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Fish larval nutrition: a review of recent advances in the roles of amino acids

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Abstract

Marine pelagic fish eggs from various latitudes contain up to 50% of the total amino acid pool as free amino acids (FAAs). The FAA pool is established during final oocyte maturation and seems to derive from the hydrolysis of a yolk protein. During yolk resorption, the FAA pool is depleted and reaches low levels at first feeding. The FAA are predominantly used as metabolic fuel, but they are also utilized for body protein synthesis. Amino acids are also important catabolic substrates after the onset of first feeding and may account for 60% or higher of the energy dissipation. Since growth is primarily an increase in body muscle mass by protein synthesis and accretion and fish larvae have very high growth rates, they have a high dietary requirement for amino acids. Fish larvae that develop stomachs late in development have a low proteolytic and absorptive capacities of the digestive systems at first feeding. In vivo studies have shown higher absorption of FAA than peptides and protein bound amino acids from the larval gut in the early stages of marine fish larvae. In the ocean, marine fish larvae obtain a large supply of FAA by consuming plankton after first feeding. The FAA composition of live feed used in aquaculture may to some extent be manipulated within rearing conditions and species and strain selection. While microdiets are a promising feed for larval fish, no satisfactory techniques have at present been developed that allows delivery of high contents of FAA. New techniques using liposomes have the potential to alleviate this problem.

Keywords: Amino acids; Egg; Larvae; Feeding and nutrition; Physiology

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1. Introduction

Many of the marine fish species considered to have potential for culture have proven difficult to rear. The reasons are predominantly related to their small size and simple digestive system during start-feeding. Unlike the salmonids, larvae of marine teleosts have not yet been mass-reared on commercial compound diets from first feeding, but instead require some form of live food during the earliest phases. This has led many investigators to focus on the basic nutritional demands of the larvae (Watanabe and Kiron, 1994).

This review focuses on the important roles amino acids play in the nutritional physiology of the early life stages of marine fish. The sources and dynamics of the amino acid pools during oocyte maturation, and their subsequent fate and function during embryonic and yolk-sac larval development will be discussed. Attention will be focused on the exogenous sources of amino acids derived from live and compound diets, and consider utilisation in relation to the functional status of the larval alimentary canal. Protein turnover, and fluxes of amino acids between the free and protein-bound pools, during growth will not be treated, since these topics have recently been reviewed by Houlihan et al. (1995) and Conceicão (1997).

2. The egg

Newly spawned marine fish eggs have a total amino acid content of 40–60% of their dry mass (Fyhn, 1989; Rønnestad and Fyhn, 1993; Thorsen et al., 1993; Finn, 1994; Rønnestad et al., 1996). This includes amino acids polymerised in proteins and other macromolecules (PAA) and those of the free pool (FAA). Of the total content, protein bound amino acids dominate, regardless of whether the eggs are pelagic or demersal (Fig. 1). However, in marine pelagic fish eggs, FAA may constitute up to 50% of the total amino acid pool (Thorsen et al., 1993). This contrasts with the situation in both marine demersal, and freshwater fish eggs, where the FAA pool only comprises 2 to 5% of the total (Dabrowski et al., 1985; Thorsen et al., 1993).

The relative composition of the FAA pool also differs significantly between pelagic and demersal eggs (Fig. 1; Thorsen et al., 1993; Rønnestad et al., 1996). In demersal eggs, the FAA pool is dominated by taurine, an amino acid analogue which is not incorporated into protein, but which is well known for its role in cell volume regulation (Huxtable, 1992). In contrast, the FAA pool in pelagic eggs is dominated by neutral amino acids such as leucine, valine, isoleucine, alanine and serine. This large pool of FAA, varying between 150–200 mM in concentration, generally represents about 50% of the yolk osmolality, and, in terms of relative composition, is highly conserved in most species studied to date (Thorsen et al., 1993; Rønnestad et al., 1996).

Despite the apparent similarity in the profiles of the FAA pools of pelagic fish eggs (Rønnestad and Fyhn, 1993) detailed investigations of 23 species representing eight families from Panama (Rønnestad et al., 1996) and 10 species representing six families from Norway (Thorsen et al., 1993) revealed some variability in the FAA pool of

