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First insights *Sabella spallanzanii* (Annelida: Polychaeta) biochemistry: a potential resource for feed industry and fishes nourishment

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ABSTRACT

To contrast the global fish stocks over-exploitation by aquaculture interest is being paid to the identification of non-traditional species with potential food value useful for reared species nourishment. In this framework in the present work we investigated the biochemical composition of the polychaete *Sabella spallanzanii*. We determined, over an annual cycle, the elemental composition, the gross protein and proximate composition, the amino-acids composition, the total lipids and fatty acids content. Glycosaminoglycans content and polychlorinated biphenyls contamination were estimated too. *Sabella spallanzanii* body is particularly high in gross protein (54.8 ± 5.8 %) and in gross energy (20.5 ± 2.2 MJ/kg) content. All samples exhibited high concentrations of Na, K, Ca, and Cl. Saturated fatty acids predominated over monounsaturated and polyunsaturated ones. A low $\omega 6/\omega 3$ ratio (1.7) was recorded. From our results we suggest the potential employment of *S. spallanzanii* as a dietary supplement for fishes nourishment. Another potential utilization is its inclusion in fish artificial diet as attractant considering its potential palatability for several fish presumably related to some amino-acids found in high amount. Finally another original aspect highlighted is the high content of glycosaminoglycans useful in pet nutrition and in nourishment of farmed fish with partially cartilaginous skeleton.

Key words: Elemental composition; gross protein; proximate composition; amino-acids composition; total lipids; fatty acids content.

INTRODUCTION

The past 30 years have seen significant advances in the commercial aquaculture production and like other animal production systems, aquaculture has developed into a highly globalized trade-dependent industry. Cultured seafood production (not including aquatic plants) has increased more than seven-fold by weight (from 5 to 55.1 million tonnes (Mt)) between 1980 and 2009 and the value generated has grown from USD 9 billion in 1984, the first year that statistics are available, to USD 52 billion in 2000 (FAO, 2010; FAO-FIGIS, 2004;). This economic growth has led to the adoption of aquaculture as a preferred development path for many nations despite its environmental and social shortcomings (Lebel et al., 2002; Primavera, 2000). By tracing and mapping patterns of trade flows globally for fishmeal it is clear the aquaculture industry's increasing use of marine ecosystems worldwide. Today, there is heightened concern about the state of the world's oceans when three-quarters of global fish stocks are fully or over-exploited (Botsford et al., 1997; Garcia and de Leiva Moreno, 2000; FAO, 2010).

At present the main ingredient in fish feed for aquaculture is fish meal and the aquafeed industry is the principal consumer of fish meal in the world (Naylor et al., 2001). In order to contrast the aquaculture consume of fish meal which is obtained mainly from herrings suitable for human consumption, several aquaculture companies and researchers are studying alternative vegetal proteins for fish feeds in aquaculture (de Oliveira 2004; De Silva 1995; Espe et al., 2007; Gatlin et al., 2007; Krogdahl et al., 2003; McGoogan et al., 1997; Naylor et al., 2001; Palmegiano et al., 2005; Tiril 2008). There are several vegetal alternative feedstuffs actually used, as soybean meal or corn gluten meal, but their introduction in fish diets causes problems considering that such vegetal compounds are not easily utilized by fish, for the presence of antinutritional compounds such as alkaloids, tannins or glucosids (Bakke-McKellep, 1999; Bakke-McKellep et al., 2000; Francis et al., 2001; Gatlin et al., 2007). In this framework the search of new alternative sources of proteins useful for reared species nourishment are needed.

