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ARTICLE

Biochemical composition of the nereidid *Perinereis cultrifera* from the Egyptian Mediterranean Coast

Composición bioquímica del nereidido *Perinereis cultrifera* de la costa Mediterránea de Egipto

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Resumen. El presente estudio documenta la composición bioquímica del poliqueto nereidido *Perinereis cultrifera* (carbohidratos, proteínas, lípidos, ácidos grasos y amino ácidos) recolectados en un área somera de la costa de Alejandría, Mediterráneo suroriental. Los resultados de este estudio muestran que *P. cultrifera* contiene 85,34-87,76% de peso húmedo como agua, 14,7-20,53% de peso seco (DW) como ceniza, 8,3-14,84% del DW como carbohidratos, 57,33-59,9% de DW como proteína y 11,59-13,36% de DW como lípidos. Los ácidos grasos totales variaron estacionalmente desde 45,9 a 100% de lípidos totales con dominancia absoluta (37,44-91,53%) del ácido eicosapentanoico (EPA) (C20:5n-3), acompañado por una pequeña cantidad de ácido esteárico (C18:0). Se identificaron 18 amino ácidos, incluyendo 10 esenciales (59,62-61,02% de proteína) y 8 no esenciales (38,98-40,38% de proteína).

Palabras clave: Carbohidratos, lípidos, ácidos grasos, amino ácidos

Abstract. The present study documents the seasonal biochemical composition of the nereidid polychaete *Perinereis cultrifera* (carbohydrates, protein, lipids, fatty acids and amino acids) collected from a shallow area on the Alexandria coast, southeast Mediterranean. The results of this study showed that *P. cultrifera* contained 85.34-87.76% of the wet weight as water, 14.7-20.53% of dry weight (DW) as ash, 8.3-14.84% of DW as carbohydrates, 57.33-59.9% of DW as protein and 11.59-13.36% of DW as lipids. The total fatty acids varied seasonally from 45.9 to 100% of the total lipids with the absolute dominance (37.44-91.53%) of the polyunsaturated eicosapentaenoic acid (EPA) (C20:5n-3), accompanied by a small amount of the saturate stearic acid (C18:0). Eighteen amino acids were identified, including 10 essential (59.62-61.02% of protein) and 8 nonessential (38.98-40.38% of protein).

Key words: Carbohydrates, lipids, fatty acids, amino acids

INTRODUCTION

The intensive harvest of wild polychaetes as food in aquaculture and as fishing bait has caused pressures on natural stocks and habitats, whereas polychaete aquaculture appears to be the best way to meet increasing demands of these animals (Poltana *et al.* 2007, Palmer 2008, 2011).

The nutritional value of polychaetes has been specifically attributed to their high content of PUFAs (Olive 1999, Shucksmith *et al.* 2006) which have been proved to be an important vector to transfer essential fatty acids to fish and crustaceans in aquaculture (Bischoff *et al.* 2009, Palmer *et al.* 2014). There have been many recent studies documenting the nutritional benefits of polychaetes for aquaculture species, such as satisfactory food intake and reproductive performance in broodstock sole (Cardinaletti *et al.* 2009), provision of PUFAs like AA, EPA, DHA, and the high n3:n6 ratio that help in fertilization, hatch rates and spawning frequency in the shrimp *Penaeus monodon* broodstock (Huang *et al.* 2008) and for

fish such as sea bream (Bell & Sargent 2003, Caballero *et al.* 2004).

The choice of a worm species for mass culture depends upon its nutritive value, which can be evaluated through the estimation of the main biochemical components such as proteins and lipids and their breakdown into specific fatty and amino acids.

