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Research Article

Gross chemical profile and calculation of nitrogen-to-protein conversion factors for nine species of fishes from coastal waters of Brazil

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ABSTRACT. The amino acid composition and contents of nitrogen, phosphorus, lipid, carbohydrate and protein were determined in muscles of *Dactylopterus volitans*, *Genypterus brasiliensis, Mullus argentinae, Paralichthys patagonicus, Percophis brasiliensis, Pinguipes brasilianus, Rhizoprionodon lalandii, Rhizoprionodon porosus*, and *Urophycis cirrata*. The samples showed low carbohydrate content (<3.5% dry weight in all species) and were rich in protein (>66% dry weight in all species). The percentage of total lipid varied widely among species, and *M. argentinae* showed the highest concentrations (16%). The percentage of nitrogen and phosphorus was high and similar among species, with overall average values of 13.3% and 1.2%, respectively. The amino acids composition was similar among the animals, with glutamic acid and lisine as the most abundant amino acid and histidine in low concentrations. Among species, the content of proteinaceous nitrogen was high, with an average of 96.8% of the total nitrogen. From data of total amino acid and total nitrogen, specific nitrogen-to-protein conversion factors were calculated for each species. The nitrogen-to-protein conversion factors ranged from 5.39 to 5.98, with an overall average of 5.71. These findings showed that the traditional conversion factor of 6.25 overestimates the actual protein content and should be avoided.

Keywords: nitrogen-to-protein conversion factors, protein, amino acid, fish, tropical environment.

Perfil químico bruto y cálculo de los factores de conversión de nitrógeno a proteína en nueve especies de peces de aguas costeras de Brasil

RESUMEN. Se determinó la composición de aminoácidos y el contenido de nitrógeno, fósforo, lípidos, carbohidratos y proteínas en los músculos de *Dactylopterus volitans*, *Genypterus brasiliensis*, *Mullus argentinae*, *Paralichthys patagonicus*, *Percophis brasiliensis*, *Pinguipes brasilianus*, *Rhizoprionodon lalandii*, *Rhizoprionodon porosus* y *Urophycis cirrata*. Las muestras mostraron un contenido bajo en carbohidratos (<3,5% en peso seco para todas las especies) y alto en proteínas (>66% en peso seco para todas las especies). El porcentaje de lípidos totales varió ampliamente entre las especies, y *M. argentinae* presentó las mayores concentraciones (16% en peso seco). El porcentaje de nitrógeno y fósforo fue alto y similar entre las especies, con valores promedios de 13,3% y 1,2%, respectivamente. La composición de aminoácidos fue similar entre los animales, los aminoácidos más abundantes fueron ácido glutámico y lisina e histidina la de menor concentración. Entre las especies, el contenido de nitrógeno proteico fue alto, con un promedio de 96,8% de nitrógeno total. En consecuencia, los peces mostraron una concentración muy baja de nitrógeno no proteico. A partir de los datos de aminoácidos totales y nitrógeno total para cada especie, se calcularon factores de conversión de nitrógeno a proteína específicos para cada especie de pez. Los factores de conversión de nitrógeno a proteína variaron de 5,39 hasta 5,98, con un promedio general de 5,71. Estos hallazgos muestran

que el factor de conversión tradicional de 6,25 sobreestima el contenido de proteína real de peces y debería ser evitado.

Palabras clave: factores de conversión de nitrógeno a proteína, proteínas, aminoácidos, peces, ambiente tropical.

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INTRODUCTION

Studies on the chemical composition of marine organisms can offer important subsidies for the development of research in the fields of physiology, biochemistry, ecology, and conservation of the species, for example (Barbarino & Lourenço, 2009). These approaches are particularly uncommon in marine biology.

Studies on the biochemical composition of wild fish populations are rarely undertaken, possibly because others are more traditional in fish studies, such as ecological research and fisheries. The knowledge on chemical composition of fish species has fundamental importance in the application of different technological processes. The chemical composition of the organisms, in general, can be influenced by many factors, such as physiological characteristics, habitat and life cycle, in addition to environmental characteristics. The chemical composition of heterotrophic organisms is also influenced by the food that they ingest, age and reproductive traits (Childress *et al*., 1990; Zaboukas *et al*., 2006).

Fish is an important component in the diet, not only as a source of protein of high nutritional quality, but as a significant source of polyunsaturated fatty acids of the omega-3 series (ω-3). These substances are thought to be very beneficial to human health (Venugopal & Shahidi, 1996; Ramos Filho *et al*., 2008). Studies with focus on the chemical composition of fish are predominantly found in food science, and in general the results are available in fresh weight basis. According to Ogawa & Maia (1999), the moisture in muscles reaches typically 80% of the weight, but Ramos-Filho *et al*. (2008) recorded values for moisture from 60 to 80%. The use of fresh weight makes comparisons and interpretations on chemical composition among different studies more difficult.

Particularly, protein data of marine fishes present many applications, involving both basic and applied research. However, comparisons of protein content among species are difficult because of methodological differences. The most common methods used for protein determination are Lowry *et al*. (1951), Bradford (1976) and the use of conversion factor of nitrogen-protein 6.25 (Jones, 1931). These two first methods are subject to interferences from many factors (Stoscheck, 1990), which are a consequence of the protein extraction and effects of some substances on specific amino acids. This happens bacause the chemical reactions which produce the protein quantification depends on the reactivity of the amino acid side groups (Legler *et al*., 1985). Although the problems with protein analyses have been known for many decades, they are very hard to avoid. In general, adaptations of the traditional techniques to specific species are necessary to run the analysis (Barbarino & Lourenço, 2005).

The total nitrogen (TN) analysis is relatively simple and easy to perform, and nitrogen-to-protein conversion factors (N-Prot factors) can be used to estimate crude protein content. The use of N-Prot factors to determine protein content has some important advantages if compared to other methodologies. Total nitrogen analysis, carried out by Kjeldhal's method (AOAC, 1990), Hach techniques (Hach *et al*., 1987) or CHN analysis, eliminates the necessity of extracting the protein content of the sample to be analyzed.

