Communications

Seasonal changes in the biochemical components of *Pseudonereis anomala* (Polychaeta, Nereididae) from the Alexandria coast, Egypt

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Abstract

The biochemical composition (carbohydrates, protein, lipids, fatty acids and amino acids) of the nereid polychaete *Pseudonereis anomala* Gravier 1901, from a shallow part of the Alexandria coast (Egypt), was studied seasonally. The results revealed that *P. anomala* had a lower water content, higher carbohydrates and protein, but approximately similar or higher lipid levels than several other polychaetes.

Fatty acids appeared to be dominated by unsaturated acids, constituting seasonally 49.6–81%, while saturated acids reached high amounts in winter and spring (23.3 and 38.3% respectively). C20:5n-3 was the major polyunsaturated fatty acid, accompanied by small amounts of C18:4n-3, C20:4n-6, C16:1n-7 and C20:1n-9. C18:0 dominated the saturated fatty acids for most of the year, except in autumn when C16:0 was the major one.

1. Introduction

The importance of polychaetes as feed in aquaculture is attributed to their potential to provide polyunsaturated fatty acids, which are essential for egg maturation in cultured prawns (Meunpol et al. 2005, Nguyen et al. 2012), spawning in hatchery-reared fish species (Dinis et al. 1996) and enhancing reproductive performance in reared prawn stocks (Huang et al. 2008).

Pseudonereis anomala Gravier 1901 is an Indo-Pacific nereid polychaete species that migrated through the Suez Canal from the Red Sea into the Mediterranean and established healthy populations (Çinar & Altun 2007, Dorgham et al. 2013). It can act as a food source for many large predators, including crabs and fishes (Çinar & Altun 2007), as it occurs in a variety of shallow water benthic habitats (Ergen & Çinar 1997, Çinar & Ergen 2005) and exhibits a wide ecological valence that enables it to extend its distributional range into different parts of the Mediterranean (Çinar & Altun 2007).

Polychaetes are widely used as bait in recreational fishing in Egypt, but they are not applied as feed in aquaculture owing to the lack of information about their nutritional value. Such information is not available because little attention has been paid to the biochemical composition of polychaetes along Egyptian coasts. Only Osman (2007) measured protein and total lipids in the Oenonid polychaete *Halla parthenopeia* from the Suez Canal.

The present study aims to measure the amount of some biochemical components in *P. anomala* in order to assess its potential as a source of fatty acids and amino acids for animal feeds in aquaculture.

2. Material and methods

The worms were collected seasonally (summer: August, autumn: October, winter: January and spring: April) from hard substrates within a depth range of 20–50 cm on the Alexandria coast from August 2009 to July 2010. The fouling samples were placed in a plastic container filled with seawater; back in the laboratory 2–3 hours later, a number of specimens of different size of *P. anomala* were isolated and divided into two subsamples. In each season one subsample was used for determining the water and ash contents, while the other one was kept frozen at -80°C in a liquid nitrogen freezer for about one month for the biochemical analysis. The number, weight and length of the specimens used in the different seasons are given in Table 1. The second subsample was subdivided into four subsamples to determine the different biochemical components. The content of the worms' guts were

Table 1. Biometric data of the worms (L – length, Wt – weight)

Date	No. of worms	L range [cm]	Wt range [g]
August 2009	12	3.60 ± 1.50	0.120 ± 0.120
October 2009	10	3.73 ± 1.33	0.108 ± 0.109
January 2010	14	5.00 ± 1.89	0.207 ± 0.230
April 2010	16	5.42 ± 1.82	0.243 ± 0.216

studied but they was not allowed to empty their guts before the biochemical analysis.

The water content was determined by drying a known weight of worms at 50–60°C for 24 h to constant weight, and the ash content was estimated by burning the sample at 500°C in a muffle furnace for six hours.