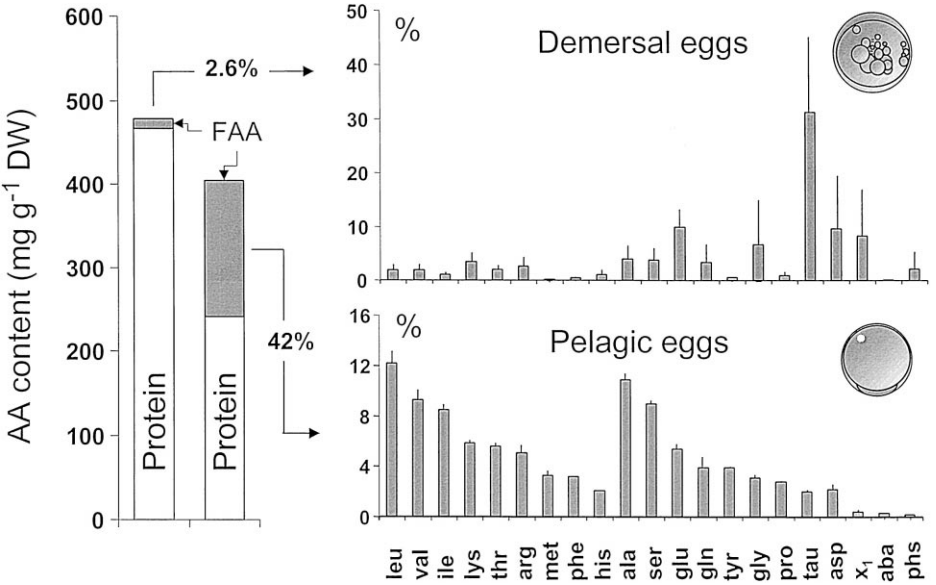
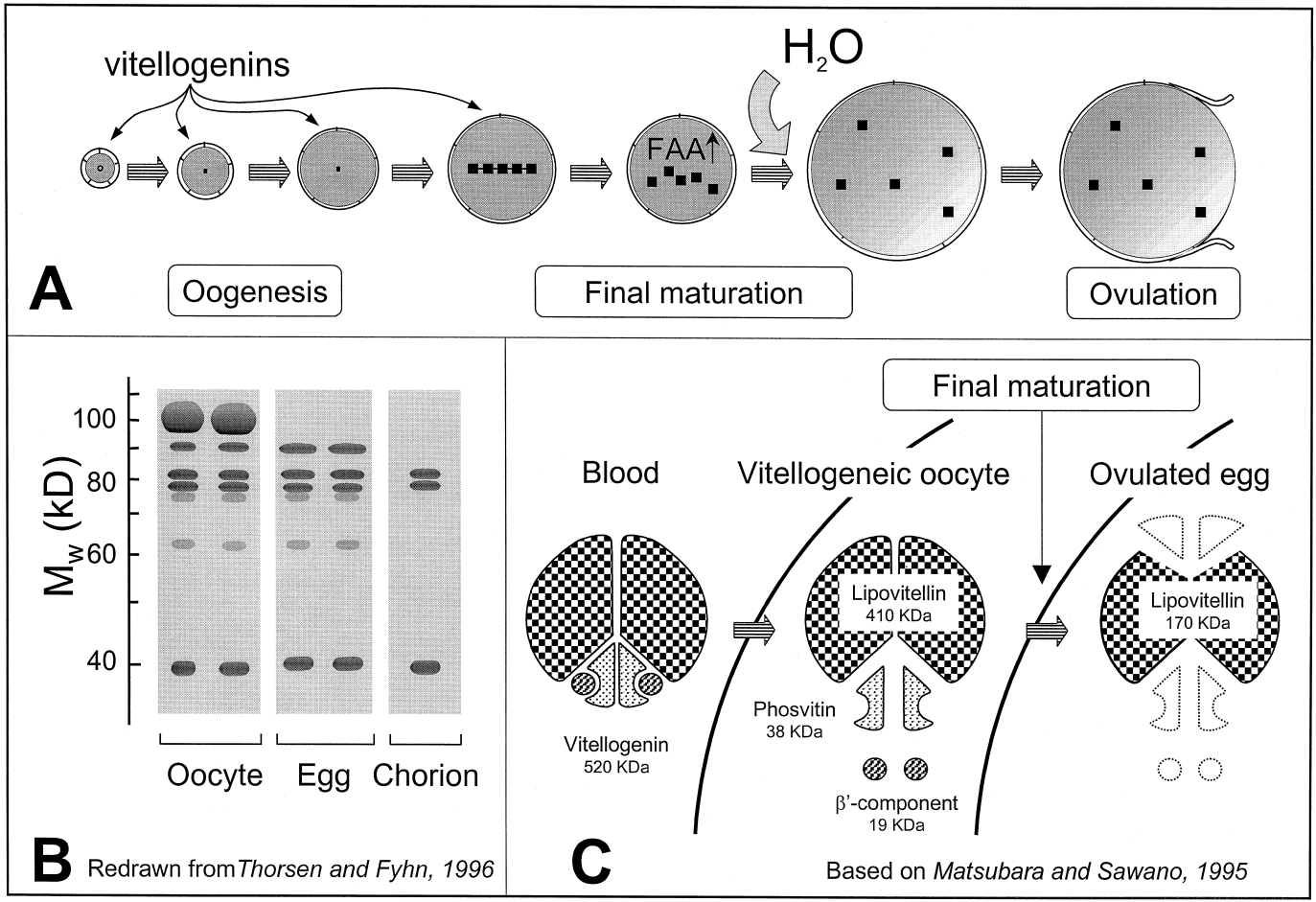


Fig. 1. Contents of protein and FAA in newly spawned marine fish eggs from 23 species from the Panama area. The average FAA profiles of pelagic and demersal eggs are also shown (±SD) (based on Rønnestad et al., 1996). leu: leucine; val: valine; ile: isoleucine; lys: lysine; thr: threonine; arg: arginine; met: methionine; phe: phenylalanine; his: histidine; ala: alanine; ser: serine; glu: glutamic acid; gln: glutamine; tyr: tyrosine; gly: glycine; pro: proline; tau: taurine; asp: aspartic acid; x_1: unknown; aba: α-amino butyric acid; phs: phosphoserine.

species from the two regions. Further studies will be carried out in order to check whether this variability is related to phylogeny or latitude.

