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Growth and Biochemical Composition of Cultured Sea Bass (*Lates calcarifer*) Larvae

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Abstract

Developing eggs and larvae of hatchery-reared sea bass (*Lates calcarifer*, Bloch) were ana-lyzed to determine the biochemical changes occurring during early development of this commer-cially important species. Mean egg diameter was 807 (±10.7) µm. Larval growth was rapid; larval length increased from 2.38 to 8.49 mm during the first 23 d of development with a corresponding increase in dry weight from 0.021 to 2.641 mg. Ash content of developing eggs was 8.0% increas-ing to 11.7-16.9% in larvae. Dry weight decreased by 43% between day 1 (developing egg) and day 2 (hatched larva). The protein content of larvae was relatively constant during development, form-ing 48.3-55.2% of the dry weight. Lipid was the major energy reserve used by developing larvae; total lipid content declined from 22.7% of the dry weight in day 2 larvae to 11.0% in day 9 larvae. The majority (62.7%) of the lipid utilized during this period was neutral lipid. Carbohydrate levels were low in developing eggs and remained low during larval development forming less than 3% of the dry weight.

Introduction

Sea bass or barramundi, *Lates calcarifer* (Bloch), inhabit coastal, estuarine and freshwater areas from the Arabian Gulf through Southeast Asia to northern Australia. The species supports important commercial and recreational fisheries throughout this range, as well as established and growing aquaculture industries in Asia and Australia (Copland and Grey 1987). A major research effort over the last decade has advanced our knowledge of the culture requirements of *L. calcarifer* and hatchery techniques are now well established (MacKinnon 1987; Ruangpanit 1987; Russell et al. 1987; Rimmer et al. 1994).

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Sea bass is a warmwater species; eggs develop rapidly at high water temperatures (approximately 28°C) and hatch approximately 17-18 h after fertilization (Russell et al. 1987). Although some previous studies have described morphological and energetic aspects of larval development in *L. calcarifer* (Moore 1982; Bagarinao 1986; Kohno et al. 1986), little is known of the biochemical or metabolic changes during development. Knowledge of larval metabolism is important in understanding factors which influence larval survival such as the rate of utilization of endogenous energy reserves. Such data are useful as a guide to the nutritional status of normally feeding larvae and in developing hatchery management techniques, such as feeding strategies.

Studies of the changes in chemical composition of eggs and larvae of marine fish are few. The majority have been conducted with temperate, coldwater species (Ehrlich 1974a, b; Cetta and Capuzzo 1982; Tocher et al. 1985) which, unlike *L. calcarifer*, have slowly developing eggs which may take days or weeks to hatch. The aim of this study was to describe the morphometric and biochemical changes during the development of *L. calcarifer* larvae reared according to standard hatchery protocol.

Materials and Methods

Eggs and larvae of *L. calcarifer* were reared at the Northern Fisheries Centre (NFC) in Cairns, Australia. Larvae were reared at densities of 10-30 per liter in 1,200-l fiberglass tanks containing 5 µm filtered water. Water temperature was maintained at approximately 28°C and salinity at 28-30‰ during larval rearing.

Rotifers were reared on microalgae (*Nannochloropsis oculata*; CSIRO isolate no. CS-179) and small quantities of yeast. They were supplemented with 'Booster' (Frippak Feeds, UK) prior to feeding to barramundi larvae (Rimmer and Reed 1991). Brine shrimp (*Artemia*) nauplii were harvested daily and starved for 24 h to allow the yolk to be fully resorbed. *L. calcarifer* larvae were fed rotifers at 10-20 ml^{-1} from day 2 (1 d after hatching) to day 14, and *Artemia* at 2 ml^{-1} from day 8, increasing to 5 ml^{-1} by day 12 and continuing at 5 ml^{-1} until day 23 (Rimmer et al. 1994). *Artemia* nauplii fed from day 13 onwards were first supplemented with 'Booster.' This feeding protocol has been shown to support very high (>90%) survival of *L. calcarifer* larvae (Rimmer et al. 1994).