Total protein was measured calorimetrically using the biuret reaction (Gornall et al. 1949). Lipids were extracted with a polar solvent mixture consisting of chloroform, methanol and water (1:2:0.8), and the fat content was determined by weighing the lipids after solvent evaporation according to Bligh & Dyer (1959). Carbohydrates were estimated according to the method described by James (1995), using the following equation:

$$\text{carbohydrates } \% = 100 - (\text{moisture } \% + \text{protein } \% + \text{lipid } \% + \text{ash } \%)\,.$$

Fatty acids were determined by dissolving lipid samples in a methanol solution of potassium hydroxide (1M) for complete conversion to FAME (fatty acid methyl esters). This mixture was then evaporated to dryness and dissolved in methanol before injection into the HPLC. The injected solution was regulated according to the optimal concentration on the calibration curve of each FAME standard. The HPLC (Agilent-1200) separation of fatty acids was done using C18 reversed-phase columns (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).

3. Results

The seasonal water contents in *P. anomala* were very similar, fluctuating between 83.65% (of wet weight) in winter and 84.8% in autumn. As shown in Figure 1, the ash content was approximately similar during all seasons (18.7%–18.9%), while total protein took the lowest value (56.2%) in autumn and the highest one (66.5%) in summer. Total lipids fluctuated between 6%

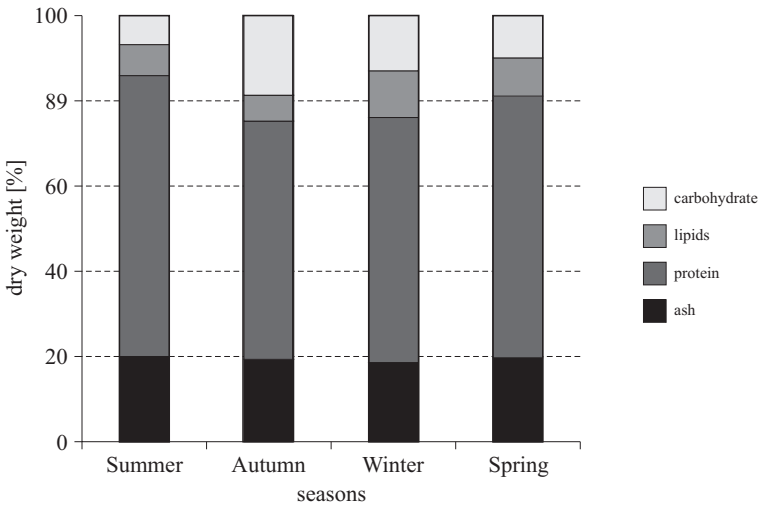

Figure 1. Seasonal contents of ash, total protein, total lipids and carbohydrates in *Pseudonereis anomala*

in autumn and 10.7% in winter and carbohydrates between 6.5% in summer and 18.7% in autumn.

The seasonal changes in fatty acids and amino acids are given in Tables 2 and 3. Polyunsaturated fatty acids (PUFA) were represented mainly by C20:5n-3, which attained the maximum percentage (76.8%) in winter and the minimum (49.6%) in summer. The fatty acid composition was mostly unsaturated (UFA), with the lowest value (49.6%) in summer and the highest (81%) in autumn. Meanwhile, saturated fatty acids (SFA) made up 2.2% in summer and reached a maximum of 38.6% in spring. The detailed composition of fatty acids was detected in autumn only; in the other seasons they were not detected by the HPLC because their concentrations were at undetectable levels, except the saturated acid C18:0 and the polyunsaturated acid C20:n5-3, which appeared in all seasons (Table 2). Other autumn PUFAs included C18:2n-6, C18:3n-3, C18:4n-3, C20:4n-3 and C22:6n-3, together constituting 24.5% of the total fatty acids (Table 2). The monounsaturated fatty acids (MUFA) formed collectively 12.54% and comprised three acids only (C16:1n-7, C18:1n-9 and C20:1n-9). Meanwhile, the SFA were dominated by C12:0, C14:0, C16:0 and C18:0 in autumn only, whereas C18:0 was dominant in most seasons, with distinctly high values in winter and spring (Table 2). However, C16:0 was the major SFA in autumn but in low amounts (4.4%). The $\omega 3/\omega 6$ ratio in autumn was 1:4.6.

