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The amino acid profiles of estuary perch, *Macquaria colonorum*, during early development at different salinities

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Abstract − Estuary perch, *Macquaria colonorum*, is a truly estuarine species and spawns naturally in salt-wedged estuaries in salinities of 20–30. In this study, results on the changes in the total amino acids (TAA) (protein bound + free) and free amino acid (FAA) pools of developing eggs and newly hatched larvae until yolk-sac resorption were evaluated at four incubation salinities (15, 20, 25 and 30). The TAA in 1-h post-fertilised eggs ranged from 207.4 ± 2.2 to 231 ± 10 nmol·egg^{-1}, at salinities of 20 and 15, respectively. The TAA content decreased significantly ($P < 0.05$) as development progressed. The FAA in eggs of estuary perch ranged from 35.5 ± 0.5 to 41.0 ± 0.9 nmol·egg^{-1} at salinities of 30 and 15, respectively, and accounted for 15.7–28.0 % of the TAA. In newly hatched larvae the TEAA accounted for between 42.0 and 43.2 % of the TAA. The FAA decreased significantly with development, it being reduced in yolk-sac resorbed larvae to 19, 16.2, 22.8 and 28.7 % of that of the original amount in fertilised eggs in salinities of 15, 20, 25 and 30, respectively. The FEAA in the FAA pool also decreased significantly with development, to approximately 33.7–35.3 % in yolk-sac resorbed larvae from that in fertilised eggs. The data suggest that estuary perch uses its FAA for energy dissipation during early ontogeny rather than for body protein synthesis. Overall, the amino acid profiles of estuary perch were close to that of pelagic eggs of marine fish, and there were no major influences of salinity on the amino acid profiles as development progressed. © 2000 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

fish eggs / fish larvae / amino acids / embryonic development / salinity / *Macquaria colonorum*

Résumé − Profils des acides aminés des œufs et des larves de la perche, *Macquaria colonorum*, à différentes salinités. La perche, *Macquaria colonorum*, est une espèce euryhaline et pond naturellement dans les estuaires à des salinités de 20 à 30. Dans cette étude, des résultats sur les changements dans les groupes d'acides aminés totaux (TAA) et des acides aminés libres (FAA) des œufs en cours de développement et des larves nouvellement écloses jusqu'à la résorption du sac vitellin, ont été évalués pour quatre salinités (15, 20, 25 et 30) lors de l'incubation. Les TAA chez les œufs (1 h après fécondation) varient de 207,4 ± 2,2 à 231 ± 10 nmol·œuf^{-1} à des salinités de 20 et 15, respectivement. Les TAA diminuent de façon significative ($p < 0,05$) au fur et à mesure du développement. Les FAA des œufs de perche représentent de 35,5 ± 0.5 à 41,0 ± 0.9 nmol·œuf^{-1} à des salinités de 30 et 15 respectivement et 15,7 à 28,0 % des TAA. Chez les larves nouvellement écloses, les TEAA représentent entre 42,0 et 43,2 % des TAA. Les FAA diminuent significativement avec le développement chez les larves de 19, 16,2, 22,8 et 28,7 % des œufs fécondés à des salinités de 15, 20, 25 et 30, respectivement. Les FEAA diminuent également de façon significative avec le développement de 33,7 à 35,3 % des FAA chez les larves. Ces données montrent que la perche utilise les FAA pour sa consommation énergétique durant l'ontogenèse plutôt que pour la synthèse des protéines corporelles. Dans l'ensemble, les profils des acides aminés de la perche sont proches de ceux des œufs pélagiques des poissons marins, et il n'y a pas d'influence majeure de la salinité sur les profils des acides aminés au cours des premières étapes du développement. © 2000 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

œufs de poissons / larves de poissons / acides aminés / développement embryonnaire / salinité / *Macquaria colonorum*

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1. INTRODUCTION

The importance of evaluating changes in the total (protein bound + free) and free amino acid (FAA) pools during early developmental stages of teleost fish, as a means of understanding nutrition and the physiology of energy partitioning during early ontogeny, were reviewed by Rønnestad and Fyhn (1993) and Rønnestad et al. (1999). Most of the previous work, however, was carried out on marine species, and that on early developmental stages of freshwater species is limited (Zeitoun et al., 1977; Dabrowski et al., 1985; Srivastava et al., 1995; Gunasekera et al., 1999). A comparable study has not been undertaken previously on truly estuarine species.

Previous studies on marine and freshwater species have indicated that there are significant differences in the amino acid pools of the two groups, and the fate of groups of amino acids can differ during early ontogeny. For example, marine teleosts that lay pelagic eggs tend to have a significantly larger free amino acid (FAA) pool which acts as an energy source through development (Rønnestad and Fyhn, 1993; Rønnestad et al., 1999). In contrast, in freshwater species the FAA pool tends to be smaller and is conserved during early development. Similarly, differences in individual amino acids are evident in marine and freshwater teleosts, such as, for example, taurine. Taurine has not been recorded in the free amino acid (FAA) pool in the eggs and/or pre-feeding larvae of freshwater species that have been investigated, for example, rainbow trout (Zeitoun et al., 1977), coregonid species (Dabrowski et al., 1985), Atlantic salmon (Srivastava et al., 1995), Nile tilapia (Gunasekera et al., 1996) and the Australian Percichthyid fish trout cod and Murray cod (Gunasekera et al., 1999). However, taurine is found in significant quantities in marine species throughout development (Conçeicão et al., 1997).

In the light of major differences between freshwater and marine teleosts and because of the paucity of work on estuarine species, the present study was instigated on *Macquaria colonorum* (Günther), a truly estuarine species (Llewellyn and MacDonald, 1980; McCarraher and McKenzie, 1986), with a view to evaluating the changes in the total and free amino acid pools in relation to early ontogeny. As the salinity in estuarine environments are subjected to rapid changes, and the developing eggs and larvae have to be physiologically capable of tolerating such changes (Day et al., 1989; Haedrich, 1992), the present study was conducted in four salinities, viz. 15, 20, 25 and 30, in which estuary perch development occurred (Newton, 1994).

2. MATERIALS AND METHODS

2.1. Broodfish

All egg and larval samples for the present study originated when mature, male and female estuary perch were angled from the lower reaches of the Hopkins River (38°23'S; 142°31'E) in October/November 1998. Female estuary perch (370 mm, 910 g and 350 mm, 500 g) with free flowing, translucent ova were stripped and dry fertilised with milt drawn from four males on each occasion. The eggs (15 mL) were distributed in approximately equal amounts using a sterilised plastic spoon (2.5 mL) into 1 L aquaria of salinities of 15, 20, 25 and 30 at 17 ± 0.2 °C. The number of eggs introduced into each salinity was estimated using sub-samples.

2.2. Egg and larval samples

Dry-fertilised eggs were introduced into experimental salinities of 15, 20, 25 and 30 (eggs introduced into 10 did not develop) maintained at 17 ± 0.2 °C, with constant aeration, ensuring that eggs do not clump together, under a 12 h/12 h light/dark regime. The eggs and larvae of the two females were reared separately, under identical conditions. After 1 h of mixing eggs and sperm, opaque eggs were removed and counted. Approximately 70 % of water in each aquarium was replenished daily with water of identical salinity, prepared by mixing glass-fibre filtered (1 µm) seawater and distilled water, the salinity being made up using a refractometer (ISSCO, USA; 0 to 100). Dead eggs (opaque) and larvae were removed daily. Hatching success and larval survival until yolk-sac resorption were estimated for each salinity for the two females. In addition, at each salinity the lengths of ten larvae were determined, after anaesthetisation (MS 2222; 1:10 000), under a Zeiss SV-6 microscope.

Egg samples were taken 1 h post-fertilisation (p-f), thereby ensuring that the sampled eggs were fertilised, from each of the experimental salinities, and thereafter at 15 and 40 h p-f, and larval samples, immediately after hatching (approximately 55–57 h p-f) and at yolk-sac resorption (66–78 h p-f). On each occasion the samples at each stage of development consisted of 60–80 units, four such samples being collected for each stage of development at each salinity, from each mother. In all cases, the samples were rinsed quickly in de-ionised water, to remove adhering salts, and frozen immediately. The frozen samples were freeze dried and all analyses were performed on triplicate on the latter.

