Fish Physiology and Biochemistry **29:** 141–147, 2003.

Nutrient and amino acid profiles of egg and larvae of Asian seabass, *Lates calcarifer* (Bloch)

J. Syama Dayal, S. Ahamad Ali, A.R. Thirunavukkarasu, M. Kailasam and R. Subburaj
Central Institute of Brackishwater Aquaculture (Indian Council of Agricultural Research) 75, Santhome High Road, Raja Annamalai Puram Chennai 600 028 India (Phone:(91 44) 2 4610565, 24618817; Fax:(91 44) 24610311; E-mail: syamdayal@rediffmail.com)

Accepted: 26 April 2004

Key words: amino acids, biochemical composition, fish egg and larvae, larval nutrition

Abstract

Fertilized eggs and developing larvae of hatchery reared Asian seabass, *Lates calcarifer* (Bloch), were analyzed to determine the changes occurring in their proximate and amino acid (AA) composition. The fertilized dry egg weighed 31 μg and contained 13.71 μg (44%) protein, 8.48 μg (27%) lipid and 0.657 J of gross energy. Dry weight decreased by 39% during hatching. The protein, lipid and carbohydrate nutrients decreased by 4.86, 4.15 and 0.09 μg, respectively from egg to 2-days post hatching (dph) larvae (pre-feeding). The protein content of the spawned eggs and larvae were hydrolysed to AA in the laboratory. The fertilized eggs had a total AA content of 42% of their dry weight. The egg contained 1.287 μg, 1.132 μg, 0.964 μg, 0.942 μg, 0.787 μg and 0.713 μg of leucine, lysine, arginine, valine, threonine and phenylalanine, respectively and these six indispensable amino acids (IAA) constituted approximately 78% of the total IAA. In the early feeding stages of *L. calcarifer* larvae, the ratio of IAA/DAA increased from 0.797 in the pre-feeding stage to 1.632 after 2 days of feeding. During larval growth of *L. calcarifer*, the percentage contribution of isoleucine and leucine to total IAA contents increased, while it decreased for lysine, phenyl alanine and arginine. *L. calcarifer* larvae were found to have proteins, which are rich in glutamic acid, leucine and lysine, and poor in threonine and histidine, suggesting high dietary leucine and lysine IAA requirement.

Introduction

The viable fry production from teleost egg depends mainly on the biochemical composition of the egg, the time to fertilization after ovulation (Kjørsvik et al. 1989; Bromage 1995) and the availability of nutritionally balanced bio/micro-diets on initial exogenous feeding (Rosenthal and Alderdice 1976; Rønnestad et al. 1999). The nutrient composition of fish eggs is species–specific and the precise sequence of consumption varies both qualitatively and quantitatively (Cetta and Capuzzo 1982; Heming and Buddington 1988; Rainuzzo 1993; Finn 1994). Fish eggs can be classified into two energetic categories with (Type II) and without (Type I) oil globule. Type I eggs support 70% of their energy dissipation via catabolism of amino acids (AA). The remaining 30% is largely derived from catabolism of phospholipids and triacylglycerols. Type II eggs derive 50% of their energy from AA and 50% from predominantly lipids such as wax esters and triacylglycerides (Finn 1994). Phosphoglycerides with their high concentration of essential (n-3) HUFAs are utilized not only for cell division, organogenesis and structural components in membrane biogenesis but also for energy generation (Rainuzzo 1993; Finn 1994; Sargent 1995).

Asian seabass, *Lates calcarifer* (Bloch) is a catadromous fish that is widely distributed within the tropical Indo-Pacific region (Greenwood 1976). It is a good candidate species for aquaculture and a production close to 20,000 tons per annum with a value of >65 million dollars (Tucker et al. 2002). The potential for *L. calcarifer* farming increased to many folds in India after successful induced breeding of this

fish at the Central Institute of Brackishwater Aquaculture, Chennai, India (Thirunavukkarasu et al. 2001). *L. calcarifer* egg belongs to the Type II category (Finn 1994). The early development of *L. calcarifer* larvae and yolk resorption based on morphometric measurements have been reported in detail by Kohno et al. (1986) and changes in proximate profiles by Southgate et al. (1994).

More recent findings, however, have shown a pool of free AA (FAA) in marine fish eggs that are available as an additional endogenous fuel in energy metabolism of marine egg and larvae (Fyhn 1989, 1990; Rønnestad and Fyhn 1993; Rønnestad et al. 1992, 1994, 1999). Sivaloganathan et al. (1998) studied the role of FAA as an energy substrate in the developing eggs and larvae of *L. calcarifer*. FAA appeared to be an important energy substrate during the embryonic stages (2 to 16 h post spawning); after hatching, the contribution of FAA to energy metabolism was less significant.