Palmer *et al.* (2014) provides a very recent comparison of some important nutritional values of commercially relevant polychaetes, and other researchers have studied how seasonal and environmental factors can affect the biochemical compositions of a range of species (Fernandez 1998, Danovaro *et al.* 1999, Mayzaud *et al.* 1999). In particular, there have been numerous studies regarding the nereidid polychaete *Perinereis cultrifera* (Grube, 1840). These studies have focused on the synthesis of neutral lipids in oocytes (Dhainaut & Belhamra 1986), their distribution in coelomic constituents

during oogenesis (Fontaine *et al.* 1984), the qualitative and quantitative evolution of carbohydrates during oogenesis (Porchet & Spike 1978), the origin of yolk protein, vitellin (Baert & Slomianny 1987), and physical-chemical and functional properties of the protein (Musmeci & Damelio 1985). Others have studied *P. cultrifera* as biomarkers for environmental quality (Guemouda *et al.* 2014), and the effect of amylase on their growth, biochemical composition and fatty acid profile (Elayaraja *et al.* 2011).

The aim of the present work was to add further knowledge about the biochemical composition of *P. cultrifera* from the Alexandria coast through seasonal study, in order to evaluate its nutritive value as a life feed in aquaculture.

MATERIALS AND METHODS

Worms were collected seasonally in summer (August), autumn (October), winter (January) and spring (April) during August 2009 to July 2010 at 07:00-08:00 h from the algae and associated fouling covering hard-bottom within a depth range of 30-60 cm at El Mex (Fig. 1), west of the Alexandria coast, Egypt (Table 1). Within 2 h after the samples collection, worms were isolated from the benthos and divided into 2 subsamples. One subsample was used for determining the water and ash contents, and the other was frozen at -80°C in a liquid nitrogen freezer prior to biochemical analyses. After thawing, the later subsample was subdivided into 4 subsamples to determine their biochemical composition.

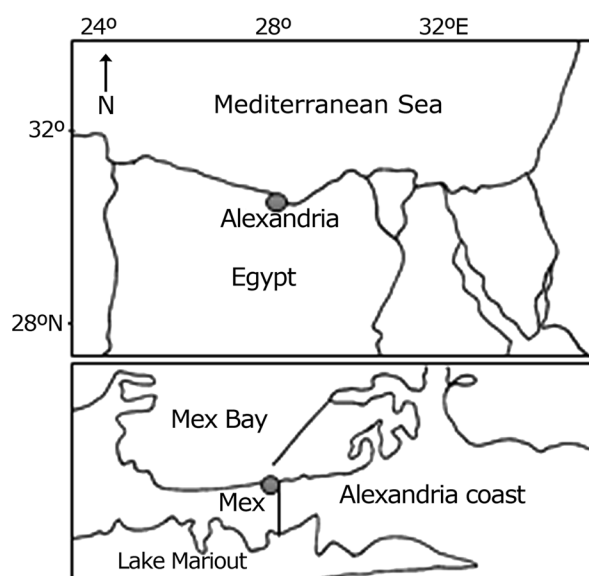


Figure 1. Location of the sampling site at Alexandria, Egypt / Ubicación del lugar de muestreo en Alejandría, Egipto

Table 1. The biometric data and number of worms used in the study (Imm: immature) / Datos biométricos y número de poliquetos utilizados en el estudio (Imm: inmaduro)

Date	Sex			Length range (cm)	Weight range (g)
	Imm.	♀	♂		
	N° of worms				
Summer (August)	3	3	1	1.85 - 3.8	0.011 - 0.108
Autumn (October)	13	1	2	2.4 - 5.0	0.023 - 0.161
Winter (January)	13	9	0	2.6 - 7.4	0.023 - 0.510
Spring (April)	7	5	0	2.0 - 7.0	0.017 - 0.501

The water content was estimated by drying a known weight of worms at 105°C for 24 h to constant weight. Ash content was determined by burning the sample at 500°C in a muffle furnace for 6 h. Total protein was extracted following Rossi *et al.* (2001) by 0.5 N NaOH for 4 h and measured calorimetrically according to Gornall *et al.* (1949).

Lipids were extracted with a polar solvent mixture consisting of chloroform, methanol and water (1:2:0.8), and fat content was determined by weighing the lipids after solvent evaporation (Bligh & Dyer 1959). The estimation of carbohydrates was undertaken according to James (1995), using the following equation:

Carbohydrates % = 100 - (moisture % + protein % + lipid % + ash %).