The use of specific N-Prot factors is widely recommended in order to get more accurate estimates of protein content (Sosulski & Imafidon, 1990). The nitrogen: protein ratio does vary according to the source considered (Mariotti *et al*., 2008). The use of N-Prot factors is particularly wide in food science, such as those calculated for certain cereals (*e.g.* 5.26 for rise, 5.47 for wheat; Fujihara *et al*., 2008), legumes (*e.g.* 4.75-5.87 for cassava root; Yeoh & Truong, 1996), mushrooms (4.70; Mattila *et al*., 2002), Cheddar cheese (6.38; Rouch *et al*., 2008) and fish and fish's products (4.94; Salo-Väänänen & Koivistoinen, 1996), among other products. The use of N-Prot factors is still uncommon in sea science, possibly because most of the scientific community ignores this methodological alternative. A few studies using N-Prot factors in sea science were performed with seaweeds (Aitken *et al*., 1991; Lourenço *et al*., 2002) and microalgae (Lourenço *et al*., 2004), which yielded specific N-Prot factors lower than 5.0.

The use of the factor 6.25, in case of high concentration of non-protein nitrogen (NPN), tends to

overestimate the protein data, since NPN and protein-N can not be distinguished in TN analysis. Despite this, several authors continue to use the factor 6.25 to estimate the content of fish protein (Polvi & Ackman, 1992; Maia *et al*., 1999; Undeland *et al*., 1999; Zaboukas *et al*., 2006; Ramos- Filho *et al*., 2008). Except for a short list of specific N-Prot factors available for marine organisms, the factor 6.25 calculated by Jones (1931) is still used for most plant and animal sources from the sea. Studies in this field are needed for fishes, since very limited information is available.

The purpose of our study was to determine specific N-Prot factors for nine species of marine fishes from coastal areas, based on the ratio of amino acid composition to total nitrogen (TN) content. In addition, we also characterized and compared the fish species regarding carbohydrate, lipid, nitrogen and phosphorus contents.

MATERIALS AND METHODS

Fishes

In this study nine fish species were analyzed. The identification of the species was carried with experts' supervision. *Dactylopterus volitans* (Linnaeus, 1758 - Dactylopteridae; common name: "flying gurnard"), *Genypterus brasiliensis* (Regan, 1903 - Ophidiidae; "cusk eels"), *Mullus argentinae* (Hubbs & Marini, 1933 - Mulidae; "Argentine goatfish"), *Paralichthys patagonicus* (Jordan, 1889 - Paralichthydae; "Patagonia flounder"), *Percophis brasiliensis* (Quay & Gaimard, 1825 - Percophidae; "Brazilian flathead"), *Pinguipes brasilianus* (Cuvier, 1829 - Pinguipedidae; "Brazilian sandperch"), *Rhizoprionodon lalandii* (Muller & Henle, 1839 - Carcharhinidae; "Brazilian sharpnose shark"), *Rhizoprionodon porosus* (Poey, 1861 – Carcharhinidae; "Caribbean sharpnose shark"), and *Urophycis cirrata* (Goode & Bean, 1896 - Phycidae; "gulf hake"). These species were selected given their ecological importance and abundance in the field.

Sampling

For each analysis, the samples were obtained from different individuals, randomly selected. Six to ten individuals were sampled, but only four of them (n = 4), were chemically analyzed for carbohydrate, protein, lipid, nitrogen, and phosphorus. Due the high cost of the amino acid analysis using the method employed here, only three replicates were analyzed for each fish species (n = 3), a widely accepted procedure.

D. volitans, *R. lalandii*, and *R. porosus* were collected in May 2004 at Niterói (Itaipu Beach, 22°52'S, 43°06'W). *M. argentinae*, *P. patagonicus*, *P. brasiliensis*, *P. brasilianus*, *U. cirrata*, and *G. brasiliensis* were sampled in June 2007 at Arraial do Cabo (22°57'S, 42°01'W). Both sites are located in Rio de Janeiro State, southeastern Brazil (Fig. 1).

Not-sexed adult fishes (with similar body sizes) were collected with bottom trawls. The animals were packed in plastic bags and kept on ice until returned to the laboratory. In the laboratory, samples were cut and the muscles were frozen at -18°C. Subsequently, the samples were freeze dried in a Terroni Fauvel, model LB1500TT device. The dried material was powdered manually using a mortar and pestle, and it was kept in desiccators containing silica-gel, under vacuum and at room temperature, until the chemical analyses were carried out.

Tissue analysis

Total carbohydrate was extracted with 80% H_2SO_4, according to Myklestad & Haug (1972). The carbohydrate concentration was determined spectrophotometrically at 485 nm, 30 min after the start of the chemical reaction, by the phenol-sulfuric acid method (Dubois *et al*., 1956), using glucose as a standard.

Total lipids was extracted according to Folch *et al.* (1957), and determined gravimetrically after solvent (chloroform) evaporation.

Total nitrogen and phosphorus were determined in muscle after peroxymonosulphuric acid digestion, using a Hach digestor (Digesdhal®, Hach Co.) (Hach *et al*., 1987). Samples were digested with concentrated sulfuric acid at 440ºC and treated with 30% hydrogen peroxide. Total nitrogen and phosphorus contents in the samples were determined spectrophotometrically after specific chemical reactions. See Lourenço *et al.* (2005) for analytical details.