Larval rearing is usually carried out under intensive conditions and larvae are fed on cultured live food (mainly Rotifer and *Artemia*). Research is being directed to improve fry quality in *L. calcarifer* through feed supplementation and to lower production cost by using alternative live or artificial feeds (Marte 2003). The indispensable AA (IAA) profile of fish has been proposed as a good index of the IAA requirements of larval fish (Watanabe and Kiron 1994). During development of larval turbot (Conceição et al. 1997), the percentage contribution of valine, isoleucine and threonine to total IAA contents tends to increase, while it decreases for lysine, phenylalanine and arginine. Sivaloganathan et al. (1998) reported the role of FAA in early life stages of *L. calcarifer* as energy substrate upto the pre-feeding stage, whereas in the present study AA are analysed upto 21 day post hatching (dph) larvae along with protein, lipid, carbohydrate and energy flow. This data provides a broader set of information on nutritional requirements in general and IAA requirements in particular, which helps in formulating balanced micro-diets for larvae of *L. calcarifer*.

Materials and methods

Maintenance of seabass larvae

Fertilized eggs of *L. calcarifer* were collected from the fish hatchery of the Central Institute of Brackishwater Aquaculture, Chennai and transferred into an incubation tank. The eggs hatched out in 18 hours after spawning. Freshly hatched healthy larvae were then reared in 2 ton capacity fibre reinforced plastic tanks at an initial stocking density of 40 l^{-1} and was gradually reduced to 10 l^{-1} by the 15th day, as described by Tiensongrusmee et al. (1989). Health of larval samples was ascertained by microbial and parasitic investigations as a part of the routine health monitoring in the hatchery. Larvae were maintained in sand filtered sea water with a salinity of 30 ppt and a temperature ranging between 27–29 °C. Water exchange was performed 30–40% daily during rearing. Larvae were fed on rotifers, *Brachionus plicatlis* from 3-dph. The concentration of 2 to 5 rotifers ml^{-1} and up to 2000 to 6000 *Artemia* nauplii l^{-1} were maintained in the rearing medium from the 3rd day to the 21 dph.

Collection and laboratory analysis of samples

Newly fertilized eggs, unfed hatchlings and larvae at day 1 and 2 were collected on 100 -μm mesh sieves. Hatchling (newly hatched larvae) measured 1.45 mm in total length and has visible eye lens and auditory vesicle, whereas a 1-day old larvae measures 2.36 mm and has pectoral fin buds. At 2-dph larvae the mouth and anus are open and they have completely pigmented eyes. The fed larvae on days 5, 9, 17 and 21-dph were kept in 100-litre tank containing sand filtered sea water (devoid of live feed) to evacuate the digestive tract, eliminating the influence of gut contents on the composition of larvae. The samples collected from triplicate tanks were washed briefly with distilled water and freeze-dried in Lyophiliser (Virtis, USA) at −40 °C for 12 hours. The dry weight was measured using groups of 100–200 eggs and larvae, depending on the larval size in a analytical balance with 0.01 mg accuracy (Sartorius, Germany).

The total protein and carbohydrates were estimated by the spectrophotometric method using a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan). The samples were homogenized in 0.5 N NaOH and colour was developed by using the Folin Ciocalteu phenol reagent for protein determination. Crystalline bovine serum albumin was used as a standard and the absorbance was measured at 691 nm for total protein determination as described by Lowry et al. (1951). The carbohydrate content of the samples was determined by phenol-sulphuric acid reagent at 490 nm using a D-glucose standard (Dubios et al. 1956). Total lipid content was determined gravimetrically after extraction in chloroform/methanol (2:1 v/v) (Folch et al. 1957).

The gross energy values of eggs and larvae were measured by using a semi-micro oxygen bomb calorimeter (Parr Instrument Company, USA Model 1425). 10 mg sample pellet was subjected to combustion in a 22 ml stainless steal bomb filled with oxygen at 35 psi. Temperatures in the calorimeter were sensed by a thermistor and read from a microprocessor based thermometer (1672, Parr Instrument Company, USA) with an accuracy of 0.0002 °C.