3. The origin of the FAA pool

The FAA pool of marine pelagic fish eggs is established during final oocyte maturation (Fig. 2a), when the opaque oocyte matures to a hyaline ovum (Thorsen et al., 1993; Thorsen, 1995). This follows a longer period of slow growth where yolk proteins, mainly vitellogenin, from the liver become incorporated into the oocyte. Shortly before ovulation, there is an abrupt increase in FAA content. Based on the data of Thorsen (1995) and the fact that the FAA profile is highly conserved in almost all species studied to date, it is suggested that the FAA pool is derived from the hydrolysis of a yolk protein. This protein is about 100 kDa in size, and it disappears during oocyte maturation (Fig. 2b). Matsubara and Sawano (1995) and Matsubara and Koya (1997) have shown that, in barfin flounder (*Verasper moseri*), vitellogenin present in the oocyte is cleaved to form three smaller subunits: lipovitellin, phosvitin and a β-component. These authors argue that it is the partial hydrolysis of these subunits during final oocyte maturation that gives rise to the FAA pool (Fig. 2c).


vitellogenins
H2O
FAA
A
Oogenesis
Final maturation
Ovulation
100
80
60
40
Mw (kD)
Oocyte
Egg
Chorion
B
Redrawn from Thorsen and Fyhn, 1996
Final maturation
Blood
Vitellogeneic oocyte
Ovulated egg
Lipovitellin
410 KDa
Lipovitellin
170 KDa
Phosvitin
38 KDa
Vitellogenin
520 KDa
β'-component
19 KDa
C
Based on Matsubara and Sawano, 1995

Concomitant with the establishment of the FAA pool, there is an osmotic influx of water (Fig. 2a) which results in a rapid increase (3–5 times) in oocyte volume (Fulton, 1898; Selman and Wallace, 1989; Thorsen and Fyhn, 1996; Thorsen et al., 1996). The resulting high water content (> 90%) is a prerequisite for increased buoyancy and prepares the embryo for development in a hyperosmotic environment during a period before osmoregulatory organs are established (Mangor-Jensen, 1987; Fyhn, 1993). Indeed, due to the very low water permeability of the vitelline membrane in a newly spawned and hardened egg, this source of water is vital for the physiological viability of the early cells (Riis-Vestergaard, 1982; Mangor-Jensen, 1987).

4. The fate of amino acids during yolk-dependent development

Although active uptake of FAA is common among marine invertebrates (Manahan, 1990) only in one case, herring eggs (Siebers and Rosenthal, 1977), has this been shown to occur in fish eggs or larvae. However, since the magnitude of the FAA uptake was only about 1% of the energy dissipation, the authors concluded that the development of herring was exclusively depended on nutrients stored in the yolk (Siebers and Rosenthal, 1977). A similar conclusion was reached in amino acids efflux studies carried out on Atlantic halibut larvae (Rønnestad, 1993). The dependence on amino acids derived from the yolk probably applies throughout the period of endogenous nutrition, until the hatched larva has sufficiently developed organ systems to commence exogenous feeding.

During the endogenous phase of development, the FAA pool declines (Fig. 3). There are, however, differences between species in the timing of the decreases in FAAs, this seeming to be related to whether or not the egg possesses an oil globule. In pelagic eggs with one or more oil globules, e.g., turbot (Rønnestad et al., 1992b; Finn et al., 1996), European sea bass (Gatesoupe, 1986; Rønnestad et al., 1998), Asian sea bass (Sivaloganathan et al., 1998), gilthead sea bream (Garcia Gallego et al., 1993; Rønnestad et al., 1994), and red sea bream (Seoka et al., 1997), the FAA mainly disappear prior to hatch, whereas in species that do not contain an oil globule, e.g., Atlantic cod (Finn et al., 1995a), Atlantic halibut (Finn et al., 1995b), and lemon sole (Rønnestad et al., 1992a), the FAA depletion continues beyond hatch.

As pointed out by Rønnestad (1995) the interpretation of ontogenetic changes in composition should be based on data presented in absolute terms (preferably in mol ind.$^{-1}$), because the use of relative data can lead to erroneous conclusions of utilisation, synthesis, bioconversion, selective retention or catabolism of various components in

Fig. 2. Sources of FAA in the oocyte. (A) Model proposed by Fyhn (1993) indicating that FAAs are first seen in the oocyte during final maturation. (B) SDS polyacrylamide gel electrophoresis of proteins in the oocyte, spawned egg and isolated chorion of plaice (*Pleuronectes platessa*) (redrawn from Thorsen and Fyhn, 1996). (C) Stage-specific cleavage of vitellogenin and hydrolysis of its subunits in barfin flounder (*V. moseri*) as suggested by Matsubara and Sawano (1995). See text for further details.