Among marine invertebrates, polychaetes worms, particularly Nerieidae, commonly named omegaworms due to their high content of omega-3 (ω -3) polyunsaturated fatty acids (PUFA) (Harrison 1991; Alonso et al., 2008), are of key importance in the diet of shrimps and are used as a food item in aquaculture (Olive 1999). As regards Sabellidae, *Sabella spallanzanii* is an ideal candidate for integrated aquaculture production and its use as bioremediator of aquaculture wastes has been already evaluated coupled with the conversion of the wastes into polychaetae protein-rich biomass of potentially marketable value (Giangrande et al., 2004; Stabili et al., 2006; Stabili et al., 2010). However, for these future applications, the biochemical composition of this species have to be clarified. Thus the aim of the present study is the determination of the biochemical composition of *S. spallanzanii* in order to consider its potential introduction as meal in fish feeds in aquaculture. In particular we evaluated, over an annual cycle, some physical and chemical properties of *S. spallanzanii* such as, elemental composition, the protein and carbohydrate content, the amino-acido composition, the total lipids and fatty acids composition and glycosaminoglycans (GAGs). This polychaete is a very common species in the Mediterranean sea and constitutes the natural diet of a great number of fish and large invertebrates as lobsters and cephalopods leading to suggest its introduction in fish feeds for aquaculture as a natural consequence. In this context there are interesting perspectives for *S. spallanzanii* meal and its utilisation fits also with modern trend for organic fish production, as this meal might represent a natural attractant that could substitute artificial ones. In aquaculture feeds and in general in all the sector of modern animal productions, some compounds used to improve feeds palatability and feeds consumption are called attractants. Attractants, whose prices are considerably higher than fish meal, are already largely used in fish feeds in particular in new species and in case of extensive substitution of fish meal with alternative vegetal protein (Allen Davis et al., 1995; Choi et al., 2004; Dias et al., 1997; Kikuchi K., 1999; Kikuchi K. et al, 2009; Shankar et al., 2008 Shimizu et al., 1990 Yilmaz E. 2005).

MATERIAL AND METHODS

Animals and samples preparation

Sampling was undertaken in the harbour of Brindisi (Southern Adriatic Sea, Italy) using SCUBA equipment (depth range = 5–15 m) over four periods: April 2006 (T1), July 2006 (T2), November 2006 (T3) and January 2007 (T4) (Figure 1). At each sampling period 200 adult specimens of *Sabella spallanzanii* were collected and transported to the laboratory under refrigeration. Here animals were immediately removed from their tubes, homogenized by a Polytron (Kinematica Typ PT/10/35) and stored at -80°C until use.

Water content

The wet weights of 15 samples for each sample period (3 replicates for each of the five groups of 40 individuals) were measured on an analytical balance. They were dehydrated overnight at 105°C and their dry weight was measured.

Determination of the inorganic composition

The inorganic composition was determined for each sample after lyophilization of sample solution at 52°C and 0.061 mbar using a LIO 5P CINQUEPASCAL freeze-dryer. Carbon, H and N analyses were performed using a 1106 Carlo Erba elemental analyser, while an AA-6200 Shimadzu atomic absorption flame emission spectrophotometer was used for the determination of Fe, Ca, Mg, Zn, Cu, K and Na. A P/N 206-17143 Shimadzu hydride vapour generator was coupled to the atomic absorption spectrophotometer in order to analyse the Sn and Se content. In general, each sample was mineralized to oxidize the organic fraction. To this end a weighted sample (~10 mg) was treated with HNO_3 (1 mL) and H_2SO_4 96% w/w (2.5 mL) at high temperature until no more fumes were released. The residue was treated again with the acids two more times. The final liquid residue was dissolved in water to give a 100 mL solution. For each element a calibration curve was obtained by using standard solutions. The quantitative analysis of phosphorous was performed

using a UV-1601 Shimadzu spectrophotometer using the method reported in the literature (Kitson & Mellon, 1944; Quinlan & Desesa, 1955). A 785 DMP Metrohm Titrino was used for the quantitative determination of the inorganic chloride using a potentiometric determination.

Determination of the PCBs content

The quantitative analysis of the PCBs was performed using a gas chromatograph equipped with a capillary column (HT-5, 25 m × 0.22 mm × 0.10 mm) and connected with a mass spectrometric detector (GC-MS). The oven temperature programme was as follows: 125°C for 1 minute; 25°C min⁻¹ to 200°C; 48°C min⁻¹ to 260°C, 60°C for 1 minute (total run time: 20 minutes) and the injector temperature was 300°C. Helium was used as the carrier gas at a constant flow of 0.7 mL min. The determination was done according to the CEN 15308 method UNI EN 15308:2008 Characterization of waste—Determination of selected PCBs in solid waste by using capillary gas chromatography with electron capture or mass spectrometric detection which take into account seven congeners of PCBs (Table 1). Molecular weights and retention times of these PCB congeners were also reported.