The protein bound AA were separated from FAA by using 6% TCA solution (Finn et al. 1995). AA were analysed after sealed tube hydrolysis with 6N HCl for 22 h at 110 °C (Spackman et al. 1958 and Finlayson 1964). After hydrolysis, the acid was evaporated in vacuum oven and the sample was kept in a NaOH desiccator to remove traces of acid. The residue was brought into 1 ml of sample diluent (pH 2.20). AA were analysed using the Shimadzu HPLC model LC-10A (Shimadzu corp., Japan). Separation of AA was performed in a column (Shimpack ISC-07/S1504 Na) packed with a strongly acidic Na^+ type cation exchange resin (Styrene-divinyl benzene copolymer with sulfinic group) under gradient elusion. The AA were detected and quantified using a fluorescent detector (FLD-6A) after post column derivitization with O-pthaladehyde and 2-mercaptoethanol. AA standard solution (Sigma-aldrich Inc., USA) for fluorescent detection was used as external standard. For every ten-sample injections one standard run was carried out. The n mole units of AA were converted to μg units by multiplying with its molecular weight $\times$ 1000. All the analyses were carried out in triplicates.

Statistical analysis

The experiment was conducted under a completely randomized design (Gomez and Gomez 1984). The analysis of variance (ANOVA) was determined by F-tests using the SPSS statistical software package.

Results

The nutrient composition of eggs and different stages of larvae of *L. calcarifer* are presented in Table 1. The mean dry weight of eggs was 31 μg and it decreased to 19 μg in hatchlings. The statistical analysis of the data revealed that the differences between replicates collected from three tanks were non-significant for all the parameters analysed in the study.

Initially the egg contained 13.71 μg protein, whereas the newly hatched larva had a protein content of 9.48 μg. The lowest protein content was observed in two days old larvae after hatching (pre-feeding). The lipid and carbohydrate levels decreased by 48 and 43 per cent from fertilized egg (8.48 μg and 0.21 μg) to 2-dph larvae (4.33 μg and 0.12 μg), respectively. A total of 41% (0.268 J) of energy was lost from fertilized eggs (0.657 J) to 2-dph larvae (0.389 J). The AA profiles estimated in spawned eggs and larvae were the laboratory hydrolysis products of the proteins, depicted in table 2. Leucine was the predominant indispensable AA (IAA) (1.287 μg) accounting for 9.9% of the total protein in the egg. Cysteine and methionine constituted only 1.24 and 2.91%, respectively of the total protein, which were the lowest among IAA. The total IAA contributed to 57.8% of the total AA in the egg, and reduced to 44.35% on 2-dph. Leucine, alanine, serine, glutamic acid, lysine, valine and isoleucine had accounted for more than 50% of the total AA in the egg, while glutamic acid was predominant among the dispensable AA (DAA). The larvae of *L. calcarifer* had proteins rich in leucine (9.57%) and lysine (8.31%), and poor in cysteine (0.63%) and histidine (2.12%), suggesting a high leucine and lysine requirement.

The relative contents of indispensable and dispensable AA were reduced by 32.8% and 21.3% in the hatchling and by 2-dph their proportion dropped by 50% and 12.4%, respectively when compared to that of the egg (Figure 1). In *L. calcarifer* larvae, leucine, valine, isoleucine and threonine were depleted by 4.86, 4.5, 4.03 and 3.69 n moles, respectively from egg to 2 dph. However, glycine, aspartic acid, tyrosine and cysteine were depleted by 0.07, 0.53, 0.6, and 0.8 n moles, respectively. The IAA profiles increased from 44% in the pre-feeding stage to 62% in the early feeding stage, indicating the use of DAA preferentially to IAA as energy substrates during this stage. The AA profile of *L. calcarifer* larvae changed with ontogenic stage during larval growth.

Discussion

Fish eggs contain necessary nutrients to support cellular growth and homeostasis during embryonic development until the larvae start feeding (Fyhn 1989). The mean dry weight decreased from 31 μg (egg) to 19 μg (hatchling) and probably was a result of the loss of the chorion and perivitelline colloids at hatching, and of the metabolic wastes as well as the catabolism of metabolic stores in the rapidly developing embryo

Table 1. Nutrient composition and gross energy values of egg and larvae of *L. calcarifer*

Age (days)	Dry matter (μg)	Protein (μg)	Lipid (μg)	Carbohydrate (μg)	Energy (J)
Egg	31 ± 0.95	13.71 ± 0.77	8.48 ± 0.05	0.21 ± 0.03	0.657 ± 0.0304
Hatch	19 ± 1.31	9.48 ± 0.33	4.81 ± 0.21	0.18 ± 0.02	0.377 ± 0.0614
1	23 ± 0.97	10.13 ± 0.17	4.78 ± 0.06	0.15 ± 0.02	0.431 ± 0.0433
2	26 ± 0.76	8.85 ± 0.3	4.33 ± 0.08	0.12 ± 0.01	0.389 ± 0.0104
5	41 ± 1.00	19.41 ± 0.61	5.66 ± 0.15	1.05 ± 0.04	0.699 ± 0.0340
9	85 ± 0.82	48.06 ± 0.24	8.85 ± 0.07	3.34 ± 0.13	1.602 ± 0.1260
17	984 ± 2.60	530.18 ± 1.4	88.56 ± 2.58	15.94 ± 1.12	16.464 ± 1.1843
21	2749 ± 4.48	1459.68 ± 4.66	210.12 ± 2.41	29.87 ± 0.93	44.685 ± 1.7021

