Aquat. Living Resour. 12 (4) (1999) 255–261

The amino acid profiles in developing eggs and larvae of the freshwater Percichthyid fishes, trout cod, *Maccullochella macquariensis* and Murray cod, *Maccullochella peelii peelii*

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Received April 19, 1999; accepted July 28, 1999

Abstract — Results on changes in the total amino acids (protein bound + free) and the free amino acids (FAA) in relation to development, from egg (unfertilised and/or fertilised) to yolk-sac resorbed larva, before first feeding, in two Percichthyid fish, trout cod, *Maccullochella macquariensis* and Murray cod, *Maccullochella peelii peelii*, which lay demersal, adhesive eggs, are presented. Throughout development, the FAA accounted for only a small proportion (0.19 % in fertilised eggs of both species) of the total amino acid pool. Nine essential amino acids (EAA) and eight non-essential amino acids (NEAA) were quantified in the amino acid pool at all stages of development. In both species, the total amino acid content decreased during the transformation (at 20 ± 1 °C) from newly hatched larva to yolk-sac resorbed larva. Overall, the changes in the TEAA and TNEAA reflected that of the amino acid pool. In trout cod, all but one EAA (lysine) and two NEAA (cysteine and glycine) decreased with ontogeny, from fertilised egg to yolk-sac resorbed larva. In Murray cod, however, the exceptions to the general decline were two NEAA (aspartic acid and glycine). In contrast, the FAA increased with development, the changes being reflected in both FEAA and FNEAA. Qualitatively, the predominant free amino acids in trout cod and Murray cod eggs were alanine, lysine, leucine and serine. Because the egg protein and the total amino acid contents declined with development, it is concluded that the rate of breakdown of yolk protein was higher than the anabolic and catabolic processes during embryogenesis. Data also suggest that in freshwater fish FAA are an unlikely primary energy substrate during embryogenesis. © 1999 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Freshwater fish / fish eggs / fish larvae / amino acids / embryonic development

Résumé — Les profils des acides aminés au cours du développement des œufs et larves de poissons d'eau douce Percichthyidés, *Maccullochella macquariensis* et *Maccullochella peelii peelii*. Sont étudiés ici les résultats des changements en acides aminés totaux (protéines associées ou non) et en acides aminés libres (FAA) en relation avec le développement ontogénétique de deux poissons d'eau douce Percichthyidés, depuis l'œuf (fécondé ou non) jusqu'au stade larvaire à la résorbtion vitelline mais précédant la première alimentation. *Maccullochella macquariensis et Maccullochella peelii peelii* ont des œufs adhésifs et les pondent sur le fond. Au cours du développement, les acides aminés libres entrent en faible proportion dans l'ensemble des acides aminés (0,19 % dans les œufs fécondés des deux espèces). Neuf acides aminés essentiels (EAA) et huit acides aminés non essentiels (NEAA) sont quantifiés à tous les stades de développement. Chez les deux espèces, la teneur en acides aminés totaux décroît durant la transformation de la larve nouvellement éclose (à 20 °C) au stade de résorption vitelline. Globalement, les changements en EAA totaux et NEAA totaux reflètent ceux de l'ensemble des acides aminés. Chez *M. macquariensis*, tous, à l'exception de la lysine (EAA), de la cystéine et de la glycine (NEAA) décroissent lors de l'ontogénie. Chez *M. peelii peelii* cependant, seuls l'acide aspartique et la glycine (NEAA) échappent à cette diminution. En revanche, les FAA augmentent lors du développement, ces changements se reportant à la fois pour les EAA libres et NEAA libres. Qualitativement, les acides aminés libres qui prédominent dans les œufs de ces poissons sont l'alanine, la lysine, la leucine et la sérine. Les protéines et la teneur en acides aminés totaux des œufs diminuant avec le développement, on conclut que la chute du taux de protéine du vitellus est plus élevée que les processus anaboliques et cataboliques durant l'embryogenèse. Ces données laissent supposer que les acides aminés libres (FAA) chez les poissons d'eau douce ne sont pas le substrat énergétique primordial durant l'embryogenèse. © 1999 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Poissons d'eau douce / œufs / larves / acides aminés / développement embryonnaire et larvaire

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1. INTRODUCTION

Information on changes in the amino acids and fatty acids in eggs and larvae is thought to permit the estimation of nutritional requirements of exogenous feeding larvae [7, 30, 37]. Such studies also provide a better understanding of utilisation of yolk reserves with early development. These aspects are important in optimising survival and improving larval quality in the artificial propagation of a species.

