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NOTE

RNA, DNA and protein concentrations and amino acid profiles of deep-sea decapod *Aristeus antennatus*: An indication for seasonal variations of nutrition and growth

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Abstract – Seasonal changes in nucleic acid concentrations and amino acid profiles in the muscle of juvenile *Aristeus antennatus* were investigated. RNA content, RNA:DNA and RNA:protein ratios varied significantly between seasons ($p < 0.05$), being the lowest and the highest values obtained in winter and spring/summer. A significant variation in the total amino acid content (TAA) from winter to summer was observed ($p < 0.05$). In fact, during this period a significant percent of increase in total non- (NEAA; 20.2%) and essential amino acids (EAA, 18.7%) contents occurred. Concomitantly, a significant decrease in the free amino acid (FAA) content from winter to summer was observed. A higher percentage of decrease with free essential amino acids (FEAA; 56.6%) in comparison to free non-essential amino acids (FNEAA; 34.2%) was attained. The significant increase in RNA and TAA contents from winter to summer may be related with protein synthesis. On the other hand, the lowest values obtained in winter may be due to a reduction in feeding activity; in this period the muscle protein must be progressively hydrolysed, which is evident with the higher FAA content. The liberated amino acids enter FAA pool and become available for energy production.

Key words: Nucleic acids / Amino acid / Growth / Nutritional condition / *Aristeus antennatus* / Red shrimp

1 Introduction

The red shrimp, *Aristeus antennatus* (Risso 1816) has a wide geographical distribution, being found throughout the Mediterranean, as well as in Eastern Atlantic, from the Portuguese south coast to the Green Cape Islands (Holthius 1980). Biochemical indicators reflecting muscle growth could be an interesting alternative to the classical methods of evaluating growth rates. Moreover, it is worth noting that muscle growth rate cannot be estimated by temporal changes in crustacean body mass, since growth is constrained by the carapace and because proteins gradually replace tissue water during growth (Passano 1960) with no appreciable change in density (Mayrand et al. 2000). Of the biochemical indices, tissue RNA concentrations/ratios are increasingly used as indices of growth rate and nutritional condition. In fact, the RNA/DNA index is based on the assumption that the amount of deoxyribonucleic acid (DNA), the primary carrier of genetic information, is stable under changing environmental situations, where as the amount of ribosomal ribonucleic acid (RNA), which serves as both as template and an organizer for protein synthesis, is affected by the nutritional state of the organism (Chícharo et al. 1998). Consequently, this ratio is susceptible to changes in the environment, which affect the physiology of the organism, e.g. low prey availability, and should be a good indicator of the condition of the individuals from wild populations. Individuals in good condition tend to have higher RNA:DNA ratios than those in poorer condition (Clemmesen 1994). Thus, the ratio has been used to give a measure of instantaneous growth in the field, thus avoiding the need for repeated measurements (Buckley et al. 1999).

Amino acids are precursors of proteins and also act as an energy source. Deficiencies or excess of one or more essential amino acids limit protein synthesis and growth or both (Litaay et al. 2001). In addition, previous research has shown that muscle proteins (protein-bound amino acids) are the main reserve depleted during starvation in Crustacea (Dall 1981; Dall and Smith 1987). According to these authors, the muscle protein is progressively hydrolysed, but with the remaining muscle maintaining its amino acid composition. In fact, during that period of starvation the abdominal muscle makes the largest contribution of protein to energy metabolism and small changes in this tissue are sufficient to make a substantial contribution to the overall animal maintenance (Barclay et al. 1983).

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The liberated amino acids, i.e. the free amino acids (FAA), constitute a labile pool available for energy production, since most are non-essential.

The objective of this study was to describe the seasonal variations in nucleic acids concentrations in the muscle of juvenile *Aristeus antennatus* and its relation to growth and nutritional condition. Concomitantly, and since greater precision in defining changes in nutritional status may be achieved by combining the nucleic acids analyses with other biochemical indicators, the total (protein bound + free) and free amino acid profiles were determined throughout the year.