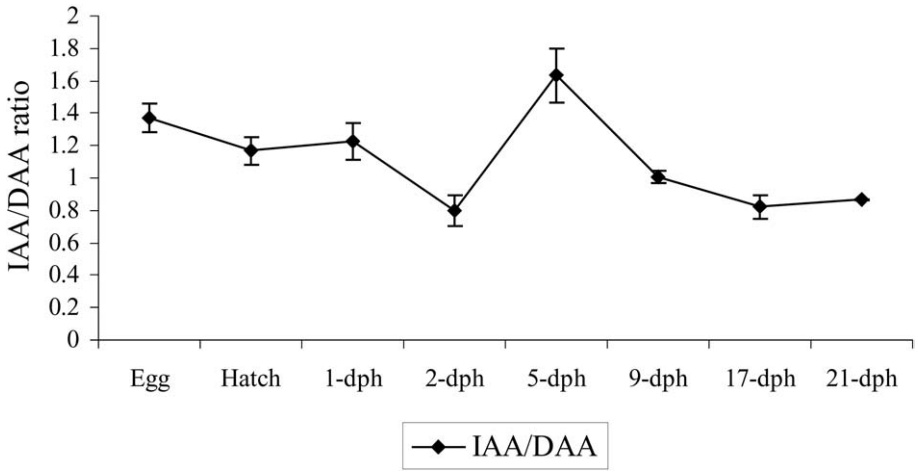

Figure 1. IAA/DAA ratio changes during larval development of *L. calcarifer*

(Rønnestad et al. 1992). The sum of protein, lipid and carbohydrate contents were 51.15% to 76.15% of dry weight in the present study; similar results of 57.07% to 76.52% were reported in *L. calcarifer* larvae (Southgate et al. 1994). Dry weight of egg and larvae includes all the organic and inorganic components. In the present study only protein, lipid and carbohydrates were measured and does not include other organic components (FAA) and inorganic components (total ash). The energy content of the fertilized *L. calcarifer* egg was 0.657 J.

In *L. calcarifer* the lipid content was 27% of the dry weight of fertilized eggs and in hatchlings it declined rapidly from 25% to 10% in 9-dph larvae (Table 1). Similar observations were made by Southgate et al. (1994) in *L. calcarifer* and by Vazquez et al. (1994) in Senegal sole, *Solea senegelensis*. The carbohydrate content decreased from 0.21 μg to 0.12 μg from fertilized egg to 2-dph *L. calcarifer* larvae. Similar reports of carbohydrate as an energy source were reported in marine fishes (Nakagawa 1970; Terner 1979; Cetta and Capuzzo 1982; Vetter et al. 1983). During the pre-feeding stage of larvae, the dry weight increased from 19 μg to 26 μg in *L. calcarifer*, which may be due to the increase of ash content as reported by Southgate et al. (1994) in *L. calcarifer* larvae. In addition to yolk, the possibility of assimilation of dissolved nutrients from water after hatch has also been suggested in the case of some fishes (Amend and Fender 1976; Lin and Arnold 1982; Wiggins et al. 1985; Peihong Zhu et al. 2003).

More recent studies, however, have shown that a pool of FAA is available as an additional endogenous fuel in marine fish egg and larvae (Fyhn 1989, 1990; Rønnestad and Fyhn 1993). The newly spawned *L. calcarifer* eggs had a total protein bound AA content of 42% of their dry mass in the present study. The composition of the AA in the eggs of *L. calcarifer*