Apart from dietary requirement studies, most of the previous studies on amino acids measured free amino acids (FAA) of eggs and larvae of marine teleosts. Rønnestad et al. [22, 23] reported the importance of free amino acids to the energy metabolism and the compartmental distribution of FAA of eggs and larvae of turbot *Psettta maxima* and in developing yolk-sac larvae of Atlantic halibut, *Hippoglossus hippoglossus,* respectively. Metabolic aspects of FAA in developing eggs and larvae of marine fish species were reviewed by Rønnestad and Fyhn [21] and Rønnestad et al. [24]. Changes in the FAA profile in relation to early development in freshwater species are relatively less understood [4, 29, 32, 38].

The amino acid composition of eggs of a number of fish species, wild caught and cultured, is known [28, 31]. Gunasekera et al. [10] observed that egg fertilisability and hatchability in the Nile tilapia, *Oreochromis niloticus*, was related to egg free amino acid content.

The Percichthyid fish of the genus *Maccullochella* is found in the Murray Darling basin in the eastern part of the Australian continent. The two species chosen for the present study are the closely related trout cod, *Maccullochella macquariensis* (Cuvier), and the Murray cod, *M. peelii peelii* (Mitchell). Their conservation status is designated as endangered [18] and threatened [25], respectively, and both species are artificially propagated for replenishing depleted wild stocks.

Although the biology and aspects of artificial propagation of the two species are well documented [5, 25–27] there is very little known on the changes in amino acids and fatty acids during early development of Australian native fish species [6], and on freshwater species in general. Results on changes in the total and free amino acid (FAA) profiles in relation to development, from egg to post yolk-sac larva, prior to onset of first feeding, in trout cod and Murray cod are presented in this paper. The study also evaluates the importance of FAA in development in freshwater fish, which is unknown.

2. MATERIALS AND METHODS

2.1. Procurement of egg and larval samples

Aspects on broodstock management, spawning of trout cod and Murray cod and larval rearing were described previously by De Silva et al. [6] and Gunasekera et al. [13], respectively. Some relevant information, however, is presented here in brief. Trout cod are artificially propagated by hypophysing mature, pond reared broodstock [5, 15] with Chorulon (1 500 IU·kg^{-1}; Intervet, Australia), in early 'southern spring'. Ovulating females were stripped during the 1997 spawning season and samples of eggs (100–140) were taken and frozen immediately. The rest of the eggs were dry fertilised using a pool of milt, drawn from a minimum of two males randomly selected from the stock, and incubated for 24 h (20 ± 1 °C). The percentage fertilisability was estimated by counting the number of translucent, developing eggs as opposed to opaque, dead eggs, 24 h after mixing eggs and milt, in a minimum of five sub-samples of about 200 eggs each. The eggs of each spawn were incubated at 20 ± 1 °C, and the hatchlings reared separately up to yolk-sac resorption. Murray cod spawn naturally in the late 'southern spring', in ponds provided with suitable spawning boxes for egg laying [25]. During the season spawning boxes were checked regularly for eggs, and the lining (fine nylon mesh) of the boxes with attached eggs was removed and taken to the hatchery for incubation (20 ± 1 °C) and subsequent larval rearing of each spawn separately. Although the percentage fertilisability could be estimated for Murray cod, it was not possible to obtain samples of pre-fertilised eggs.

For each spawn, of both species, the diameter of a minimum of 30 eggs was determined under a dissecting microscope using an eye piece fitted with a graticule. Samples of eggs (unfertilised and/or fertilised), newly hatched larvae (100–140) and yolk-sac resorbed larvae (100–140; prior to first feeding), from each spawn of trout cod and Murray cod were taken and frozen and stored at −35 °C for amino acid analysis. A separate set of samples (40–60 eggs and/or larvae) from each spawn was taken for proximate analysis. Each sample was divided into two subsamples, treated identically and analysed separately in duplicate. As eggs and larvae from only those spawns which resulted in yolk-sac resorbed larvae were used the results of this study are based on eight and four spawns of trout cod and Murray cod, respectively.