2.3. Amino acid analysis

Sample preparation for total and free amino acid determinations were carried out according to Gunasekeraet al. (1998, 1999) and details are given in these publications. Briefly, total amino acid content of egg and larval samples was determined on two subsamples from each salinity (each in triplicate). The method of analysis was the same as in our previous studies (Gunasekera et al., 1998, 1999). In the present instance also, for both total and free amino acids, L-hydroxyproline was used as an internal standard, and they were analysed by a pre-column fluorescence derivative method using a fully automated, GBC LC

Table I. Total (protein bound + free) amino acid content (± SE) in nmol·egg^{-1} or larva^{-1} of estuary perch at different stages of development at a salinity of 15. Values with the same superscript in each row are not significantly different ($P > 0.05$)*.

Amino acid	Fertilised eggs			Larvae
	1 h	15 h	40 h	Newly hatched (57 h)
Arginine	$9.9^{b} \pm 0.9$	$7.7^{a} \pm 0.1$	$8.4^{a} \pm 0.1$	$7.0^{a} \pm 0.1$
Histidine	3.8 ± 0.7	4.4 ± 0.8	5.6 ± 0.8	3.7 ± 0.4
Isoleucine	$8.6^{c} \pm 0.4$	$6.9^{b} \pm 0.2$	$7.0^{b} \pm 0.1$	$5.9^{a} \pm 0.2$
Leucine	$22.2^{b} \pm 1.4$	$18.9^{a} \pm 0.2$	$18.9^{a} \pm 0.4$	$16.6^{a} \pm 0.4$
Lysine	14.0 ± 0.2	13.3 ± 0.5	14.9 ± 1.6	11.3 ± 0.4
Methionine	6.4 ± 1.6	4.7 ± 0.3	5.3 ± 0.6	4.1 ± 0.4
Phenylalanine	8.2 ± 1.1	7.0 ± 0.3	7.1 ± 0.1	6.2 ± 0.1
Threonine	$12.1^{b} \pm 0.3$	$10.7^{a} \pm 0.1$	$11.9^{b} \pm 0.2$	$10.1^{a} \pm 0.1$
Valine	$11.6^{b} \pm 1.2$	$8.9^{a} \pm 0.3$	$9.6^{a} \pm 0.2$	$7.7^{a} \pm 0.1$
Σ TEAA	$97.0^{c} \pm 6.1$	$82.9^{b} \pm 0.8$	$88.8^{bc} \pm 1.8$	$72.9^{a} \pm 0.5$
Alanine	25.9 ± 2.7	22.4 ± 1.9	22.6 ± 2.0	22.1 ± 2.5
Aspartic acid	15.5 ± 1.3	13.6 ± 0.7	13.0 ± 0.6	11.9 ± 0.8
Cystine	$8.6^{b} \pm 0.2$	$8.2^{b} \pm 0.1$	$7.8^{b} \pm 0.2$	$6.8^{a} \pm 0.3$
Glutamic acid	$23.6^{c} \pm 1.1$	$20.9^{b} \pm 0.5$	$20.5^{b} \pm 1.0$	$17.0^{a} \pm 0.5$
Glycine	$12.6^{b} \pm 0.5$	$10.7^{a} \pm 0.1$	$12.5^{b} \pm 0.2$	$11.2^{a} \pm 0.2$
Proline (+ taurine)	$20.2^{b} \pm 1.8$	$18.0^{b} \pm 1.6$	$20.6^{b} \pm 1.5$	$13.0^{a} \pm 2.1$
Serine	$20.1^{b} \pm 0.6$	$17.0^{b} \pm 0.7$	$18.1^{b} \pm 0.7$	$14.9^{a} \pm 0.4$
Tyrosine	$7.4^{b} \pm 0.6$	$7.1^{b} \pm 0.2$	$7.4^{b} \pm 0.2$	$5.8^{a} \pm 0.1$
Σ TNEAA	$134.0^{c} \pm 4.8$	$119.2^{b} \pm 2.6$	$122.6^{b} \pm 2.6$	$103.0^{a} \pm 2.4$
Σ TAA	$231.0^{c} \pm 9.8$	$202.2^{b} \pm 1.9$	$211.5^{b} \pm 2.8$	$175.9^{a} \pm 2.1$

* At each developmental stage the analysis was based on four samples, two from each female. Each sample was analysed in triplicate. The yolk-sac resorbed larval samples were not available for TAA analysis.

1150 HPLC (GBC Scientific Equipment, Australia). Resulting peaks were analysed using a Winchrom software package (GBC Scientific Equipment, Australia). All determinations were carried out in triplicate. Tryptophan was not estimated in this study. A portion of the yolk-sac resorbed larval samples at a salinity of 15 was lost and was sufficient for estimating only the FAA content.

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

3. RESULTS

The estuary perch eggs, 1 h post-fertilised, weighed 1 ± 0.1 mg and the mean diameter was 1.01 ± 0.02 mm. The eggs were spherical and semi adhesive. The yolk was unsegmented and one or more oil globules were present.

The mean percent hatchability of eggs incubated in the four experimental salinities was 22.8 % (salinity of 15), 37.2 % (20), 52.5 % (25) and 56.0 % (30). The hatchability at salinities of 25 and 30 was significantly higher ($P < 0.05$) than at the two lower salinities. However, the larval survival, until yolk-sac resorption, in salinities of 20, 25 and 30 was over 90 %, and differed significantly ($P < 0.05$) from that at 15 (58 %). The mean hatchability and/or larval survival between the two mothers, at any of the experimental salinities, were not significantly different ($P > 0.05$). The time to hatch ranged from 55 to 57 h, and to yolk-sac resorption from 66 to 78 h at salinities of 30 to 15, respectively. The mean times to hatch and yolk-resorption at the different salinities are given in *tables I–IV*. The larval length at hatching was significantly lower at a salinity of 15 (2.54 ± 0.04 mm) compared to that at the higher salinities (e.g. at 25, 2.67 ± 0.03 mm).

3.1. Total amino acids

The total amino acid (TAA) content of eggs of estuary perch, 1 h after fertilisation ranged from 207.4 ± 2.2 to 231.0 ± 9.8 nmol·egg^{-1}, in salinities ranging from 15 to 30 (*tables I–IV*). However, at all four salinities tested (15, 20, 25 and 30), the TAA decreased as development proceeded, and in all instances, the decrease was significant ($P < 0.05$) from 1 h post-fertilised eggs to yolk-sac resorbed larvae. In fertilised eggs, at all salinities the total essential amino acids (TEAA) and the total non-essential amino acids (TNEAA) of the TAA were dominated by leucine, lysine, threonine and valine, and alanine, glutamic acid, proline and serine, respectively. The above dominant amino acids, irrespective of the stage of development and/or salinity, accounted for 60–68 % of the total of each category (*tables I–IV*). At salinities of 20, 25 and 30 the amount of individual amino acids in yolk-sac resorbed larvae was significantly lower ($P < 0.05$) than in post-fertilised eggs, except in the case of histidine. However, at a salinity of 15 in addition to

Table II. Total (protein bound + free) amino acid content (± SE) in nmol·egg^{-1} or larva^{-1} of estuary perch at different stages of development at a salinity of 20. Values with the same superscript in each row are not significantly different ($P > 0.05$)*.