Table 2. Amino acid profiles of the egg and larvae of *L. calcarifer*

Amino	Egg		Hatch		2-dph		5-dph		9-dph		17-dph		21-dph	
acid	μg	%	μg	%	μg	%	μg	%	μg	%	μg	%	μg	%
Indispensable amino acids (IAA)														
ARG	0.964	7.42	0.668	7.14	0.527	6.11	1.051	6.04	3.069	6.50	31.19	6.02	76.42	5.31
CYS	0.161	1.24	0.124	1.33	0.059	0.68	1.010	5.80	1.029	2.18	3.050	0.59	9.070	0.63
HIS	0.437	3.36	0.301	3.22	0.198	2.29	0.528	3.03	1.037	2.20	10.82	2.09	30.51	2.12
ILE	0.710	5.46	0.416	4.44	0.181	2.10	1.021	5.86	2.559	5.42	20.66	3.99	67.08	4.66
LEU	1.287	9.90	0.795	8.49	0.650	7.53	1.927	11.1	3.442	7.29	40.93	7.91	137.7	9.57
LYS	1.132	8.71	0.817	8.73	0.796	9.22	1.351	7.76	3.970	8.41	48.81	9.43	119.6	8.31
MET	0.378	2.91	0.251	2.68	0.196	2.27	0.981	5.64	0.748	1.59	8.750	1.69	25.02	1.74
PHE	0.713	5.49	0.628	6.71	0.453	5.25	0.812	4.66	2.882	6.11	25.68	4.96	78.35	5.45
THR	0.787	6.06	0.513	5.48	0.348	4.03	0.851	4.89	2.219	4.70	18.20	3.52	45.63	3.17
VAL	0.942	7.25	0.530	5.66	0.419	4.86	1.268	7.28	2.730	5.78	25.68	4.96	78.90	5.48
Total IAA	**7.511**	**57.8**	**5.043**	**54.0**	**3.827**	**44.3**	**10.8**	**62.0**	**23.685**	**50.2**	**233.77**	**45.2**	**668.28**	**46.4**
Dispensable amino acids (DAA)														
ALA	1.243	9.57	1.050	11.2	1.089	12.6	1.350	7.75	3.485	7.38	32.47	6.27	100.9	7.01
ASP	0.645	4.96	0.441	4.71	0.575	6.66	0.721	4.14	4.123	8.73	50.28	9.71	134.4	9.34
GLU	1.209	9.30	0.942	10.0	1.113	12.9	1.690	9.71	5.593	11.9	77.83	15.0	191.9	13.3
GLY	0.264	2.03	0.208	2.22	0.259	3.00	0.297	1.71	3.987	8.45	43.59	8.41	119.9	8.33
PRO	0.358	2.74	0.297	3.17	0.409	4.74	0.481	2.76	2.295	4.86	29.32	5.66	70.10	4.87
SER	1.212	9.33	0.985	10.5	0.913	10.6	1.420	8.16	1.734	3.67	21.25	4.10	66.80	4.64
TYR	0.555	4.27	0.394	4.21	0.446	5.17	0.658	3.78	2.304	4.88	29.22	5.64	87.14	6.06
Total DAA	**5.486**	**42.2**	**4.317**	**46.0**	**4.804**	**55.7**	**6.617**	**38.0**	**23.521**	**49.8**	**283.96**	**54.8**	**771.14**	**53.6**
TOTAL	**13.00**	**100**	**9.360**	**100**	**8.631**	**100**	**17.42**	**100**	**47.20**	**100**	**517.8**	**100**	**1439**	**100**

presented in this study is fairly consistent with those of other teleost species of both fresh and marine origins (Ketola 1982). Although tryptophan was not quantified in the present study, there were no detectable differences in the amount of the IAA and DAA when compared to other marine fish eggs (Tamaru et al. 1992). It is reported that marine fish eggs have a total of AA content in the range of 40–60% (Fyhn 1989; Rønnestad and Fyhn 1993; Thorsen et al. 1993; Finn 1994; Rønnestad et al. 1996). Sivaloganathan et al. (1998) studied the role of FAA as an energy substrate in the developing eggs and larvae of *L. calcarifer*. FAA appeared to be an important energy substrate during the embryonic stages (2 to 16 h post-spawning); after hatching, the contribution of FAA to energy metabolism becomes less significant. In the present study 4.37 μg of AA were lost during embryogenesis and early larval development of *L. calcarifer*. The dependence on AA from the yolk probably applies throughout the endogenous nutrition, until the larvae commence feeding. Glycine, aspartic acid, tyrosine and cysteine had lower depletion rates before complete yolk absorption than leucine, valine, isoleucine and threonine in *L. calcarifer* larvae, similar to that reported in *Claria gariepinus* larva by Conceição et al. (1998). The commencement of the external feeding phase seems to be the most critical in the life of fish. At this time the larvae have an active search behavior to catch and ingest prey, whilst relying on the digestive and absorptive capacity of the gut to assimilate the ingested nutrients for the purposes of growth and energy dissipation (Rønnestad et al. 1999). The implications of the absence of a stomach in early larvae was clearly demonstrated in a study on *L. calcarifer* by Walford and Lam (1993) and suggested that trypsin is a key digestive enzyme, because it activates other pancreatic proteases. Fish larvae that lack a stomach at first feeding initially absorb FAA more efficiently than AA in proteins (Rust et al. 1993). During the first weeks of exogenous feeding, the energy metabolism is highly geared toward the catabolism of AA (Rønnestad and Naas 1993).

