Amino Acids (2009) 36:177–183
DOI 10.1007/s00726-008-0045-6

ORIGINAL ARTICLE

How does fish metamorphosis affect aromatic amino acid metabolism?

Wilson Pinto · Luís Figueira · Maria Teresa Dinis · Cláudia Aragão

Received: 17 October 2007 / Accepted: 11 February 2008 / Published online: 26 February 2008

Abstract Aromatic amino acids (AAs, phenylalanine and tyrosine) may be specifically required during fish metamorphosis, since they are the precursors of thyroid hormones which regulate this process. This project attempted to evaluate aromatic AA metabolism during the ontogenesis of fish species with a marked (Senegalese sole; *Solea senegalensis*) and a less accentuated metamorphosis (gilthead seabream; *Sparus aurata*). Fish were tube-fed with three L-[U-^{14}C] AA solutions at pre-metamorphic, metamorphic and post-metamorphic stages of development: controlled AA mixture (Mix), phenylalanine (Phe) and tyrosine (Tyr). Results showed a preferential aromatic AA retention during the metamorphosis of Senegalese sole, rather than in gilthead seabream. Senegalese sole's highly accentuated metamorphosis seems to increase aromatic AA physiological requirements, possibly for thyroid hormone production. Thus, Senegalese sole seems to be especially susceptible to dietary aromatic AA deficiencies during the metamorphosis period, and these findings may be important for physiologists, fish nutritionists and the flatfish aquaculture industry.

Keywords *Solea senegalensis* · *Sparus aurata* · Metamorphosis · Aromatic amino acids · Amino acid metabolism · Tyrosine

Introduction

Sub-optimal performance and poor larval quality frequently observed in marine fish aquaculture are often related to dietary imbalances. Growth optimization can be achieved by manipulating the dietary nitrogen profile to fulfil larval amino acid (AA) requirements (Aragão et al. 2004a, 2007). Being the building blocks of protein synthesis, AAs in fish are also used in energy production or for other metabolic purposes (Rønnestad et al. 2001a; Weltzien et al. 1999). Amino acid requirements may change during fish ontogeny, probably due to different velocities in tissue and organ development (Oikawa and Itazawa 1984). These changes may implicate different dietary AA requirements during the early life stages of fish (Aragão et al. 2004b).

In fish larvae, metamorphosis has traditionally been considered to be a critical rearing point, and special AA supplies may be crucial to meet both growth and physiological requirements at this developmental stage (Aragão et al. 2004b). For instance, aromatic AAs (phenylalanine and tyrosine) are used as precursor molecules for the synthesis of thyroid hormones, melanin, dopamine and catecholamines.

Thyroid hormones (thyroxin—T4 and thriiodothyronine—T3) are endocrine factors of the thyroid gland in all vertebrates (Power et al. 2001). In amphibians, these hormones are continuously required throughout metamorphosis for this process to be successfully completed (Schreiber and Specker 1998). In fish, these hormones also regulate metamorphosis, a process that achieves a higher degree of complexity in flatfish (Pleuronectiformes), where every organ system experiences major changes. Hence, the metamorphosis period may be a developmental stage where fish have special requirements of thyroid hormone precursors and special amounts of dietary tyrosine and phenylalanine may have to be supplied for a successful transition from larvae to juvenile stage (Schreiber and Specker 1998).

Senegalese sole (*Solea senegalensis*) is a flatfish species recently becoming important for South-eastern aquaculture

W. Pinto (✉) · L. Figueira · M. T. Dinis · C. Aragão
CCMAR, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
e-mail: wpinto@ualg.pt

(Conceição et al. 2007; Imsland et al. 2003), and suggestions have been made concerning ontogenic changes on its ideal dietary AA profile (Aragão et al. 2004b). For instance, Senegalese sole larvae may be especially susceptible to dietary AA imbalances during metamorphosis (Aragão et al. 2004b), although active feeding is maintained at a constant ingestion rate during this process (Fernández-Diaz et al. 2001; Parra and Yúfera 2001). The marked metamorphosis of Senegalese sole is undergone around 12–20 days after hatching (DAH), reaching its climax from 14 to 17 DAH. During this stage, the internal organs are rearranged and the digestive tract is reorganized. These body conformational changes involve movement of the anus towards the pelvic fin, migration of the left eye and a 90° rotation in body position (Ribeiro et al. 1999), resulting in an asymmetric benthic postlarvae. These changes are more pronounced in Senegalese sole than, for instance, in gilthead seabream (*Sparus aurata*) which has a much less complex metamorphosis that starts around 32 to 35 DAH. In this species, as opposed to Senegalese sole, no spatial reorganization of the digestive system occurs, while metamorphosis is a much more diffuse and gradual process involving no changes in the body bilateral symmetry. In species such as Senegalese sole, the marked metamorphosis may increase specific AA requirements such as aromatic AA, which seem to be important during this stage of development.

