




*Short Communication*

## Variations in the concentration of free amino acids during the early development of the Pacific red snapper *Lutjanus peru*

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**ABSTRACT.** The free amino acids (FAAs) profile and its variations during the embryonic development and yolk-sac larvae of Pacific red snapper *Lutjanus peru* are described. The concentration of 15 FAAs (nine essentials and six non-essentials) was evaluated by reverse-phase high-performance liquid chromatography. The fertilized eggs of *L. peru* contain a small concentration of FAAs ( $4.77 \pm 0.89$  nmol ind<sup>-1</sup>). However, the variations in the FAAs pool during the early development are similar to the changes reported in other species with small pelagic eggs. The essential free amino acids are more abundant than the non-essential free amino acids throughout the studied period. During the egg stage, an increase in the FAAs was observed, and after hatching, isoleucine, leucine, lysine, and alanine were the FAAs that decreased the most during the yolk-sac larvae stage. The hatching rate was correlated with the total FAAs, the fraction of essential free amino acids, and the concentrations of glutamic acid, threonine, and valine in the egg stage, suggesting a possible role as a determinant in egg quality. Further studies are required to assess the role of FAAs in other aspects of egg development, like fertilization rate and egg buoyancy.

**Keywords:** *Lutjanus peru*; Pacific red snapper; free amino acids; early development; yolk-sac larvae

The Pacific red snapper *Lutjanus peru* is an important marine species in the local fisheries of Mexico's Pacific coast. To date, final maturation and spawning in captivity have been achieved by our laboratory (Pelcastre-Campos 2006). Like other subtropical and tropical species, *L. peru* spawns small eggs (diameter of <1 mm) at 26°C; hatching occurs 24 h after fertilization. The yolk-sac larvae have a fast rate of development, and in only 2-3 days after hatching, the nutritional sources are depleted, and the beginning of the exogenous feeding occurs (Peña et al. 2014).

The biochemical composition of the newly spawned eggs and its variations during embryonic and early development play a major role in determining egg quality

and yolk-sac larvae survival (Mommens et al. 2013). Amino acids are an important component in the egg. They are in a polymerized form in proteins and as a free pool. The pool of free amino acids (FAAs) may constitute up to 50% of the total amino acids in marine pelagic fish eggs (Thorsen & Fyhn 1996). Some studies have shown that FAAs have important functions acting as a major substrate for energy production of developing marine fish eggs and larvae (Fyhn & Serigstad 1987), in protein synthesis, carbohydrate, and cellular lipid metabolism, and function as signaling factors (Finn & Fyhn 2010).

The study of the FAAs profile during early development has been a valuable tool for gaining more

knowledge on egg quality (Bromage 1995, Seoka et al. 2004, Ceccon-Lanes et al. 2012) and the nutritional requirements in marine fish larvae (Fyhn 1989, Zakeri et al. 2014, Saavedra et al. 2015). It has also been used to recommend the nutritional composition of the food organisms or artificial feed used in first-feeding marine fish larvae. The FAAs profile and variations during the early development have been described in eggs and yolk-sac larvae of other marine fish species (Brown et al. 2005, Saavedra et al. 2015). The main objective of the present study was to evaluate the profile of the FAAs in eggs and yolk-sac larvae of *L. peru*, describe their fluctuations during early development and evaluate their implications on the egg quality of this species.

Animal manipulation in the present study was conducted under good laboratory animal care principles, according to the Official Mexican Standard NOM-033-SAG/ZOO-2014.