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Total lipids and fatty acid analyses

Total lipids from each worm sample were extracted according to the method of Folch et al. (1957). The sample, was homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of the sample. After centrifugation and siphoning of the upper phase, the lower chloroform phase contained the lipids. Total lipid content was determined by the colorimetric enzymatic method (Zöllner & Kirsch, 1962) using commercial kit (FAR—Verona, Italy). Fatty acid composition was determined as described by Budge & Parrish (2003). In this method, fatty acid methyl esters (FAME) of lipid extracts of the sample were prepared using 14% BF₃-MeOH mixture. Lipid extracts were dissolved in 0.5 ml hexane and 1.5 ml 14% BF₃-MeOH mixture. The samples were flushed with nitrogen and heated for 90 minutes at 85°C. After cooling to room

temperature, FAME were extracted three times with a mixture of 2 ml hexane and 2 ml water, followed by centrifugation at 2000g for 2 minutes. Hexane layers, which contained FAME were separated, combined, and evaporated under a stream of nitrogen at room temperature. Analysis of fatty acid methyl esters was performed by gas–liquid chromatography using a 6890 Hewlett Packard series gas chromatograph equipped with an Omegawax 250 capillary column (Supelco, USA). The column temperature programming was as follows: from 150 to 250°C at 4°C min⁻¹ and maintained at 250°C. Helium was used as the carrier gas at a flow of 1 ml min⁻¹. Methyl esters of fatty acid were identified by a FAME mix (Supelco, USA) as standard, by comparison of retention times, and the results were reported as percentages of total identified methyl esters fatty acids.

Triglycerides total cholesterol and phospholipids

Triglycerides total cholesterol and phospholipides were measured by the colorimetric enzymatic (Trinder, 1969; Bucolo & David, 1973) using the commercial kit (SGM, Rome, Italy).

Proximate composition

A sample of 100 g for each sampling period was used for chemical composition. Upon the arrival in the laboratory, samples were kept frozen. All samples were analyzed to determine the proximate composition according to standard methods (AOAC, 1997). The gross energy content (GE) was determined using an adiabatic calorimetric bomb (IKA C7000, Staufen, Germany). Total nitrogen content was determined using a nitrogen analyzer (Rapid N III, Elementar Analysensysteme GmbH, Germany) according to the Dumas method and the crude protein was calculated as total N×6.25.

Amino acids composition

The amino acids composition was measured by an amino acid analyser via acid hydrolysis using a Beckman System Gold HPLC system, (Beckman System Gold, Palo Alto, Ca, USA), according to standard analytical methods (AOAC, 1997) as described by Cavallarin et al. (2005).

Glycosaminoglycans (GAGs)

For GAGs biochemical investigations, tissues were homogenized, digested by papain and deproteinized with trichloroacetic acid; GAGs were precipitated by adding 4 vol of cold ethanol, lyophilized and dissolved in distilled water (Cappelletti et al., 1980). GAGs were quantified by the carbazole method using glucuronelactone as standard (Bitter and Muir 1962); the concentration was expressed as glucuronic acid per g of wet tissue.

Statistical analysis

All the results were presented as the arithmetic mean of three replications and standard deviation. Analysis of variance (ANOVA) was used in order to evaluate differences among the mean values of the measured parameters at the different sampling periods. The analyses were performed by using the Statsoft STATISTICA v. 6.0 (Statsoft, Inc., 2001).

RESULTS

Water and inorganic content

The mean water content of *S. spallanzanii* was $79.3 \pm 0.3\%$ (Figure 2a). In particular it was $79.1 \pm 0.2\%$ in spring, $78 \pm 0.3\%$ in summer, $84 \pm 0.4\%$ in autumn. The lowest value was recorded in winter corresponding to $76.1 \pm 0.5\%$. The residual part of the dried sample is mainly represented by inorganic salts left over from evaporating seawater.

The results obtained from the elemental analyses (Table 2) showed that the ~ 70% of the dry weight was composed of inorganic elements. In particular the data collected reveal that C, H and N represent almost 58% of the lyophilised samples. In the samples collected no significant changes of the values of the elements investigated were found. All samples exhibited low concentrations of Cu, Se and Sn, and high contents of Na, K, Ca, and Cl. The PCBs content estimated using the CEN method 15308 was $< 0.01 \mu\text{g/g}$.