The present work aimed to evaluate the metabolism of aromatic AA in species with a marked (Senegalese sole) and with a less complex (gilthead seabream) metamorphosis. Focusing especially on the metamorphosis period, this paper also meant to verify whether aromatic AA metabolism changes during Senegalese sole and gilthead seabream larval development.

Materials and methods

Larval rearing

Senegalese sole (*S. senegalensis*) and gilthead seabream (*S. aurata*) eggs were obtained from a wild broodstock acclimated to captivity and maintained at CCMAR facilities (Faro, Portugal) and from a local aquaculture (Viveiros Vila Nova, SA, Portugal), respectively. The larvae of both species were reared at CCMAR facilities in duplicate 200 l conical cylindrical tanks using a closed water recirculating system (4 to 5 initial daily water renewals), using a light intensity of 600 l× and light/dark cycles of 10:14 and 14:10-h, respectively, for Senegalese sole and gilthead seabream. Fish larvae were fed with rotifers (*Brachionus plicatilis*) and *Artemia* enriched with commercial products according to standard rearing procedures (Dinis et al. 1999; Moretti et al. 1999). Initial larval density for Senegalese sole was 45 larvae l^{-1}. Water temperature (20.3 ± 0.4°C; mean ± standard deviation), oxygen saturation level (93.1 ± 3.5%) and salinity (36.1 ± 1.0 g l^{-1}) were measured in a daily basis with commercial probes. Eighteen days after hatching (DAH), post-metamorphosed Senegalese sole were transferred to two 21 l sand-coloured, fibreglass raceways (0.21 m^2, 10 cm water depth) at a density of 3,000 post-larvae m^{-2} and maintained until 34 DAH in a closed water recirculating system (two water renewals h^{-1}). Light intensity was 200 l× and a 12:12-h light/dark cycle was adopted. Water temperature (20.4 ± 0.5°C), oxygen saturation levels (94.8 ± 2.3%) and salinity (32.1 ± 0.8 g l^{-1}) values were recorded daily.

The initial larval density for gilthead seabream was 165 larvae l^{-1}. Water temperature (20.5 ± 1.2°C), oxygen saturation level (88.2 ± 3.0%) and salinity (35.4 ± 0.8 g l^{-1}) were recorded daily. Seabream larvae were reared until 46 DAH.

Tube-feeding trials

The method of in vivo controlled tube feeding described by Rust et al. (1993) and modified by Rønnestad et al. (2001b) for marine fish larvae was used in Senegalese sole and gilthead seabream at pre-metamorphic, metamorphic and post-metamorphic stages of development. Senegalese sole and gilthead seabream were tube fed with L-[U-^{14}C] AA solutions at 11 (pre-metamorphic), 16 (metamorphic), 23 and 34 (post-metamorphic) and 26 (pre-metamorphic), 33 (metamorphic) and 46 (post-metamorphic) DAH, respectively. Three amino acid solutions were used: protein hydrolysate, a controlled mixture of 16 AA (Mix—Amersham Biosciences, UK; alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine), phenylalanine (Phe—Amersham Biosciences) and tyrosine (Tyr—American Radiolabeled Chemicals, USA). Specific activities for Mix, Phe and Tyr solutions were 50, 50 and 55 μCi ml^{-1}, respectively.

Before the tube-feeding experiments, fish were acclimatized to the room where the experiments were conducted. Fish were maintained in small white trays with seawater and airflow for 12 h. On the day of the experiments, larvae were anaesthetized with 33 μM MS-222 and tube-fed with one of the AA solutions using a 0.19-mm diameter plastic capillary. In all tube-feeding experiments, each AA solution was tube-fed to 20 larvae. Injection volumes were previously determined by tube-feeding larvae with an innocuous colorant and concomitant observation of stomach distension. These results varied from 4.6 to 27.6 and from 4.6 to 9.2 nl in Senegalese sole and gilthead seabream, respectively. After capillary withdrawal, larvae were gently rinsed for