Developing eggs (ca. 15 h after fertilization), and larvae at 2, 9, 13, 17, 21 and 23 d after fertilization, were collected on 100-µm mesh sieves, washed briefly with distilled water and freeze-dried for biochemical analysis. Corresponding to each dried sample, approximately 100 larvae were preserved in 4% formalin solution from which 30 d were selected at random for length measurements. Dry weights were measured in triplicate on a Cahn 21 Electrobalance, using groups of 5-40 individuals, depending on larval size. Ash was determined by heating to a constant weight at 500°C. The ash-free dry weight (AFDW) was calculated as the difference between dry weight and ash weight.

For biochemical analysis, dried samples (10-15 mg) were homogenized in distilled water. Protein was precipitated from the homogenate by the addition of perchloric acid (PCA) to a concentration of 5% (v/v). The precipitate was then

dissolved in 1M sodium hydroxide and protein determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard. Carbohydrate was determined from the PCA-soluble fraction by the method of Dubois et al. (1956) using D-glucose as the standard. Lipid was extracted by the method of Folch et al. (1957) and dried. The dried lipid residue was taken up in chloroform and an aliquot of this solution was removed and dried for assay of total lipid. To remove polar lipids, activated silicic acid was added to the remaining lipid solution which was shaken and held at 0°C for 15 minutes. After centrifugation, an aliquot of the lipid solution was removed and dried for neutral lipid assay. Total lipid and neutral lipid were assayed by charring with sulphuric acid (Marsh and Weinstein 1966) using tripalmitin as the standard. Energy values were calculated using the caloric equivalents of 36.42 (8.7), 23.86 (5.7) and 17.16 $kJ \cdot g^{-1}$ (4.1 $kcal \cdot g^{-1}$), for lipid, protein and carbohydrate, respectively (Brett and Groves 1979).

Results

Morphometric data for the *L. calcarifer* larvae used in this study are shown in Table 1. Mean diameter of 15-h old fertilized eggs was 807 (±10.7) μm. Mean dry weight decreased by 43% (AFDW by 45%) from 37 to 21 μg between day 1 (developing eggs) and day 2 (hatched larvae) probably as a result of the loss of the chorion at hatching. Mean larval length increased from 2.38 to 8.49 mm during the first 23 d of development with a corresponding increase in dry weight from 0.021 to 2.641 mg. AFDW increased from 0.018 to 2.19 mg over the same period.

Biochemical compositions and energy contents of developing eggs and larvae are shown in Table 2. The relative changes in protein, total lipid, neutral lipid and carbohydrate during development are shown in Fig. 1. Lipid made up 23.2% of the dry weight of developing eggs and declined rapidly from 22.7% of the dry weight in day 2 larvae to 11.0% in day 9 larvae (Fig. 1). Larval lipid content continued to decline and made up 6.4% of the dry weight of day 23 larvae. Protein content was relatively constant during development and made up 49.9% of the dry weight in developing eggs and 50.2% of the dry weight in day 23 larvae. The carbohydrate content of developing eggs was low and remained low (<3%) during larval development.

The energy content of developing eggs was 20.50 $kJ \cdot g^{-1}$. Larval energy content decreased from 21.06 $kJ \cdot g^{-1}$ on day 2 to 16.35 $kJ \cdot g^{-1}$ on day 9 (Table 2), corresponding with the major drop in lipid content during this period (Fig. 1). Changes in protein, lipid and carbohydrate content between days 2 and 9 are shown in Table 3. Larvae utilized five times more lipid (116.7 $mg \cdot g^{-1}$ dry weight) than protein (23.16 $mg \cdot g^{-1}$ dry weight) during this period; however, on a caloric basis, lipid provided almost eight times the energy derived from protein. The majority (62.7%) of the lipid utilized between days 2 and 9 was neutral lipid (Table 3, Fig. 1). Carbohydrate content of larvae increased only marginally between days 2 and 9.