Determination of fatty acids was carried out by dissolving lipid samples in a methanol solution of potassium hydroxide (1M) and the produced mixture (fatty acid methyl esters) was evaporated to dryness, then dissolved in methanol and injected into the HPLC. The separation of fatty acids was done in HPLC (Agilent-1200) using C18 reversed-phase column (25 cm) and a UV detector at a flow rate of 1 ml min⁻¹ at room temperature of a 97:3 methanol:water eluent mixture. Amino acids were determined using Dionex (ICS-3000) following the technique describe by Crafts *et al.* (2012). Amino acids are expressed as a percentage of the total protein, and specific fatty acids are expressed as a percentage of the total lipids.

Some environmental parameters of the surrounding water were measured concurrently with the polychaete sampling, including temperature, salinity, pH, dissolved oxygen (DO) and biochemical oxygen demand (BOD). The water temperature and pH were determined in the field, using a digital portable HANNA 10pH⁺, pH - °C Meter, while salinity was measured by a calibrated Beckman Induction Salinometer (Model RS-7C). Dissolved oxygen (DO) and biochemical oxygen demand (BOD) were measured according to the Winkler method described by Strickland & Parsons (1975).

Correlations between the biochemical constituents and environmental parameters were calculated. The univariate statistical analysis was used to calculate the mean and standard deviation (SD) of the seasonal values of different biochemical constituents.

RESULTS

PHYSICO-CHEMICAL CONDITIONS

As shown in Table 2, water temperature displayed wide seasonal variations, from 18.9°C in winter and 28.9°C in summer. Salinity fluctuated between a minimum of 29.7 in summer and a maximum of 39.8 in autumn, mainly due to the discharge of the brackish water from the adjacent Lake Mariout. Neither pH nor DO showed seasonal variations, except for the relatively low values of DO in autumn. The BOD was also relatively low in autumn and spring, increasing pronouncedly in winter and reaching the maximum value in summer (Table 2).

BIOCHEMICAL ANALYSES

The water content in *P. cultrifera* showed slight seasonal differences (85.34–87.76% of wet weight) (Fig. 2), while greater comparative differences were recorded in ash contents between the minimum (14.7%) in autumn and the maximum (20.53%) in spring (Fig 3). Total lipids varied within a seasonal range of 11.6–13.4% of DW, displaying the lowest value in winter and the highest in summer. Total protein had narrow seasonal variations, with the highest value (59.9%) in winter, and slightly lower values in the other seasons (spring: 55.1%, summer: 55.6%, autumn: 57.3%). Carbohydrate showed similarly narrow seasonal variations, being 14.8% of DW in autumn, decreased to 8.3% in winter, and then sustained comparatively higher similar values (12.5 and 12.6%) in spring and summer respectively (Fig. 3). Carbohydrates displayed negative significant correlation with DO ($r = -0.9966$, $P < 0.05$).

Table 2. Seasonal values of the physico-chemical parameters at El Mex (DO: dissolved oxygen, BOD: biochemical oxygen demand) / Valores estacionales de los parámetros físico-químicos en El Mex (DO: oxígeno disuelto, BOD: demanda bioquímica de oxígeno)

Season	Temp (°C)	Salinity	pH	DO (mg l ⁻¹)	BOD (mg l ⁻¹)
Summer (August)	28.9	29.7	8.21	8.53	6.21
Autumn (October)	26.3	39.8	8.10	4.40	1.11
Winter (January)	18.9	38.7	8.30	8.90	4.38
Spring (April)	22.6	32.6	8.20	8.20	1.77