any spillage through three successive wells with clean seawater and transferred into 7.5 ml seawater filled incubation chambers (water fraction). These chambers were considered to contain all labelled ^{14}C resultant from fish evacuation. A connection through gentle airflow was provided between each incubation chamber and a KOH trap (5 ml, 0.5 M) to collect $^{14}CO_2$ produced by labelled AA oxidation. Once the set incubation period was over (8 and 6 h for Senegalese sole and gilthead seabream, respectively), the fish were tested and the $^{14}CO_2$ in the remaining water was collected as described by Rønnestad et al. (2001b) (trap—catabolized fraction). Fish bodies were treated with trichloroacetic acid (TCA; 500 µl, 6% w/v) for 24 h at 4°C to remove ^{14}C-labelled free AA (FAA fraction). Subsequently, bodies were solubilized with 500 µl Solvable (PerkinElmer, USA) for 48 h at 50°C for ^{14}C-labelled protein determination (body fraction).

DPM from all samples were determined by adding Ultima Gold XR (PerkinElmer, USA) scintillation liquid and counting in a Beckman LS 6000IC liquid scintillation counter (Fullerton, CA, USA).

Data analysis

The metabolic budgets were calculated after subtraction of the blanks of each fraction (water, trap, FAA and body). Absorbed (trap, FAA and body) and evacuated (water) proportions were expressed as a percentage of total tracer fed (i.e. the sum of the DPM in all compartments of a given fish). Catabolized (trap) and retained (FAA and body) fractions were expressed as a percentage of total absorbed proportion (trap, FAA and body).

The results were expressed as means ± standard deviation (SD). Data were tested using one-way ANOVA followed by Tukey's multiple comparison tests. The significance level used was $P \leq 0.05$. All results expressed as a percentage were previously arcsine transformed (Zar 1999).

Results

All tube-fed Senegalese sole were alive and with normal appearance at the end of the incubation period at 11, 16, 23 and 34 DAH. In gilthead seabream with 26, 33 and 46 DAH survival rates varied between 65 and 70%, 65 and 90%, and 85 and 100%, respectively.

Senegalese sole and gilthead seabream results showed different evacuation fractions (water; Tables 1 and 2) between treatments. Data show that in Senegalese sole, ^{14}C evacuation (water) values were significatively higher for aromatic AA than for the protein hydrolysate (Mix) at pre-metamorphic (11 DAH) and post-metamorphic (23 DAH)

Table 1 Metabolic budgets for *S. senegalensis* tube-fed with L-[U-^{14}C] protein hydrolysate (Mix), phenylalanine (Phe) and tyrosine (Tyr) at pre-metamorphic (11 days after hatching—DAH), metamorphic (16 DAH) and post-metamorphic (23 and 34 DAH) stages of development

	DAH	Mix	Phe	Tyr
Water	11	20.2 ± 21.4^{a}	48.4 ± 22.5^{b}	22.9 ± 23.3^{a}
	16	51.1 ± 27.8	52.8 ± 24.7	50.1 ± 28.4
	23	42.3 ± 29.3^{a}	68.6 ± 28.5^{b}	61.9 ± 16.8^{a,b}
	34	46.4 ± 29.5	39.7 ± 23.2	55.6 ± 25.3
Trap	11	50.9 ± 22.7^{d}	27.2 ± 12.8^{c}	18.8 ± 11.8^{c}
	16	20.0 ± 7.8^{d}	14.7 ± 9.0^{d}	4.4 ± 3.7^{c}
	23	21.0 ± 9.3^{d}	5.6 ± 3.3^{c}	18.5 ± 8.2^{d}
	34	15.3 ± 11.4^{d}	5.6 ± 4.8^{c}	11.6 ± 9.0^{c,d}
FAA	11	1.9 ± 3.0	3.3 ± 3.4	3.1 ± 2.5
	16	8.1 ± 6.8	12.4 ± 12.6	10.4 ± 10.4
	23	7.6 ± 8.0	6.4 ± 11.5	5.6 ± 9.0
	34	13.3 ± 13.7	12.9 ± 10.5	8.8 ± 9.2
Body	11	27.1 ± 21.7^{e}	21.2 ± 14.0^{e}	55.2 ± 24.5^{f}
	16	20.8 ± 21.2	20.2 ± 12.7	35.2 ± 26.9
	23	29.1 ± 22.7	19.4 ± 23.5	14.0 ± 12.0
	34	25.0 ± 20.5	41.8 ± 22.3	24.0 ± 14.2