2.2. Sample analysis

Sample preparation for total and free amino acid determinations was carried out according to Gunasekera et al. [12, 13]. Briefly, total amino acid content of egg and larval samples was determined on two subsamples each of about 20–30 eggs or larvae, from each spawn. The samples were hydrolysed for 24 h at 100 °C with 6 N HCl in sealed glass tubes replaced with nitrogen. An aliquot of an appropriate amount of the hydrolysate was taken, diluted with 0.25 M borate buffer, pH adjusted to 8.5, and was filtered through a 25 µm membrane filter. Free amino acids were separated from separate egg and larval samples (20–30 in each case) by homogenising (Ika-labortechnik homogenizer) in ice-cold 6 % trichloroacetic acid at a speed of 24 000 rpm, and centrifuging for 20 min at 8 400 *g*. The supernatant was freeze dried, dissolved in 0.25 M

borate buffer, pH adjusted to 8.5, and was filtered through a 25 μm membrane filter.

The pH adjusted samples were reacted with 9-fluroenylmethyl chloroformate (FMOC) to form amino acid FMOC derivatives using an automated GBC LC 1610 Autosampler, with a Hypersil column (150 mm L × 4.6 mm in internal diameter). For both total and free amino acids L-hydroxyproline was used as an internal standard, and they were analysed by the pre-column fluorescence derivative method using a fully automated, GBC LC 1150 HPLC (GBC Scientific Equipment, Australia). Resulting peaks were analysed using a Winchrom software package (GBC Scientific Equipment, Australia). All determinations were performed in duplicate. Tryptophan was not estimated in this study.

Proximate analysis (moisture, protein and total lipid) of egg and larval samples were carried out according to the methods specified by the AOAC [2].

All data were subjected to ANOVA followed by Duncan's multiple range test for comparison of the means among different developmental stages studied in each species. Analyses were conducted using the SPSS PC+ software package.

3. RESULTS

3.1. Size of females spawned

The present study was conducted on samples obtained from spawns of trout cod (8) and Murray cod (4). The length and weight of trout cod and Murray cod females from which spawns were used in this study ranged from 48.5 to 65.5 cm and 1.62 to 4.76 kg, and from 59.0 to 82.0 cm and 3.0 to 8.32 kg, respectively.

3.2. Proximate composition

In both species eggs start to hatch about 5 days after fertilisation, and peak hatching occurs after 7 days. The changes in weight and proximate composition at each stage of development in trout cod and Murray cod are given in *table I*. In both Percichthyids the protein content decreased ($P < 0.05$) with development, but not the lipid content.

3.3. Total amino acid pool

The mean amount of individual total essential amino acids (TEAA), and total non-essential amino acids (TNEAA) in the total amino acid (TAA) pool (protein bound + free) for each stage of development in trout cod and Murray cod is given in *table II*. Nine EAA and eight NEAA were quantified in the TAA pool at all stages of development. In trout cod all but one EAA (lysine) and two NEAA (cystine and glycine) decreased ($P < 0.05$) with ontogeny, from fertilised egg to yolk-sac resorbed larva. In Murray cod, however, such a decrease was absent only in the case of two NEAA (aspartic acid and glycine).

Table I. Mean wet weight and proximate composition (± SE) of unfertilised (not for Murray cod) and fertilised eggs, and newly hatched and yolk-sac resorbed larvae of trout cod and Murray cod. Mean weights are based on a minimum of 30 measurements of each stage from each spawn. Moisture, protein and lipid values are based on determinations on two sub-samples (30–40 eggs and/or larvae per sub-sample and analysed in duplicate) from each stage of development from each spawn. For each species, for any one parameter, values with the same superscript are not significantly different ($P > 0.05$).

