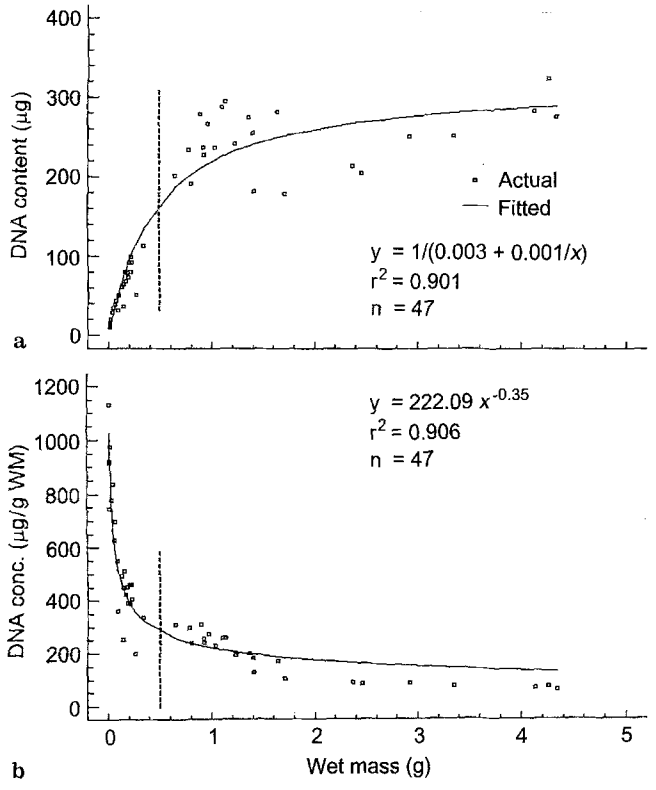

Fig. 8 *Ariosoma balearicum.* Regression of DNA content against wet mass (**a**) and of DNA concentration against wet mass (**b**)

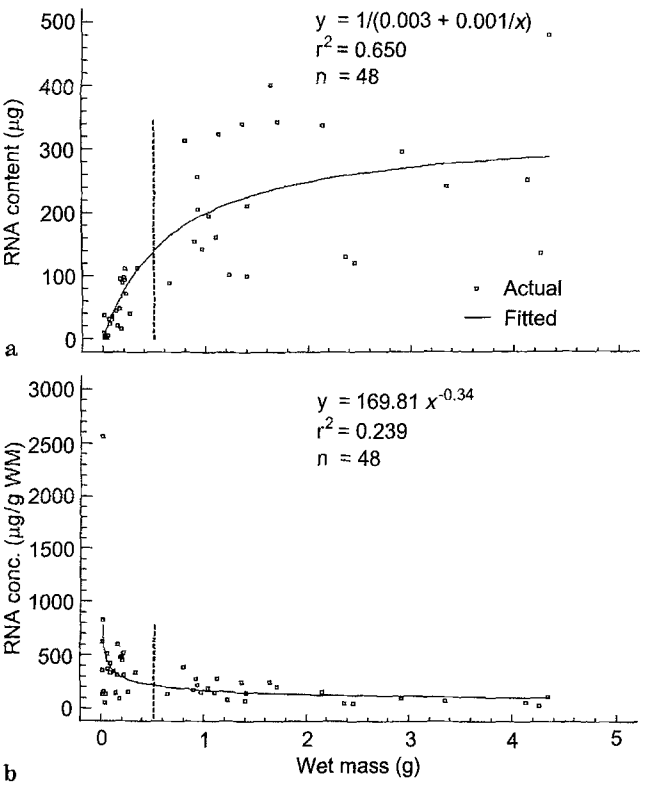

Fig. 9 *Ariosoma balearicum.* Regression of RNA content against wet mass (**a**) and of RNA concentration against wet mass (**b**)

content was more variable, especially in specimens larger than $\simeq 0.8$ g WM (Fig. 9a). The relationship between RNA concentration and WM showed a significant multiplicative decrease; however, the regression was not as strongly supported in small specimens (Fig. 9b). As a function of AFDM, trends in RNA concentration were similar to those in DNA concentration (Table 1). The ratio of RNA to DNA was low in all larvae (mean $\pm$ SD $= 0.86 \pm 0.58$, $n = 47$) and showed no trends as specimen length or mass increased. In only one sample did the RNA:DNA ratio exceed 2.0.