Proximate and amino acids composition

S. spallanzanii composition is particularly high in gross protein on dry matter (54.8 ± 5.8 %) (Figure 2c) and in gross energy content ($GE = 20.5 \pm 2.2$ Mj/kg). Looking at measure variability, the values are comparable in the different collection periods with no significant statistical differences.

In order to investigate the protein quality, amino acids composition was determined (Anderson et al., 1995). As shown in Table 3 in *S. spallanzanii*, compared with fish meal, there is a slight deficiency of lysine (- 12%), leucine (- 9%) and phenylalanine (- 8%) while there is an excess of glycine (+ 154%), arginine (+ 65%), cysteine (+ 45%), histidine (+ 28 %) and glutamic acid (+ 24 %)

Glycosaminoglycans (GAGs)

Results concerning Glycosaminoglycans (GAGs) content in *S. spallanzanii* are reported in Table 4. There are noticeable differences in GAGs contents between the considered collection periods and the highest value was recorded in summer .

Total lipids and fatty acid analyses

The results of the mean lipidic content are presented in Figure 2c. The total mean lipid content of *S. spallanzanii* was 8% of the organic part the in spring (8g/100g), 9% in summer, 4% in autumn and 6% in winter. The fatty acids profile of *S. spallanzanii* is shown in Table 5. In spring the palmitic acid (16:0) was the predominant saturated fatty acid (23%), while, among monounsaturated fatty acids palmitoleic acid (16:1) prevailed (8%). Finally among polyunsaturated fatty acids, 16-docosadienoic acid (22:2, n-6) was abundant (6.4%).

In summer the palmitic acid (16:0) reached a concentration of 36.6%, followed by the 11% of myristic acid (14:0) and stearic acid (18:0). The eicosenoic acid (20:1) accounted to 4% and docosadienoic acid (22:2) the 3%.

In autumn, the palmitic acid (16:0) showed a percentage of 23%, the behenic acid (22:0) equal to 10%, myristic acid (14:0), stearic acid (18:0) and vaccenic acid (18:1 n-7) were equal to about 7.5% and finally the acid 16-docosadienoic (22:2, n-6) was present with a percentage of 3%.

In winter the palmitic acid (16:0) reached a value of 22%, the acid docosanoic (22:0) and the stearic acid (18:0) of 8.0 % and finally the acid docosadienoic (22:2) of 5.5 %.

In addition, in spring the fatty acids consisted of saturated fatty acids for 52.5%, of monounsaturated fatty acids for 23.6% and of polyunsaturated fatty acid for 23.9%. In summer fatty acids present were made for 69.1% of saturated fatty acids, for 20.2% of monounsaturated fatty acids and for 10.7% of polyunsaturated fatty acids. In autumn fatty acids were made for 54.7% of saturated fatty acids, for 31.7% of monounsaturated fatty acids and for 13.6% of polyunsaturated fatty acids. Finally, in winter fatty acids present were composed for 52.8% of saturated fatty acids, for 30.3% of monounsaturated fatty acids and for 16.9% of polyunsaturated fatty acids.

The triglycerides (Figure 3) represented only a small fraction of the total lipids corresponding to 0.3% in autumn and spring, 0.43% in summer and the highest value was recorded in winter (0.57% of total lipids). Cholesterol (Figure 3) was present with a mean percentage of 0.32% of total lipids with the highest value in spring (0.42%) and the lowest in autumn (0.27%).

Concerning the long-chain polyunsaturated fatty acids of the Omega-3 (ω -3) values were 7.37 % in spring, 4.2% in summer, 5.46% in autumn and 5.74% in winter with a mean of 5.7 ± 1.3 %.