Parameter (mg·individual^{-1})	Eggs		Larvae	
	Unfertilised	Fertilised	Newly hatched	Yolk-sac resorbed
Trout cod				
Weight	15.6 ± 1.0^{a}	16.3 ± 0.8^{a}	15.5 ± 0.8^{a}	19.4 ± 0.9^{b}
Moisture	10.5 ± 0.9^{a}	10.9 ± 0.7^{a}	10.6 ± 0.6^{a}	15.7 ± 0.7^{b}
Protein	3.7 ± 0.3^{b}	3.8 ± 0.2^{b}	3.1 ± 0.2^{b}	2.3 ± 0.1^{a}
Lipid	0.8 ± 0.0^{a}	1.1 ± 0.0^{b}	1.1 ± 0.0^{b}	0.7 ± 0.0^{a}
Murray cod				
Weight	na	13.5 ± 0.9^{ab}	13.1 ± 0.7^{a}	16.5 ± 1.0^{b}
Moisture	na	9.2 ± 0.5^{a}	9.1 ± 0.5^{a}	12.9 ± 0.7^{b}
Protein	na	3.2 ± 0.2^{b}	2.8 ± 0.3^{ab}	2.0 ± 0.2^{a}
Lipid	na	1.0 ± 0.0^{ab}	1.1 ± 0.1^{b}	0.8 ± 0.0^{a}

na: not available; note that in Murray cod only fertilised eggs were collected from nest boxes.

The amount of individual amino acids present in the different developmental stages were not always significantly different from each other (*table II*). For example, in Percichthyids the amount of arginine, histidine, phenylalanine and threonine in yolk-sac resorbed larvae was lower ($P < 0.05$) than in all the other developmental stages, but the differences amongst unfertilised eggs, fertilised eggs and newly hatched larvae were not. In the case of some amino acids (isoleucine and valine) the amount in newly hatched larvae was higher than in fertilised eggs in trout cod, but not so in Murray cod (*table II*).

3.4. Free amino acid pool

The changes in the individual free essential amino acids (FEAA), and non-essential free amino acids (FNEAA) in the free amino acid (FAA) pool with development in trout cod and Murray cod are given in *table III*. During ontogeny from fertilised egg to yolk-sac resorbed larva the FAA content increased ($P < 0.05$) from 46 to 382 n·mol·ind^{-1} and from 36 to 217 n·mol·ind^{-1} in trout cod and Murray cod, respectively (*table III*). This trend was reflected in both FEAA and FNEAA of the FAA pool.

In the FAA pool of trout cod the individual FEAA occurring in highest amounts, in all the development stages studied, were leucine and lysine. In Murray cod leucine and lysine were found in highest quantities in fertilised eggs, but in yolk-sac resorbed larvae threonine was the highest (*table III*). Of the FNEAA alanine was the highest in all the development stages investigated in both species. Unlike in the case of individual

Table II. Total (protein bound + free) amino acid content (± SE) in μ moles per egg or larva of unfertilised eggs (UFE), fertilised eggs (FE), newly hatched larvae (NHL) and yolk-sac resorbed larvae (YSRL) of trout cod and Murray cod (without UFE). Values with the same superscript in each row, for each species, are not significantly different ($P > 0.05$).