2 Material and methods

2.1 Sampling

Juvenile specimens (immatures with <24 mm of carapace length and <12 g of total weight) were taken seasonally aboard the commercial trawl vessel "Costa Sul" in Portimão (south coast of Portugal). In each season, one or two sampling periods were done (Autumn – October 2000; Winter – January and February 2001; Spring – April and May 2001; Summer – July and August 2001), consisting of twenty to thirty immature shrimps (per season). Approximately 500 mg of muscle tissue was excised from each specimen, pooled in triplicate (with a manual homogeniser) and frozen immediately in liquid nitrogen and then stored at −80 °C in the laboratory. The maturation scale of Arculeo et al. (1995) was used. Since in crustaceans moulting hormones may stimulate synthesis of both DNA and RNA, this study was carried out using intermoult individuals. RNA concentration has also been shown to change when organisms are acclimated to different temperatures (Parslow-Williams et al. 2001). In the present study, the specimens were in caught between 200 and 450 m; at these depths the seasonal fluctuations of sea temperature in the south coast of Portugal are not relevant (<0.3 °C between 250 and 500 m). It is worth noting that in this coast the deep sea environment is influenced by the Mediterranean waters outflow, which runs below the 500 m isobath, along the south coast (Fiúza et al. 1998).

The nucleic acids and protein concentrations were determined in triplicate per sampling period ($n = 6$ per season; with the exception of autumn, $n = 3$) and the amino acids profiles were done in triplicate in one sampling period (Autumn – October; Winter – February; Spring – May; Summer – August).

2.2 Nucleic acids analyses and protein determination

Nucleic acids were extracted from the muscle using a modified Schmidt-Thannhauser method described by Moss (1994). RNA concentrations were determined using orcinol method (Mejbaum 1939). Samples (250 mg of fresh-frozen tissue) were placed in centrifuge tubes and 4 ml of cold 0.22 M perchloric acid (PCA) were added. After being stored at 4 °C for 15 min, the samples were centrifuged at 3000 × g (4 °C) for 15 min. The supernatant was discarded, and the precipitate was washed twice PCA using the above procedure. The resulting precipitate was dissolved in 4 ml 0.3 N potassium hydroxide (KOH) while incubating in a hot-water bath (38 °C) for 2 h. Standards of Torula yeast (Sigma) were prepared in the same manner. The tubes containing the samples and standards were cooled prior to the addition of 1 ml PCA and further centrifugation. RNA was determined by adding 2 ml orcinol reagent to 1ml of the supernatant, which was then incubated in a hot-water bath (98 to 100 °C) for 20 min. RNA samples were cooled and the absorbance values at 665 nm were determined using a spectrophotometer.

DNA concentrations were determined using the dual wavelength method (Wilder and Stanley 1983). Samples (50 mg) of tissue and standards (calf thymus DNA, Sigma) were placed in centrifuge tubes to which 5 ml of 0.5N PCA were added and the tubes were heated at 70 °C for 30 min. After cooling and centrifugation (3000 g, 15 min), the absorbance of the samples at wavelengths of 232 and 260 nm was measured.

Protein concentration was determined on 100 mg of wet tissue using using the Bio-Rad protein assay (BIO-RAD), which is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Bovine gamma globulin (BIO-RAD) was used as a standard.

2.3 Amino acid analyses

In order to determinate the total amino acid profile, proteins were hydrolysed with 6 N hydrochloric acid (containing 0.1% phenol) in a MLS-1200 Mega Microwave System (Milestone), at 800 W, 160 °C for 10 min. The hydrolysis was performed under inert and anaerobic conditions to prevent oxidative degradation of amino acids. The hydrolysates were filtered and dissolved in sodium citrate buffer pH 2.2. Amino acids were separated by ion exchange liquid chromatography in an automatic analyser Biochrom 20 (Amersham Biosciences), equipped with a column filled with a polysulphonated resin (250 × 4.6 mm), using three sodium citrate buffers – pH 3.20, 4.25 and 6.45 (Amersham Biosciences) and three different temperatures (50 °C, 58 °C and 95 °C).

The extraction of the free amino acids was done according to the method described by Gras et al. (1978). Samples were precipitated with a solution of 10% trichloroacetic acid. Following centrifugation, the supernatant was filtered and the pH was adjusted to 2.80 with 1M lithium hydroxide. Free amino acids were also separated by ion exchange liquid chromatography (Biochrom 20), using five lithium citrate buffers – pH 2.80, 3.00, 3.15, 3.50 and 3.55 (Amersham Biosciences) and three different temperatures (34 °C, 76 °C and 85 °C). The detection of amino acids was done at 440 nm and 570 nm after reaction with ninhydrin (Amersham Biosciences). Amino acids were identified by comparison of their retention time with those of specific standards (Sigma) and quantified with the software EZChrom™ Chromatography Data System, vers. 6.7. (Scientific Software Inc.) using norleucine (Sigma) as internal standard.