Amino acid	Fertilised eggs			Larvae	
	1 h	15 h	40 h	Newly hatched (56 h)	Yolk-resorbed (72 h)
Arginine	$8.1^{b} \pm 0.0$	$8.3^{b} \pm 0.1$	$8.3^{b} \pm 0.1$	$6.8^{a} \pm 0.1$	$7.5^{b} \pm 0.4$
Histidine	3.5 ± 0.8	4.8 ± 0.3	4.1 ± 0.5	3.8 ± 0.8	4.1 ± 0.9
Isoleucine	$8.3^{d} \pm 0.5$	$7.9^{cd} \pm 0.2$	$7.1^{c} \pm 0.1$	$5.7^{b} \pm 0.1$	$3.3^{a} \pm 0.2$
Leucine	$19.0^{c} \pm 0.3$	$21.2^{d} \pm 0.3$	$19.1^{c} \pm 0.3$	$15.7^{b} \pm 0.1$	$13.2^{a} \pm 0.5$
Lysine	$14.7^{ab} \pm 1.3$	$13.9^{ab} \pm 0.5$	$18.8^{c} \pm 1.6$	$11.6^{a} \pm 0.5$	$17.5^{bc} \pm 1.5$
Methionine	$3.8^{ab} \pm 1.1$	$4.4^{ab} \pm 0.3$	$5.1^{b} \pm 0.3$	$4.2^{ab} \pm 0.3$	$2.5^{a} \pm 0.1$
Phenylalanine	$6.9^{c} \pm 0.2$	$7.5^{c} \pm 0.1$	$7.4^{c} \pm 0.0$	$5.9^{b} \pm 0.0$	$5.3^{a} \pm 0.2$
Threonine	$11.2^{b} \pm 0.0$	$11.8^{c} \pm 0.1$	$12.0^{c} \pm 0.1$	$9.7^{a} \pm 0.2$	$9.3^{a} \pm 0.2$
Valine	$10.2^{d} \pm 0.3$	$9.5^{c} \pm 0.2$	$9.2^{c} \pm 0.1$	$7.6^{b} \pm 0.2$	$4.8^{a} \pm 0.1$
Σ TEAA	$85.8^{b} \pm 2.7$	$89.5^{b} \pm 1.5$	$91.2^{b} \pm 1.7$	$71.0^{a} \pm 1.5$	$67.6^{a} \pm 1.6$
Alanine	$23.1^{b} \pm 2.7$	$24.1^{b} \pm 1.7$	$24.5^{b} \pm 1.6$	$21.3^{b} \pm 2.5$	$10.7^{a} \pm 0.2$
Aspartic acid	12.9 ± 1.2	14.1 ± 0.4	13.8 ± 0.4	11.4 ± 0.9	12.0 ± 1.1
Cysteine	$8.6^{d} \pm 0.2$	$7.8^{c} \pm 0.3$	$7.1^{c} \pm 0.3$	$6.3^{b} \pm 0.2$	$4.1^{a} \pm 0.1$
Glutamic a.	$20.7^{b} \pm 0.9$	$22.3^{b} \pm 0.1$	$21.2^{b} \pm 0.6$	$16.5^{a} \pm 0.6$	$18.1^{a} \pm 0.8$
Glycine	$11.5^{ab} \pm 0.6$	$11.4^{ab} \pm 0.1$	$12.5^{bc} \pm 0.1$	$10.9^{a} \pm 0.1$	$13.2^{c} \pm 0.1$
Proline (+ taurine)	$18.2^{b} \pm 1.5$	$19.7^{b} \pm 1.6$	$18.3^{b} \pm 1.6$	$9.3^{a} \pm 0.3$	$12.5^{a} \pm 1.4$
Serine	$19.2^{b} \pm 0.9$	$19.7^{b} \pm 0.1$	$19.3^{b} \pm 0.4$	$13.6^{a} \pm 0.4$	$12.0^{a} \pm 0.7$
Tyrosine	$6.5^{b} \pm 0.4$	$7.7^{c} \pm 0.1$	$7.7^{c} \pm 0.1$	$5.2^{a} \pm 0.2$	$4.6^{a} \pm 0.1$
Σ TNEAA	$121.5^{b} \pm 44$	$127.1^{b} \pm 12$	$124.6^{b} \pm 2.5$	$94.6^{a} \pm 3.9$	$87.3^{a} \pm 2.2$
Σ TAA	$207.4^{c} \pm 2.2$	$216.6^{c} \pm 2.5$	$215.9^{c} \pm 2.1$	$165.7^{b} \pm 4.9$	$154.9^{a} \pm 2.2$

* At each developmental stage the analysis was based on four samples, two from each female. Each sample was analysed in triplicate.

histidine, lysine, methionine and phenylalanine of the TEAA, and alanine of the TNEAA, did not decrease as development progressed from fertilised egg to newly hatched larva.

The TAA decreased significantly with development at the four salinities studied (*tables I–IV*). At 1 h post-fertilisation the EAA in the TAA ranged from approximately 41 to 46.6 %. However, as development

Table III. Total (protein bound + free) amino acid content (± SE) in nmol·egg^{-1} or larva^{-1} of estuary perch at different stages of development at a salinity of 25. Values with the same superscript in each row are not significantly different ($P > 0.05$)*.

	Eggs			Larvae	
	1 h	15 h	40 h	Newly hatched (55 h)	Yolk-resorbed (66 h)
Arginine	$7.9^{b} \pm 0.1$	$10.0^{c} \pm 0.1$	$8.3^{b} \pm 0.1$	$6.6^{a} \pm 0.1$	$6.1^{a} \pm 0.3$
Histidine	3.3 ± 1.1	4.6 ± 0.4	3.7 ± 0.5	4.4 ± 0.3	3.0 ± 1.3
Isoleucine	$9.0^{d} \pm 0.9$	$9.3^{d} \pm 0.1$	$6.7^{c} \pm 0.1$	$5.3^{b} \pm 0.0$	$3.2^{a} \pm 0.0$
Leucine	$18.9^{c} \pm 0.6$	$24.6^{d} \pm 0.5$	$19.3^{c} \pm 0.2$	$14.8^{b} \pm 0.3$	$11.3^{a} \pm 0.4$
Lysine	$16.2^{b} \pm 1.5$	$16.8^{b} \pm 0.7$	$14.9^{b} \pm 1.5$	$13.9^{b} \pm 2.2$	$8.7^{a} \pm 0.4$
Methionine	$3.8^{b} \pm 1.0$	$6.8^{c} \pm 0.3$	$5.8^{c} \pm 0.9$	$3.4^{ab} \pm 0.4$	$1.9^{a} \pm 0.1$
Phenylalanine	$6.7^{c} \pm 0.2$	$8.6^{e} \pm 0.1$	$7.4^{d} \pm 0.1$	$5.7^{b} \pm 0.1$	$4.5^{a} \pm 0.1$
Threonine	$11.2^{c} \pm 0.1$	$13.9^{d} \pm 0.2$	$11.9^{c} \pm 0.2$	$9.4^{b} \pm 0.2$	$7.7^{a} \pm 0.1$
Valine	$10.2^{d} \pm 0.3$	$11.9^{d} \pm 0.3$	$9.5^{c} \pm 0.3$	$7.0^{b} \pm 0.1$	$3.8^{a} \pm 0.1$
Σ TEAA	$87.5^{c} \pm 2.1$	$106.8^{d} \pm 0.7$	$87.8^{c} \pm 2.1$	$70.7^{b} \pm 2.8$	$50.6^{a} \pm 2.6$
Alanine	$25.3^{bc} \pm 0.3$	$30.0^{c} \pm 1.6$	$22.4^{b} \pm 0.9$	$20.9^{b} \pm 2.0$	$7.9^{a} \pm 0.1$
Aspartic acid	$14.3^{c} \pm 1.0$	$17.0^{d} \pm 0.6$	$13.4^{c} \pm 0.3$	$11.3^{b} \pm 0.6$	$9.1^{a} \pm 0.9$
Cysteine	$8.4^{c} \pm 0.6$	$8.1^{c} \pm 0.1$	$7.6^{bc} \pm 0.2$	$6.6^{b} \pm 0.3$	$3.9^{b} \pm 0.2$
Glutamic acid	$22.2^{c} \pm 0.7$	$26.6^{d} \pm 0.8$	$21.5^{c} \pm 0.6$	$16.3^{b} \pm 0.2$	$13.8^{a} \pm 0.8$
Glycine	$11.8^{b} \pm 0.6$	$13.9^{c} \pm 0.1$	$12.6^{b} \pm 0.1$	$10.7^{a} \pm 0.2$	$10.8^{a} \pm 0.3$
Proline (+ taurine)	$17.8^{b} \pm 1.8$	$22.3^{b} \pm 1.8$	$21.2^{b} \pm 1.0$	$10.1^{a} \pm 1.0$	$11.8^{a} \pm 0.1$
Serine	$18.5^{c} \pm 0.9$	$23.1^{d} \pm 0.5$	$18.7^{c} \pm 0.4$	$12.7^{b} \pm 0.5$	$10.4^{a} \pm 0.8$
Tyrosine	$6.7^{c} \pm 0.4$	$9.3^{d} \pm 0.1$	$7.1^{c} \pm 0.1$	$5.3^{b} \pm 0.1$	$3.9^{a} \pm 0.1$
Σ TNEAA	$125.2^{c} \pm 4.9$	$150.5^{d} \pm 2.7$	$124.6^{c} \pm 1.8$	$93.9^{b} \pm 2.6$	$71.9^{a} \pm 2.4$
Σ TAA	$212.7^{e} \pm 1.5$	$257.3^{d} \pm 2.7$	$212.5^{c} \pm 2.8$	$164.6^{b} \pm 4.6$	$122.5^{a} \pm 2.9$