Data are expressed as percentage (%) of the total tube-fed ^{14}C-labelled solution. Means ± SD ($n = 20$). Different superscripts within each stage indicate significant differences among the three amino acid solutions for that compartment. The lack of superscripts indicates that no differences were observed within ages of the same compartment for the different amino acid solutions

Table 2 Metabolic budgets for *S. aurata* tube-fed with L-[U-^{14}C] protein hydrolysate (Mix), phenylalanine (Phe) and tyrosine (Tyr) at pre-metamorphic (26 days after hatching—DAH), metamorphic (33 DAH) and post-metamorphic (46 DAH) stages of development

	DAH	Mix	Phe	Tyr
Water	26	84.5 ± 16.7^{c}	28.6 ± 27.5^{a}	57.3 ± 24.7^{b}
	33	61.2 ± 25.8	65.4 ± 34.4	54.9 ± 31.6
	46	11.5 ± 18.6^{a}	36.2 ± 28.3^{b}	27.8 ± 17.1^{b}
Trap	26	8.8 ± 2.0	9.9 ± 4.4	14.2 ± 8.3
	33	18.9 ± 12.8^{d}	6.9 ± 5.7^{e}	16.71 ± 1.3^{d}
	46	14.4 ± 6.0^{d}	10.9 ± 13.0^{e}	12.8 ± 4.7^{d}
FAA	26	0.7 ± 0.6 ^{f}	18.0 ± 14.6^{h}	9.0 ± 6.2 ^{g}
	33	7.6 ± 6.9	5.9 ± 6.3	6.2 ± 5.1
	46	25.5 ± 6.4^{f}	11.1 ± 5.2^{g}	13.8 ± 3.8^{g}
Body	26	6.1 ± 18.7^{i}	43.5 ± 23.0^{k}	19.5 ± 17.5^{j}
	33	12.2 ± 11.4	21.8 ± 25.3	22.2 ± 17.7
	46	48.6 ± 13.3	41.7 ± 30.0	45.5 ± 11.9

Data are expressed as a percentage (%) of the total tube-fed ^{14}C-labelled solution. Means ± SD ($n = 20$). Different superscripts within each stage indicate significant differences among the three amino acid solutions for that compartment. The lack of superscripts indicates that no differences were observed within ages of the same compartment for the different amino acid solutions