A total of 10 spawns of *L. peru* were obtained from mature wild-captured broodstock induced by hormonal injection, according to Pelcastre-Campos (2006). In every spawn, viable and non-viable eggs were separated by the float method using a 1 L test tube. The eggs were incubated in one 120-L incubation tank filled with mechanically (1  $\mu\text{m}$ ) filtered and UV-treated seawater at 26-27°C, 35 salinity, and natural photoperiod, with slight aeration from the bottom of the tank and water flow of 500 mL  $\text{min}^{-1}$ . The hatching rate (HR) and survival at first feeding (48 h after hatching, HAH) were evaluated in each spawn. Six random samples of 100-150 embryos were taken from the incubation tank after 10 h post-fertilization and placed in each of six 1 L plastic incubators filled with 500 mL of filtered and UV-sterilized seawater at the same conditions as in the incubation tank. After hatching, the organisms of three incubators were sampled and anesthetized with a 4% phenoxyethanol solution. Hatched larvae were counted using a dissection microscope, and the hatching percentage was evaluated. Around 48 HAH, the larvae in the other three incubators were sampled, and survival at first feeding was evaluated by identifying dead and live larvae.

Samples of eggs and larvae were taken in triplicate at different developmental stages in each spawn. Starting at fertilized eggs (2 h into incubation) and in blastula, gastrula, and newly-hatch larvae (hatching), at 24 and 48 HAH. All samples corresponded to floating (viable) eggs and were taken directly from the incubation tank using a 100  $\mu\text{m}$  sieve. Each sample was dried and weighted to the nearest 0.0001 g using a digital balance and stored at -80°C until analysis.

Before the amino acids analysis, all samples were lyophilized for 24 h. A total of 15 FAAs, nine essential (EFAAs): histidine (His), threonine (Thr), arginine (Arg), methionine (Met), valine (Val), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), and lysine (Lys); and six non-essential (NEFAAs): aspartate (Asp), glutamate (Glu), serine (Ser), glycine (Gly), alanine (Ala) and tyrosine (Tyr) were analyzed by reverse-phase high-performance liquid chromatography according to the methodology reported by Vázquez-Ortiz et al. (1995). Concentrations of FAAs are reported as  $\text{nmol ind}^{-1}$ . A one-way analysis of variance was performed to evaluate the differences in FAAs during the different developmental stages. Tukey's test was performed when significant differences among developmental stages were detected. The significance level in all the tests was set at  $P < 0.05$ . Additionally, Pearson's correlation considering hatching rate and survival at first feeding as the dependent variable and FAAs concentration during the different developmental stages as the independent variable was performed to evaluate the role of FAAs in these egg quality criteria.

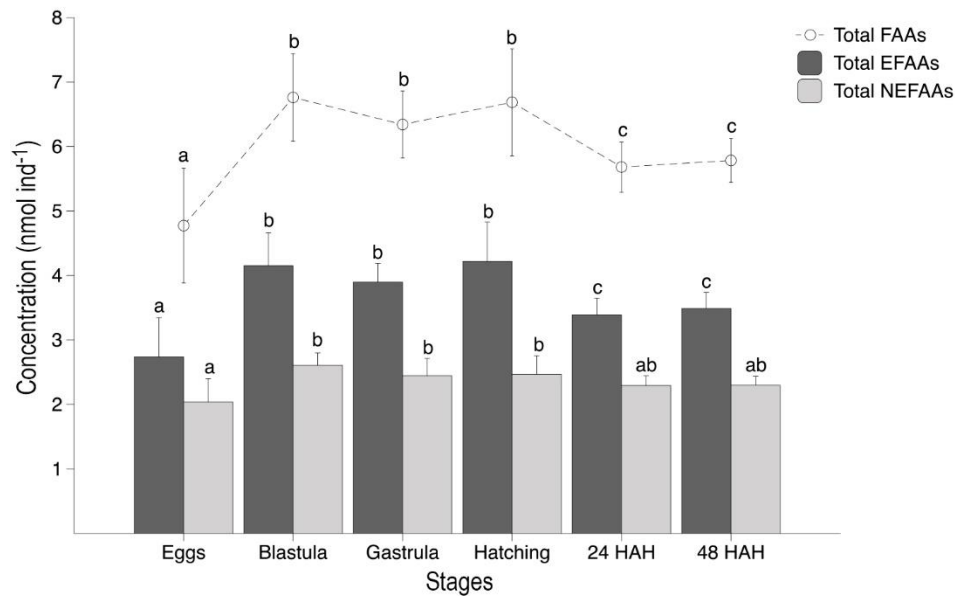
The general characteristics of the spawns of *L. peru* are presented (Table 1). The total egg volume ranged from 50 to 120 mL. The viable egg fraction was higher than the non-viable fraction in all the spawns. The hatching percentage ranged from 16.6-96.2%, mean of 68.1%, and yolk-sac larvae stage survival at first feeding fluctuated from 5 to 92%, mean of 48.5%.