* At each developmental stage the analysis was based on four samples, two from each female. Each sample was analysed in triplicate.

Table IV. Total (protein bound + free) amino acid content (± SE) in nmol·egg^{-1} or larva^{-1} of estuary perch at different stages of development at a salinity of 30. Values with the same superscript in each row are not significantly different ($P > 0.05$)*.

	Eggs			Larvae	
	1 h	15 h	40 h	Newly hatched (55 h)	Yolk-resorbed (66 h)
Arginine	$8.3^{b} \pm 0.2$	$8.3^{b} \pm 0.1$	$8.5^{b} \pm 0.1$	$6.5^{a} \pm 0.3$	$6.1^{a} \pm 0.4$
Histidine	3.8 ± 0.2	2.4 ± 0.2	4.5 ± 0.7	4.7 ± 1.2	4.4 ± 1.1
Isoleucine	$7.9^{d} \pm 0.3$	$8.7^{d} \pm 0.6$	$6.6^{c} \pm 0.1$	$4.9^{b} \pm 0.1$	$3.0^{a} \pm 0.1$
Leucine	$20.8^{c} \pm 0.5$	$19.9^{c} \pm 0.9$	$20.2^{c} \pm 0.8$	$14.6^{b} \pm 0.6$	$11.0^{a} \pm 0.5$
Lysine	$28.9^{b} \pm 2.3$	$14.3^{a} \pm 0.8$	$11.6^{a} \pm 0.6$	$10.4^{a} \pm 1.3$	$9.6^{a} \pm 0.8$
Methionine	$6.4^{b} \pm 0.5$	$4.4^{ab} \pm 1.0$	$4.1^{ab} \pm 0.5$	$3.5^{a} \pm 0.2$	$2.0^{a} \pm 0.3$
Phenylalanine	$7.5^{c} \pm 0.5$	$6.9^{e} \pm 0.3$	$7.5^{c} \pm 0.1$	$5.6^{b} \pm 0.2$	$4.3^{a} \pm 0.1$
Threonine	$11.5^{e} \pm 0.1$	$11.6^{e} \pm 0.3$	$12.2^{c} \pm 0.1$	$9.5^{b} \pm 0.2$	$7.7^{a} \pm 0.2$
Valine	$10.1^{c} \pm 0.5$	$9.9^{c} \pm 0.2$	$9.3^{c} \pm 0.2$	$6.6^{b} \pm 0.1$	$3.4^{a} \pm 0.1$
Σ TEAA	$105.6^{d} \pm 1.5$	$86.8^{c} \pm 2.4$	$84.8^{c} \pm 1.3$	$66.6^{b} \pm 3.0$	$51.7^{a} \pm 2.5$
Alanine	$22.6^{b} \pm 1.8$	$23.1^{b} \pm 2.2$	$21.9^{b} \pm 0.6$	$16.9^{b} \pm 0.7$	$8.2^{a} \pm 0.4$
Aspartic acid	$13.5^{b} \pm 1.4$	$12.7^{b} \pm 0.9$	$14.3^{b} \pm 0.5$	$10.2^{a} \pm 0.3$	$9.2^{a} \pm 0.5$
Cysteine	$8.5^{c} \pm 0.5$	$6.4^{abc} \pm 0.6$	$7.1^{bc} \pm 0.5$	$5.3^{ab} \pm 0.6$	$4.6^{a} \pm 0.2$
Glutamic acid	$21.9^{b} \pm 0.2$	$20.7^{b} \pm 0.8$	$22.4^{b} \pm 0.2$	$15.6^{a} \pm 0.6$	$14.2^{a} \pm 0.6$
Glycine	$11.0^{a} \pm 0.2$	$12.0^{ab} \pm 0.3$	$13.1^{b} \pm 0.5$	$10.7^{a} \pm 0.2$	$10.7^{a} \pm 0.3$
Proline (+ taurine)	$20.5^{b} \pm 1.3$	$19.7^{b} \pm 1.8$	$20.0^{b} \pm 1.6$	$12.0^{a} \pm 2.8$	$10.3^{a} \pm 1.5$
Serine	$18.4^{b} \pm 0.5$	$19.4^{b} \pm 0.9$	$19.1^{b} \pm 0.7$	$12.0^{a} \pm 0.9$	$10.6^{a} \pm 0.8$
Tyrosine	$7.5^{c} \pm 0.3$	$7.4^{c} \pm 0.1$	$7.1^{c} \pm 0.2$	$4.8^{b} \pm 0.1$	$3.9^{a} \pm 0.1$
Σ TNEAA	$121.1^{c} \pm 4.3$	$121.7^{c} \pm 2.9$	$125.1^{c} \pm 1.4$	$87.6^{b} \pm 2.6$	$72.0^{a} \pm 1.6$
Σ TAA	$226.7^{d} \pm 0.1$	$208.5^{c} \pm 3.7$	$209.9^{c} \pm 1.5$	$154.3^{b} \pm 2.9$	$123.7^{a} \pm 4.0$

* At each developmental stage the analysis was based on four samples, two from each female. Each sample was analysed in triplicate.

progressed the proportion of EAA in the TAA did not change significantly, except at 30 when the EAA in TAA decreased from 46.6 to 41.8 %.

3.2. Free amino acids

The FAA in developing eggs and larvae of the estuary perch accounted for 15.7–27.9 % of the TAA, in 1-h post-fertilised eggs at salinities of 30 and 20, respectively, The FAA content in fertilised eggs in the different salinities ranged from 35.5 to 58.1 nmol·egg^{-1}, and that of newly hatched and yolk-sac resorbed larvae from 15.9 to 20.8 and 7.8 to 10.2 nmol·larva^{-1}, respectively. The changes in the percent FAA in TAA with development at the four experimental salinities are shown in *figure 1*. It is evident from the figure that in all instances there was a gradual decline in the amount of FAA in the TAA, and in newly hatched larvae it was in the range 9.7–10.4 %, and no clear trend in relation to differences in the rearing salinities was evident.