Total amino acid was determined by high performance liquid chromatography with pre-column derivatization with AccQ.Fluor® reagent (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate), reverse phase column C_{18}AccQ.Tag® Nova-Pak (150x3.9 mm; 4 µm), ternary mobile phase in gradient elution composed by sodium acetate 140 mM + TEA 17 mM pH 5.05 (solvent A), acetonitrile (solvent B) and water (solvent C), flow 1 mL min^{-1} (Cohen & De Antonis, 1994). A Waters, model Alliance 2695 chromatograph was used, equipped with a fluorescence detector Waters® 2475 ($\mu_{ex.}$ 250 nm, $\mu_{em.}$ 395 nm). Analytical conditions were suitable to determine all amino acids, except tryptophan, cysteine + cistine and methionine. The percent of nitrogen in each amino acid was used to calculate nitrogen recovered from total amino acid analysis. Aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine,

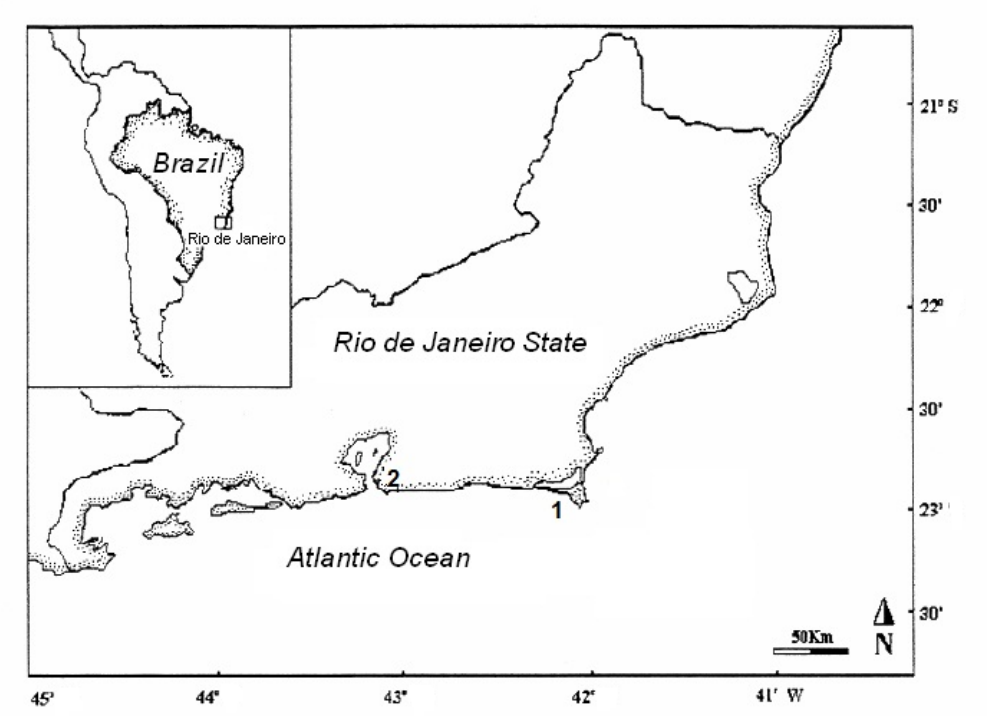

Figure 1. Map showing the sampling sites in Rio de Janeiro State, Brazil. 1: Arraial do Cabo, 2: Niterói.

tyrosine, phenylalanine, histidine, lysine, and arginine contents were multiplied by 0.106, 0.118, 0.134, 0.096, 0.123, 0.188, 0.158, 0.120, 0.108, 0.108, 0.078, 0.085, 0.271, 0.193, and 0.322, respectively (Diniz *et al*., 2011).

Calculation of N-Prot factors

N-Prot factors were determined for each species by the ratio of amino acid residues (AA-Res) to total nitrogen (TN) of the sample: N-Prot factor = AA-Res / TN. Thus, for a 100 g (dry weight) sample having 16.21 g of amino acid residues and 3.48 g of TN, a N-Prot factor of 4.66 is calculated.

The amino acid residues of the samples was calculated by summing up the amino acid masses retrieved after acid hydrolysis (total amino acids), minus the water mass (18 H_2O mol^{-1} of amino acid) incorporated into each amino acid after the disruption of the peptide bonds (Mossé, 1990).

Statistical analysis

The results were analyzed by one-way analysis of variance (One Way ANOVA) with significance level $\alpha = 0.05$ (Zar, 1996), followed, where applicable, with a Tukey's multiple comparison test. The raw data were tested for normality and homoscedasticity and no transformation was needed.

RESULTS

Carbohydrate was the less abundant organic substance measured in all species, ranging from 1.03% (*M. argentinae*) to 3.45% (*D. volitans*) of the dry weight The value of *D. volitans* was significantly higher than the other species ($P < 0.001$). The percentage of total lipid showed wide variations among species, ranging from 4.40% (*R. porosus*) to 16.1% (*D. volitans*). The lowest lipid concentrations were recorded in elasmobranch fishes (*R. porosus* and *R. lalandii)*. *M. argentinae* showed significantly higher lipid concentration than other species ($P < 0.001$) (Table 1).

The fishes showed high values of total nitrogen, ranging from 11.6% (*M. argetinae*) to 14.9% (*R. porosus*) of the dry weight. Elasmobranch fishes showed the highest concentrations of total nitrogen in the muscles (Table 1). The concentrations of phosphorus also varied widely among species. *P. brasiliensis* showed the highest value (1.45%, $P < 0.001$) and *D. volitans* showed the lowest concentrations (0.94%, $P < 0.001$).

Following the trends described for nitrogen concentration, the muscles showed high values of hydrosoluble protein. The values ranging from 45.7% (*R. porosus*) to 56% (*G. brasiliensis*). The higher concentrations were recorded in *G. brasiliensis* and

Table 1. Gross chemical composition of nine species of fishes sampled in tropical sites of Brazil. Values are expressed as percentage of the dry mass and represent the mean of four replicates ± standard deviation (n = 4)[#].