Discussion

In the light of the unique morphology and developmental strategy of leptocephalus larvae, it is not surprising to find their biochemical composition to be markedly different from that of typical larval fish. Most notably, leptocephalus larvae have higher concentrations of water, ash, carbohydrate, and hexosamine – attributes that are maintained throughout the premetamorphic period as a direct consequence of the high proportion of gelatinous body tissue. High water and ash concentrations result in a length to dry-mass relationship with a slope of 2.85 (Table 1). In contrast, declining water concentrations with growth and a low ash concentration (e.g. 10% DM) in non-leptocephalus larvae result in length to dry-mass relationships in which slope values range from $\simeq 3.5$ to 4.7 (Balbontin et al. 1973; Laurence 1979). Although carbohydrate and hexosamine are present at low concentrations in non-leptocephalus larvae (e.g. 5% DM, Balbontin et al. 1973; Ehrlich 1974a, b), these components are major constituents of the proteoglycans comprising the acellular gelatinous matrix that is a large portion of the organic mass in premetamorphic leptocephali. As noted by Pfeiler (1986), the gelatinous matrix serves as an energy depot to help fuel metamorphosis.

Our finding that protein constitutes the major organic component of *Ariosoma balearicum* larvae is consistent with that found for other larval fishes (Balbontin et al. 1973; Ehrlich 1974a, b, 1975; Cetta and Capuzzo 1982). However, because of the much lower ratio of AFDM to WM in leptocephali, their protein concentration (%WM and%AFDM) is notably lower than that of non-leptocephalus teleost larvae. Hulet et al. (1972) estimated protein concentrations from total nitrogen content for three leptocephali specimens (0.1 to 0.3 g WM) and reported an average value of 6.3% WM, a concentration notably higher than the concentration we found for similarly sized specimens (mean $\pm$ SD $= 2.5 \pm 0.70$, WM < 0.3 g, $n = 13$). Hulet et al.'s (1972) values are probably too high because the percentage of nonprotein nitrogen (in the form of hexosamines and glycosaminoglycans) is high in leptocephali (Pfeiler and

Luna 1984), so protein values cannot be accurately estimated from nitrogen content.

All of the assayed organic constituents increased in absolute amount, but at varying rates, during Phase I growth, resulting in differing patterns in the concentrations of each as a function of AFDM. For example, protein always constituted the greatest proportion of AFDM, even though concentrations declined in larger individuals. In contrast, lipid concentrations were highest in small individuals and initially declined with specimen mass due to a lower rate of increase in lipid content relative to increases in total AFDM. When lipid deposition subsequently increased, the rate of deposition was sufficient to maintain a constant lipid concentration for the remainder of development. Only hexosamine maintained a relatively constant proportion of the AFDM throughout Phase I.

In quantitative estimates of proximate composition derived from biochemical analyses, it is important that a particular procedure efficiently assays the tissue in question and that the standard used reflects the actual tissue composition. For this data set, the percentage of AFDM recovered showed a steady decline with increasing mass. The decreased %AFDM recovered in larger specimens was principally attributable to lower protein and carbohydrate values, an observation that may only reflect decreased tissue reactivity due to an increased complexation of these components within the extracellular matrix during development, and not an actual decrease in the concentrations of these components. Also, we may have slightly underestimated hexosamine content because of our use of D-glucosamine as a standard. The principal glycosaminoglycan (GAG) components in *Ariosoma balearicum* are chondroitin and chondroitin sulfate (Pfeiler 1991, 1993), compounds that contain N-acetyl-D-galactosamine as the primary aminated sugar (Hascall and Hascall 1981). Tsuji et al. (1969) found that galactosamine has a slightly lower molar extinction coefficient than glucosamine, which results in a slightly higher regression slope for glucosamine and thus slightly higher predicted concentrations for the same absorbance. It is unknown whether GAG composition in *A. balearicum* changes during Phase I development, a condition that would affect not only estimates of hexosamine content but also the observed pattern of a relatively constant mass-percent with increasing specimen size. Changes in GAG composition during development have been reported for bonefish (*Albula* sp.; Pfeiler 1993).