The free essential amino acids (FEAA) in developing eggs of estuary perch were dominated by lysine, leucine, valine and isoleucine. These four amino acids accounted for 66.3–69.0 % and 58.5–61.2 % of the FEAA in 1-h post-fertilised eggs, and newly hatched larvae, at the four salinities investigated. However, in yolk-sac resorbed larvae these amino acids accounted for only 40.7, 48.5, 53.1 and 50.0 % of the FEAA at salinities of 15, 20, 25 and 30, respectively. All nine FEAA identified in this study decreased significantly ($P < 0.05$) with development, from fertilised egg to yolk-sac resorbed larva, at all four experimental salinities (*tables V–VIII*), except lysine. The amount of lysine in eggs reared at a salinity of 30 decreased significantly between 1 and 15 h post-fertilisation, but not necessarily so as development progressed further. It is also important to note that at salinities of 25 and 30 there was a tendency to conserve lysine to a much greater extent than at 15 and 20 (*tables V–VIII*),

Eight free non-essential amino acids (FNEAA) were identified during development in estuary perch. The FNEAA were dominated by proline + taurine and serine, which collectively accounted for approximately 51.7–53.0 %, 44.7–50.6 % and 49.0–53.0 % of the FNEAA, in eggs 1 h p-f, newly hatched larvae and yolk-sac resorbed larvae, respectively, at the four salinities investigated. Cysteine was not present in newly fertilised eggs, but at all four salinities, the amount of free cysteine increased significantly ($P < 0.05$) as development progressed, where as all the FNEAA decreased significantly.

Overall, there was a significant decrease in FEAA in the FAA, when the percentage of the former decreased from approximately 55 % to nearly 35 % (*figure 2*). At all salinities the trend in this decline was similar and at each stage of development investigated there was no significant ($P > 0.05$) difference in the proportion of EFAA in the FAA pool.

4. DISCUSSION

The present study is the first conducted on the changes in the amino acid profiles in relation to development in a truly estuarine fish. Generally, there

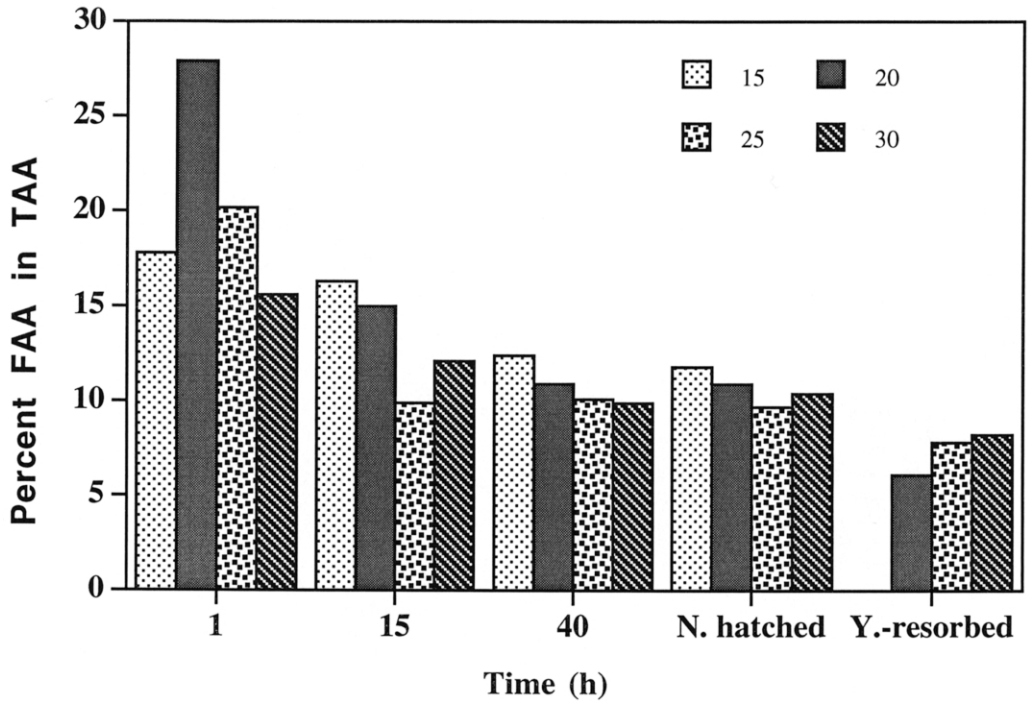

Figure 1. The changes in percentage free amino acids (FAA) in total amino acid (TAA) in relation to development at the four experimental salinities of 15, 20, 25 and 30. Please note that at a salinity of 15 the data were not available as the TAA could not be estimated.

is a paucity of studies on the amino acid profiles of estuarine fish. Thorsen et al. (1996) compared the FAA profiles of brackish water and marine eggs of the brackish water cod, and found that the FAA content of

Table V. Free amino acid content (± SE) in nmol·egg^{-1} or larva^{-1} of estuary perch at different stages of development at a salinity of 15. Values with the same superscript in each row are not significantly different ($P > 0.05$)*.

Amino acid	Fertilised eggs			Larvae	
	1 h	15 h	40 h	Newly hatched (57 h)	Yolk-resorbed (78 h)
Arginine	$1.6^{e} \pm 0.01$	$1.3^{d} \pm 0.01$	$1.1^{c} \pm 0.01$	$0.8^{b} \pm 0.00$	$0.2^{a} \pm 0.00$
Histidine	$1.0^{d} \pm 0.01$	$0.8^{c} \pm 0.01$	$0.7^{c} \pm 0.01$	$0.5^{b} \pm 0.01$	$0.2^{a} \pm 0.01$
Isoleucine	$2.6^{e} \pm 0.01$	$2.0^{d} \pm 0.02$	$1.6^{c} \pm 0.01$	$1.1^{b} \pm 0.01$	$0.1^{a} \pm 0.00$
Leucine	$4.5^{e} \pm 0.001$	$3.6^{d} \pm 0.01$	$2.5^{c} \pm 0.01$	$1.4^{b} \pm 0.01$	$0.2^{a} \pm 0.01$
Lysine	$4.8^{c} \pm 0.40$	$4.6^{c} \pm 0.12$	$2.1^{b} \pm 0.15$	$1.5^{b} \pm 0.01$	$0.6^{a} \pm 0.01$
Methionine	$1.6^{d} \pm 0.05$	$1.3^{c} \pm 0.05$	$1.2^{c} \pm 0.06$	$0.9^{b} \pm 0.06$	$0.2^{a} \pm 0.00$
Phenylalanine	$1.0^{d} \pm 0.02$	$0.8^{c} \pm 0.01$	$0.8^{c} \pm 0.02$	$0.6^{b} \pm 0.01$	$0.4^{a} \pm 0.01$
Threonine	$2.2^{e} \pm 0.05$	$1.8^{d} \pm 0.04$	$1.5^{c} \pm 0.08$	$1.1^{b} \pm 0.03$	$0.5^{a} \pm 0.00$
Valine	$3.3^{e} \pm 0.07$	$2.4^{d} \pm 0.03$	$1.9^{c} \pm 0.13$	$1.5^{b} \pm 0.07$	$0.2^{a} \pm 0.02$
Σ FEAA	$22.5^{e} \pm 0.54$	$17.8^{d} \pm 0.86$	$13.4^{c} \pm 0.43$	$9.4^{b} \pm 0.05$	$2.7^{a} \pm 0,08$
Alanine	$3.3^{c} \pm 0.24$	$2.3^{b} \pm 0.10$	$1.8^{b} \pm 0.13$	$1.8^{b} \pm 0.09$	$0.3^{a} \pm 0.00$
Aspartic acid	$0.7^{c} \pm 0.02$	$0.6^{c} \pm 0.04$	$0.3^{b} \pm 0.02$	$0.2^{a} \pm 0.01$	$0.1^{a} \pm 0.00$
Cysteine	nd	$0.3^{a} \pm 0.01$	$0.7^{b} \pm 0.07$	1.0 ± 0.20	1.0 ± 0.03
Glutamic acid	$1.7^{d} \pm 0.09$	$1.1^{c} \pm 0.06$	$0.6^{b} \pm 0.04$	$0.5^{ab} \pm 0.02$	$0.3^{a} \pm 0.00$
Glycine	$1.3^{d} \pm 0.09$	$1.0^{b} \pm 0.01$	$1.1^{c} \pm 0.03$	$1.1^{c} \pm 0.02$	$0.3^{a} \pm 0.02$
Proline + taurine	$4.8^{d} \pm 0.01$	$4.1^{c} \pm 0.07$	$3.9^{c} \pm 0.05$	$3.2^{b} \pm 0.02$	$2.2^{a} \pm 0.04$
Serine	$5.0^{e} \pm 0.10$	$3.5^{d} \pm 0.08$	$2.7^{c} \pm 0.10$	$1.9^{b} \pm 0.05$	$0.3^{a} \pm 0.01$
Tyrosine	$1.8^{c} \pm 0.02$	$1.5^{bc} \pm 0.06$	$1.5^{bc} \pm 0.16$	$1.2^{b} \pm 0.03$	$0.6^{a} \pm 0.01$
Σ FNEAA	$18.5^{d} \pm 0.46$	14.4 ± 0.30	$13.0^{bc} \pm 011$	$11.4^{b} \pm 0.24$	$5.1^{a} \pm 0.05$
Σ FAA	$41.0^{e} \pm 0.93$	$33.0^{d} \pm 0.30$	$26.1^{c} \pm 1.10$	$20.8^{b} \pm 0.20$	$7.8^{a} \pm 0.12$

* At each developmental stage the analysis was based on four samples, two from each female. Each sample was analysed in triplicate.