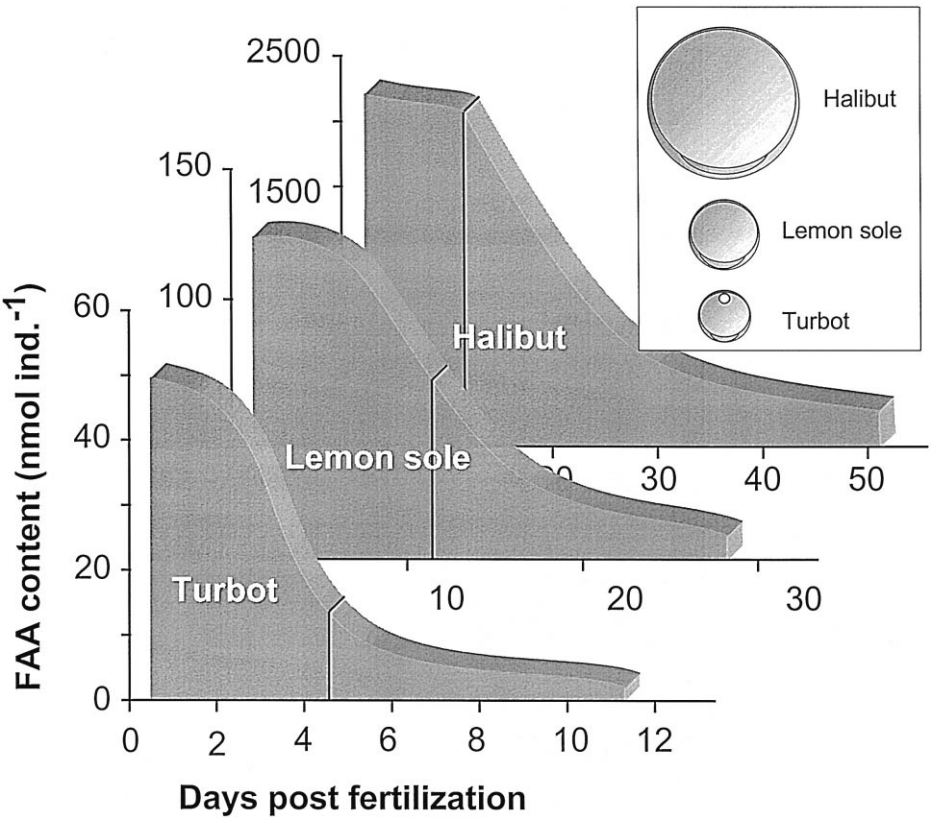


Fig. 3. Ontogenetic changes in the FAA pool of pelagic eggs of three fish species. Hatching is represented by vertical lines. The relative size of three newly fertilised eggs is indicated (modified from Rønnestad and Fyhn, 1993).

developing eggs and larvae (Rønnestad, 1995). Stoichiometric studies employing the former approach have shown that the disappearing FAA (Fig. 4) comprise the major substrate of aerobic metabolism during the earliest developmental phases (Finn, 1994). Moreover, when the pool of FAA is exhausted, PAA are recruited to sustain the larva's metabolic demand.

To summarise the situation for egg types with (Type II) and without (Type I) oil globules (Finn, 1994) Type I eggs support 70% of their energy dissipation via catabolism of amino acids. The remaining 30% is largely derived from catabolism of phospholipids and triacylglycerols. Type II eggs derive 50% of their energy from amino acids and 50% from predominantly neutral lipids such as wax esters and triacylglycerols.

Once yolk nutrients are no longer sufficient to support the metabolic demand of the larvae, they must initiate exogenous feeding. The commencement of this phase seems to be the most critical in the life of the fish. At this time the larvae must initiate an active search behaviour to catch and ingest prey, whilst relying on the digestive and absorptive