By contrast the long-chain polyunsaturated fatty acids of the Omega-6 (ω -6) the highest value was recorded in spring (16.19%) and the lowest in summer (6.3%) with a mean of 9.9 ± 0.98

DISCUSSION

The bioavailability of macro and micro nutrients such as proteins and lipids is important to define the biochemical and nutritional value of a species (Guillaume J. 2001; Kolkovski et al., 2000, Kolkovski 2001; Parrish, 2009). Fish and edible marine invertebrates constitute an important food source, supplying proteins of high nutritional value, polyunsaturated fatty acids, vitamins and minerals for human and animal nutrition. However, the availability of traditional seafood products has undergone significant changes in recent decades; particularly as a result of over-fishing. Increasing interest is thus being paid to the identification of non-traditional species with potential food value. In this framework a number of studies have been carried out on the biochemical composition of several species of marine invertebrates including crustaceans and molluscs of the Mediterranean Sea (Alkanani et al.2007; Beukema and de Bruin 1977; Freiteset al., 2002a; 2002b King et al., 1990, Klingensmith, and Stillway 1982). By contrast studies on annelid worms are scarce and the available information concerns only few species such as *Nereis diversicolor*, *Nereis virens*, *Nephtys incisa* and some species of polar environments such as *Yoldia hyperborea*, *Nephtys ciliata* and *Artacama proboscidea*. (Luis et Passos 1995, Parrish et al., 1996, Pocock et al., 1971; Garcia-Alonso et al., 2008).The present work provides a preliminary step on this topic describing some aspects of the biochemical composition of the polychaete *Sabella spallanzanii*.

It is noteworthy that we recorded a *S. spallanzanii* mean gross protein content corresponding to 54.8 ± 5.8 %,on DM. This value is particularly relevant compared to other marine invertebrates which showed lower protein concentrations, equal to 8% in *Mytilus galloprovincialis*, 11% in *Anemonia viridis* and 19-20% in *Nephtys norvegicus* (Gonzales et al., 2001; Orban et al., 2002; Fuentes et al., 2009).

The average lipid concentration of *S. spallanzanii* (7%) lies in the range of values observed for other marine invertebrates including the polychaete *Artacama proboscidea* (Parrish et al., 1996) but lower than in *Nereis diversicolor* (Luis et Passos 1995).

Lipids are divided broadly into two categories: namely, neutral lipid (NL), which is the stored fat and is mainly composed of triglycerides, and phospholipids (PL) and cholesterol, which are building blocks of membranes. Identification of lipid composition is important for physiological studies. Furthermore, PCBs and other organochlorine contaminants are known to accumulate in tissue, and the information for lipid composition is helpful to explain the mechanism for the accumulation of these chemicals. (Hites et al., 2004; Warnau et al., 1998)

Our results showed that the main lipids in *S. spallanzanii* were triglycerides (TG), fatty acids (FA), cholesterol (CT), and phospholipids (PL). The ratios of these compounds to total lipid were 0.4 % for TG, 0.32 % for CT and 99.28 % for FA and PL. Among lipids triglycerides and phospholipids were more abundant in winter than in spring. Also in mussels (Cautadella and Bronzi 2001) these lipids undergo changes that can be affected by the quantity and quality of fat in the diet and vary depending on location and season. Results of analysis by the Iatroscan TLC showed that the main lipids in *Mytilus trossulus*, were triglyceride (TG), free fatty acid (FFA), sterol (ST), and phospholipid (PL). The ratios of these compounds to total lipid were 10 - 23% for TG, 24 - 37% for FFA, 4 - 7% for ST, and 36 - 55% for PL

Fatty acids are the principal components in lipids. Their diversity in terms of chain length, degree of unsaturation, geometry, and position of the double bonds is responsible for the definitive characteristics of lipids for different organisms (Gutnikov 1995). In *S. spallanzanii* the saturated fatty acids accounted for 57% of the total fatty acids. Palmitic acid methyl ester (16:0) was the predominant saturated fatty acid with the highest value (36.6%) in summer, whilst, among monounsaturated fatty acids, palmitoleic acid (16:1) prevailed with the maximum in autumn (10%). Palmitic acid has been reported in numerous studies, as the major saturated fatty acids also in mussels (Alkanani et al. 2007; Freitas et al., 2002a, 2002b; Karakoltsidis et al. 1995; King et al., 1990; Orban et al., 2002; Otlés & Sengor, 2005; Vernocchi et al., 2007). Generally, the lipids of marine animals are the most unsaturated of the animal kingdom. These lipids contain a high proportion of polyunsaturated fatty acids, principally omega-3 fatty acids. The lipid composition of