Species	Total nitrogen	Total phosphorus	Total carbohydrate	Total lipid	Hydrosoluble protein
	***	***	***	***	***
Dactylopterus volitans	11.7 ± 0.23[b]	0.94 ± 0.05[d]	3.45 ± 0.25[a]	7.81 ± 0.84[b]	47.1 ± 1.32[b]
Genypterus brasiliensis	14.0 ± 0.67[a]	1.29 ± 0.08[ab]	1.11 ± 0.03[de]	8.28 ± 0.72[b]	56.0 ± 1.79[a]
Mullus argentinae	11.6 ± 0.72[b]	1.12 ± 0.08[c]	1.03 ± 0.10[e]	16.1 ± 3.68[a]	47.7 ± 2.24[b]
Paralichthys patagonicus	13.4 ± 0.33[a]	1.21 ± 0.06[bc]	1.38 ± 0.09[bc]	5.62 ± 0.55[bc]	53.9 ± 0.75[a]
Percophis brasiliensis	13.7 ± 0.08[a]	1.45 ± 0.10[a]	1.15 ± 0.07[cde]	5.50 ± 1.42[bc]	55.9 ± 1.23[a]
Pinguipes brasilianus	12.6 ± 0.53[b]	1.30 ± 0.10[ab]	1.46 ± 0.21[b]	8.03 ± 1.19[b]	53.3 ± 3.11[a]
Rhizoprionodon lalandii	14.4 ± 0.41[a]	1.15 ± 0.04[bc]	1.40 ± 0.09[bc]	4.40 ± 0.37[c]	48.2 ± 1.39[b]
Rhizoprionodon porosus	14.9 ± 0.28[a]	1.10 ± 0.01[cd]	1.17 ± 0.04[cde]	5.28 ± 0.33[bc]	45.7 ± 0.30[b]
Urophycis cirrata	13.8 ± 0.67[a]	1.41 ± 0.09[a]	1.36 ± 0.10[bcd]	5.54 ± 1.30[bc]	55.5 ± 1.62[a]

[#]Mean values significantly different: ***$P < 0.001$, a > b > c > d > e. Identical superscript letters (a, a; b, b) or absence of letters indicate that mean values are not significantly different

P. brasiliensis. The lower concentrations of hydrosoluble protein were observed in the elasmobranch fishes *R. porosus* and *R. lalandii* and to bone fishes *M. argentinae* and *D. volitans.*

The amino acid profiles of the samples were very similar and are presented in Table 2. Glutamic acid was the most abundant amino acid in all species. The highest concentration of glutamic acid (14% of total amino acid) was found in *D. volitans*, while *M. argentinae* had the lowest (12.5%) concentrations. The fish muscles were also rich in lysine. These values varied from 9.63% (*D. volitans*) to 11.1% (*P. patagonicus*). The sum of the most abundant amino acids (glutamic acid and lysine) represented more than 20% of total amino acid. The percentage of histidine was the lowest in all species, with an average value of 2.12%. The higher concentration of arginine was recorded in *D. volitans*. Percentages of the other amino acids were similar among all species.

The total protein content of the samples is showed in Table 3 as total amino acid residues. The marine fish muscles showed high protein concentration, varying from 66.2% (*M. argentinae*) to 81.5% (*G. brasiliensis*) of the dry weight.

Nitrogen mass within total amino acid ranged from 11.4% (*M. argentinae*) to 13.9% (*G. brasiliensis*). The relative percentage of protein nitrogen was estimated as the ratio of nitrogen recovered from amino acid to total nitrogen (Table 1). High percentages of protein nitrogen were estimated, ranging from 91.4% (*R. porosus*) to 101.4% (*D. volitans*). The elasmobranch fishes showed the higher percentages of NPN (average = 7.85%, estimated from Table 3, as [100% - protein-N]).

From the ratio of the mass of amino acid residues to total nitrogen we calculated specific N-Prot factors for the species. The N-Prot factors ranged from 5.39 (*R. porosus*) to 5.98 (*D. volitans*). The elasmobranch species (*R. porosus* and *R. lalandii*) recorded the lowest values of N-Prot factors. An overall average N-Prot factor = 5.71 was calculated from the data for all species (Table 3).

DISCUSSION

Despite the remarkable importance of marine fishes, these organisms use to be chemically studied only in food science, covering species of commercial importance as food. The present study is also potentially useful in food science, but it includes ecologically important fish species, independent of their possible use as food. In addition, despite the low number of replicates used, the interpretation of the results is strengthened by the low dispersion of the data, as shown in Tables 1-3.

All fish species analyzed showed low content of carbohydrate. According to Ogawa & Maia (1999), in general, the presence of glycogen in the muscles is low, varying from 1.5 to 5% of carbohydrate (values converted into a dry mass basis). There is a remarkable lack of data in the literature on carbohydrates in fishes, probably due the small importance of carbohydrate for the nutritional value of fishes. The majority of the chemical studies on fishes are focused on both protein and lipid composition (Puwastien *et al*., 1999; Vila-Nova *et al*., 2005; Simões *et al*., 2007).

Table 2. Total amino acid composition of nine species of fishes. Results are expressed as grams of amino acid measured in 100 g of fish protein and represent the actual recovery of amino acids after acid hydrolysis. Values are the mean of three replicates ± SD (n = 3).