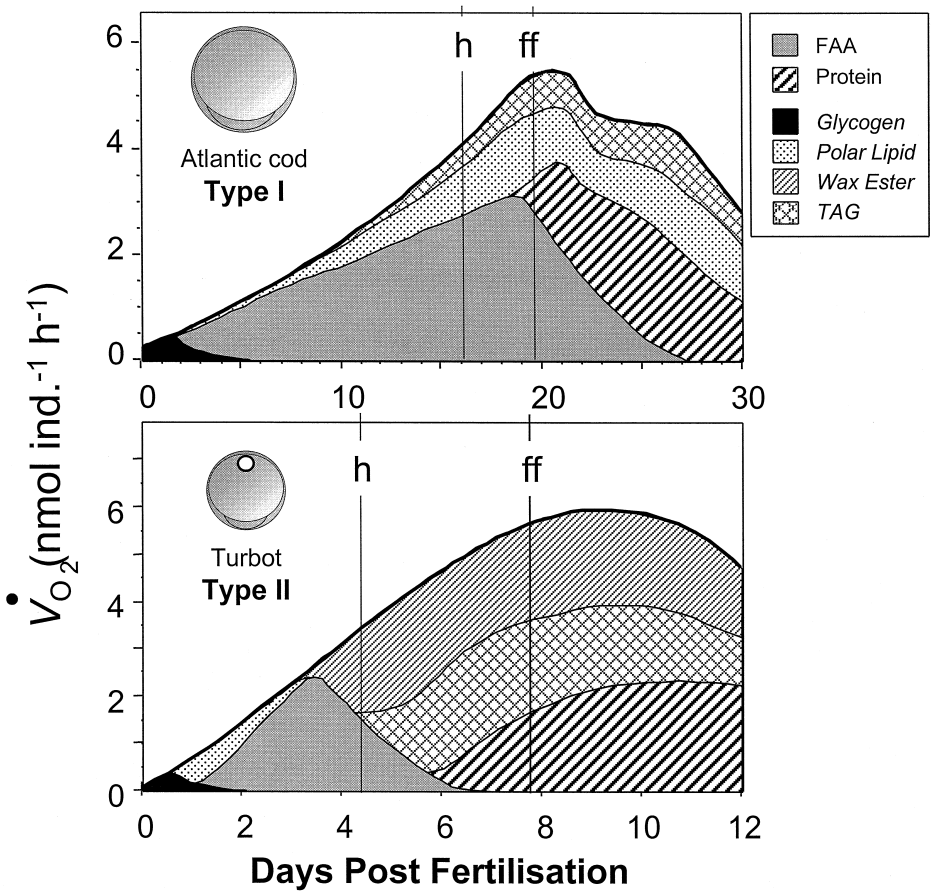
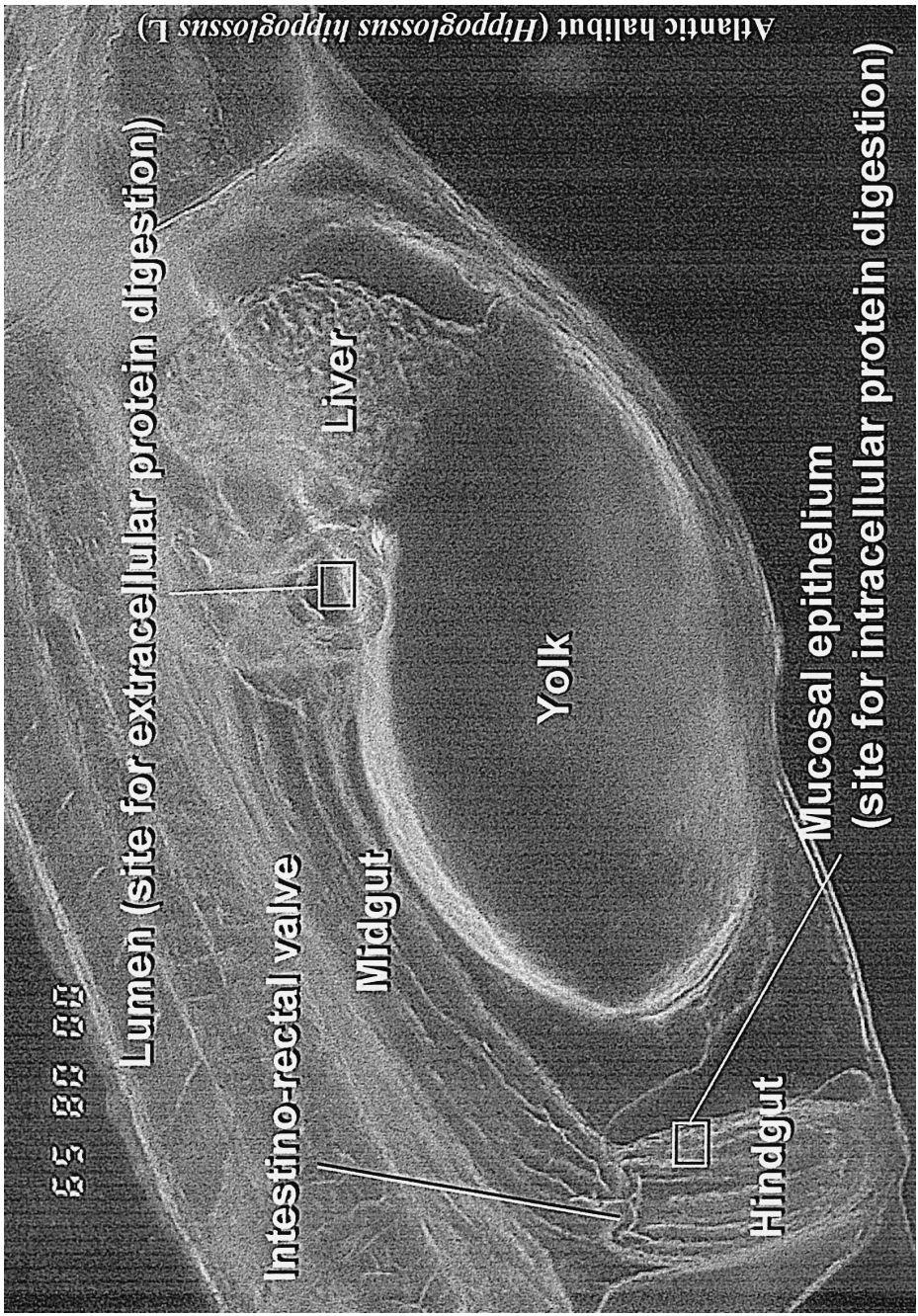