Amino acid	Trout cod (n = 8)				Murray cod (n= 4)		
	UFE	FE	NHL	YSRL	FE	NHL	YSRL
Essential amino acids (protein bound + free)							
Arginine	1.1 ± 0.1^{b}	1.1 ± 0.0^{b}	1.2 ± 0.1^{b}	0.6 ± 0.0^{a}	1.0 ± 0.3^{b}	0.8 ± 0.1^{b}	0.7 ± 0.0^{a}
Histidine	0.7 ± 0.0^{b}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}	0.3 ± 0.0^{a}	0.5 ± 0.0^{b}	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}
Isoleucine	0.8 ± 0.1^{b}	1.0 ± 0.0^{c}	1.3 ± 0.1^{d}	0.5 ± 0.0^{a}	0.9 ± 0.0^{b}	0.8 ± 0.1^{b}	0.6 ± 0.0^{a}
Leucine	2.3 ± 0.2^{b}	2.3 ± 0.1^{b}	2.4 ± 0.1^{b}	1.3 ± 0.1^{a}	1.9 ± 0.1^{b}	1.7 ± 0.1^{b}	1.3 ± 0.1^{a}
Lysine	1.6 ± 0.2^{ab}	1.6 ± 0.1^{ab}	1.7 ± 0.1^{b}	1.3 ± 0.1^{a}	1.3 ± 0.1^{b}	1.1 ± 0.1^{a}	0.9 ± 0.1^{a}
Methionine	0.9 ± 0.1^{c}	0.8 ± 0.0^{bc}	0.7 ± 0.0^{b}	0.4 ± 0.0^{a}	0.7 ± 0.0^{c}	0.6 ± 0.0^{b}	0.5 ± 0.0^{a}
Phenylalanine	0.7 ± 0.1^{b}	0.7 ± 0.0^{b}	0.8 ± 0.0^{b}	0.5 ± 0.0^{a}	0.6 ± 0.0^{b}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}
Threonine	1.4 ± 0.1^{b}	1.4 ± 0.1^{b}	1.5 ± 0.1^{b}	0.9 ± 0.0^{a}	1.2 ± 0.0^{c}	1.0 ± 0.1^{b}	0.8 ± 0.1^{a}
Valine	1.1 ± 0.1^{b}	1.1 ± 0.0^{b}	1.4 ± 0.1^{c}	0.6 ± 0.0^{a}	1.0 ± 0.0^{c}	0.8 ± 0.1^{b}	0.6 ± 0.0^{a}
Σ TEAA	10.7 ± 0.8^{b}	10.7 ± 0.4^{b}	11.5 ± 0.5^{b}	6.4 ± 0.3^{a}	9.0 ± 0.3^{c}	7.7 ± 0.5^{b}	6.1 ± 0.4^{a}
Non-essential amino acids (protein bound + free)							
Alanine	2.8 ± 0.2^{b}	2.8 ± 0.1^{b}	2.6 ± 0.1^{b}	1.6 ± 0.1^{a}	2.2 ± 0.1^{b}	2.0 ± 0.1^{b}	1.5 ± 0.1^{a}
Aspartic	1.7 ± 0.1^{b}	1.5 ± 0.1^{ab}	1.3 ± 0.1^{a}	1.3 ± 0.1^{a}	1.1 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}
Cystine	0.6 ± 0.0^{a}	1.0 ± 0.0^{b}	0.6 ± 0.0^{a}	0.6 ± 0.1^{a}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}	0.4 ± 0.0^{a}
Glutamic	2.2 ± 0.2^{c}	2.1 ± 0.0^{bc}	1.9 ± 0.1^{ab}	1.6 ± 0.1^{a}	1.6 ± 0.1^{b}	1.2 ± 0.1^{a}	1.3 ± 0.1^{a}
Glycine	1.3 ± 0.1^{ab}	1.3 ± 0.0^{ab}	1.4 ± 0.1^{b}	1.2 ± 0.0^{a}	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}	1.1 ± 0.0^{a}
Proline	1.9 ± 0.1^{d}	1.7 ± 0.1^{c}	1.3 ± 0.7^{b}	0.8 ± 0.0^{a}	1.3 ± 0.1^{b}	0.9 ± 0.1^{a}	0.8 ± 0.0^{a}
Serine	1.8 ± 0.1^{b}	1.8 ± 0.1^{b}	1.9 ± 0.1^{b}	1.1 ± 0.1^{a}	1.5 ± 0.0^{b}	1.3 ± 0.1^{b}	1.1 ± 0.1^{a}
Tyrosine	0.8 ± 0.1^{b}	0.8 ± 0.0^{b}	0.8 ± 0.0^{b}	0.5 ± 0.0^{a}	0.7 ± 0.0^{c}	0.5 ± 0.0^{b}	0.4 ± 0.0^{a}
Σ TNEAA	13.2 ± 0.9^{b}	12.8 ± 0.5^{b}	11.7 ± 0.5^{b}	8.7 ± 0.3^{a}	10.0 ± 0.2^{b}	8.5 ± 0.5^{a}	7.6 ± 0.4^{a}
Σ TAA	23.9 ± 1.7^{b}	23.5 ± 0.9^{b}	23.2 ± 1.0^{b}	15.1 ± 0.6^{a}	19.0 ± 0.5^{c}	16.2 ± 1.0^{b}	13.7 ± 0.8^{a}

TEAA, total essential amino acids; TNEAA, total non-essential amino acids; TAA, total amino acids.

amino acids in the TAA pool, increases in FEAA and FNEAA of the FAA pool in the transformation from fertilised egg to newly hatched larva and from newly hatched larva to yolk-sac resorbed larva were significant ($P < 0.05$), except in the case of methionine in Murray cod. The FAA pool in both Percichthyids was only a small proportion of the TAA pool. The percentage of FAA in the TAA pool ranged from 0.19 to 2.57 % and from 0.19 to 1.64 % (*table III*) in fertilised eggs and yolk-sac resorbed larvae of trout cod and Murray cod, respectively, and increased significantly at each stage of development from fertilised egg through newly hatched larva to yolk-sac resorbed larva in trout cod. In Murray cod a significant increase was evident from egg to newly hatched larva only.