Amino acids	*Dactylopterus volitans*	*Genypterus brasiliensis*	*Mullus argentinae*	*Paralichthys patagonicus*	*Percophis brasiliensis*	*Pinguipes brasilianus*	*Rhizoprionodon lalandii*	*Rhizoprionodon porosus*	*Urophycis cirrata*
Aspartic acid	8.41 ± 0.45	9.63 ± 0.11	9.20 ± 0.45	9.78 ± 1.04	8.22 ± 0.09	9.99 ± 0.38	8.96 ± 0.12	8.66 ± 0.43	8.45 ± 2.23
Threonine	5.26 ± 0.27	4.50 ± 0.09	4.44 ± 0.13	4.39 ± 0.22	4.46 ± 0.09	4.67 ± 0.01	4.72 ± 0.05	5.24 ± 0.31	4.64 ± 0.17
Serine	4.26 ± 0.12	4.33 ± 0.02	4.32 ± 0.08	4.78 ± 0.12	4.22 ± 0.09	4.32 ± 0.04	4.59 ± 0.05	4.33 ± 0.29	5.03 ± 0.70
Glutamic acid	14.0 ± 0.72	13.0 ± 0.18	12.5 ± 0.17	13.4 ± 0.85	13.9 ± 0.16	13.0 ± 0.25	13.3 ± 0.27	14.8 ± 0.71	12.0 ± 2.29
Proline	4.49 ± 0.25	3.65 ± 0.05	3.63 ± 0.08	3.81 ± 0.09	3.90 ± 0.01	3.66 ± 0.06	3.54 ± 0.03	4.02 ± 0.46	3.43 ± 0.26
Glycine	3.89 ± 2.13	4.66 ± 0.20	4.77 ± 0.25	3.35 ± 0.59	6.04 ± 0.13	4.68 ±0.10	5.14 ± 0.16	5.11 ± 1.96	4.93 ± 0.25
Alanine	6.45 ± 0.14	5.81 ± 0.06	6.14 ± 0.02	5.94 ± 0.05	6.13 ± 0.07	5.86 ± 0.06	5.69 ± 0.07	5.97 ± 0.12	6.06 ± 0.26
Valine	6.34 ± 0.07	5.43 ± 0.14	5.68 ± 0.08	5.83 ± 0.13	5.89 ± 0.14	5.59 ± 0.03	5.50 ± 0.13	5.92 ± 0.09	6.14 ± 0.52
Isoleucine	5.85 ± 0.04	5.33 ± 0.14	5.41 ± 0.10	5.57 ± 0.09	5.55 ± 0.12	5.38 ± 0.06	5.76 ± 0.09	6.11 ± 0.02	5.98 ± 0.63
Leucine	9.62 ± 0.09	8.62 ± 0.19	8.74 ± 0.15	9.46 ± 0.14	9.35 ± 0.18	8.53 ± 0.11	8.78 ± 0.13	9.60 ± 0.14	9.40 ± 0.92
Tyrosine	3.69 ± 0.24	3.49 ± 0.17	3.53 ± 0.12	3.76 ± 0.28	3.34 ± 0.06	3.64 ± 0.08	2.45 ± 0.08	2.18 ± 0.06	3.30 ± 0.17
Phenylalanine	5.42 ± 0.37	4.83 ± 0.08	5.12 ± 0.06	5.65 ± 0.57	4.85 ± 0.12	4.94 ± 0.07	5.73 ± 0.01	5.71 ± 0.68	5.11 ± 2.07
Histidine	1.13 ± 1.56	2.60 ± 0.01	2.48 ± 0.07	1.67 ± 0.90	2.29 ± 0.05	2.28 ± 0.01	2.47 ± 0.01	1.70 ± 1.48	2.45 ± 0.26
Lysine	9.63 ± 0.89	9.82 ± 0.19	10.4 ± 0.15	11.1 ± 0.23	9.85 ± 0.05	10.2 ± 0.06	10.6 ± 0.14	10.7 ± 0.80	10.6 ± 0.35
Arginine	11.0 ± 1.07	9.45 ± 0.27	10.2 ± 0.08	7.17 ± 0.91	8.30 ± 0.08	8.85 ± 0.13	8.16 ± 0.05	9.66 ± 1.50	9.59 ± 0.08
Total	99.5 ± 8.42	95.2 ± 1.90	96.6 ± 1.99	95.7 ± 6.21	96.3 ± 1.46	95.6 ± 1.44	95.4 ± 1.39	99.7 ± 9.06	97.1 ± 11.1

Table 3. Calculation of nitrogen-to-protein conversion factors for nine species of fishes based on the amino acid residues to total nitrogen ratio. Values are expressed as percentage of the dry matter, except for the nitrogen-to-protein factors (no units). Results represent the mean of three replicates ± SD (n = 3).

Species	Total amino acid	Amino acid residues	Amino acid-N	Protein-N	N-Prot factor
Dactylopterus volitans	81.5 ± 9.08	70.2 ± 7.82	11.9 ± 1.33	101.4 ± 11.30	5.98 ± 0.67
Genypterus brasiliensis	94.7 ± 1.98	81.5 ± 1.70	13.9 ± 0.29	99.3 ± 2.08	5.80 ± 0.12
Mullus argentinae	76.9 ± 1.46	66.2 ± 1.26	11.4 ± 0.22	98.5 ± 1.87	5.69 ± 0.11
Paralichthys patagonicus	91.7 ± 7.16	78.9 ± 6.16	12.9 ± 1.00	95.8 ± 7.48	5.88 ± 0.46
Percophis brasiliensis	91.0 ± 1.29	78.1 ± 1.11	13.2 ± 0.19	96.2 ± 1.37	5.69 ± 0.08
Pinguipes brasilianus	86.0 ± 1.19	74.0 ± 1.02	12.5 ± 0.17	99.0 ± 1.37	5.85 ± 0.08
Rhizoprionodon lalandii	92.3 ± 1.24	79.3 ± 1.06	13.4 ± 0.18	92.9 ± 1.24	5.50 ± 0.07
Rhizoprionodon porosus	93.6 ± 11.3	80.5 ± 9.69	13.6 ± 1.64	91.4 ± 11.0	5.39 ± 0.65
Urophycis cirrata	91.0 ± 8.01	78.2 ± 6.89	13.5 ± 1.19	97.2 ± 8.56	5.65 ± 0.50