The total concentration of FAAs varied during the egg and yolk-sac larvae stages of *L. peru*. The lowest concentration of FAAs was detected at the egg stage ( $4.77 \pm 0.89 \text{ nmol ind}^{-1}$ ), while the highest FAAs concentration was observed during the blastula stage ( $6.76 \pm 0.68 \text{ nmol ind}^{-1}$ ). However, no significant difference ( $P > 0.05$ ) between the gastrula stage and the newly hatched larvae was detected (Fig. 1). During the yolk-sac larvae stage, a significant ( $P < 0.05$ ) decrease in total FAAs was detected. It remained relatively constant at 24 and 48 HAH. The EFAAs fraction was higher than the NEFAAs fraction during all the developmental stages. Overall, the changes in concentration in the EFAAs were similar to those in the total FAAs: a low concentration in the egg stage, an increase during the embryonic development, and a decrease after hatching (Fig. 1). The NEFAAs concentration was low at the egg stage, increased during the embryonic development and remained relatively constant during all developmental stages and no significant differences were detected ( $P > 0.05$ ) (Fig. 1).

The variations of the individual amino acids are shown (Table 2). The concentrations of His, Thr, Arg,

**Table 1.** General characteristic of the spawns of the Pacific red snapper *Lutjanus peru* broodstock. FF: first feeding, SD: standard deviation.

	Spawn										Mean $\pm$ SD
	1	2	3	4	5	6	7	8	9	10	
Volume (mL)	110	120	100	90	85	90	100	50	80	70	89.5 $\pm$ 19.03
Viable eggs (mL)	90	80	85	70	50	60	70	30	50	40	62.5 $\pm$ 18.87
Non-viable eggs (mL)	20	40	15	20	35	30	30	20	30	30	27.0 $\pm$ 7.48
Hatching (%)	81.22	90.62	96.21	91.28	77.13	16.66	51.76	24.1	60.23	39.72	68.1 $\pm$ 27.45
Survival at FF (%)	92	73	42	32	85	0	28	0	80	53	48.5 $\pm$ 32.06

**Figure 1.** Variations of total free amino acids (FAAs), essential free amino acids (EFAAs), and non-essential free amino acids (NEFAAs) during early development of Pacific red snapper *Lutjanus peru*. Data are mean (n = 10). Vertical bars are standard deviations. Different superscript denotes significant differences at  $P < 0.05$ . HAH: hours after hatching.

Met, Val, Phe, Ile, Leu, and Lys was lowest at the egg stage. Except for Leu and Ile, the concentration of all other EFAAs showed a significant ( $P < 0.05$ ) increase at the blastula stage, followed by mild variations during embryonic development and hatching. At 24 HAH, a reduction in the concentration of all the EFAAs was observed. However, only in the case of Ile, Leu, and Lys was significant ( $P < 0.05$ ) (Table 2). No significant ( $P > 0.05$ ) difference was observed between their concentrations at 24 and 48 HAH.

Val and Leu were the most abundant EFAAs at the egg stage, representing 7.96 and 10.64% of the total FAAs. At the time of hatching, the most abundant EFAAs were Thr, Val, and Leu being 8.41, 8.63, and 8.33% of the FAAs, respectively. At 48 HAH, when the yolk sac was depleted, Thr and Val were the most abundant (8.41 and 8.80%, respectively). The EFAA

that changed the most was Leu decreasing from 10.64% in the egg stage to 6.27% at 48 HAH.