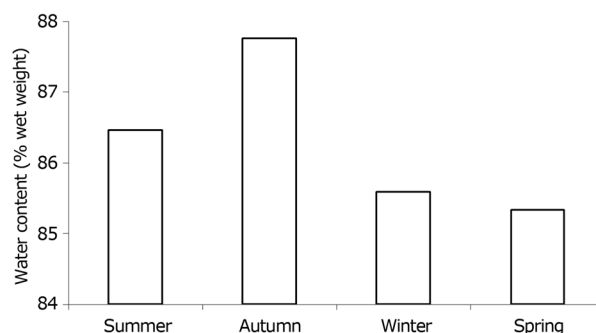


Figure 2. Seasonal percent of water content of *Perinereis cultrifera* at El Mex, Alexandria, Egypt / Porcentaje estacional del contenido de agua de *Perinereis cultrifera* en El Mex, Alejandria, Egipto

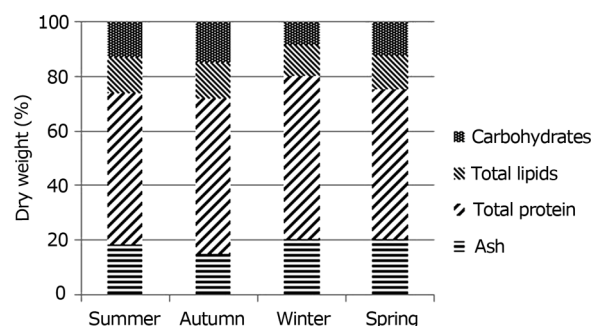


Figure 3. Seasonal percent of contents of ash, total protein, total lipids and carbohydrates in *Perinereis cultrifera* at El Mex, Alexandria, Egypt / Porcentaje estacional de los contenidos de ceniza, proteína total, lípidos totales y carbohidratos en *Perinereis cultrifera* en El Mex, Alejandria, Egipto

The profile of the amino acids in *P. cultrifera* (Table 3) reported 10 essential amino acids (EEA) and 8 nonessential amino acids (NEEA), with the dominance of EAA (59.6–61% of protein) against NEAA (39.4–40.4% of protein). Leucine sustained the highest concentration among the EAA, but without observable seasonal differences (32.2–33.8%), but other EEA displayed relatively high concentrations, like lysine (7.3–8.3%), methionine (5.2–6%) and histidine (2.9–4.2%). The NEAA were dominated by serine, which showed no seasonal differences (16.2–17.1%), and to a lesser degree glycine (9.8–10.1%) and alanine (4.9–5.7%) (Table 3). Amino acids in *P. cultrifera* had no significant correlation with the environmental conditions, except that of tyrosine and proline with temperature at $P < 0.05$ ($r = 0.976$ and -0.968), aspartate with pH ($r = -0.956$) and glycine with DO (0.954) and Cystine and Glutamate with BOD (-0.992 and 0.960).

Table 3. Seasonal ranges and mean of the amino acids contents (% of protein) in *Perinereis cultrifera* at El Mex (SD= standard deviation) / Rangos y medias estacionales del contenido de amino ácidos (% de proteína) en *Perinereis cultrifera* en El Mex (SD= desviación estándar)

Amino acid	Range	Mean \pm SD
EAA		
Isoleucine	1.3 - 2.07	1.67 \pm 0.342
Leucine	32.21 - 33.70	32.988 \pm 0.641
Lysine	7.29 - 8.31	7.723 \pm 0.438
Methionine	5.19 - 6.03	5.6 \pm 0.356
Phenylalanine	0.26 - 1.30	0.865 \pm 0.457
Threonine	2.33 - 3.39	2.798 \pm 0.452
Tryptophan	0.78 - 2.08	1.435 \pm 0.538
Valine	2.34 - 3.38	2.848 \pm 0.481
Histidine	2.86 - 4.15	3.42 \pm 0.591
Tyrosine	0.52 - 1.82	1.105 \pm 0.577
Total	59.62 - 61.02	60.448 \pm 0.591
NEAA		
Arginine	0.78 - 1.82	1.328 \pm 0.457
Alanine	4.92 - 5.73	5.35 \pm 0.338
Aspartate	2.07 - 3.38	2.743 \pm 0.551
Cysteine	0.26 - 1.04	0.585 \pm 0.390
Glutamate	0.78 - 1.55	1.188 \pm 0.345
Glycine	9.09 - 10.10	9.69 \pm 0.445
Proline	1.3 - 2.59	2.015 \pm 0.572
Serine	16.21 - 17.10	16.65 \pm 0.364
Total	38.99 - 40.36	39.55 \pm 0.579
EAA / NEAA	1.5 - 1.60	1.525 \pm 0.050