The lipid concentrations were higher than the carbohydrates for all species. This trend suggests that the animals concentrations store energy as fat content, converting the excess of sugar in fatty tissues. In general, data for lipid showed wider variations among the species. According to Mathew *et al*. (1999), Zaboukas *et al*. (2006) and Özogul & Özogul (2007), the lipid percentages are more influenced by the environmental conditions, physiological conditions and feeding than the carbohydrate and protein percentages. Henderson & Tocher (1987), Ramos-Filho *et al*. (2008) and Mathew *et al*. (1999) state that the concentration of lipids in the muscles vary from 0.3 to 20% fresh weight. In the present study, the average value for the lipid content of the nine species was 6.3% (dry weight). Only *M. argentinae* showed a high lipid concentration (16.1% dry weight), which possibly reflects species-specific physiological traits. The lowest concentrations of lipid were recorded in elasmobranch fishes (*R. lalandii* and *R. porosus*), which may be related to the fact that these fishes store fat mainly in the liver (Ogawa & Maia, 1999). According to Pigott & Tucker (1990), fish can be classified by their percentage of fat (fresh weight) as fish with low (< 2% fresh weight), moderate (from 2 to 5%), and high (> 5%) content of fat. Following this classification, the species analyzed in the present study would be of low fat content, except *M. argentinae*, with moderate content of fat.

Analysis of the phosphorus content in the organisms may be useful to interpret metabolic speed. High concentrations of phosphorus could be related to the characteristics of muscle tissues. Muscles of vertebrates have storage of high potential phosphorus in the form of creatinine-phosphate, which can quickly transfer its phosphate group to ATP (Stryer, 1996). Ogawa & Maia (1999) state that the animals that move fast spend more energy and consequently use more ATP, requiring a greater supply of phosphorus in the muscles than animals of more limited movements. The use of muscles in our analysis explains the typical high content of phosphorus found for all fish species analyzed here.

Our data indicate high protein concentrations in the species, and this agrees with the typical muscle characteristics. Concentrations of total protein were similar to the results reported by Zaboukas *et al*. (2006) and Simões *et al*. (2007), who state that total protein in fish fluctuates *ca*. 75% of dry weight. The lowest protein concentration was found in *M. argentinae*, which can be related to the high lipid content recorded in this species. According to Ogawa & Maia (1999) there is an inverse relationship between lipid and protein contents in fishes.

The protein quantification, by the method of Lowry *et al*. (1951), and by the sum of the amino acid residues, showed remarkable differences. For all species, the values for protein measured by the method of Lowry *et al*. (1951) were lower (*ca*. 35%) than results estimated by the sum of AA-Res. The possible cause for these differences could be related to the more difficult extraction of protein from freeze-dried samples according to Barbarino & Lourenço (2005). This could suggest a lower efficiency on extraction with the Lowry method. On the other hand, total amino acid analysis involves the acidic hydrolysis of the samples, which eliminates problems with protein extraction. It is widely accepted in the literature that the best estimation of protein content is the sum of AA-Res, which represents the actual protein in each sample (Sosulski & Imafidon, 1990; Yeoh & Truong, 1996; Fujihara *et al*., 2008; Diniz *et al*., 2011).

Proteins are composed of one or more chains of amino acids and the nutritional quality of a protein is basically determined by their content, proportion and availability of amino acids (Taboada *et al*., 2010). The main findings of amino acid composition of fish proteins described here are in agreement with previous studies (Uhe *et al*., 1991; Ogawa & Maia, 1999). All fish samples exhibited similar amino acid patterns. In general, all species are rich in glutamic acid and lysine, and poor in histidine.

The high nitrogen concentration observed in the muscles can be related with the high proportion of protein in the tissues, and by the presence of OTMA (oxydeoftrimethylamine), a nitrogenous substance widely found in marine animals (Ogawa & Maia, 1999). Sterner & George (2000) and Dantas & Attayde (2006) found similar values of TN in freshwater fishes compared to those recorded in the present study, with values fluctuating from 9.5% to 10.35% of dry weight.

More than 90% of the total nitrogen present in fish muscles are in proteins. In cartilaginous fishes, a smaller concentration of protein nitrogen (PN) was found, which may be related to the high concentration of OTMA. This substance is associated to urea in the control of the osmotic pressure; a high concentration of urea can be found in the muscles, achieving up to 2% (Ogawa & Maia, 1999). As a consequence of a high NPN, the budget of nitrogen is particularly affected in these species.

According to Puwastein *et al*. (1999), the quantity of NPN in muscles can vary from 6 to 14% of the total nitrogen. NPN in animals is present in the constitution of several substances, such as OTMA, amines, guanidines, nucleotides and their degrading products such as urea and ammonia. Other non-proteinaceous, substances with nitrogen that can be present are glycilbetaine, carnitine and homarine (Ogawa & Maia, 1999). The flavor of seafoods depends on the species, the fat content, and the presence as well as the type of nonprotein nitrogenous compounds (Venugopal & Shahidi, 1996).

In most studies of the protein content of fishes N-Prot factors were used. However, the factor used in these studies was the traditional factor 6.25 calculated by Jones (1931), from animal muscles. The use of the factor 6.25 is based on the assumption that samples contain protein with 16% nitrogen and an insignificant amount of non-protein nitrogen (Coklin-Brittain *et al*., 1999). However, the amino acid composition varies from one protein source to another, existing different N content in each amino acid. Moreover, this assumption is invalid for organisms that contain high concentrations of other nitrogenous compounds, such as nucleic acids, amines, urea, inorganic intracellular nitrogen (ammonium, nitrate and nitrite), vitamins and alkaloids (Fujihara *et al*., 2001). The contribution of non-protein nitrogenous compounds to the total crude protein content of different kinds of seafood depends on the composition (species) of the raw material, and ranges from 10 to 40% (Venugopal & Shahidi, 1996).