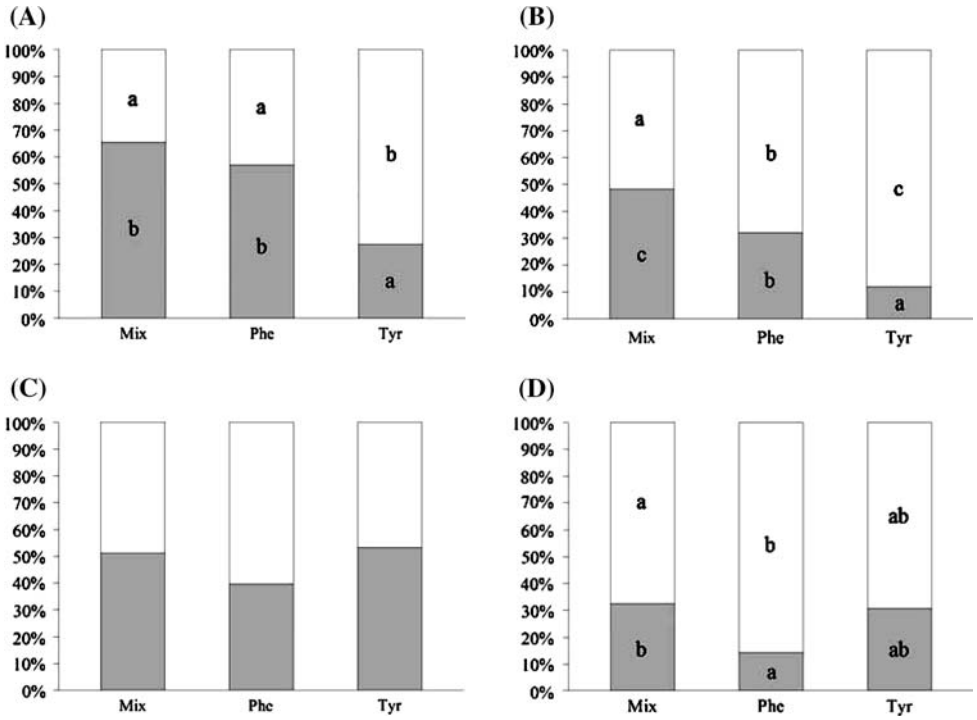

Fig. 1 Percentage of the absorbed tube-fed ^{14}C-labelled solution that was catabolized (*shaded square*) or retained (*open square*) by *S. senegalensis* at pre-metamorphic (11 days after hatching—DAH; **a**), metamorphic (16 DAH; **b**), and post-metamorphic (23 and 34 DAH; **c** and **d**, respectively) stages of development. Means ± SD ($n = 20$). Different letters within the same compartment represent significant differences among tube-fed solutions. *Mix* L-[U-^{14}C] protein hydrolysate; *Phe* L-[U-^{14}C] phenylalanine; and *Tyr* L-[U-^{14}C] tyrosine

stages of development. In gilthead seabream, ^{14}C evacuation values were also different between treatments in pre-metamorphic (26 DAH) and post-metamorphic (46 DAH) stages of development. During the pre-metamorphosis state in gilthead seabream, ^{14}C aromatic AAs were less evacuated than the protein hydrolysate (Mix), while during the post-metamorphosis state an inverse tendency occurred.

Results from the FAA fraction during Senegalese sole's ontogeny were not significantly different between treatments, while in gilthead seabream these differences occurred during the pre-metamorphosis (26 DAH) and post-metamorphosis (46 DAH) periods (Tables 1 and 2). During the pre-metamorphic state, ^{14}C FAA values were significatively higher in aromatic AA than in the protein hydrolysate (Mix), while during the post-metamorphic state these values were lower than the Mix treatment.

Differences obtained for Senegalese sole and gilthead seabream evacuation portion turned the analysis of the absorbed fraction rather difficult, since it makes comparisons among the three AA solutions tested more complicated. Therefore, to facilitate this analysis, results were analyzed according to Aragão et al. (2004a). In this analysis, ^{14}C evacuation values were eliminated and the absorbed portion was recalculated. From the absorbed portion, results were divided into the labelled AA that were catabolized (trap) or retained, where FAA were considered together with the body portion. The retained fraction (FAA and body) was considered in such way because no major differences were obtained within the results of the FAA compartment. Therefore, the changes whereby obtained, essentially reflect differences in the body (mostly protein) compartment.

Concerning Senegalese sole, the retained fraction was significantly higher in Phe than in Mix solution at 16 (metamorphosis) and 34 DAH (post-metamorphosis; Fig. 1). The retained fraction at pre-metamorphosis (11 DAH) and metamorphosis (16 DAH) stages was higher in larvae tube-fed with Tyr than with Mix solution. In the post-metamorphosis stages (23 and 34 DAH) no significant differences were observed between Tyr and both Mix and Phe solutions.

In gilthead seabream, the retained fraction was significantly higher in Phe than in Mix and Tyr solutions at pre-metamorphosis state (26 DAH; Fig. 2). At this age, Tyr solution was proportionally more retained than Mix solution. Gilthead seabream with 33 (metamorphosis) and 46 DAH (post-metamorphosis) showed no significant differences for catabolized and retained fractions among the different AA solutions.

Some differences in the retained fraction of the AA solutions were also obtained along the ontogeny of both species. In Senegalese sole tube-fed with Mix and Phe, the retained fraction increased along development, achieving the highest value at post-metamorphosis (34 DAH). Concerning Tyr, the retained fraction was significantly higher during the metamorphosis period (16 DAH) than at all the other stages tested. In gilthead seabream, the retained fraction in Mix solution increased with development, being highest at the post-metamorphic state (46 DAH). Regarding Tyr, the retained fraction was not significantly