(EAA): essential amino acids

(NEEA): nonessential amino acids

Fatty acids were estimated as total amounts with broad groupings into saturates and unsaturates. As shown in Table 4 the unsaturates were the major component of fatty acids, and were dominated by the polyunsaturate eicosapentaenoic acid, C20:5n-3, which experienced wide seasonal variation (37.4-91.5% \pm 23.38 of total fatty acids), and attained the highest value in autumn and the lowest in spring. The saturates were pronouncedly lower than the unsaturates, having values from 5.5-8.7 \pm 1.527% and were represented mainly by stearic acid (C18:0) which sustained similar levels in most seasons (8.47-8.7%), and comparatively low one in winter (5.5%).

Table 4. Seasonal ranges and mean of the fatty acids contents (% of total fatty acids) in *Perinereis cultrifera* at El Mex (SD= standard deviation) / Rangos y medias estacionales del contenido de ácidos grasos (% de ácidos grasos totales) en *Perinereis cultrifera* en El Mex (SD= desviación estándar)

	Range	Mean \pm SD
SFA	5.5 - 8.7	7.79 \pm 1.527
UFA	37.44 - 91.53	68.34 \pm 23.388
Total fatty acids	45.91 - 100	76.125 \pm 23.932

SFA: saturated fatty acids

UFA: unsaturated fatty acids

DISCUSSION

The intensive use of marine polychaetes as fishing bait and for enhancing reproduction in shrimp and fish broodstock in numerous world countries (Palmer *et al.* 2014) represents a stress on the biodiversity of the benthic communities, particularly polychaete community. These conditions promote the mass culture of polychaetes for commercial use (Palmer 2010, Limsuwatthanathamrong *et al.* 2012) in aquaculture, whereas they form 5-33% of shrimp diets (Meunpol *et al.* 2005, Coman *et al.* 2007), relative to their advantages and preferences for use over other broodstock feeds (Palmer *et al.* 2014). The preference of polychaetes as feed for crustaceans is attributed to their contents of a suitable balance of nutrients and several other factors promoting crustacean reproduction (Palmer *et al.* 2014). Several authors documented the crucial role of high concentrations of polyunsaturated fatty acids in membrane structures, metabolic processes and in vivo supply of sex steroids (*e.g.*, Izquierdo *et al.* 2001, Nguyen *et al.* 2012, Palmer 2014). However, the nutritional value of polychaetes as well as other diets can vary with species, season of harvest and life stage (Chimsung 2014).

The present study revealed that *P. cultrifera* can have a similar biochemical composition to that of many other commercially relevant polychaete species. Its water content (85.34-87.76% of wet weight) was slightly higher than in *Pseudonereis anomala* (83.65-84.8%: Dorgham *et al.* 2014) but lower than that found in deep pelagic polychaetes, like *Poebius meseres*, *Pelagobia* sp., *Tomopteris pacifica*, *Traviopsis lobifera* (89.4-95%: Thuesen & Childress 1993). Lower water contents were found in *Perinereis helleri*, varying between 804.7 g kg⁻¹ of wet weight in small worms and 792 g kg⁻¹ in large worms (Palmer *et al.* 2014).

Carbohydrates in *P. cultrifera* displayed levels (8.3-14.8%) within the ranges reported for other polychaete species, including *Laonereis ankyloseta* (9.4%: Balasubramanian *et al.* 2012), *P. anomala* (6.5-18.7%: Dorgham *et al.* 2014) and

Nereis virens (130-170 g kg⁻¹: Brown *et al.* 2011). For *P. helleri*, Palmer *et al.* (2014) recorded lower values (60-80 g kg⁻¹).