4. DISCUSSION

During embryonic and larval development, yolk proteins are broken down into amino acids which are then either utilised in organogenesis or catabolised for energy production [35]. Therefore, until first feeding developing eggs and larvae depend entirely on the nutritional material in the yolk. It is evident that in the two Percichthyids the amount of protein in an egg and/or in a larva decreased ($P < 0.05$) as development progressed (*table I*) between fertilised eggs and yolk-sac resorbed, pre-feeding larvae. However, the decrease between the intermediate stages investigated was not significant. Such a trend was not observed in total lipid. This indicates that both trout cod and Murray cod utilise the available protein in preference to other main energy sources such as lipids during early ontogeny. The decline in total amino acids with development in the two Percichthyids is consistent with the above observation. The reduction in total amino acids following hatching was also reported by Srivastava et al. [29] in eggs and alevins of cultured and wild Atlantic salmon *Salmo salar*.

The amount of total amino acids in eggs of trout cod and Murray cod are comparable to that reported for striped mullet [31], white sturgeon [17] and Nile tilapia [11] but is considerably lower than that in Atlantic salmon [29]. This difference is probably a reflection of the large egg size and consequently the larger amount of yolk in the latter.

Qualitatively, the predominant amino acids in the FAA pool of eggs in trout cod and Murray cod were alanine, lysine, leucine and serine. This is comparable to that reported for dentex [34], Atlantic halibut [23] and turbot [22], but differs from that for Atlantic salmon [29] and Nile tilapia [11]. In Atlantic salmon and Nile tilapia the predominant amino acids in the FAA pool were aspartic acid, glutamic acid, cysteine and serine, and proline, tyrosine, histidine, valine and glycine, respectively. Therefore, it is difficult to discern a particular pattern in the FAA of fish in relation to habitat and related life history traits.

Table III. Free amino acid content (± SE) in n moles per egg or larva of unfertilised eggs (UFE), fertilised eggs (FE), newly hatched larvae (NHL) and yolk-sac resorbed larvae (YSRL) of trout cod and Murray cod (without UFE). Values with the same superscript in each row, for each species, are not significantly different ($P > 0.05$).