In the early feeding stages of *L. calcarifer* larvae, the ratio of IAA/DAA increased from 0.797 in the pre-feeding stage to 1.632 (Figure 1) after two days of feeding. The variation in AA profile of *L. calcarifer* larvae during this period may be due to ontogenic changes in the functional capacity and type of essential intestinal transporters (Buddington and Diamond 1989; Buddington 1992) and changes in the levels of whole body activity of the AA catabolism enzymes (Segner and Verreth 1995). This may also be a resuet of the use of DAA preferentially to IAA as energy substrates at this stage, suggesting that the fish have the capacity to spare IAA at the expense of DAA. These results indicate that fish larvae have a better control of AA catabolism than previously assumed (Conceição et al. 1997, 1998), being comparable to that of juvenile fish (Cowey and Sargent 1979; Kim et al. 1992). A high relative bio- availability for IAA means that it was retained more efficiently than the DAA. It is important to note that relative bio-availabilities may change during development for a given species. The two IAA, arginine and lysine, were retained in larval body in greater proportion, as these were less used for oxidation relative to two DAA, alanine and glutamate, when tube-fed with AA solution containing ^{14}C-tracers in *Hippoglossus hippoglossus* larvae (Applebaum and Rønnestad 2003) and in post-larvae of *S. senegalensis* (Rønnestad et al. 2003).

A more precise estimate of an ideal dietary IAA profile requires the knowledge of the relative bioavailabilities of the individual AA and metabolic budgets (including unabsorbed AA, AA oxidation and AA retention). These can be estimated using an *in vivo* method based on controlled tube-feeding of AA mixtures containing a ^{14}C-labelled AA as reported by Conceição et al. (2003). In the absence of above systematic studies in *L.calcarifer*, the larval IAA profile is an indicator of IAA requirements. Further studies are needed for defining the ideal dietary IAA profile for *L. calcarifer* on the factors affecting the relative bio-availability of IAA, such as temperature and the molecular form(s) in which nitrogen is present in the diet. Furthermore, it is important to study the impact of eventual imbalances in dietary IAA profiles on growth and survival of fish larvae.

Acknowledgements

The authors express their sincere thanks to Dr. Mathew Abraham, Director, Central Institute of Brackishwater Aquaculture, Chennai for his encouragement in carrying out this work.

References

Amend, D.F. and Fender, D.C. 1976. Uptake of bovine serum albumin by rainbow trout from hyperosmotic solutions: A model for vaccinating fish. Science 192: 793–794.

Applebaum, S.L. and Rønnestad, I. 2003. Absorption, assimilation and catabolism of individual free amino acids by larval Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 230: 313–322.

Bromage, N.R. 1995. Broodstock management and seed quality – general considerations. *In*: Broodstock Management and Egg and Larval quality. pp. 1–24. Edited by N.R. Bromage and R.J. Roberts. Blackwell Science, London.

Buddington, R.K. 1992. Intestinal nutrient transport during ontogeny of vertebrates. Am. J. Physiol. 262: R503–R509.

Buddington, R.K. and Diamond, J.M. 1989. Ontogenic development of intestinal transports. Am. Rev. Physiol. 51: 601–619.

Cetta, C.M. and Capuzzo, J.M. 1982. Physiological and biochemical aspects of embryonic and larval development of the winter flounder, *Pseudopleuronectes americanus*. Mar. Biol. 71: 327–337.

Conceição, L.E.C., Grasdalen, H. and Rønnestad, I. 2003. Amino acid requirements of fish larvae and post larvae: new tools and recent findings. Aquaculture 227: 221–232.

Conceição, L.E.C., Ozorio, R.O.A., Suurd, E.A. and Verreth, J.A.J. 1998. Amino acid profiles and amino acid utilization in larval catfish (*Claria gariepinus*): effects of ontogeny and temperature. Fish Physiol. Biochem. 19: 43–57.

Conceição, L.E.C., Vander Meeren, T., Verreth, J.A.J., Evjen, M.S., Houlihan, D.F. and Fyhn, H.J. 1997. Amino acid metabolism and protein turn over in larval turbot (*Scophthalmus maximus*) from first feeding until metamorphosis, fed natural zooplankton or *Artemia.* Mar. Biol. 129: 255–265.

Cowey, C.B. and Sargent, J.R. 1979. Nutrition. *In*: Fish Physiology. Vol. III pp. 1–70. Edited by W. S. Hoar and D.J. Randall. Academic Press, New York.

Dubios, M., Gillies, K.A., Hamilton, J.K., Reberts, P.A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350–356.