Fig. 4. Proposed scheme for the sequence of catabolic substrate oxidation in Type I eggs and Type II eggs (Finn et al., 1995a,b, 1996). h = hatch, ff = first feeding.

capacity of the gut to assimilate the ingested nutrients for the purposes of growth and energy dissipation.

5. The early larval gut

The digestive system of an Atlantic halibut larva is used to illustrate the gut at the stage when pelagic marine fish larvae are ready to commence first-feeding (Fig. 5). At this stage, the larvae possess an intestine which is divided into foregut, midgut and hindgut. The larvae also possess pancreatic tissue, a gall bladder and liver, but lack a stomach (Kjørsvik and Reiersen, 1992; Segner et al., 1994). This lack of a stomach does not mean that the digestive tract is non-functional, but the challenge is to determine which nutrients, molecules or particles the gut can be digested and absorbed.


Atlantic halibut (Hippoglossus hippoglossus L)
Lumen (site for extracellular protein digestion)
Liver
Intestino-rectal valve
Midgut
Yolk
Hindgut
Mucosal epithelium
(site for intracellular protein digestion)

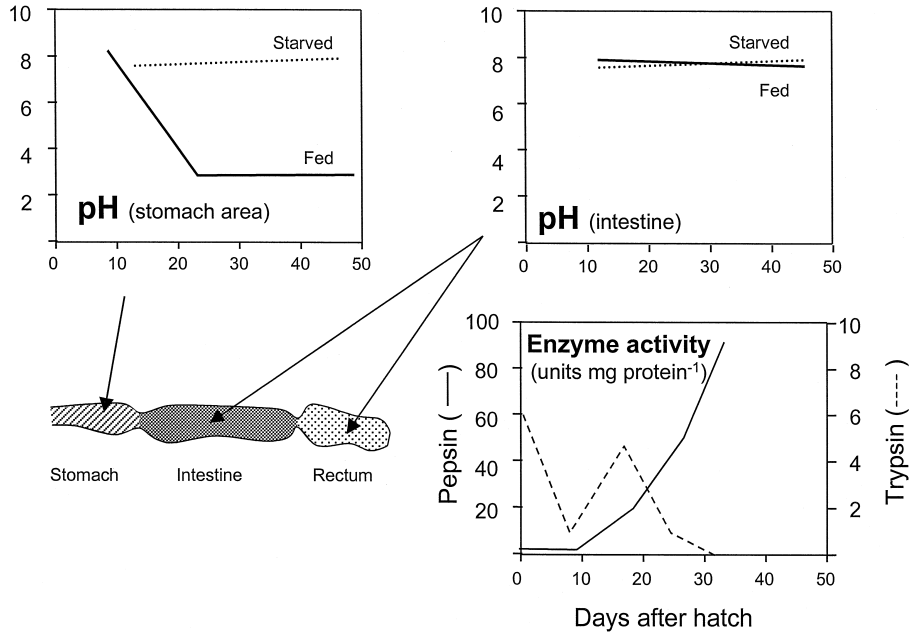


Fig. 6. Ontogeny of digestive function in marine fish larvae. Enzyme activity (homogenate of whole fed larva) and pH (fed and starved larvae) in different areas of the digestive tract of sea bass, *L. calcarifer* (based on Walford and Lam, 1993). See text for further explanations.

With regard to the digestion of proteins in early larvae, it is generally believed that the major site of absorption is the epithelial mucosa of the hindgut. There may be pinocytosis followed by intracellular lysosomal digestion (Govoni et al., 1986). The implication of the early absence of a stomach was clearly demonstrated in a study on Asian seabass (*Lates calcarifer*) by Walford and Lam (1993). These authors showed that prior to day 14 there was no acid secretion in the presumptive stomach. A marked decrease in the pH from about 8 to about 3.7 in the presumptive stomach from day 14 to 22 in fed larvae shows increasing ability to secrete acid during this period (Fig. 6). By contrast, the pH of the presumptive stomach of starving control larvae remained stable around 8 throughout development. Furthermore, the pH of the anterio-median intestine, as well as in the hindgut area, remained stable around 8 regardless of whether the larvae were fed or not during the same phase of development. Data for proteolytic enzymes in their studies (Walford and Lam, 1993) showed that, at hatching, pepsin-like enzymes were very low, but began to increase in parallel with stomach differentiation and the declining pH of the fed larvae. Since natural folded proteins present a smaller surface area than denatured proteins, they may be more less readily attacked by alkaline

Fig. 5. Digestive tract of Atlantic halibut larva at 23 days post hatch. Histological (Kjørsvik and Reiersen, 1992) and behavioural studies (Pittman, 1991; Skiftesvik et al., 1994) suggest that the larva is ready to commence feeding at this developmental stage.

proteases (Jany, 1976). Thus, due to the absence of a stomach and its HCl and pepsin secreting cells, there is no preparatory acid denaturation of ingested proteins in the early feeding larvae.