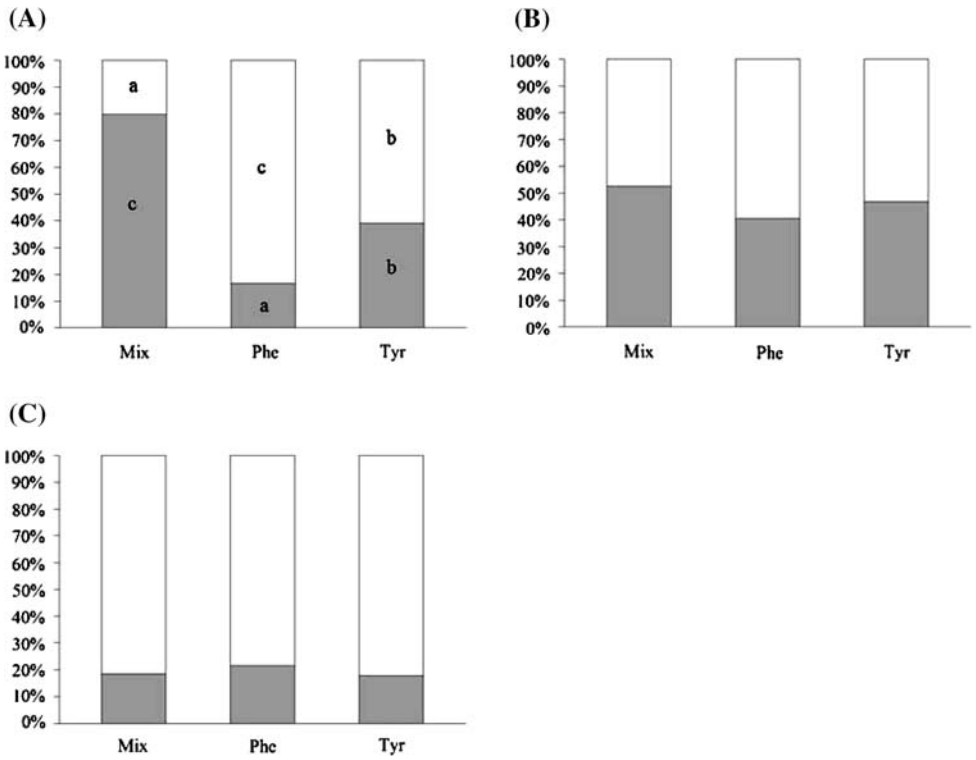

Fig. 2 Percentage of the absorbed tube-fed ^{14}C-labelled solution that was catabolized (*shaded square*) or retained (*open square*) by *S. aurata* at pre-metamorphic (26 days after hatching—DAH; **a**), metamorphic (33 DAH; **b**), and post-metamorphic (46 DAH; **c**) stages of development. Means ± SD ($n = 20$). *Different letters* within each compartment represent significant differences among tube-fed solutions. *Mix* L-[U-^{14}C] protein hydrolysate; *Phe* L-[U-^{14}C] phenylalanine; and *Tyr* L-[U-^{14}C] tyrosine

altered before the post-metamorphic state (46 DAH), suffering a significant increase at this stage.

Discussion

Different aromatic AA metabolism patterns were obtained during Senegalese sole and gilthead seabream ontogenesis. In Senegalese sole, data indicate high levels of tyrosine and phenylalanine retention in metamorphosing larvae (16 DAH). In gilthead seabream, the same trend was not observed, as highest aromatic AA retention values were achieved in post-metamorphic larvae (46 DAH). These differences likely reflect variations in the metamorphosis complexity that both species undergo. In Senegalese sole, the metamorphosis period is characterized by thyroid gland activation (Delgado et al. 2006). These authors suggested that in this species, the thyroid gland becomes functional during metamorphosis, being able to secrete and release hormones (T4 and T3) that are important for general metabolism and larval metamorphosis completion. However, in gilthead seabream, thyroid hormone levels were found to be stable throughout metamorphosis, increasing only after 52 DAH (Szisch et al. 2005). Being the backbone of thyroid hormones (Hamre et al. 2005) and high constituents of thyroid proteins (Delgado et al. 2006), phenylalanine and tyrosine may be of special importance for the fish larvae when high thyroid hormone secretion occurs.

Inui and Miwa (1985) showed that thyroid hormone treatment accelerated metamorphosis in Japanese flounder, while thyroid blockage with thiourea induced metamorphosis stasis. Since thyroid hormone production is dependent on the available amount of phenylalanine and tyrosine (Solbakken et al. 1999), it seems reasonable to consider that these amino acids may indirectly affect fish metamorphosis by influencing thyroid hormone production (Hamre et al. 2005; MacKenzie et al. 1998,). It is possible that particularly high aromatic AA retention values obtained during Senegalese sole's accentuated metamorphosis are related to an increase in thyroid hormone production. In gilthead seabream, the less complex metamorphosis is concomitant with a stable thyroid hormone production, and lower levels of aromatic AA retention indicate that these AAs assume less importance during this process.