Finlayson, A.J. 1964. Amino acid recovering in the analysis of some feed samples. Can. J. Plant Sci. 45: 184–188.

Finn, R.N. 1994. Physiological Energetics of Developing Marine Fish Embryos and Larvae. Dr. Scient. Thesis, University of Bergen, Norway.

Finn, R.N., Fyhn, H.J. and Evjen, M.S. 1995. Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen metabolism. Mar. Biol. 124: 355–369.

Folch, J., Lees, H. and Sloane-stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 726: 497–509.

Fyhn, H.J. 1989. First feeding of marine fish larvae. Are free amino acids the source of energy? Aquaculture 88: 111–120.

Fyhn, H.J. 1990. Energy production in marine fish larvae with emphasis on free amino acids as a potential fuel. In: Nutrition in wild and domestic animals Vol. 5. pp. 176–192. Edited by J. Mellinger. Karger, Basel.

Gomez, K.A. and Gomez, A.A. 1984. Statistical procedures for agricultural research 2nd Edition pp. 8–19. John Wiley and Sons, New York.

Greenwood, P.H. 1976. A review of the family centropomidae (Pisces Perciformes). Bull. Br. Mus. Nat. Hist. (Zoology). 29: 1–81.

Heming, T.A. and Buddington, R.K. 1988. Yolk absorption in embryonic and larval fishes. *In*: Fish Physiology. Vol XI. part A pp. 407–446. Edited by W.S. Hoar, D.J. Randall and J.R. Brett. Academic Press, New York.

Ketola, G.H. 1982. Amino acid nutrition of fishes: requirement and supplementation of diets. Comp. Biochem. Physiol. 73B: 17–24.

Kim, K., Grimshaw, T.W., Kayes, T.B. and Amudson, C.H. 1992. Effect of fasting or feeding diets containing different levels of protein or amino acids on the activities of the liver amino acid –degrading enzymes and amino acid oxidation in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 107: 89–105.

Kjørsvik, E., Mangor-jensen, A. and Holmefjord, I. 1989. Egg quality in marine fishes. Ny Fisk. Rapp. L. Nr. 5/89, Norway.

Kohno, H., Hara, S. and Taki, Y. 1986. Early larval development of the seabass *Lates calcarifer* with emphasis on the transition of energy sources. Bull. Jap. Soc. Sci. Fish. 52: 1719–1725.

Lin, M. and Arnold, C. 1982. Transfer of glucose from seawater to the blood of red fish. Proc. 66th Ann. Meet. Fed. Am. Soc. Exp. Biol. 41: 489.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265–275.

Marte, C.L. 2003. Larviculture of marine species in Southeast Asia: current research and industry prospects. Aquaculture 227: 293–304.

Nakagawa, H. 1970. Studies on rainbow trout egg *Salmo gairdneri irideus*. II. Carbohydrate in the egg protein. J. Fac. Fish. Anim. Husb. Hiroshima Univ. 9: 57–63.

Peihong Zhu, Christopher, C.P. and Brown, J.A. 2003. Lipid and amino acid metabolism during early development of Atlantic halibut (*Hippoglossus hippoglossus*). Aquacult. Int. 11: 43–52.

Rainuzzo, J.R. 1993. Lipid in Early stages of Marine Fish. Dr. Scient. Thesis, University of Trondheim, Norway.

Rønnestad, I. and Fyhn, H.J. 1993. Metabolic aspects of free amino acids in developing marine fish eggs and larvae. Rev. Fish. Sci. 1: 239–259.

Rønnestad, I. and Naas, K.E. 1993. Routine metabolism in Atlantic halibut at first feeding- a first step towards an energetic model. *In*: Physiology and Biochemistry of marine fish larval development. pp. 279–284. Edited by B.T. Walther and H.J. Fyhn. University of Bergen, Norway.

Rønnestad, I., Finn, R.N., Groot, E.P. and Fyhn, H.J. 1992. Utilization of free amino acids related to energy metabolism of developing eggs and larvae of lemon sole, *Microstomus kitt*, reared in the laboratory. Mar. Ecol. Prog. Ser. 88: 195–205.

Rønnestad, I., Koven, W.M., Tandler, A. and Harel, M. 1994. Energy metabolism during development of eggs and larvae of gilt head sea bream (*Sparus aurata*). Mar. Biol. 120: 187–196.

Rønnestad, I., Robertson, R.R. and Fyhn, H.J. 1996. Free amino acids and protein content in pelagic and demersal eggs of tropical marine fishes. In: The Fish Egg. pp. 81–84. Edited by MacKinlay, D.D. and Eldridge, M. American Fisheries Society, Physiology Section. Bethesda.