In the study of Walford and Lam (1993) trypsin-like enzymes, which include the hatching enzyme, were present from hatching but showed variable contents, and indeed trypsin-like activity had apparently decreased to undetectable levels by day 30. Trypsin is a key digestive enzyme, because it activates other pancreatic proteases. Both the relative activity and content of trypsin-like enzymes may peak during initiation of first-feeding, but then decline and remain low until metamorphosis when the stomach becomes functional (Hjelmeland et al., 1984, 1988; Pedersen et al., 1987; Pedersen and Hjelmeland, 1988; Hjelmeland, 1995). Current data available for brush border enzymes (Cahu and Zambonino Infante, 1997) indicate that enzyme secretion is related to the type of food ingested, with compound diets inducing a lower secretion than live prey such as *Artemia nauplii*. Thus, the fish larval gut may have limited extracellular ability to digest high molecular weight molecules, particularly complex and long polymers like proteins.

To specifically test the functionality of the digestive system, Rust et al. (1993) force-fed marine fish larvae of different ages and determined nutrient absorption rates. This revealed that fish larvae that lack a stomach at first-feeding initially absorb free amino acids (FAAs) more efficiently than amino acids in polymerised form (Fig. 7).

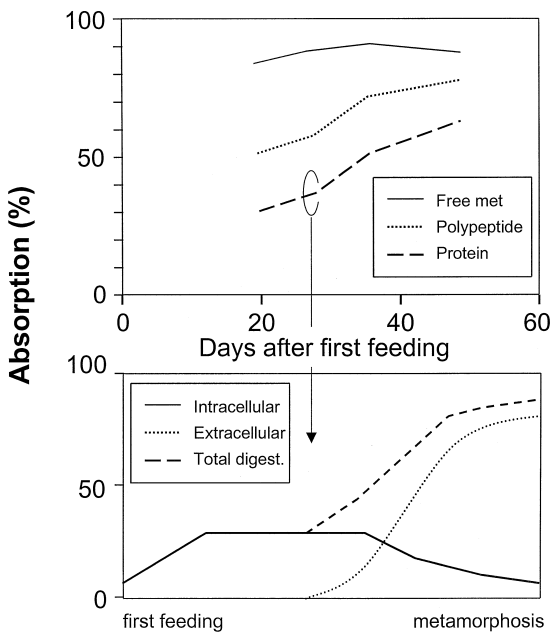


Fig. 7. Upper panel: Nutrient absorption in striped bass larvae force-fed protein containing ^{35}S-methionine, polypeptide containing ^{35}S-methionine or free ^{35}S-methionine. Lower panel: A model changes in for protein digestion during the development of marine fish larvae that lack stomach at first feeding (based on Rust, 1995).

Walford et al. (1991) have also shown that first feeding sea bass larvae are unable to digest protein membrane microcapsules. The ability to digest and absorb protein increase as the larvae approach metamorphosis (Walford et al., 1991; Rust et al., 1993). Rust (1995) also supported the model of Govoni et al. (1986) that protein digestion in the early larvae is dependent on intracellular digestion in the hindgut, with extracellular proteolytic capacity only developing when the larvae approach metamorphosis. Taken together, these data support the notion that FAA (Fyhn, 1989) or low molecular peptides (Walford and Lam, 1993) are vital components of the diet of first-feeding marine fish larvae. Indeed, data collected for five species of marine fish larvae (Finn, unpublished data), indicate that nitrogen quotients are very high during the first weeks of exogenous feeding, which suggests that energy metabolism is highly geared towards the catabolism of amino acids. Rønnestad and Naas (1993) concluded that fed Atlantic halibut larvae derived about 60% of its energy need from amino acids during the first month of exogenous feeding.

Thus, amino acids, preferably in their free form, should probably comprise a major component of the diets of early larvae. These molecules are highly water soluble so they readily leach down their diffusion gradient to the culture medium. This creates retention problems when amino acids and small peptides are included in particulate feeds designed for presentation to small first feeding larvae.

6. Amino acids and live prey

Modern teleosts have evolved in the oceans and the majority of larvae feed on zooplankton and, perhaps, phytoplankton in the euphotic zone. Live foods such as phytoplanktons, rotifers, *Artemia* and various zooplanktons all contain a significant pool of FAA (Frolov et al., 1991; Fyhn et al., 1993, 1995; Helland, 1995), although the mass-specific content of FAA varies between the various live foods. For example, some zooplanktons, such as calanoid copepods, contain more than twice the amount of FAA per gram wet mass than *Artemia* (Næss et al., 1995). Further, the FAA pool of a given species may vary with life-stage and rearing salinity (Fyhn et al., 1995) and FAA content may also be amenable to modulation via adjustments to the culture environment. Marine invertebrates are generally isosmotic to sea water and dynamically regulate cell volume by adjusting their osmolyte concentration in response to changing salinity. Marine invertebrates achieve this almost exclusively through regulation of the organic osmolytes, mostly FAA (Yancey et al., 1982). When marine invertebrates are faced with an hyperosmotic challenge the intracellular pool of FAA is increased via synthesis or via transmembrane transport and in hypoosmotic environments, the FAA are either catabolised or excreted (Hawkins and Hilbish, 1992).