2.4 Statistical analysis

Data were analysed by one-way ANOVA, using the software Statistica (version 4.5). Previously, normality and

homogeneity of variances were verified by Kolmogorov-Smirnov and Bartlett tests, respectively. When data did not meet the assumptions of ANOVA, the nonparametric ANOVA equivalent (Kruskal-Wallis test) was performed. Having demonstrated a significant difference within the groups with the ANOVA and Kruskal-Wallis test, the Tukey Test or the Dunn Test were applied, respectively, to find out where those differences were (Zar 1996).

3 Results

3.1 Nucleic acids, RNA:protein and RNA:DNA ratios

The seasonal variation in the nucleic acids contents and RNA:protein and RNA:DNA ratios of the muscle of *Aristeus antennatus* is showed in Figure 1. RNA content varied significantly between seasons ($F_{3,20} = 20.68$, $p < 0.05$), being the highest value attained in summer (375.7 μg 100 mg^{-1} wet weight). In both RNA:DNA and RNA:protein ratios significant seasonal differences occurred ($F_{3,20} = 13.48$ and 29.12, $p < 0.05$, respectively) being the lowest and the highest values obtained in winter and spring/summer, respectively.

3.2 Total and free amino acids

Seasonal variations of total (protein bound + free) amino acid composition (% wet weight) in the muscle of *Aristeus antennatus* are presented in Table 1. A significant increase from winter to summer was observed ($p < 0.05$) (statistical differences are summarized in the table with superscripts). The major total essential amino acids (EAA) were, by order of decreasing magnitude, arginine, histidine and leucine; in relation to total non-essential amino acids (NEAA), the quantitatively most important were glutamic acid, aspartic acid, proline and glycine. From winter to summer, a significant variation in respect to NEAA content occurred (20.2%; $p < 0.05$), mainly due to the significant increase of glycine (36.6%) and serine (22.7%) ($p < 0.05$). EAA content also increased significantly (18.7%) ($p < 0.05$), due to the significant raise of arginine (30.5%), histidine (28.2%) and methionine (22.8%).

The free amino acid (FAA) content varied significantly ($p < 0.05$) throughout the year (Table 2). A significant decrease was observed from winter to summer ($p < 0.05$; statistical differences are summarized in the table with superscripts). The quantitatively most important free non-essential amino acids (FNEAA) were proline, glycine, taurine and glutamine. The free essential amino acids (FEAA) also revealed significant variations ($p < 0.05$) and the most important were arginine, leucine and histidine. From winter to summer a higher percentage of decrease was attained with FEAA (56.6%) in comparison to FNEAA (34.2%), mainly due to the significant decrease of isoleucine (96.3%), histidine (84.1%), phenylalanine (78.2%) and leucine (75.9%).

4 Discussion

Food and temperature are the two main environmental parameters affecting growth rates (Gage and Tyler 1991).