In the current experiment, data showed higher retention values in the body portion of the tyrosine treatment for pre-metamorphic and metamorphic Senegalese sole larvae, as opposed to the FAA proportion where no differences were obtained. Whether tyrosine was converted to body lipids and carbohydrates or to protein is still an unresolved issue. However, based on the results from previous studies with Senegalese sole, it is more likely that the major part of tyrosine is retained in the form of protein. During the metamorphosing period Senegalese sole larval body is essentially constituted by protein (Ribeiro 2003). Moreover, Aragão et al. (2004b) found

that before and during the metamorphosing period the protein profile of Senegalese sole larvae is richer in tyrosine than after metamorphosis.

Although Senegalese sole larvae experience a series of complex changes in all organ systems and body conformation during the transition to the post-larval stage, they are known to constantly continue to feed and grow during metamorphosis (Fernández-Diaz et al. 2001; Parra and Yúfera 2001). At this stage, the reorganization of the digestive tract provides an increasing efficiency in digestive and absorption capacities (Ribeiro et al. 1999). High aromatic AA retention prior and during metamorphosis does not seem to be related with an inability to feed or to digest aromatic AA, but with an increase in the physiological requirements for these AA. Thus, it would also be interesting to verify if tyrosine and/or phenylalanine dietary supplementation prior and during metamorphosis would reduce metamorphosis-related problems in Senegalese sole rearing, such as pigmentation defects, arrest of metamorphosis or migration of the wrong eye (Power et al. 2001), and thereby optimizing larval performance.

It has been shown that fish larvae are able to discriminate dispensable (DAA) from indispensable (IAA) amino acids (Aragão et al. 2004a; Conceição et al. 2002; Rønnestad et al. 2001a). While DAA are preferentially used as energy substrates, IAAs are most commonly spared for growth. Although tyrosine is considered to be semi-IAA because it can only be synthesized from phenylalanine, it remains unclear if tyrosine conversion from phenylalanine is existent in early fish larvae. High tyrosine retention during Senegalese sole metamorphosis is an indicator that tyrosine may be conditionally indispensable during this species metamorphosis. Hence, it is possible that dietary improvements can be conducted in Senegalese sole rearing during this critical stage of development. In other flatfish, where metamorphosis is also a critical rearing point, these improvements can also be feasibly performed, possibly improving larval development. However, posterior clarification with biosynthesis studies is required to understand tyrosine conversion from phenylalanine in Senegalese sole and other flatfish early stage larvae.

In summary, differences were obtained for the aromatic AA metabolism during the ontogeny of Senegalese sole and gilthead seabream. While Senegalese sole showed high levels of phenylalanine and tyrosine retention during metamorphosis, gilthead seabream displayed more stable proportions of catabolism and retention of aromatic AA along ontogenesis. These differences probably reflect variations in the complexity of the metamorphosis that each species undergoes. In Senegalese sole, a more complex metamorphosis may increase specific physiological aromatic AA requirements during this critical period, probably for thyroid hormone production.

Acknowledgments The authors would like to acknowledge Viveiros Vila Nova, S.A. (Vila Nova de Mil Fontes, Portugal) for kindly donating seabream eggs which made these experiments possible. The authors are also deeply thankful to Julie Rowntree for a careful linguistic review of the manuscript. This work was supported by project POCI/CVT/60176/2004—SULFAAR, financed by POCI 2010 (FCT, Portugal), which is co-financed by FEDER. Wilson Pinto and Cláudia Aragão benefit from grants SFRH/BD/27889/2006 and SFRH/BPD/37197/2007 (FCT, Portugal), respectively.

References

Aragão C, Conceição LEC, Martins D, Rønnestad I, Gomes E, Dinis MT (2004a) A balanced dietary amino acid profile improves amino acid retention in post-larval Senegalese sole (*Solea senegalensis*). Aquaculture 233:293–304

Aragão C, Conceição LEC, Fyhn HJ, Dinis MT (2004b) Estimated amino acid requirements during early ontogeny in fish with different life styles: gilthead seabream (*Sparus aurata*) and Senegalese sole (*Solea senegalensis*). Aquaculture 242:589–605

Aragão C, Conceição LEC, Lacuisse M, Yúfera M, Dinis MT (2007) Do dietary amino acid profiles affect performance of larval gilthead seabream? Aquat Living Resour 20:155–161

Conceição LEC, Rønnestad I, Tonheim SK (2002) Metabolic budgets for lysine and glutamate in unfed herring (*Clupea harengus*) larvae. Aquaculture 206:305–312