Rønnestad, I., Thorsen, A. and Finn, R.N. 1999. Fish larval nutrition: a review of recent advances in the roles of amino acids. Aquaculture 177: 201–216.

Rønnestad, I., Tonheim, S.K., Fyhn, H.J., Rojas-Garcia, C.R., Kamisaka, Y., Koven, W., Finn, R.N., Terjesen, B.F., Barr, Y. and Conceição, L.E.C. 2003. The supply of amino acids during early feeding stages of marine fish larvae: a review of recent findings. Aquaculture 227: 147–164.

Rosenthal, H. and Alderdice, D.F. 1976. Sub lethal effects of environmental stressors, natural and pollutional on marine fish eggs and larvae. J. Fish. Res. Board Can. 33: 2047–2065.

Rust, M.B., Hardy, R.W. and Stickney, R.R. 1993. A new method for force-feeding larval fish. Aquaculture 116: 341–352.

Sargent, J.R. 1995. Origin and functions of egg lipids: nutritional implications. *In*: Broodstock management and Egg and Larval Quality. pp. 353–372. Edited by N.R. Bromage and R.J. Roberts. Blackwell Science, London.

Segner, H. and Verreth, J. 1995. Metabolic enzyme activities in larvae of the African catfish, *Clarias gariepinus*: changes in relation to age and nutrition. Fish Physiol. Biochem. 14: 385–398.

Sivaloganathan, A., Walford, J., Ip, Y.K. and Lam, T.J. 1998. Free amino acids and energy metabolism in eggs and larvae of seabass, *Lates calcarifer*. Mar. Biol. 131: 695–702.

Southgate, P.C., Lee, P.S. and Rimmer, M.A. 1994. Growth and biochemical composition of cultured seabass (*Lates calcarifer*) larvae. Asian Fish. Sci. 7: 241–247.

Spackman, D.H., Stein, W.H. and Moore, S. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30: 1190–1195.

Tamaru, C. S., Harry, A. and Lee, C.S. 1992. Fatty acid and amino acid profiles of spawned eggs of striped mullet, *Mugil cephalus* L. Aquaculture 105: 83–94.

Terner, C. 1979. Metabolism and energy conversion during early development. *In*: Fish Physiology. Vol III pp. 261–268. Edited by W.S. Hoar and D.J. Randall. Academic Press, New York.

Thirunavukkarasu, A.R., Kailasam, M., Kishore Chandra, P., Shiranee, P., Mathew Abraham, Charles, A.V.K. and Subburaj, R. 2001. Captive broodstock development and breeding of Seabass, *Lates calcarifer* (Bloch) in India. *In*: Perspectives in Mariculture. pp. 111–124. Edited by N.G. Menon and P.P. Pillai. The Marine Biological Association of India. Cochin.

Thorsen, A., Fyhn, H.J. and Wallace, R. 1993. Free amino acids as osmotic effectors for oocyte hydration in marine fishes. *In*: Physiology and Biochemistry of Fish Larval Development. pp. 94–98. Edited by B.T. Walther and H.J. Fyhn. University of Bergen, Bergen.

Tiensongrusmee, B., Chantarasri, S., Budileksonos, S., Yowono, S.K. and Santoso, H. 1989. Propagation of seabass, *Lates calcarifer*, in captivity *In*: Sea farming Development Project. pp. 1–55. INS/81/008. UNDP, FAO. Jakarta, Indonesia.

Tucker, J.W., Russell, D.J. and Rimmer, M.A. 2002. Barramundi culture: a success story for aquaculture in Asia and Australia. World Aquacult. 33: 53–59.

Vazquez, R., Gonzalez, S., Rodriguez, A. and Mourente, G. 1994. Biochemical composition and fatty acid content of fertilized eggs, yolk sac stage larvae of Senegal sole, (*Solea senegalensis* Kaup). Aquaculture 119: 273–286.

Vetter, R.D., Houdson, R.E. and Arnold, C. 1983. Energy metabolism in a rapidly developing marine fish egg, the Red drum, S*cianops ocellata.* Can. J. Fish. Aquat. Sciences. 40: 627–634.

Walford, J. and Lam, T.J. 1993. Development of digestive tract and proteolytic enzyme activity in seabass (*Lates calcarifer*). Aquaculture 109: 187–205.

Watanabe, T. and Kiron, V. 1994. Review-Prospects in larval fish dietetics. Aquaculture 124: 223–251.

Wiggins, T.A., Bender, T.R., Mudrack, V.A. and Coll, J.A. 1985. The development, feeding, growth and survival of cultured American shad larvae through the transition from endogenous to exogenous nutrition. Prog. Fish-Cult. 47: 87–93.