There are different ways to calculate N-Prot conversion factors. Several studies calculate the N-Prot factors as the ratio between AA-Res and NT (Levey *et al*., 2000; Matilla *et al*., 2002; Fujihara *et al*., 2008), such as it has been done in the present study. On the other hand, many studies determined conversion factors taking into account the proportion between total amino acid and the recovery of nitrogen from the amino acids (AA-N) (Mossé, 1990; Sosulski & Imafidon, 1990; Yeoh & Wee, 1994). Salo-Väänänen & Koivistoinen (1996) calculated a conversion factor of 4.94 for fish and fish products based on AA-N. However, the application of the conversion factor calculated only by AA-N can overestimate the actual protein content of species with high NPN. This happens because the factor would multiply the total nitrogen content to calculate the percentage of protein: it is not possible to distinguish protein nitrogen and NPN in total nitrogen. For a more accurate determination of protein using conversion factors, the quantity of TN should be corrected according to the NPN. Thus, the use of conversion factors based on AA-N does not have a practical value.

The total amino acid content represents not only amino acids derived from proteins but also those in free form. Thus. the presence of free amino acids contributes to an overestimation of the total protein. However, according to Mossé (1990), the use of data of total amino acid, without determination of free amino acids, is a widely accepted procedure to estimate protein, since in acid hydrolysis some amino acids are partially or totally destroyed (*e.g.* tryptophan, cystine, methionine and serine). The loss during acid hydrolysis might compensate for the influence of free amino acids in the quantification of protein by the sum of the total amino acid residues

The overall mean N-Prot factors calculated in this report was 5.71. Conversion factors calculated in this study were very similar among the species. Values for the elasmobranch fishes were smaller, achieving 5.39 and 5.5, in *R. porosus* e *R. lalandii*, respectively. This is possibly related to the higher concentration of NPN than in the other species. The high N-Prot factors calculated in this study reflect the low concentrations of NPN in the muscles. Despite the high concentrations of PN, all conversion factors were lower than the traditional factor 6.25.

The best estimate of the protein content is the sum of AA-Res, representing the actual protein content in the samples. A comparison of the values obtained using the N-Prot factor and the values obtained by the sum of AA-Res shows clearly that the factor 6.25 overestimates the protein content in fish samples in *ca*. 9% (14% for elasmobranchs). Such differences are not negligible, which means that the presence of non-proteinaceous substances with nitrogen affects the use of nitrogen-to-protein conversion factors in fish samples.

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REFERENCES

Aitken, K.A., L.D. Melton & M.T. Brown. 1991. Seasonal protein variation in the New Zealand seaweeds *Porphyra columbina* Mont. & *Porphyra-subtumens* J. Ag. (Rhodophyceae). Jap. J. Phycol., 39: 307-317.

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis. Washington, D.C., 556 pp.

Barbarino, E. & S.O. Lourenço. 2005. An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. J. Appl. Phycol., 17: 447-460.

Barbarino, E. & S.O. Lourenço. 2009. Comparison of CHN analysis and Hach acid digestion to quantify total nitrogen in marine organisms. Limnol. Oceanogr: Methods, 7: 751-760.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal. Biochem., 72: 248-254.

Childress, J.J., M.H. Price, J. Favuzzi & D. Cowles. 1990. Chemical composition of midwater fishes as a function of depth of occurrence off the Hawaiian Islands: food availability as a selective factor? Mar. Biol., 105: 235-246.

Cohen, S.A. & K.M. De Antonis. 1994. Applications of amino acid derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. Analysis of feed grains, intravenous solutions and glycoproteins. J. Chromatogr., 661: 25-34.

Coklin-Brittain, N.L., E.S. Dierenfeld, R.W. Wranghan, M. Norconk & S.C. Silver. 1999. Chemical protein analysis: a comparasion of Kjeldhal crude protein and total ninhydrin protein from wild, tropical vegetation. J. Chem. Ecol., 25: 2601-2622.

Dantas, M.C. & J.L. Attayde. 2006. Nitrogen and phosphorus content of some temperate and tropical freshwater fishes. J. Fish Biol., 70: 100-108.

Diniz, G.S., E. Barbarino, J. Oiano-Neto, S. Pacheco & S.O. Lourenço. 2011. Gross chemical profile and calculation of nitrogen-to-protein conversion factors for five tropical seaweeds. Am. J. Plant Sci., 2: 287-296.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Reberts & F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Folch, J., M. Lees & G.H. Sloanne-Stanley. 1957. A simple method for the isolation and purification of total lipid from animal tissue. J. Biol. Chem., 226: 497-509.

Fujihara, S., A. Kasuga & Y. Aoyagi. 2001. Nitrogen-to-protein conversion factors for common vegetables in Japan. J. Food Sci., 66(3): 412-415.

Fujihara, S., H. Sasaki, Y. Aoyagi & T. Sugahara. 2008. Nitrogen-to-protein conversion factors for some cereal products in Japan. J. Food Sci., 73: 204-209.

Hach, C.C., B.K. Bowden, A.B. Kopelove & S.T. Brayton. 1987. More powerful peroxide Kjeldhal digestion method. J. Assoc. Anal. Chem., 70: 783-787.

Henderson, R.J. & D.R. Tocher. 1987. The lipid composition and biochemistry of fresh water fish. Prog. Lipid Res., 26(4): 281-347.

Jones, D.B. 1931. Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. USDA Circ., 183: 1-21.

Legler, G., C.M. Müller-Platz, M. Mentges-Hettkamp, G. Pflieger & E. Jülich. 1985. On the chemical basis of the Lowry protein determination. Anal. Biochem., 150: 278-287.

Levey, D.J., H.A. Bissell & S.F. O'Keefe. 2000. Conversion of nitrogen to protein and amino acids in wild fruits. J. Chem. Ecol., 26: 1749-1763.

Lourenço, S.O., E. Barbarino, A. Nascimento & R. Paranhos. 2005. Seasonal variations in tissue nitrogen

and phosphorus of eight macroalgae from a tropical hypersaline coastal environment. Cryptog. Algol., 26(4): 355-371.

Lourenço, S.O., E. Barbarino, J.C. De-Paula, L.O.S. Pereira & U.M. Lanfer-Márquez. 2002. Amino acid composition, protein content, and calculation of nitrogen-to-protein conversion factors for nineteen tropical seaweeds. Phycol. Res., 50(3): 233-241.