Amino acid	Trout cod (n = 8)				Murray cod (n = 4)		
	UFE	FE	NHL	YSRL	FE	NHL	YSRL
Free essential amino acids							
Arginine	2.2 ± 0.2^a	3.4 ± 0.3^b	10.7 ± 0.3^c	14.2 ± 0.4^d	2.5 ± 0.4^a	12.3 ± 1.5^b	11.8 ± 1.5^b
Histidine	1.0 ± 0.1^a	1.3 ± 0.1^a	10.0 ± 0.3^b	27.2 ± 0.8^c	1.8 ± 0.3^a	7.3 ± 1.0^b	16.3 ± 1.3^c
Isoleucine	1.1 ± 0.1^a	1.9 ± 0.2^b	9.4 ± 0.3^c	15.1 ± 0.3^d	1.7 ± 0.3^a	14.2 ± 1.8^b	14.3 ± 1.7^b
Leucine	2.2 ± 0.2^a	4.4 ± 0.4^b	18.4 ± 0.6^c	23.5 ± 0.5^d	3.4 ± 0.4^a	19.0 ± 2.5^b	15.9 ± 1.5^b
Lysine	4.2 ± 0.4^a	5.7 ± 0.4^a	17.7 ± 0.7^b	33.6 ± 1.8^c	3.4 ± 0.5^a	14.0 ± 1.9^b	14.9 ± 1.1^b
Methionine	0.8 ± 0.1^a	1.7 ± 0.2^b	9.21 ± 0.3^c	9.3 ± 0.4^c	1.2 ± 0.2^a	10.1 ± 1.2^c	7.5 ± 0.9^b
Phenyla	0.8 ± 0.1^a	1.7 ± 0.2^b	7.4 ± 0.3^c	9.6 ± 0.3^d	1.1 ± 0.2^a	7.9 ± 1.0^b	8.2 ± 1.0^b
Threonine	2.0 ± 0.2^a	3.2 ± 0.3^b	9.2 ± 0.2^c	17.2 ± 0.3^d	1.5 ± 0.2^a	11.0 ± 1.9^b	18.2 ± 1.7^c
Valine	1.9 ± 0.2^a	3.4 ± 0.3^b	11.8 ± 0.3^c	19.6 ± 0.4^d	2.6 ± 0.5^a	12.1 ± 1.7^b	13.7 ± 1.5^b
Σ FEAA	16.2 ± 1.4^a	26.7 ± 2.2^b	103.9 ± 2.6^c	169.2 ± 3.9^d	19.3 ± 2.9^a	107.9 ± 14.2^b	120.8 ± 11.5^b
Free non-essential amino acids							
Alanine	7.9 ± 0.5^a	7.2 ± 0.5^a	33.9 ± 0.6^b	64.8 ± 1.1^c	5.7 ± 0.8^a	25.8 ± 3.5^b	40.1 ± 3.1^c
Aspartic	2.7 ± 0.4^a	1.9 ± 0.2^a	5.8 ± 0.2^b	18.3 ± 1.0^c	1.2 ± 0.2^a	3.5 ± 0.3^b	5.2 ± 0.5^c
Cysteine	tr	tr	tr	tr	tr	tr	tr
Glutamic	2.6 ± 0.2^a	2.3 ± 0.1^a	6.8 ± 0.3^b	34.2 ± 1.2^c	2.6 ± 0.3^a	5.3 ± 0.8^b	11.0 ± 1.1^c
Glycine	1.0 ± 0.1^a	0.7 ± 0.1^a	13.3 ± 0.3^b	41.7 ± 1.1^c	1.8 ± 0.4^a	8.1 ± 1.3^b	9.5 ± 0.9^b
Proline	0.7 ± 0.1^a	0.7 ± 0.1^a	5.0 ± 0.5^b	12.0 ± 1.0^c	0.7 ± 0.1^a	2.5 ± 0.5^b	2.7 ± 0.3^b
Serine	2.7 ± 0.3^a	3.8 ± 0.3^a	11.3 ± 0.5^b	31.0 ± 0.9^c	3.4 ± 0.6^a	13.3 ± 1.7^b	17.3 ± 1.7^b
Tyrosine	1.1 ± 0.1^a	2.1 ± 0.2^b	10.7 ± 0.4^c	10.2 ± 0.5^c	1.7 ± 0.4^a	10.4 ± 1.5^b	10.8 ± 1.4^b
FNEAA	18.8 ± 1.5^a	18.8 ± 1.3^a	86.8 ± 1.8^b	212.3 ± 4.5^c	16.9 ± 2.5^a	68.9 ± 9.2^b	96.6 ± 7.4^c
FAA	35.1 ± 2.9^a	45.6 ± 3.5^a	190.6 ± 4.3^b	381.5 ± 8.3^c	36.2 ± 5.3^a	$176.8 \pm 23.^b$	$217.4 \pm 18.^b$
FAA/TAA (%)	0.15^a	0.19^a	0.84^b	2.57^c	0.19^a	1.18^b	1.64^b

FEAA, free essential amino acids; FNEAA, free non-essential amino acids; FAA, free amino acids; TAA, total amino acids (includes protein bound and free amino acids). tr, trace amount only.

In trout cod and Murray cod, during early ontogeny, the non-essential amino acid taurine was not detected in the FAA pool. The absence of taurine has also been reported in eggs of other freshwater and/or anadromous species such as rainbow trout [38], coregonid species [4], Atlantic salmon [29] and Nile tilapia [11]. On the other hand, considerable amounts of taurine have been detected in the FAA pool in larvae of marine species [3, 22, 23]. Conçeicão et al. [3] observed that the taurine content increased significantly during early ontogeny in turbot, and suggested that its primary role in early ontogeny may be osmolytic. It may be that taurine is less important to freshwater teleosts during early ontogeny, compared to larvae of marine teleosts. It will be of interest to investigate whether taurine appears in yolk-sac resorbed, first feeding larvae of freshwater fish when the liver becomes functional, particularly because taurine is thought to be the sole amino acid that conjugates with cholic acid to produce bile salt in teleosts [36].