In the case of the NEFAAs, the concentrations of Asp, Glu, Ser, Gly, Ala, and Tyr increased from the egg stage to the blastula stage, although only Ala showed a significant increase ( $P < 0.05$ ) (Table 2). After the gastrula stage, the concentration of Asp showed an increase. On the other hand, the concentrations of Glu and Ser showed a decrease after the gastrula stage. The concentration of Gly showed a significant increase ( $P < 0.05$ ) during the yolk-sac larvae stage. The concentration of Ala diminished from the gastrula stage, although only a significant decrease was registered from 24 HAH. Tyr was the less abundant NEFAA and remained relatively constant throughout the early development (Table 2).

**Table 2.** Concentration of free amino acids (nmol ind<sup>-1</sup>) during early development of the Pacific red snapper *Lutjanus peru*. Data are the mean (n = 10) ± SD (standard deviation). Different superscript denotes significant differences (P < 0.05) between developmental stages. HAH: hours after hatching. His: histidine, Thr: threonine, Arg: arginine, Met: methionine, Val: valine, Phe: phenylalanine, Ile: isoleucine, Leu: leucine, Lys: lysine, Asp: aspartate, Glu: glutamate, Ser: serine, Gly: glycine, Ala: alanine, and Tyr: tyrosine.

Amino acid	Stages					
	Eggs	Blastula	Gastrula	Hatching	24 HAH	48 HAH
Essential (E)						
His	0.173 ± 0.06 <sup>a</sup>	0.423 ± 0.05 <sup>b</sup>	0.399 ± 0.03 <sup>b</sup>	0.431 ± 0.07 <sup>b</sup>	0.370 ± 0.06 <sup>b</sup>	0.393 ± 0.04 <sup>b</sup>
Thr	0.254 ± 0.08 <sup>a</sup>	0.552 ± 0.06 <sup>b</sup>	0.520 ± 0.03 <sup>b</sup>	0.562 ± 0.09 <sup>b</sup>	0.483 ± 0.04 <sup>b</sup>	0.486 ± 0.04 <sup>b</sup>
Arg	0.218 ± 0.08 <sup>a</sup>	0.377 ± 0.04 <sup>b</sup>	0.356 ± 0.02 <sup>b</sup>	0.383 ± 0.05 <sup>b</sup>	0.330 ± 0.03 <sup>b</sup>	0.338 ± 0.03 <sup>b</sup>
Met	0.250 ± 0.09 <sup>a</sup>	0.440 ± 0.06 <sup>b</sup>	0.415 ± 0.03 <sup>b</sup>	0.457 ± 0.07 <sup>b</sup>	0.386 ± 0.05 <sup>b</sup>	0.401 ± 0.03 <sup>b</sup>
Val	0.380 ± 0.15 <sup>a</sup>	0.560 ± 0.06 <sup>b</sup>	0.529 ± 0.03 <sup>b</sup>	0.577 ± 0.08 <sup>b</sup>	0.491 ± 0.04 <sup>b</sup>	0.509 ± 0.04 <sup>b</sup>
Phe	0.276 ± 0.09 <sup>a</sup>	0.397 ± 0.05 <sup>b</sup>	0.375 ± 0.03 <sup>b</sup>	0.438 ± 0.09 <sup>b</sup>	0.348 ± 0.03 <sup>b</sup>	0.357 ± 0.03 <sup>b</sup>
Ile	0.331 ± 0.05 <sup>a</sup>	0.366 ± 0.06 <sup>a</sup>	0.334 ± 0.04 <sup>a</sup>	0.348 ± 0.05 <sup>a</sup>	0.211 ± 0.02 <sup>b</sup>	0.235 ± 0.01 <sup>b</sup>
Leu	0.508 ± 0.13 <sup>a</sup>	0.589 ± 0.10 <sup>a</sup>	0.544 ± 0.06 <sup>a</sup>	0.557 ± 0.06 <sup>a</sup>	0.375 ± 0.03 <sup>b</sup>	0.363 ± 0.03 <sup>b</sup>
Lys	0.347 ± 0.07 <sup>a</sup>	0.448 ± 0.05 <sup>b</sup>	0.424 ± 0.02 <sup>b</sup>	0.465 ± 0.06 <sup>b</sup>	0.394 ± 0.03 <sup>a</sup>	0.404 ± 0.03 <sup>a</sup>