Eighteen amino acids were found in *P. anomala*, 10 essential ones (EAA) and 8 non-essential ones (NEAA). The latter group made up 72.77%–73.47%

Table 2. Seasonal contents (% of dry weight) of fatty acids in *Pseudonereis anomala*. (SFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids)

	Summer	Autumn	Winter	Spring
SFA				
C12:0	–	0.29	–	–
C14:0	–	1.92	–	–
C16:0	–	4.44	–	–
C18:0	2.22	0.08	23.25	38.64
MUFA				
C16:1n-7	–	6.23	–	–
C18:1n-9	–	1.70	–	–
C20:1n-9	–	4.61	–	–
PUFA				
C18:2n-6	–	5.81	–	–
C18:3n-3	–	0.23	–	–
C18:4n-3	–	11.21	–	–
C20:4n-6 (AA)	–	6.47	–	–
*C20:5n-3 (EPA)	49.59	43.92	76.75	61.36
*C22:6n-3 (DHA)	–	0.78	–	–
SFA	2.22	6.73	23.25	38.64
USFA	49.59	80.96	76.75	61.36
MUSFA	0.00	12.54	0.00	0.00
PUSFA	49.59	68.42	76.75	61.36
n-3	49.59	56.14	76.75	61.36
n-6	0.00	12.28	0.00	0.00
n-9	0.00	6.31	0.00	0.00

Unidentified peaks were not considered in the computations.
ω-3: C18:3n-3, C18:4n-3, C20:5n-3, C22:6n-3.
ω-6: C18:2n-6, C20:4n-6.
ω-9: C18:1n-9, C20:1n-9.
EFA: C18:2n-6, C18:3n-3, C20:4n-6, C20:5n-3, C22:6n-3.

of the total amino acids. Aspartate was the dominant one, fluctuating seasonally between 26.9% and 27.9% of the total, followed by alanine (19.2%–20.6%). Other NEAA, like glycine, arginine, serine and glutamate, were found in relatively high percentages (mostly $< 8\%$) (Table 3). In contrast, the percentages of all the EAA were low except leucine (4.6%–5.5%). The EAA:NEAA ratio fluctuated within a narrow seasonal range (0.36%–0.37%).

Table 3. Seasonal contents [%] of amino acids in *Pseudonereis anomala* (EAA = essential amino acids, NEEA = non-essential amino acids)

	Summer	Autumn	Winter	Spring
EAA				
Isoleucine	1.45	1.73	1.25	1.64
Leucine	4.97	5.20	5.52	4.62
Lysine	1.25	1.63	1.14	1.44
Methionine	1.45	1.22	1.77	1.64
Phenylalanine	1.09	0.71	1.35	1.54
Threonine	2.74	2.34	3.12	3.18
Tryptophan	2.80	3.26	2.19	2.57
Valine	2.12	1.63	2.50	1.75
Histidine	6.93	7.34	6.04	6.47
Tyrosine	1.76	2.14	1.66	1.95
Total	26.56	27.20	26.54	26.80
NEAA				
Arginine	6.26	5.91	6.66	6.26
Alanine	19.83	19.16	20.19	20.64
Aspartate	27.9	27.83	26.85	27.10
Cysteine	0.21	0.10	0.62	0.31
Glutamate	4.98	4.28	5.52	4.21
Glycine	7.66	8.66	7.18	7.80
Proline	1.15	0.82	1.46	1.03
Serine	5.46	6.01	4.99	5.85
Total	73.45	72.77	73.47	73.20
EAA/NEAA	0.36	0.37	0.36	0.37

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