In both trout cod and Murray cod the FAA content of fertilised eggs was about 0.19 % of the total amino acid (TAA) content (of the eggs). In fish eggs the proportion of FAA in TAA is very variable [21]. The FAA pool constituted 20–50 % of the total amino acids in the pelagic fish eggs but in the case of marine demersal eggs this was only about 2–3 % [21]. In eggs of freshwater fish the proportion of FAA in the TAA pool varied between 4.4 and 4.7 % in Coregonid fishes [4], 2 % in diadromous Atlantic salmon [29] and 0.53 % in Nile tilapia [10].

Unlike in the case of marine fish species, the role of FAA in early development is relatively less understood in freshwater species. In both Percichthyid fish investigated in this study the proportion of FAA in the TAA pool, as well as the absolute amount of FAA in an individual increased significantly during development. Comparable observations have been reported for Atlantic salmon [29]. In a study on rainbow trout eggs it was reported that free amino acid concentrations declined just after fertilisation, increased to near initial values in the blastula stage and then nearly doubled at hatching [38]. Terjesen et al. [32] reported that during early ontogeny in African catfish, *Clarias gariepinus*, the protein content in the egg decreased and the FAA content increased until hatching, as in the case of the two Percichthyids. On the other hand, in marine species that lay pelagic eggs, such as, for example, cod [9], Atlantic halibut [23] and turbot [22], the FAA content decreased with ontogeny.

FAA in fish eggs were initially studied with a view to evaluating their role as osmolytes [14, 19, 24]. However, investigations during the last decade have demonstrated FAA as a fuel in the energy metabolism

of developing marine fish eggs and larvae, particularly in pelagic eggs [8, 21]. The present observations on trout cod and Murray cod, together with those on salmonids [29, 38] suggest that in freshwater fish species FAA are not utilised as an energy source during early ontogeny. Increase in the FAA pool with development have been reported for all freshwater fish studied, except in coregonids [4]. In the latter instance, however, it was reported that all the FNEAA (except serine) showed a significant increase during ontogeny but not the FEAA. Taken together, evidence therefore suggest that in freshwater fish FAA are an unlikely primary energy source during early ontogeny, as opposed to that in marine fish.

During development, in trout cod and Murray cod there was a decline in protein with a concomitant decline in TAA and an increase FAA. According to Srivastava et al. [29], based on their study on Atlantic salmon, such a trend suggests a higher protein breakdown rate than the rate of utilisation of amino acids for building new body tissues or catabolised for energy production. Zeitoun et al. [38] suggested higher rates of yolk protein breakdown may be required to activate the protein biosynthesis in the embryos.

During early ontogeny the FAA may also have an osmolytic role. In marine pelagic fish eggs, during final maturation there is an accumulation of FAA which brings about an osmotic influx of water that swells the oocytes prior to ovulation and spawning [33]. The resulting high water content prepares the embryo for development in the hyperosmotic medium. During this period the embryo depends entirely on endogenous water sources [16, 20, 21], which consequently results in a decrease in the moisture content as development proceeds [20].

Overall, our understanding of the osmoregulatory mechanisms in eggs of freshwater fish is much less than in marine fish [1]. In eggs of freshwater fish, unlike in marine fish eggs, a higher FAA pool is not established during ovulation, possibly because the eggs will not be laid in a hyperosmotic environment. Although Zeitoun et al. [38] and Srivastava et al. [29] postulated the possible reasons for the increase of FAA during early ontogeny in freshwater fish, they did not hypothesise a possible physiological role/function for the accumulated FAA in the developing embryo. It may be that the accumulated FAA add to the solute pool in the developing embryo and contribute to the osmoregulatory potential of larvae which have to depend on exogenous water sources during development. In one of our previous study [13] on the 'swollen yolk-sac syndrome' in Murray cod, it was shown that a deficiency of certain FAA in the eggs was the likely causative factor of this syndrome. This syndrome was thought to be due to an osmotic imbalance which resulted in the death of eggs and hatchlings. These observations provide further evidence in favour of the present hypothesis with regard to possible function of FAA in increasing the osmolarity during embryogenesis.

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