Although Palacios *et al.* (1998, 1999) related carbohydrates to egg glucose levels with larval quality and broodstock condition, Meena *et al.* (2013) supposed that carbohydrates may be not essential for shrimp broodstock diets. However, carbohydrates have a role in glycogen accumulation in the hepatopancreas and acting as binders and in transport of nutrients in the hemolymph (Harrison 1997).

The protein content in *P. cultrifera* (55.1-59.9% of DW) was clearly higher than those in *Nereis virens* (3.6-3.9%: Lemieux *et al.* 1997) and *L. ankyloseta* (7.7%: Balasubramanian *et al.* 2012). In other worms protein seems to be lower, like in *P. anomala* (56.2-66.5%: Dorgham *et al.* 2014), in small and large worms of *P. helleri* (670-690 g kg⁻¹ and 620-6460 g kg⁻¹, respectively: Palmer *et al.* 2014), in *Perinereis* sp. and *Marphysa* sp. (64% DM and 510 g kg⁻¹ DM respectively: Meunpol *et al.* 2005), in *Australonuphis* sp. and *Marphysa sanguinea* (750 g kg⁻¹ and 690 g kg⁻¹: Marsden *et al.* 1992), and in cultured *N. virens* (570-620 g kg⁻¹: Brown *et al.* 2011). Proteins are an index of food quality since they generally reflect the availability of amino acids (Marsh & Tenore 1990, Gremare *et al.* 1997).

The total lipid content appeared to be variable in different polychaetes, amounting to 11.6-13.4% in *P. cultrifera* (present study), 13.4% in *Perinereis nuntia* (Limesuwathanathamrong *et al.* 2012), 9.1-13% in *P. helleri* (Palmer *et al.* 2014), 6-10.7% in *P. anomala* (Dorgham *et al.* 2014), 10.0% in *Diopatra neapolitana* (Luis & Passos 1995), 9.1% in *Paradiopatra* sp. (Drazen *et al.* 2008), 5% in *Laetmonice* sp. and 3% in *Travisia* sp. (Drazen *et al.* 2008). Other lipid contents (140 g kg⁻¹ and 50 g kg⁻¹ DW) were found in *Perinereis* sp. and *Marphysa* sp. (Meunpol *et al.* 2005), 30 g kg⁻¹ and 40 g kg⁻¹ in wild *Australonuphis* sp. and *M. sanguinea* (Marsden *et al.* 1992), 240 g kg⁻¹ in *N. virens* (Brown *et al.* 2011), 190 g kg⁻¹ and 200 g kg⁻¹ in wild and cultured *Nereis diversicolor* (Luis & Passos 1995, Costa *et al.* 2000). Although the variation in total lipids may be related to differences in the maturity stage of the nereidid species, the ecological stress in the area of study may inhibit the accumulation of lipids in *P. cultrifera*, particularly during the maturation time. This is in agreement with Balasubramanian *et al.* (2012) who proposed that the variations in the physicochemical properties influence the biochemical composition of the annelid *L. ankyloseta* and the state of estuarine water. The diet content of total lipid may show adverse effect on ovarian maturation and feed consumption (Wouters *et al.* 2001), or it may be not important, as the average lipid level in commercial broodstock diets (10%) appears to be around 3% higher than in

grower feeds used in commercial culture ponds (Meena *et al.* 2013).

The profile of amino acids in *P. cultrifera* was different from that documented for other polychaete species. The EAA leucine and to a less extent lysine and methionine were the dominant in *P. cultrifera*, while the NEAA dominated in other species, like aspartate and alanine in *P. anomala* (Dorgham *et al.* 2014), glutamate, aspartate and glycine in *P. helleri* (Palmer 2008, Palmer *et al.* 2014) and alanine in *Arenicola marina* (Hoeger & Abe 2004). However, both NEAA (beta-alanine, glycine, glutamate) and EAA (lysine) were abundant in *Nereis japonica* (Hoeger & Abe 2004). Furthermore, arginine was among the prevalent amino acid in *P. helleri* and the predominant in both *Australonuphis* sp. and *M. sanguinea* (Marsden *et al.* 1992), while it was less important in *P. cultrifera*.