Table VI. Free amino acid content (± SE) in nmol·egg^{-1} or larva^{-1} of estuary perch at different stages of development at a salinity of 20. Values with the same superscript in each row are not significantly different ($P > 0.05$)*.

	Eggs			Larvae	
	1 h	15 h	40 h	Newly hatched (56 h)	Yolk-resorbed (72 h)
Arginine	$2.2^{e} \pm 0.02$	$1.2^{d} \pm 0.05$	$1.1^{c} \pm 0.02$	$0.6^{b} \pm 0.01$	$0.3^{a} \pm 0.00$
Histidine	$1.4^{d} \pm 0.01$	$0.8^{c} \pm 0.13$	$0.7^{c} \pm 0.04$	$0.4^{b} \pm 0.03$	$0.1^{a} \pm 0.00$
Isoleucine	$3.6^{e} \pm 0.04$	$2.0^{d} \pm 0.04$	$1.5^{c} \pm 0.06$	$0.9^{b} \pm 0.07$	$0.7^{a} \pm 0.00$
Leucine	$6.5^{e} \pm 0.13$	$3.8^{d} \pm 0.04$	$2.1^{c} \pm 0.10$	$1.1^{b} \pm 0.02$	$0.3^{a} \pm 0.00$
Lysine	$7.1^{d} \pm 0.20$	$3.8^{c} \pm 0.83$	$1.4^{ab} \pm 0.07$	$1.9^{b} \pm 0.12$	$0.4^{a} \pm 0.03$
Methionine	$2.2^{e} \pm 0.01$	$1.3^{d} \pm 0.1$	$1.1^{c} \pm 0.00$	$0.7^{b} \pm 0.00$	$0.3^{a} \pm 0.00$
Phenylalanine	$1.5^{c} \pm 0.14$	$0.8^{b} \pm 0.01$	$0.8^{b} \pm 0.00$	$0.6^{a} \pm 0.00$	$0.6^{ab} \pm 0.01$
Threonine	$3.0^{e} \pm 0.05$	$1.8^{d} \pm 0.02$	$1.3^{c} \pm 0.00$	$1.0^{b} \pm 0.02$	$0.7^{a} \pm 0.02$
Valine	$4.6^{e} \pm 0.03$	$2.4^{d} \pm 0.04$	$1.7^{c} \pm 0.03$	$1.3^{b} \pm 0.06$	$0.2^{a} \pm 0.00$
Σ FEAA	$32.2^{e} \pm 0.53$	$18.1^{d} \pm 0.64$	$11.8^{c} \pm 0.05$	$8.5^{b} \pm 0.03$	$3.3^{a} \pm 0.09$
Alanine	$4.8^{d} \pm 0.19$	$2.4^{c} \pm 0.07$	$1.4^{b} \pm 0.00$	$1.4^{b} \pm 0.00$	$0.4^{a} \pm 0.00$
Aspartic acid	$1.0^{d} \pm 0.04$	$0.6^{c} \pm 0.04$	$0.2^{b} \pm 0.00$	$0.1^{a} \pm 0.00$	$0.1^{a} \pm 0.00$
Cysteine	nd	$0.4^{a} \pm 0.02$	$0.8^{b} \pm 0.02$	$0.7^{b} \pm 0.04$	$1.0^{c} \pm 0.04$
Glutamic acid	$2.2^{c} \pm 0.10$	$1.1^{b} \pm 0.06$	$0.4^{a} \pm 0.00$	$0.3^{a} \pm 0.00$	$0.2^{a} \pm 0.01$
Glycine	$1.7^{c} \pm 0.01$	$1.0^{b} \pm 0.01$	$1.1^{b} \pm 0.00$	$1.0^{b} \pm 0.02$	$0.5^{a} \pm 0.05$
Proline + taurine	$6.7^{e} \pm 0.05$	$4.3^{d} \pm 0.05$	$3.6^{c} \pm 0.03$	$3.0^{b} \pm 0.14$	$2.8^{a} \pm 0.03$
Serine	$6.7^{e} \pm 0.07$	$3.4^{d} \pm 0.05$	$2.4^{c} \pm 0.00$	$1.8^{b} \pm 0.02$	$0.2^{a} \pm 0.00$
Tyrosine	$2.4^{d} \pm 0.07$	$1.4^{b} \pm 0.22$	$1.8^{c} \pm 0.02$	$1.2^{b} \pm 0.04$	$0.8^{a} \pm 0.02$
Σ FNEAA	$25.9^{e} \pm 0.38$	$14.5^{d} \pm 0.37$	$11.7^{c} \pm 0.05$	$9.6^{b} \pm 0.02$	$6.1^{a} \pm 0.15$
Σ FAA	$58.1^{e} \pm 0.76$	$32.5^{d} \pm 0.43$	$23.4^{c} \pm 0.06$	$18.1^{b} \pm 0.32$	$9.4^{a} \pm 0.24$

* At each developmental stage the analysis was based on four samples, two from each female. Each sample was analysed in triplicate.

eggs in marine eggs was higher. The present study complements those of our own on fresh water Percichthyid fishes (Gunasekera et al., 1998, 1999) and those on marine species, which were recently reviewed (Rønnestad et al., 1999).

The survival of the embryo prior to hatching in euryhaline species is affected by the response of the eggs to the salinity of the external medium. The estuarine perch is known to spawn in the Hopkins estuary (38°23'S 142°31'E), a salt-wedge-type estuary

Table VII. Free amino acid content (± SE) in nmol·egg^{-1} or larva^{-1} of estuary perch at different stages of development at a salinity of 25. Values with the same superscript in each row are not significantly different ($P > 0.05$)*.