Total E	2.736 ± 0.61 <sup>a</sup>	4.151 ± 0.51 <sup>b</sup>	3.896 ± 0.29 <sup>b</sup>	4.218 ± 0.61 <sup>b</sup>	3.389 ± 0.26 <sup>c</sup>	3.486 ± 0.25 <sup>c</sup>
Non-essential (NE)						
Asp	0.250 ± 0.04 <sup>a</sup>	0.307 ± 0.03 <sup>ab</sup>	0.291 ± 0.03 <sup>ab</sup>	0.314 ± 0.03 <sup>ab</sup>	0.344 ± 0.04 <sup>b</sup>	0.349 ± 0.03 <sup>b</sup>
Glu	0.541 ± 0.12 <sup>ab</sup>	0.697 ± 0.10 <sup>a</sup>	0.647 ± 0.08 <sup>ab</sup>	0.608 ± 0.11 <sup>ab</sup>	0.550 ± 0.04 <sup>b</sup>	0.558 ± 0.04 <sup>b</sup>
Ser	0.210 ± 0.07 <sup>a</sup>	0.275 ± 0.06 <sup>a</sup>	0.278 ± 0.08 <sup>a</sup>	0.271 ± 0.09 <sup>a</sup>	0.212 ± 0.02 <sup>a</sup>	0.197 ± 0.02 <sup>a</sup>
Gly	0.352 ± 0.07 <sup>a</sup>	0.441 ± 0.07 <sup>a</sup>	0.404 ± 0.04 <sup>a</sup>	0.478 ± 0.13 <sup>a</sup>	0.600 ± 0.05 <sup>b</sup>	0.611 ± 0.06 <sup>b</sup>
Ala	0.552 ± 0.08 <sup>a</sup>	0.730 ± 0.09 <sup>b</sup>	0.665 ± 0.05 <sup>b</sup>	0.636 ± 0.08 <sup>ab</sup>	0.430 ± 0.05 <sup>c</sup>	0.446 ± 0.02 <sup>c</sup>
Tyr	0.132 ± 0.02 <sup>a</sup>	0.160 ± 0.02 <sup>a</sup>	0.160 ± 0.04 <sup>a</sup>	0.160 ± 0.05 <sup>a</sup>	0.157 ± 0.02 <sup>a</sup>	0.136 ± 0.02 <sup>a</sup>
Total NE	2.037 ± 0.03 <sup>a</sup>	2.609 ± 0.19 <sup>b</sup>	2.444 ± 0.27 <sup>b</sup>	2.467 ± 0.29 <sup>b</sup>	2.293 ± 0.15 <sup>ab</sup>	2.297 ± 0.14 <sup>ab</sup>

At the egg stage, the most abundant NEFAAs were Glu and Ala, representing 11.34 and 11.57% of the total FAAs. At hatching, they represented 9.10 and 9.51%, respectively. By 48 HAH, the most abundant NEFAAs were Glu and Gly, representing 9.65 and 10.57% of the total FAAs. The most drastic change of NEFAAs was in the concentration of Ala, which changed from 11.57% at the egg stage to 7.72% at 48 HAH. On the other hand, the Gly abundance increased from 7.37% at the egg stage to 10.57% at 48 HAH.

Pearson's correlations (*r*) were performed between the concentration of the FAAs during the different stages of early development and the floating rate, hatching rate, and larval survival at first feeding. The Arg concentration in the egg stage was the only FAA significantly correlated to the floating rate (*r* = 0.717). In the egg stage, the concentrations of total FAAs and EFAAs were negatively correlated with the hatching rate (*r* = 0.726 and *r* = 0.714, respectively). Among the NEFAAs, the concentration of Glu was the only one correlated with the hatching rate (*r* = 0.801). Two EFAAs were correlated with the hatching rate: Tre (*r* = 0.844) and Val (*r* = 0.718). In the gastrula stage, four EFAAs were correlated with the hatching rate being

Tre (*r* = 0.674), Arg (*r* = 0.636), Val (*r* = 0.663) and Lys (*r* = 0.649).