Conceição LEC, Ribeiro L, Engrola S, Aragão C, Morais S, Lacuisse M, Soares F, Dinis MT (2007) Nutritional physiology during development of Senegalese sole (*Solea senegalensis*). Aquaculture 268:64–81

Delgado JBO, Ruane NM, Pousão-Ferreira P, Dinis MT, Sarasquete C (2006) Thyroid gland development in Senegalese sole (*Solea senegalensis* Kaup 1858) during early life stages: a histochemical and immunohistochemical approach. Aquaculture 260:346–356

Dinis MT, Ribeiro L, Soares F, Sarasquete C (1999) A review on the cultivation potential of *Solea senegalensis* in Spain and in Portugal. Aquaculture 176:27–38

Fernández-Diaz C, Yúfera M, Canavate JP, Moyano FJ, Alarcón FJ, Díaz M (2001) Growth and physiological changes during metamorphosis of Senegal sole reared in the laboratory. J Fish Biol 58:1086–1097

Hamre K, Moren M, Solbakken J, Opstad I, Pittman K (2005) The impact of nutrition on metamorphosis in Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquaculture 250:555–565

Imsland AK, Foss A, Conceição LEC, Dinis MT, Delbare D, Schram E, Kamstra A, Rema P, White P (2003) A review of the culture potential of *Solea solea* and *S-senegalensis*. Rev Fish Biol Fish 13:379–407

Inui Y, Miwa S (1985) Thyroid hormone induces metamorphosis in Flounder larvae. Gen Comp Endocr 60:450–454

MacKenzie DS, VanPutte CM, Leiner KA (1998) Nutrient regulation of endocrine function in fish. Aquaculture 161:3–25

Moretti A, Criado MPF, Cittolin G, Guidastri R (1999) Manual on hatchery production of seabass and gilthead seabream. Food and Agriculture Organization of the United Nations, Rome, 194 pp

Oikawa S, Itazawa Y (1984) Relative growth of organs and parts of the Carp, *Cyprinus Carpio*, with Special Reference to the metabolism–size Relationship. Copeia, 800–803

Parra G, Yúfera M (2001) Comparative energetics during the early development of two marine fish species, *Solea senegalensis* (Kaup) and *Sparus aurata* (L). J Exp Biol 204:2175–2183

Power DM, Llewellyn L, Faustino M, Nowell MA, Bjornsson BT, Einarsdottir IE, Canario AVM, Sweeney GE (2001) Thyroid hormones in growth and development of fish. Comp Biochem Phys C 130:447–459

Ribeiro L (2003) Ontogeny of *Solea senegalensis*: digestive system and nutritional aspects. Ph.D. thesis. Universidade do Algarve, Faculdade de Ciências do Mar e do Ambiente, Faro

Ribeiro L, Sarasquete C, Dinis MT (1999) Histological and histochemical development of the digestive system of *Solea senegalensis* (Kaup, 1858) larvae. Aquaculture 171:293–308

Rønnestad I, Conceição LEC, Aragão C, Dinis MT (2001a) Assimilation and catabolism of dispensable and indispensable free amino acids in post-larval Senegal sole *Solea senegalensis*. Comp Biochem Phys C 130:461–466

Rønnestad I, Rojas-Garcia CR, Tonheim SK, Conceição LEC (2001b) In vivo studies of digestion and nutrient assimilation in marine fish larvae. Aquaculture 201:161–175

Rust MB, Hardy RW, Stickney RR (1993) A new method for force-feeding larval fish. Aquaculture 116:341–352

Schreiber AM, Specker JL (1998) Metamorphosis in the summer flounder (*Paralichthys dentatus*). Stage-specific developmental response to altered thyroid status. Gen Comp Endocr 111: 156–166

Solbakken JS, Norberg B, Watanabe K, Pittman K (1999) Thyroxine as a mediator of metamorphosis of Atlantic Halibut, *Hippoglossus hippoglossus*. Environ Biol Fish 56:53–65

Szisch V, Papandroulakis N, Fanouraki E, Pavlidis M (2005) Ontogeny of the thyroid hormones and cortisol in the gilthead sea bream, *Sparus aurata*. Gen Comp Endocr 142:186–192

Weltzien FA, Planas M, Cunha I, Evjen MS, Fyhn HJ (1999) Free amino acid and protein contents of start-feeding larvae of turbot (*Scophthalmus maximus*) at three temperatures. Mar Biol 133:327–336

Zar JH (1999) Biostatistical analysis. Prentice-Hall International Editions, IL 662 pp