	Eggs			Larvae	
	1 h	15 h	40 h	Newly hatched (55 h)	Yolk-resorbed (66 h)
Arginine	$1.6^{e} \pm 0.01$	$1.1^{d} \pm 0.03$	$1.0^{c} \pm 0.03$	$0.4^{b} \pm 0.06$	$0.1^{a} \pm 0.00$
Histidine	$1.0^{c} \pm 0.06$	$0.7^{b} \pm 0.02$	$0.6^{b} \pm 0.03$	$0.4^{a} \pm 0.03$	$0.4^{a} \pm 0.01$
Isoleucine	$2.7^{e} \pm 0.07$	$1.7^{d} \pm 0.09$	$1.3^{c} \pm 0.08$	$0.7^{b} \pm 0.03$	$0.1^{a} \pm 0.00$
Leucine	$4.7^{e} \pm 0.10$	$2.9^{d} \pm 0.17$	$1.8^{c} \pm 0.06$	$0.8^{b} \pm 0.04$	$0.2^{a} \pm 0.00$
Lysine	$5.9^{b} \pm 0.10$	$1.7^{a} \pm 0.30$	$1.2^{a} \pm 0.07$	$1.4^{a} \pm 0.11$	$1.2^{a} \pm 0.11$
Methionine	$1.6^{e} \pm 0.04$	$1.2^{d} \pm 0.05$	$1.0^{c} \pm 0.03$	$0.7^{b} \pm 0.05$	$0.2^{a} \pm 0.02$
Phenylalanine	$1.0^{e} \pm 0.00$	$0.8^{d} \pm 0.00$	$0.8^{c} \pm 0.00$	$0.5^{b} \pm 0.00$	$0.4^{a} \pm 0.00$
Threonine	$2.2^{e} \pm 0.05$	$1.5^{d} \pm 0.06$	$1.2^{c} \pm 0.03$	$0.8^{b} \pm 0.02$	$0.3^{a} \pm 0.00$
Valine	$3.4^{e} \pm 0.05$	$2.0^{d} \pm 0.09$	$1.5^{c} \pm 0.07$	$1.3^{b} \pm 0.04$	$0.2^{a} \pm 0.00$
Σ FEAA	$24.2^{e} \pm 0.43$	$13.7^{d} \pm 0.64$	$10.5^{c} \pm 0.25$	$7.0^{b} \pm 0.11$	$3.2^{a} \pm 0.09$
Alanine	$3.4^{d} \pm 0.14$	$1.4^{bc} \pm 0.09$	$1.2^{b} \pm 0.06$	$1.6^{c} \pm 0.06$	$0.4^{a} \pm 0.03$
Aspartic acid	$0.6^{c} \pm 0.05$	$0.3^{b} \pm 0.02$	$0.2^{a} \pm 0.00$	$0.1^{a} \pm 0.00$	$0.2^{a} \pm 0.01$
Cysteine	nd	$0.4^{a} \pm 0.04$	$0.7^{c} \pm 0.02$	$0.8^{c} \pm 0.02$	$0.5^{b} \pm 0.00$
Glutamic acid	$1.6^{c} \pm 0.09$	$0.6^{a} \pm 0.04$	$0.4^{a} \pm 0.02$	$0.4^{a} \pm 0.00$	$0.7^{b} \pm 0.00$
Glycine	$1.2^{d} \pm 0.01$	$0.9^{b} \pm 0.02$	$1.1^{c} \pm 0.03$	$0.9^{b} \pm 0.00$	$0.5^{a} \pm 0.00$
Proline + taurine	$5.0^{d} \pm 0.07$	$3.9^{c} \pm 0.09$	$3.5^{b} \pm 0.02$	$2.9^{a} \pm 0.16$	$2.8^{a} \pm 0.06$
Serine	$4.9^{e} \pm 0.13$	$2.6^{d} \pm 0.13$	$2.2^{c} \pm 0.07$	$1.5^{b} \pm 0.01$	$0.5^{a} \pm 0.02$
Tyrosine	$1.8^{b} \pm 0.02$	$1.7^{b} \pm 0.05$	$1.7^{b} \pm 0.02$	$0.6^{a} \pm 0.02$	$0.6^{a} \pm 0.01$
Σ FNEAA	$18.7^{e} \pm 0.48$	$11.9^{d} \pm 0.21$	$11.0^{c} \pm 0.22$	$8.9^{b} \pm 0.06$	$6.3^{a} \pm 0.02$
Σ FAA	$42.9^{e} \pm 0.65$	$25.6^{d} \pm 0.85$	$21.4^{c} \pm 0.46$	$15.9^{b} \pm 0.13$	$9.5^{a} \pm 0.08$

* At each developmental stage the analysis was based on four samples, two from each female. Each sample was analysed in triplicate.

Table VIII. Free amino acid content (± SE) in nmol·egg^{-1} or larva^{-1} of estuary perch at different stages of development at a salinity of 30. Values with the same superscript in each row are not significantly different ($P > 0.05$)*.

	Eggs			Larvae	
	1 h	15 h	40 h	Newly hatched (55 h)	Yolk-resorbed (66 h)
Arginine	$1.4^{e} \pm 0.02$	$1.1^{d} \pm 0.07$	$0.7^{c} \pm 0.01$	$0.5^{b} \pm 0.06$	$0.2^{a} \pm 0.00$
Histidine	$0.9^{d} \pm 0.03$	$0.7^{c} \pm 0.05$	$0.4^{a} \pm 0.03$	$0.4^{a} \pm 0.02$	$0.6^{b} \pm 0.01$
Isoleucine	$2.3^{e} \pm 0.04$	$1.7^{d} \pm 0.09$	$1.1^{c} \pm 0.07$	$0.6^{b} \pm 0.04$	$0.1^{a} \pm 0.00$
Leucine	$4.1^{e} \pm 0.10$	$2.8^{d} \pm 0.17$	$1.7^{c} \pm 0.05$	$0.8^{b} \pm 0.02$	$0.3^{a} \pm 0.05$
Lysine	$3.4^{b} \pm 0.55$	$2.0^{a} \pm 0.16$	$2.0^{a} \pm 0.19$	$1.6^{a} \pm 0.19$	$1.2^{a} \pm 0.08$
Methionine	$1.4^{d} \pm 0.04$	$1.2^{c} \pm 0.07$	$0.7^{b} \pm 0.06$	$0.7^{b} \pm 0.05$	$0.2^{a} \pm 0.01$
Phenylalanine	$0.9^{d} \pm 0.02$	$0.8^{c} \pm 0.03$	$0.6^{b} \pm 0.08$	$0.4^{a} \pm 0.03$	$0.4^{a} \pm 0.00$
Threonine	$1.9^{e} \pm 0.03$	$1.4^{d} \pm 0.09$	$1.0^{c} \pm 0.03$	$0.9^{b} \pm 0.02$	$0.3^{a} \pm 0.00$
Valine	$3.0^{e} \pm 0.04$	$1.9^{d} \pm 0.11$	$1.5^{c} \pm 0.06$	$1.2^{b} \pm 0.04$	$0.2^{a} \pm 0.01$
Σ FEAA	$19.3^{e} \pm 0.32$	$13.8^{d} \pm 0.59$	$9.9^{c} \pm 0.31$	$7.0^{b} \pm 0.23$	$3.6^{a} \pm 0.13$
Alanine	$2.9^{d} \pm 0.13$	$1.4^{b} \pm 0.10$	$1.8^{c} \pm 0.14$	$1.5^{bc} \pm 0.05$	$0.5^{a} \pm 0.03$
Aspartic acid	$0.5^{c} \pm 0.04$	$0.3^{b} \pm 0.03$	$0.3^{b} \pm 0.03$	$0.1^{a} \pm 0.01$	$0.2^{a} \pm 0.01$
Cysteine	nd	$0.4^{a} \pm 0.04$	$0.7^{b} \pm 0.00$	$0.8^{c} \pm 0.01$	$0.5^{a} \pm 0.05$
Glutamic acid	$1.4^{d} \pm 0.08$	$0.6^{b} \pm 0.03$	$0.7^{b} \pm 0.04$	$0.4^{a} \pm 0.03$	$0.8^{c} \pm 0.03$
Glycine	$1.1^{c} \pm 0.02$	$0.8^{b} \pm 0.05$	$0.8^{b} \pm 0.02$	$1.0^{c} \pm 0.01$	$0.5^{a} \pm 0.00$
Proline + taurine	$4.2^{c} \pm 0.07$	$3.8^{b} \pm 0.16$	$2.8^{a} \pm 0.10$	$2.9^{a} \pm 0.14$	$3.0^{a} \pm 0.09$
Serine	$4.3^{e} \pm 0.09$	$2.6^{d} \pm 0.15$	$2.1^{c} \pm 0.07$	$1.5^{b} \pm 0.02$	$0.5^{a} \pm 0.01$
Tyrosine	$1.6^{d} \pm 0.02$	$1.7^{d} \pm 0.07$	$1.1^{c} \pm 0.02$	$0.9^{b} \pm 0.14$	$0.5^{a} \pm 0.01$
Σ FNEAA	$16.2^{d} \pm 0.43$	$11.6^{c} \pm 0.56$	$10.9^{c} \pm 0.84$	$9.1^{b} \pm 0.07$	$6.6^{a} \pm 0.13$
Σ FAA	$35.5^{e} \pm 0.53$	$25.5^{d} \pm 1.18$	$20.9^{c} \pm 1.14$	$16.1^{b} \pm 0.23$	$10.2^{a} \pm 0.27$

* At each developmental stage the analysis was based on eight samples, four from each female.

which is characterised by two seasonal discharges, high and low flow and three phases of salt-wedge dynamics. The estuary displays marked seasonal variations in the position and physio-chemical properties of its salt wedge (Sherwood and Backhouse, 1982). Hatching and the subsequent development of fertilised eggs of estuary perch occurred best between salinities of 20 and 30, which corresponds to the normal salinities of the salt-wedge. These observations are similar to those reported previously for the closely related Australian bass *Macquaria novemaculeata*, another estuarine species (McCarraher and McKenzie, 1986). Estuary perch and Australian bass are known to hybridise in natural populations when their distribution overlaps (Jerry et al., 1999). However, the western most distribution of Australian bass is about 146°50'E, whereas the estuary perch in the present study was sampled from Hopkins estuary which is considered to be the western most (142°E) limit of its natural distribution (Cadwallader and Backhouse, 1983).