The survival at first feeding was correlated with the total concentration of the FAAs, the EFAAs, and the NEFAAs in the gastrula stage (*r* = 0.808; *r* = 0.791 and *r* = 0.698, respectively). The concentrations of the NEFAAs Glu and Gly in the gastrula stage were also correlated with survival at first feeding (*r* = 0.764 and *r* = 0.761, respectively). The concentrations of the EFAAs His (*r* = 0.891), Tre (*r* = 0.908), Arg (*r* = 0.830), Met (*r* = 0.865), Val (*r* = 0.866) and Lys (*r* = 0.817) also in the gastrula stage, were significantly correlated with larval survival at first feeding.

The newly fertilized *L. peru* eggs with an average diameter of 0.8 mm (Peña et al. 2014) show a total FAAs concentration of 4.7 nmol ind<sup>-1</sup>, considerably lower than those reported in other species. However, several factors have been described to affect the FAAs concentration in the egg stage, but particularly important have been the egg size and the presence of an oil globule (Finn & Fyhn 2010).

In the case of the *L. peru* eggs, the FAAs concentration is much lower than the concentration reported in the red snapper *Lutjanus campechanus*

(21.7 nmol egg<sup>-1</sup>) (Hastey et al. 2010). However, at 24 HAH, the FAAs concentration in *L. campechanus* newly hatched larvae ( $4.12 \pm 0.56$  nmol ind<sup>-1</sup>) is lower than the reported in the present study at 24 HAH ( $5.68 \pm 0.39$  nmol ind<sup>-1</sup>). Also, in *Dentex dentex* eggs, the initial concentration of FAAs was 53.6 nmol egg<sup>-1</sup> and in 1-day larvae, the concentration decreased to 6.0 nmol ind<sup>-1</sup> (Tulli & Tibaldi 1997). Similar results were reported in *Lates calcarifer* (Sivaloganathan et al. 1998), when after an initial concentration of 25.3 nmol egg<sup>-1</sup> at 40 h after spawning, the concentration decreased to 3.8 nmol ind<sup>-1</sup>. In both cases, the FAA concentration is lower than that recorded in the present study for *L. peru* at 24 and 48 HAH, respectively. Several factors may influence the differences in the total FAAs concentration in the eggs, including if the spawn was spontaneous or hormonally induced like in our study, the broodstock size, age, nutritional condition, the culture conditions of the broodstock, feeding conditions, tropical or temperate affinity (Bromage 1995), developmental stage of the eggs at sampling (Rønnestad et al. 1999, Moran et al. 2007), parental origin and analytical methodologies (Samae et al. 2010). In the case of *Anguilla japonica* eggs, Seoka et al. (2004) suggested that most of the proteins accumulated during vitellogenesis are not degraded to FAAs but remained as small proteins and peptides, which may be a reason for the low content of FAAs in the egg.

Interestingly, despite the big differences in FAAs concentration between the eggs of the species reported in many marine fish species, the FAAs pool's relative composition shows high similarities. In *L. peru* eggs, the most abundant EFAAs are Val and Lys, while the most abundant NEFAAs are Glu and Ala. These same FAAs are among the most abundant in eggs of many species. It has been suggested that the hydrolysis of a common protein may explain this consistency in FAAs composition among fish eggs at the time of oocyte hydration (Fyhn et al. 1999), since the FAAs pool is established during the final maturation of the oocyte to initiate an osmotic influx that swells the oocyte prior to ovulation and spawning (Rønnestad et al. 1999). Also, the high proportions of the same FAAs in fish eggs of very different species may respond to their role in the same developmental processes (Li et al. 2009).