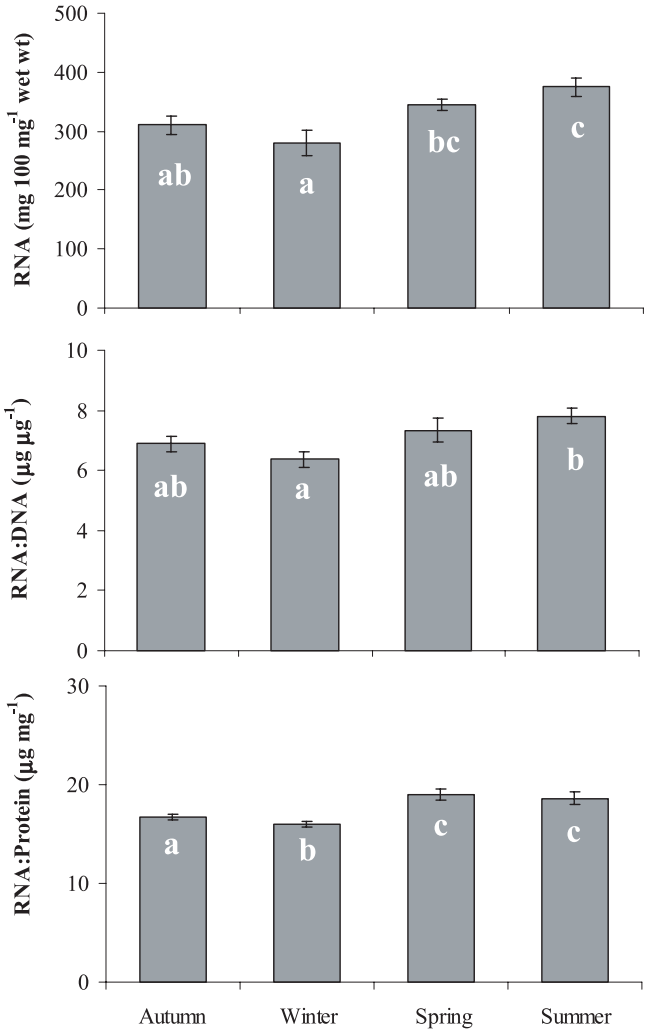

Fig. 1. Seasonal variation in nucleic acids contents (μg 100 mg^{-1} wet weight) and RNA:Protein (μg mg^{-1}) and RNA:DNA (μg μg^{-1}) ratios of the muscle of juvenile *Aristeus antennatus*. Values are means ± S.D. ($n = 6$). Different letters represent statistically significant differences ($p < 0.05$).

In crustaceans, the amount and/or quality of food and the water temperature influence the duration of the intermoult period and the moult size increment, and both cause an increase or decrease in growth rates, with faster growth rates at higher food concentrations and higher temperatures (Hartnoll 1983). The biological response to this seasonal variability has profound affects on the biochemical composition of the organisms (Rosa and Nunes 2003).

Due to the fact that deep-sea is characterized by environmental stability, the influence of seasonal temperature fluctuations may be not significant (<0.3 °C in the study area) to explain the seasonal differences in nucleic acid concentrations of *Aristeus antennatus*. Consequently, growth and nutritional condition of juvenile *A. antennatus* seem to be influenced primarily by biotic factors (e.g. food availability or food intake). Positive relationships between the RNA:DNA ratio and feeding level have been reported in several crustacean species (Parslow-Williams et al. 2001). While these studies were done

Table 1. Seasonal variation of total (protein bound + free) amino acid composition (% wet weight) in the muscle of *Aristeus antennatus.* Values are means of triplicate samples ± S.D. Different superscript letters within rows represent significant differences ($p < 0.05$).

Amino acids	Autumn	Winter	Spring	Summer
Essential (EAA)				
Threonine	0.7 ± 0.0^{a}	0.6 ± 0.0^{b}	0.7 ± 0.0^{a}	0.7 ± 0.0^{a}
Methionine	$0.5 \pm 0.0^{a,b}$	0.5 ± 0.0^{a}	$0.5 \pm 0.0^{a,b}$	0.6 ± 0.0^{b}
Isoleucine	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.1
Leucine	1.4 ± 0.1^{a}	1.3 ± 0.1^{b}	1.4 ± 0.1^{a}	1.5 ± 0.0^{a}
Phenylalanine	$0.7 \pm 0.0^{a,b}$	0.7 ± 0.0^{a}	$0.8 \pm 0.0^{a,b}$	0.8 ± 0.0^{b}
Lysine	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
Histidine	$1.6 \pm 0.1^{a,b}$	1.5 ± 0.1^{b}	$1.6 \pm 0.1^{a,b}$	1.7 ± 0.0^{b}
Arginine	1.7 ± 0.1^{a}	1.7 ± 0.0^{a}	$1.8 \pm 0.1^{a,b}$	2.2 ± 0.1^{b}
Σ EAA	$7.6 \pm 0.2^{a,b}$	7.1 ± 0.2^{a}	$7.8 \pm 0.4^{a,b}$	8.5 ± 0.2^{b}
Non-essential (NEAA)				
Aspartic acid	1.8 ± 0.1^{a}	1.7 ± 0.1^{b}	1.7 ± 0.0^{a}	2.0 ± 0.0^{c}
Serine	$0.7 \pm 0.1^{a,b}$	0.6 ± 0.0^{a}	$0.7 \pm 0.1^{a,b}$	0.8 ± 0.0^{b}
Glutamic acid	$2.8 \pm 0.1^{a,b}$	2.7 ± 0.1^{b}	$2.9 \pm 0.1^{a,b}$	3.1 ± 0.1^{b}
Glycine	1.1 ± 0.1^{a}	1.0 ± 0.1^{b}	1.2 ± 0.1^{a}	1.4 ± 0.1^{c}
Alanine	1.0 ± 0.1^{a}	0.9 ± 0.1^{b}	1.0 ± 0.1^{a}	1.1 ± 0.0^{a}
Valine	0.8 ± 0.1	0.7 ± 0.0	0.8 ± 0.1	0.8 ± 0.1
Cystine	0.0 ± 0.0^{a}	0.1 ± 0.0^{b}	0.0 ± 0.0^{a}	$0.1 \pm 0.0^{a,b}$
Tyrosine	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.1
Proline	$1.4 \pm 0.1^{a,c}$	1.2 ± 0.0^{b}	$1.4 \pm 0.1^{a,c}$	1.4 ± 0.1^{c}
Σ NEAA	10.4 ± 0.2^{a}	9.5 ± 0.2^{b}	10.6 ± 0.2^{a}	11.3 ± 0.1^{c}
Σ TAA	18.0 ± 0.4^{a}	16.7 ± 0.3^{b}	18.3 ± 0.2^{a}	19.8 ± 0.2^{c}