Despite the correlation between the amino acids in *N. japonica* and the ambient salinities of 12-35 (Hoeger & Abe 2004), this correlation was not observed for *P. cultrifera* in our study, except that at $P < 0.05$ of tyrosine and proline with temperature ($r = 0.976$ and -0.968), aspartate with pH ($r = -0.956$), glycine with DO (0.954), Cystine and Glutamate with BOD. Meanwhile, the amino acids exhibited slight seasonal differences in the study area regardless of the wide variations of both temperature (18.9-28.9°C) and salinity (24.4-39.8). The negligible variations in some amino acids in marine bivalves indicate their stability at salinity above 20 (Kube *et al.* 2007).

Essential amino acids like leucine can stimulate protein synthesis (Volek 2009), lysine and tyrosine have essential roles in reproductive functions and nutrition of developing gametes (Fischer *et al.* 1996, Hoeger & Abe 2004). On the other hand, the NEAAs alanine, proline and glycine are considered as osmolytes in *P. cultrifera* (Jeuniaux *et al.* 1961) and in other polychaetes and marine invertebrates (Hoeger & Abe 2004, Kube *et al.* 2007). Alternatively, serine is involved in methyl group transfer, which is responsible for different processes in the cell and contributes to the formation of S-adenosylmethionine, the methyl donor (Amelio *et al.* 2014).

The abundance of fatty acids in *P. cultrifera* during the present study appeared to be affected by the temperature, particularly the unsaturated acid, C20:5n-3 (EPA), which increased with decreasing temperature during autumn-winter and vice versa in spring-summer. Luis & Passos (1995) reported that the degree of unsaturation in *N. diversicolor* seems to be affected by environmental temperature, while Palmer *et al.* (2014) proposed that higher ratios of EPA occurred under natural conditions. On the other hand, the feeding habit of polychaetes may influence the amount of their fatty acid contents, particularly EPA that is common in marine diatoms and algae (*e.g.*, Wen & Chen 2003,

Pratoomyot *et al.* 2005, Dawczynski *et al.* 2007, Van Ginneken *et al.* 2011, Pereira *et al.* 2012). Algae and diatoms were frequently recorded as the natural food of *P. cultrifera* in different localities (Fauchald & Jumars 1979, Marsh & Tenore 1990, Trueblood *et al.* 1994) and in our area, and this explains the high amount of EPA during the present study. High level of EPA in *P. cultrifera* was reported also in *P. helleri* grown in sand filters of mariculture wastewater and feed predominantly on microalgae (Palmer *et al.* 2014).

The present study revealed that *P. cultrifera* was characterized by the highest content of EPA when compared with other polychaete species (see table 10 given by Palmer *et al.* 2014). Such condition may assign this worm as a preferable source of EPA. However, it is necessary to take into consideration not only the individual levels of HUFAs but also the correct ratio among them (ARA/EPA/ DHA) (Suloma & Ogata 2012). Highly unsaturated fatty acids (HUFA), especially 20:5n-3 and 22:6n-3, may be important component of live and formulated maturation diets causing their abundance in ovarian tissues (Meena *et al.* 2013) and diets deficient in n-3 HUFA displayed a negative effect on ovarian development, fecundity and egg quality (Wouters *et al.* 1999).

Palmitic acid was reported as the most prevalent saturates in many polychaete species followed by stearic acid (Palmer *et al.* 2014), but stearic acid was the dominant saturates in *P. cultrifera*, particularly due to the environmental stress of the terrestrial discharge on the area of study.

The present study revealed that the biochemical composition of *P. cultrifera* collected from the Alexandria coast was quite different from other polychaete species but generally it may be very suitable for use as feed in aquaculture. It had high levels of carbohydrate, lipids, and protein that were comparable with our previous work on *P. anomala* from the same area. *Perinereis cultrifera* was characterized by having particularly high levels of EPA at certain times of the year, making it a useful supplement in this fatty acid for a vast array of species currently cultured around the world.

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