After fertilization, the FAAs are an important energy source during embryonic development. In this regard, glycolytic metabolism is an important component of energy production during the early embryogenesis of fishes, particularly during gastrulation, where high levels of lactate dehydrogenase activity have been

reported (Boulekbache 1981). In *L. peru* embryo, high lactate dehydrogenase and glucose-6-phosphatase have been reported during early segmentation and blastula stages (Moguel-Hernández et al. 2015).

After hatching, the concentration of FAAs continuously decreased until the depletion of the yolk sac. Overall, using FAAs as a substrate for metabolic energy varies from 70-90% in the early embryo to around 10-20% during the last part of the yolk sac larvae (Rønnestad et al. 1992b, Sivaloganathan et al. 1998). Nevertheless, it was suggested that the amount, and therefore the role of the FAAs as energy substrates might be larger in species which depend exclusively on the yolk sac than in the species where an oil globule is present and that it may be thermo-dependent (Rønnestad et al. 1994, Sivaloganathan et al. 1998). Based on the initial increment from fertilized eggs to the blastula stage and the small variations of the FAAs pool during the embryonic stage observed in our study, it is likely that FAAs were not used as the major energy source during *L. peru* early development, suggesting that these species may use different energy substrates like carbohydrates or lipids since dispersed glycogen or lipids droplets have been reported in the yolk (Cetta & Capuzzo 1982, Finn et al. 1996).

The FAAs that showed a significant decrease in concentration during the yolk-sac larvae stage in *L. peru* were the EFAAs Ile, Leu, and Lys. In the NEFAA fraction, only the Ala concentration significantly decreased, supporting the hypothesis of differential use of amino acids in the same metabolic and energetic processes during the early larval development (Li et al. 2009). Only Asp and Tyr concentrations did not decrease and remained relatively constant after hatching. The same trend was reported in cod *Gadus morhua* (Fyhn & Serigstad 1987), turbot *Scophthalmus maximus* (Rønnestad et al. 1992b), lemon sole *Microstomus kitt* (Rønnestad et al. 1992a), and the seabass *L. calcarifer* (Sivaloganathan et al. 1998).

Numerous criteria have been used to assess, control or predict egg quality (Peña 2015). Some criteria to evaluate egg quality include floating rate, hatching rate, and larval survival. The proportion of sinking and floating eggs in the spawns is considered a quality criterion. The hatching rate was correlated with the total FAAs and EFAAs concentration and with Glu, Tre, Val in the egg stage and the concentration of Tre, Arg, Val, and Lys in the gastrula stage, suggesting that FAAs may have a role in the hatching rate of *L. peru* eggs. In *Hippoglossus hippoglossus*, Ser, Arg, and Val concentrations were correlated with the hatching rate (Mommens et al. 2013). In the common dentex *D.*

*dentex*, the total content of FAAs and the Glu concentration in the eggs was also correlated with the hatching rate (Samaee et al. 2010). A series of ratios of different FAAs were significantly correlated with the hatching rate, the survival rate, and the floating rate. The authors suggested the importance of the FAAs' interrelations during fish development compared to the effect of each FAA individually. In the analyzed *L. peru* spawns, the floating rate varied between 57 to 85%, and only the Arg concentration in the eggs showed a significant correlation ( $P = -0.717$ ) with the floating rate. In *D. dentex*, the total concentration of FAAs was correlated with the floating rate (Samaee et al. 2010).

In conclusion, the variations in the FAAs during the early development of *L. peru* are similar to other species with small eggs and the presence of an oil globule despite the big difference in the initial concentration. The EFAAs are the most abundant fraction during early development. Ile, Leu, Lys, and Ala are the FAAs that decreased the most during the yolk-sac larvae stage. The hatching rate correlates with the total FAAs, the EFAAs fraction, and Glu, Tre, and Val concentrations in the egg stage. However, further studies are required to evaluate the role of FAAs in other aspects of the embryonic development in Pacific red snapper, like fertilization rate and egg buoyancy, and as potential biomarkers to predict egg quality.

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