with whole animals, Houlihan et al. (1990) examined changes in muscle and found that the RNA concentration in this tissue matches the short-term effect of starvation and refeeding in *Carcinus maenas*.

Deep-water habitats are characterized by relative scarcity of available food resources (Labropoulou and Kostikas 1999). Though there is no evidence of a decreasing feeding activity during this period of the year, the diet composition of *Aristeus antennatus* should vary significantly between seasons like the other deep-sea crustaceans species, since these changes correspond basically to the period of abundance of the different dietary group in the deep-sea environment (Cartes and Sardà 1989). Moreover, examination and analyses of the stomach contents of other deep-sea decapod species revealed significant seasonal variations in the feeding activity through the year in the Portuguese south coast (Cristo and Cartes 1998).

The seasonal variations in the amino acid profiles of *Aristeus antennatus* appear to corroborate the findings obtained with nucleic acids analysis. In fact, the significant increase in RNA and TAA contents from winter to spring may be related with growth and protein synthesis. On the other hand, the lowest values obtained in winter may be due to a reduction in feeding activity; in this period the muscle protein must be progressively hydrolysed. Protein muscle loss during starvation has been observed in *Nephrops norvegicus* (Dall 1981). It appears that, because of a lack of lipid and carbohydrates muscle reserves, the hydrolysis of the abdominal-muscle hydrolysis replenish the FAA pool. The liberated amino acids enter the FAA pool and become available for energy production.

Helland et al. (2003) showed in *Artemia franciscana* an initial increase in FAA content at the beginning of the starvation period and a consistent reduction of the protein content. However, the protein content continued to diminish at a fairly even rate throughout the experimental period as the total FAA content showed a reduction and then stabile amount, suggesting that the amino acids were rapidly catabolised. According to Mente et al. (2002) findings with *Litopenaeus vannamei*, although there was some variation in the FAA pool concentration of individual amino acids, the total level of FEAA and FNEAA in the muscle pool remain stable. Thus, tissue free pools are to some extent defended against sudden changes in concentration. Dall and Smith (1987) showed the apparent lability during starvation of the more abundant FAA in *Penaeus esculentus*, particularly proline, which virtually disappeared from the FAA pool after 15 d starvation. Barclay et al. (1983) verified a reduction of TAA in the muscle during starvation in *P. esculentus*. The role of certain FAA in energy metabolism is well established in invertebrates, like arginine in crustaceans (Beis and Newshome 1975) and proline in cephalopods (Hochachka et al. 1983). However, there is few information regarding possible roles of other FAA in energy metabolism. For example, the metabolic role of glycine, one

Table 2. Seasonal variation in free amino acid composition (mg/100 g wet weight) in the muscle of *Aristeus antennatus*. Values are means of triplicate samples ± S.D. Different superscript letters within rows represent significant differences ($p < 0.05$).