Table 1. Morphometric data, ash content and ash-free dry weight (mean ±SD) for developing eggs and larvae of *Lates calcarifer*.

Age[a]	Length (mm)	Dry weight (mg)	Ash (%)	AFDW (mg)
Egg[b]	0.807[c] (±0.01)	0.037 (±0.003)	8.05 (±0.68)	0.034 (±0.003)
2	2.38 (±0.10)	0.021 (±0.001)	13.80 (±0.67)	0.018 (±0.002)
9	3.24 (±0.17)	0.052 (±0.003)	13.54 (±2.32)	0.045 (±0.004)
13	4.81 (±0.43)	0.463 (±0.011)	11.72 (±1.51)	0.409 (±0.009)
17	5.52 (±1.04)	0.893 (±0.200)	12.93 (±0.15)	0.777 (±0.174)
21	8.12 (±0.93)	2.514 (±0.587)	13.94 (±2.12)	2.164 (±0.533)
23	8.49 (±0.89)	2.641 (±0.472)	16.94 (±0.25)	2.194 (±0.398)

[a]D after fertilization
[b]Ca. 15 h after fertilization
[c]Egg diameter

Table 2. Proximate biochemical composition (mean ±SD of three determinations) and energy contents of developing eggs and larvae of *Lates calcarifer*.

Age[a]	Biochemical composition (µg egg+larva^{-1})				Energy content (kJ·g^{-1})
	Protein	Carbohydrate lipid	Total lipid	Neutral	
Egg[b]	18.49 (±1.67)	0.26 (±0.02)	8.59 (±0.18)	4.18 (±0.11)	20.50
2	11.16 (±0.59)	0.15 (±0.01)	4.76 (±0.12)	2.26 (±0.13)	21.06
9	26.43 (±1.12)	0.65 (±0.02)	5.72 (±0.42)	1.79 (±0.25)	16.35
13	223.70 (±21.26)	13.30 (±0.22)	49.55 (±4.47)	16.48 (±0.93)	15.92
17	492.80 (±12.80)	12.24 (±0.30)	81.60 (±6.61)	22.50 (±3.37)	16.73
21	1,363.20 (±70.41)	16.80 (±0.90)	202.40 (±17.60)	56.50 (±8.04)	15.99
23	1,324.80 (±51.33)	12.12 (±0.48)	170.40 (±14.41)	49.00 (±4.51)	14.40

[a]D after fertilization
[b]Ca. 15 h after fertilization

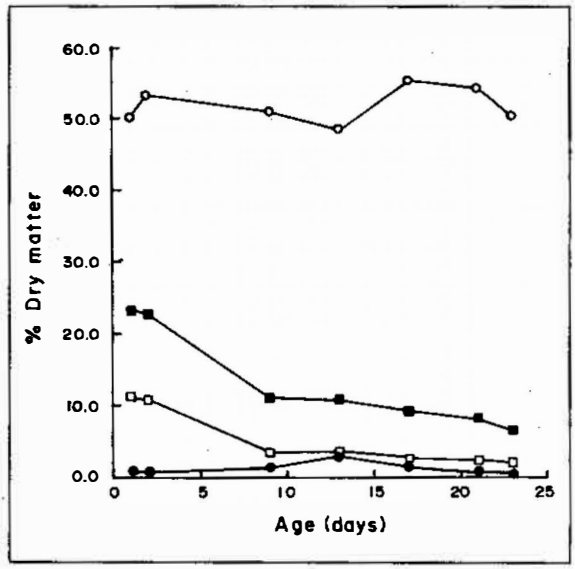

Fig. 1. Relative changes in the protein (○), total lipid (■), neutral lipid (□) and carbohydrate (●) content of *Lates calcarifer* during larval development.