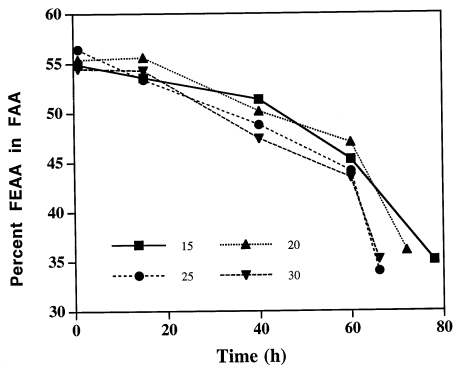

Figure 2. The percentage of free essential amino acids (FEAA) in the free amino acid (FAA) pool at each stage of development in estuary perch egg/ larvae, at salinities of 15, 20, 25 and 30.

In the estuary perch the TAA in 1-h post-fertilised eggs is considerably lower than that in eggs of freshwater percichthyids (Gunasekera et al., 1999) and other freshwater fish hitherto investigated (Zeitoun et al., 1977; Dabrowski et al., 1985; Srivastava et al., 1995; Gunasekera et al., 1999). These differences, however, can be at least partially attributed to egg size, that of estuary perch, for example, being much smaller than those of Murray cod and trout cod (Gunasekera et al., 1999). On the other hand, the FAA of eggs of estuary perch is in the range of those reported for some marine species, such as *Scophthalmus maximus*, *Gadiculus thori* and *Platichthys flesus*, all of which lay pelagic eggs (Rønnestad and Fyhn, 1993; Rønnestad et al., 1999), which are also known to have small, pelagic eggs, often with oil globules.

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 (Tyler et al., 1988; Rønnestad et al., 1999). Therefore, until first feeding developing eggs and larvae depend entirely on the nutritional material in the yolk. It is evident that in estuary perch the TAA in an egg and/or larva decreased ($P < 0.05$) as development progressed, at all four experimental salinities (*tables I–IV*), until pre-feeding larvae. How-

ever, the decreases between the intermediate stages investigated were not always significant. Although there was a trend for the TAA to decrease with development, it was interesting to note that the percentage of EAA, at each stage of development in the TAA, did not vary significantly ($P > 0.05$) from that in 1-h post-fertilised eggs.

In estuary perch the FAA content expressed as a percentage of TAA ranged between 15.7 (30) and 28.0 % (20) at the four salinities tested. The relatively higher FAA content in eggs at a salinity of 20 remains unexplainable, however. The FAA content decreased as development progressed being around 10% in yolk-resorbed larvae (*figure 1*). Further more, this trend was not salinity dependent. It has been shown that marine pelagic eggs, with one exception (Rønnestad et al., 1996), have a relatively high percentage of FAA (mean 35.7 % ± 3.5), as opposed to marine demersal eggs (2.6 % ± 0.39). It is therefore evident that the estuary perch, which lays semi-adhesive, pelagic eggs that ride the higher salinity deeper water in the salt-wedge (Newton, 1994), has a FAA level much lower than in the pelagic eggs of marine species but considerably higher than demersal eggs of marine species and/or eggs of freshwater fish (Dabrowski et al., 1985; Srivastava et al., 1995; Gunasekera et al., 1996, 1999).

In estuary perch the eggs also showed other features that may be interpreted as of intermediate character between eggs of freshwater and marine teleosts. A notable difference compared to eggs of freshwater fish was the occurrence of a peak corresponding to the physiological amino acids taurine and proline (we were unable to separate the two peaks). The taurine + proline contents combined accounted for approximately 11.5–12.0 % of FAA, a level that is equivalent to that reported for pelagic eggs of marine fish (Rønnestad et al., 1999). Proline and taurine are physiological amino acids. A number of functions have been attributed to taurine in particular (Huxtable, 1992) of which osmo-regulation is thought to be one of the most important in fish (Conçeicão et al., 1997) as well as other aquatic animals (Paynter et al., 1995). However, the absence of these amino acids in freshwater fish hitherto investigated (Zeitoun et al., 1977; Dabrowski et al., 1985, Srivastava et al., 1995; Gunasekera et al., 1996, 1998, 1999) may indicate that these amino acids do not have an osmo-regulatory role in freshwater, or at least they are not crucial functionally in early ontogeny in freshwater species.

The other feature of estuary perch eggs that is comparable to that of pelagic, marine eggs is the almost complete utilisation of the FAA with development when more than 70 % of the FAA pool is utilised to the yolk-sac resorption. In contrast, however, the TAA in eggs was reduced to only 76.1, 74.6, 57.5 and 54.6 % of that in 1-h post-fertilised eggs, at salinities of 15, 20, 25 and 30, respectively. On the other hand, there was no significant reduction in the EAA in TAA. These observations suggest that as in the case of eggs of marine fish, and unlike in the case of freshwater fish, estuary perch utilises its FAA for energy dissipation during egg and yolk-sac stages rather than for body protein synthesis. With regard to the latter the higher utilisation of TAA at the two higher salinities may be a reflection of the faster development at the higher salinities and the larger size of the larvae.

The present study also enabled a comparison amongst closely related species; viz. Murray cod and trout cod, but with different ecology and reproductive biology based on previous studies (Cadwallader and Backhouse, 1983; Llwellyn and MacDonald, 1980). Also, the eggs of the two freshwater percichthyids were considerably larger than in estuary perch, being 13.5 ± 0.9 and 16.3 ± 0.8 mg in weight and 3.52 ± 0.07 and 3.45 ± 0.07 mm in diameter in Murray cod and trout cod, respectively (Gunasekera et al., 1999). It was evident that in spite of the close phylogeny of the three species, the freshwater species differed in almost all the key traits of egg and larval amino acid profiles, except in the % FEAA in FAA. However, these were significantly lower in yolk-sac resorbed larvae of estuary perch, suggesting that in estuary perch FAA are not preferentially conserved in early ontogeny unlike in Murray cod and trout cod.

In conclusion it is evident that the eggs of estuary perch tend to conform to those features seen in pelagic, marine eggs in most respects (Rønnestad and Fyhn, 1996; Rønnestad et al., 1999). This is to be expected as the estuary perch lays its eggs in deeper high saline waters in salt-wedge estuaries. However, it is also important to note that there could be major exceptions to such generalisation, as shown for turbot, *Scophthalmus maximus* (Weltzein et al., 1999). It was also evident that the incubation salinity has minimal influence in the qualitative changes in the TAA and FAA of eggs and larvae during early ontogeny. The differences are mostly quantitative, and are related to the rate of development of eggs, which in the case of estuary perch appears to be most rapid in salinities of 25 and 30. Also, the balance of evidence suggests that the amino acid profiles of eggs and the changes associated thereof during early ontogeny until pre-feeding are not entirely phylogeny dependent but are related to reproductive biology, in particular the nature of the eggs: size, pelagic or demersal, and type of environment amongst others.

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