Our data support the conclusion by Hulet et al. (1972) that water concentration does not change with growth in Phase I *Ariosoma balearicum*. In addition, the linear increase in water content confirms the deduction by Pfeiler (1986) that leptocephali "load water" during premetamorphic growth. The variability in water concentration we observed may have been caused by difficulties associated with measuring wet mass in specimens with high water contents.

The increase in ash content, at least during most of Phase I, is also consistent with Hulet et al.'s (1972) finding that the principal seawater ions increase with mass in leptocephalus larvae, presumably a consequence of underdeveloped osmoregulatory capabilities and the steady production of an extracellular proteoglycan matrix. The initial decline in ash concentration for specimens smaller than $\simeq 90$ mm TL, even though total ash content increased, suggests that production of organic material during this period accounts for a greater proportion of the increase in DM. Hulet et al. (1972) showed that electrolyte concentrations vary with larval size. They concluded that "ionic regulation and the development of an osmotic concentration gradient between body fluids and sea water are evident in large leptocephali near to metamorphosis.". The leveling-off of ash content and the resultant declines in ash concentration we observed in *Ariosoma balearicum* specimens larger than $\simeq 165$ mm TL support this conclusion.

Our results indicate that growth of Phase I *Ariosoma balearicum* occurs in two subphases (Ia and Ib). During Phase Ia, there is high cellular proliferation, with increased synthesis of protein and carbohydrate relative to lipid. Growth occurs in length rather than mass; up to 40% of the maximum length is attained during this period, whereas only 5 to 7% of the maximum mass (wet, dry, or ash-free dry) is generated. During Phase Ib there is a leveling-off of DNA and RNA content, an increase in the rate of lipid deposition, and an exponential increase in mass (wet, dry, and ash-free dry). Declines in DNA and RNA concentrations reflect the greater proportion of acellular tissue in Phase Ib relative to Phase Ia, although increases in cell volume (Love 1970) or decreases in the rate of protein synthesis (Bulow 1970; Sutcliffe 1970; Fukuda et al. 1986) may also be contributing factors. For *A. balearicum*, the transition from Phase Ia to Phase Ib occurs around 90 mm TL (indicated in Figs. 1 to 9 by a dotted vertical line).

A pattern of preferential protein and carbohydrate deposition relative to lipid during the period immediately after yolk-sac absorption has been reported for non-leptocephalus larvae (May 1971; Ehrlich 1974a, b; Cetta and Capuzzo 1982; Fukuda et al. 1986) and, as noted by Ehrlich (1974a), reflects the fact that rapid initial growth is advantageous to newly hatched planktonic larvae. For non-leptocephalus larvae, one potential advantage is an increased prey spectrum (Blaxter and Hempel 1963), a scenario that at first seems inapplicable to leptocephali. This need not necessarily be the case. Mass increases with length in early-Phase I leptocephali are meager and involve very little organic tissue. Thus, a rapid increase in length greatly increases the ratio of surface area to dry mass, and at a low energetic cost. Since it is widely speculated that leptocephali obtain some portion of their nutrition via uptake of dissolved material across the epithelium (Hulet 1978; Pfeiler 1986; Hulet and Robins 1989;

Otake et al. 1993), a higher area-to-mass ratio would increase the rate at which nutrients could be available to the growing larva.

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