Lourenço, S.O., E. Barbarino, P.L. Lavín, U.M.L. Marquez & E. Aidar. 2004. Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. Eur. J. Phycol., 39(1): 17-32.

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

Maia, E.L., C.C.S. Oliveira, A.P. Santiago, F.E.A. Cunha, F.C.A.F. Holanda & J.A. Sousa. 1999. Composição química e classes de lipídios em peixe de água doce curimatã comum, *Prochilodus cearensis*. Ciênc. Tecnol. Aliment., 19(3): 433-437.

Mariotti, F., D. Tomé & P.P. Mirand. 2008. Converting nitrogen into protein-beyond 6.25 and Jones' factors. Crit. Rev. Food Sci. Nutr., 48(2): 177-184.

Mathew, S., K. Ammu, P.G. Viswanathan Nair & K. Devadasan. 1999. Cholesterol content of Indian fish and shellfish. Food Chem., 66: 455-461.

Mattila, P., P. Salo-Väänänen, K. Könkö, H. Aro & T. Jalava. 2002. Basic composition and amino acid contents of mushrooms cultivated in Finland. J. Agr. Food Chem., 50: 6419-6422.

Mossé, J. 1990. Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed proteic content. J. Agric. Food Chem., 38: 18-24.

Myklestad, S. & A. Haug. 1972. Production of carbohydrates by the marine diatom *Chaetoceros affinis* var. *willei* (Gran) Hustedt. I. Effect of the concentration of nutrients in the culture medium. J. Exp. Mar. Biol. Ecol., 9: 125-136.

Ogawa, M. & E.L. Maia. 1999. Manual de pesca-Ciência e tecnologia do pescado. Vol. 1, Editora Varela, São Paulo, 430 pp.

Özogul, Y. & F. Özogul. 2007. Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black seas. Food Chem., 100: 1634-1638.

Pigott, G.M. & B.W. Tucker. 1990. Seafood effects of technology on nutrition. Marcel Dekker, New York, 362 pp.

Polvi, S.M. & R.G. Ackman. 1992. Atlantic salmon (*Salmo salar*) muscle lipids and their response to alternative dietary fatty acid sources. J. Agric. Food Chem., 40(6): 1001-1007.

Puwastien, P., K. Judprasong, E. Kettwan, K. Vasanachitt, Y. Nakngamanong & L. Bhattacharjee. 1999. Proximate composition of raw and cooked Thai freshwater and marine fish. J. Food Comp. Anal., 12: 9-16.

Ramos-Filho, M.M., M.I.L. Ramos, P.A. Hiane & E.M.T. Souza. 2008. Perfil lipídico de quatro espécies de peixes da região pantaneira de Mato Grosso do Sul. Ciênc. Tecnol. Aliment., 28(2): 361-365

Rouch, D.A., H. Roginski, M.L. Britz & P. Roupas. 2008. Determination of a nitrogen conversion factor for protein content in Cheddar cheese. Inter. Dairy. J., 18: 216-220.

Salo-Väänänen, P.P. & P.E. Koivistoinen. 1996. Determination of protein in foods: comparison of net protein and crude protein (N x 6.25) values. Food Chem., 57: 27-31.

Simões, M.R., C.F.A. Ribeiro, S.C.A. Ribeiro, K.J. Park & E.X. Murr. 2007. Composição físico-química, microbiológica e rendimento do filé de tilápia tailandesa (*Oreochromis niloticus*). Ciênc. Tecnol. Aliment., 27(3): 608-613.

Sosulski, F.W. & G.I. Imafidon. 1990. Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J. Agric. Food. Chem., 38: 1351-1356.

Sterner, R.W. & N.B. George. 2000. Carbon, nitrogen and phosphorus stoichiometry of Cyprinid fishes. Ecology., 81: 127-140.

Stoscheck, C.M. 1990. Quantification of protein. Methods Enzimol., 182: 50-68.

Stryer, L. 1996. Bioquímica. Guanabara Koogan, Rio de Janeiro, 1000 pp.

Taboada, C., R. Millán & I. Miguez. 2010. Composition, nutritional aspects and effect on serum parameters of marine algae *Ulva rigida*. J. Sci. Food Agric., 90: 445-449.

Uhe, A.M., G.R. Coller & K. O'Dea. 1991. A comparison of the effects of beef, chicken and fish protein on satiety and amino acid profiles in lean male subjects. J. Nutr., 122(3): 467-472.

Undeland, I., G. Hall & H. Lingnert. 1999. Lipid oxidation in fillets of herring (*Clupea harengus*) during ice storage. J. Agric. Food Chem., 47: 524-532.

Venugopal, V. & F. Shahidi. 1996. Structure and composition of fish muscle. Food Rev. Int., 12(2): 175-197.

Vila-Nova, C.M.V.M., H.T. Godoy & M.L. Aldrigue. 2005. Composição química, teor de colesterol e caracterização dos lipídios totais de tilápia (*Oreochromis niloticus*) e pargo (*Lutjanus purpureus*). Ciênc. Tecnol. Aliment., 25(3): 430-436.

Yeoh, H.H. & V.D. Truong. 1996. Protein contents, amino acid compositions and nitrogen-to-protein conversion factors for cassava roots. J. Sci. Food Agric., 70: 51-54.

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Yeoh, H.H. & Y.C. Wee. 1994. Leaf protein contents and nitrogen-to-protein conversion factors for 90 plant species. Food Chem., 49: 245-250.

Zaboukas, N., H. Miliou, P. Megalofonou & M. Moraitou-Apostolopoulou. 2006. Biochemical composition of the Atlantic bonito *Sarda sarda* from the Aegean Sea (eastern Mediterranean Sea) in the different stages of sexual maturity. J. Fish Biol., 69: 347-362.

Zar, J.H. 1996. Biostatistical analysis. Prentice Hall, Upper Saddle River, 920 pp.