Table 3. Changes in protein, lipid and carbohydrate content of *Lates calcarifer* larvae between days 2 and 9 and their energy values.

Component	Change[a] ($mg \cdot g^{-1}$ dry weight)	Energy value (Joules)
Protein	(–) 23.16	(–) 552.8
Total lipid	(–) 116.68	(–) 4,250.0
Neutral lipid	(–) 73.19	(–) 2,665.8
Carbohydrate	(+) 5.26	(+) 90.2

[a](–) denotes loss, (+) denotes gain

Discussion

Protein and lipid serve as the major energy reserves of developing fish larvae (Heming and Buddington 1988). The lipid content of *L. calcarifer* larvae decreased by 51.5% between days 2 and 9 while protein content decreased by only 4.3% over the same period; clearly, lipid is the major energy reserve of *L. calcarifer* larvae. Utilization of lipid as the major energy source has also been shown during embryonic development of marine red drum, *Sciaenops ocellata* (Vetter et al. 1983), in developing eggs and pre-feeding larvae of the common dolphinfish, *Coryphaena hippurus* (Ostrowski and Divakaran 1991), and in developing eggs and yolk-sac larvae of Senegal sole, *Solea senegalensis* (Vazquez et al. 1994). In common with *L. calcarifer*, *S. ocellata*, *C. hippurus* and *S. senegalensis* are warmwater species which undergo rapid embryonic development (1-2 d). It is likely that utilization of lipid as an energy reserve is more suited to the metabolic requirements of warmwater marine fish which undergo rapid embryonic and larval development (Ostrowski and Divakaran 1991; Vazquez et al. 1994).

Kohno et al. (1986) described early larval growth and yolk absorption in *L. calcarifer*. They showed that the yolk was largely utilized by approximately 70 h

after hatching, while absorption of the oil droplet was not complete until 145 h after hatching (equivalent to ca. day 7 in this study). The biochemical data presented here supports these observations; the sharp decline in lipid content between days 2 and 9 reflecting utilization of stored reserves. Sixty-two percent of the lipid utilized during this period was neutral lipid. This is consistent with the recognized metabolic role of neutral lipid (predominantly triglyceride) as the principal energy store in fish eggs and larvae: triglycerides make up the majority of the yolk lipids in fish eggs (Temer 1979). Polar lipids, on the other hand, have a predominantly structural role associated with membranes, and are generally conserved during larval development (Heming and Buddington 1988). However, as neutral lipid accounted for just 62% of the total lipid lost between days 2 and 9, catabolism of some polar lipid components during this period is indicated. Neutral lipid accounted for only 35% of the total lipid loss between days 9 and 23 indicating that utilization of polar lipid increased in older larvae, perhaps in response to the depletion of neutral lipid reserves. Studies with other species have shown that, in general, neutral lipid reserves are utilized in preference to polar lipids (Heming and Buddington 1988; Ostrowski and Divakaran 1991; Vazquez et al. 1994).

The composition of yolk sac reserves, and the biochemical changes which occur in developing eggs and pre-feeding larvae, may be a good indicator of the nutritional requirements of first-feeding fish larvae (Dendrinos and Thorpe 1987; Heming and Buddington 1988; Ostrowski and Divakaran 1991). Thus, in larvae where lipid is the primary endogenous reserve, it might be expected that dietary lipid containing fatty acids in similar proportions to those present in egg and yolk sac reserves, would be of high nutritional value. Now that the importance of lipid during embryonic and early larval development of *L. calcarifer* has been shown, it would be appropriate for future studies to consider changes in lipid class and fatty acid composition during this period. From an aquaculture perspective, such studies will allow a greater understanding of the nutritional requirements of *L. calcarifer* larvae which, in turn, may allow the development of more effective larval diets. Previous studies have shown that dietary fatty acid composition may greatly influence growth and survival of *L. calcarifer* larvae (Dhert et al. 1990; Rimmer and Reed 1991; Rimmer et al. 1994).

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