The species used as live food organisms in larval marine fish culture regulate their cell volume as described above and, with few exceptions increase FAA content in response to increased salinity (Fig. 8). The data presented in Fig. 8 illustrate a potential avenue for manipulation in systems reliant on such organisms. A point worth noting, regarding *Artemia*, is that different strains contain significantly different levels of FAA.

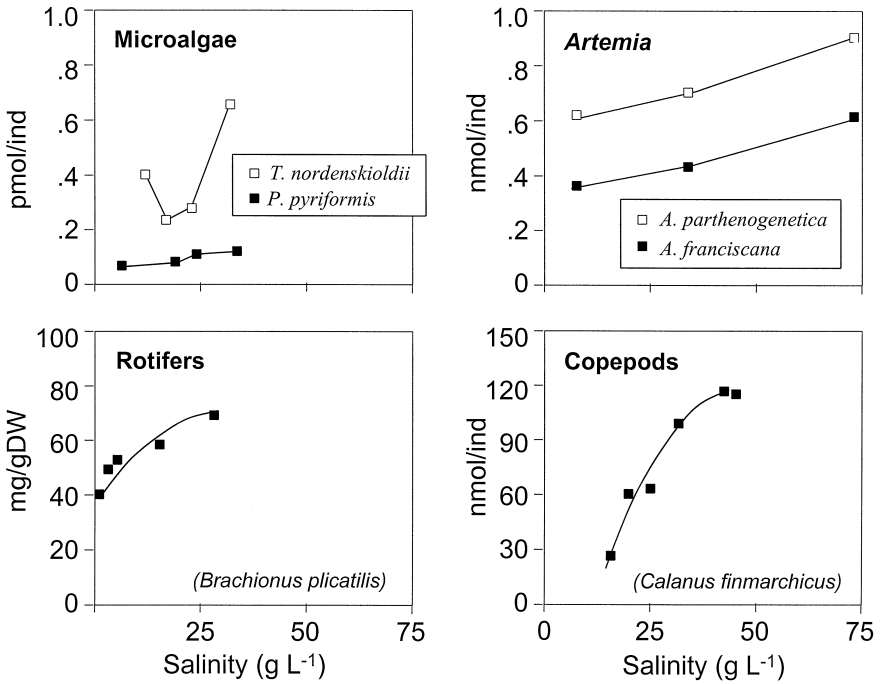


Fig. 8. Contents of FAA in various live prey organisms currently used in aquaculture: microalgae and copepods (Fyhn et al., 1995); rotifers (Frolov et al., 1991); *Artemia* (Helland, 1995).

The strain of *Artemia* most commonly used in fish culture facilities, *A. francisciana*, contains significantly less FAA than do some other strains (Helland, 1995; Fig. 8), with some populations of *A. francisciana* containing less than 20% of the FAA found in *A. partheogenetica* (Helland, 1995).

7. Amino acids and compound diets

Incorporation of FAA into formulated diets has proven difficult due to the small feed particle size and the problem of rapid leaching to the environment. Lopez-Alvarado and Kanazawa (1994) examined various microdiets and found that the retention of FAA after a 2-min immersion in water was related to the type of coating. Of the diets tested, only liposomes (lipid walled particles) showed promise for the delivery of FAA and other water-soluble molecules, to first-feeding larvae. These data are encouraging, but since liposomes are commonly in the nm or μm range, they are unlikely to form the direct food particle of first-feeding fish larvae. Two approaches to solving this problem have been reported. Ozkizilcik and Chu (1994) fed liposomes to *Artemia* and concluded that this was a viable means of enriching the live food with phospholipids and FAA. The

second approach has been to include phospholipid-rich liposomes in a microdiet and then feed this diet to the fish larvae. Koven (Centre for Mariculture Research, Eilat, personal communication) and Homme, (University of Tromsø, personal communication) have adopted this approach, but to the authors knowledge, no published data exist where diets containing FAA-enriched liposomes have been fed to fish larvae. Fernández-Díaz and Yúfera (1997) have recently fed FAA enriched microcapsules to gilthead seabream, and recorded a slow growth until day 15 post hatch although the survival was low at 11.6%.

8. Perspectives

FAAs are important nutritional components influencing the viability of early stages of marine fish. As such, greater emphasis must be placed on understanding the physiological mechanisms involved in the ontogeny of the intestinal digestion and absorption of proteins and amino acids. Similarly, studies directed towards the modulation of the nutritional composition of novel diets, and existing live food organisms, to match the functional capacity of the larval intestine would seem to be a prerequisite for increased success.

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