Free amino acids	Autumn	Winter	Spring	Summer
Essential				
Threonine	70.5 ± 8.3[a]	95.2 ± 12.1[a]	34.4 ± 13.5[b]	23.6 ± 6.1[b]
Methionine	39.3 ± 11.0[a]	56.9 ± 8.1[a]	22.8 ± 8.8[b]	17.1 ± 3.3[b]
Isoleucine	45.0 ± 8.7[a]	62.7 ± 6.6[b]	3.3 ± 0.5[c]	2.3 ± 0.3[c]
Leucine	76.7 ± 13.0[a]	118.3 ± 19.0[b]	38.3 ± 13.4[c]	28.5 ± 7.0[c]
Phenylalanine	22.0 ± 4.9[a]	48.9 ± 7.5[b]	14.8 ± 6.6[a,c]	10.6 ± 3.0[c]
Lysine	33.8 ± 7.4[a,b]	44.9 ± 5.2[a]	21.9 ± 6.8[b]	19.2 ± 5.2[b]
Histidine	139.3 ± 12.3[a]	157.8 ± 19.8[a]	32.2 ± 10.7[b]	25.1 ± 5.6[b]
Arginine	961.2 ± 62.8[a]	1328.4 ± 45.4[b]	799.9 ± 55.3[b]	706.3 ± 68.5[b]
Σ FEAA	1387.7 ± 78.2[a]	1913.1 ± 83.1[b]	967.5 ± 64.5[c]	830.4 ± 96.7[c]
Non-essential				
Phosphoserine	16.7 ± 4.2[a]	16.0 ± 2.3[a]	2.5 ± 1.6[b]	2.1 ± 1.9[b]
Taurine	302.1 ± 33.9	272.3 ± 31.3	269.1 ± 42.4	258.5 ± 48.4
Aspartic acid	5.7 ± 3.8[a]	3.5 ± 0.9[b]	tr	tr
Serine	94.0 ± 25.5[a]	48.0 ± 5.3[b]	51.6 ± 16.5[b]	40.3 ± 9.1[b]
Asparagine	71.6 ± 9.5[a]	88.4 ± 8.9[a]	35.4 ± 10.7[b]	31.5 ± 7.6[b]
Glutamic acid	66.6 ± 6.4[a]	52.0 ± 3.8[a]	40.1 ± 5.6[a,b]	28.9 ± 9.4[b]
Glutamine	140.4 ± 25.9[a]	146.0 ± 17.6[a]	182.8 ± 23.2[b]	132.0 ± 19.0[a]
Glycine	702.9 ± 64.8[a]	430.9 ± 55.5[b]	502.3 ± 49.8[b]	473.5 ± 36.6[b]
Alanine	280.0 ± 54.5[a]	247.5 ± 27.4[a]	178.0 ± 41.6[b]	153.0 ± 37.6[b]
α- Amino-n-butyric acid	8.7 ± 0.8[a]	17.0 ± 1.2[b]	3.0 ± 0.9[c]	3.1 ± 1.5[c]
Valine	67.9 ± 7.4[a]	62.2 ± 4.6[a]	38.5 ± 12.3[b]	29.1 ± 6.9[b]
Cystine	2.1 ± 1.3[a]	0.3 ± 0.3[b]	2.6 ± 0.9[a]	3.4 ± 0.5[a]
Tyrosine	47.2 ± 8.7[a]	91.3 ± 19.3[b]	28.2 ± 9.8[c]	19.7 ± 4.5[d]
β- Aminoisobutyric acid	5.0 ± 1.2[a]	19.6 ± 10.5[b]	2.3 ± 1.0[a,c]	0.8 ± 1.3[c]
Homocystine	1.3 ± 0.2[a]	6.5 ± 2.1[b]	tr	tr
Ornithine	5.8 ± 1.3[a]	11.1 ± 1.5[b]	2.8 ± 0.8[a]	2.1 ± 1.0[a]
Hydroxyproline	2.3 ± 1.4[a]	10.4 ± 3.8[b]	3.2 ± 1.9[a]	4.0 ± 2.1[a]
Proline	620.7 ± 45.8[a]	946.0 ± 55.1[b]	624.8 ± 87.8[a]	429.1 ± 64.3[c]
Σ FNEAA	2445.9 ± 103.7[a]	2469.2 ± 81.9[a]	1967.2 ± 75.9[b]	1624.9 ± 92.6[c]
Σ FAA	3833.6 ± 81.3[a]	4382.3 ± 124.1[b]	2934.7 ± 70.4[c]	2455.3 ± 117.3[d]

tr - trace.

of the most abundant amino acids and is the simplest, is uncertain (Claybrook 1983).

5 Conclusion

It seemed evident that the seasonal cycle in activities such as feeding, which has an effect on the nutritional condition of the organisms, and growth was noticed with nucleic acids analyses. Moreover, a corroboration of those findings with the amino acid analyses was attained. In fact, the determination of these nitrogen compounds provides a greater precision in defining changes in nutritional status and growth of *Aristeus antennatus*. Further work is necessary to improve the knowledge in protein synthesis, accretion and degradation and amino acid flux in deep-sea crustaceans.

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