S. spallanzanii is similar to that of other marine invertebrates including sea anemones (Nechev et al., 2006), with a high proportion of polyunsaturated fatty acids (mean value 16%). Omega-3 fatty acids notably docosadienoic acid (22:2 n-6) and docosahexaenoic acid (22:6n-3), accounted to 6.4% in spring, 3% in summer and 4.3 in autumn-winter. The composition of polyunsaturated fatty acids is biologically important, since they are associated with reduced risk of cardiovascular disease (Kromhout, Bosschieter, & Lezenne, 1985), volatile compounds are also responsible for the aroma of food products, one of the most important parameters in the evaluation of fish quality. *S. spallanzanii* PUFAs profile, particularly regarding the ω 3 and ω 6 acids is interesting also considering that World Health Organization (WHO) currently recommends that the ω 6: ω 3 ratio should be no higher than 10 in the human diet. Although the ω 3 values were lower than that recorded for *Nereis diversicolor* (Garcia-Alonso et al., 2008) the presently studied polychaetes showed a low ω 6/ ω 3 ratio (total ω 6 is 9.9% and ω 3 is 5.7%) with a ratio 1.7, of thus suggesting that this marine invertebrate may be used as a natural source of ω 3 useful for the reduction of ω 6/ ω 3 ratio (Mahan and Escott-Stump 2000). In addition, also the occurrence of the essential linolenic acid (18:3) in *S. spallanzanii* is important for both human and fish nutrition since these organisms are not able to synthesize them (Sánchez-Machado et al. 2004).

The reduction of lipid content of *S. spallanzanii* in the autumn and winter could be explained in relation to its life cycle. Lipid variation indeed has principally been related to gamete development (Martinez 1991) with the highest levels of lipids during the period when gonads are ripe. *S. spallanzanii* reproduces in the Mediterranean in the period from January to March (Giangrande et al., 2000). Our results are also in agreement with those observed by Lomovasky 2004 in the clam *Eurhomalea exalbida* showing low values of total lipids in the autumn-winter period associated with an increase of the proteins in November. Temperate bivalves are also known to accumulate lipids or glycogen during the growing season and utilize them for reproduction and maintenance metabolism during the rest of the year. Lipid levels of 10 to >18% have frequently been reported for temperate bivalves during the reproductive season (De Moreno et al. 1976, Beukema & De Bruin

1977, Pollero et al. 1979, Zandee et al. 1980, Klingensmith & Stillway 1982, Davis & Wilson 1983, Wenne & Polak 1989).

Therefore, on the basis of the obtained results and in particular considering the proximate and essential amino acids composition as well as $\omega 6/\omega 3$ ratio we can suggest the potential employment of *S. spallanzanii* as a dietary supplement for fish artificial diets. The utilization of worm meal in fish feeds has been already considered for other species of farmed fish (Salze et al., 2010). There are some species of commercial interest already farmed, as rag worm (*Nereis virens*) which is actually farmed in northern Europe (Olive et al., 2002). Another potential utilization of *Sabella spallanzanii* is its inclusion in fish artificial diet as attractant considering its potential palatability for several species of fish. Some of the amino acids found in high amount could contribute to the intense odor of these worms that might influence their palatability for fishes after an inclusion in fish feed, since glutamic acid is a known flavour enhancer, followed by arginine for bitter and glycine for sweet. The employment of *S. spallanzanii* as attractant might be a great advantage for artificial diet for new fish species, as meagre (*Argyrosomus regius*) or white seabream (*Dipodus sargus*) or sheephead bream (*Puntazzo puntazzo*) that consume typically benthonic annelids in the wild and these species are not prone to consume artificial diets rich in vegetal protein as soybean meal. An interesting aspect emerged from this research is the glycosaminoglycans (GAGs) content that could have an application in pet nutrition. GAGs are cartilage precursors and GAGs extract from New Zealand green mussel (*Perna canaliculus*) are already used in dog nutrition as arthritis preventive (Dobenecker et al., 2002; Sicuro et al., 2010) and in green mussels GAGs are also known for their beneficial effect in human natural medicament. These preliminary analyses on *S. spallanzanii* show that this polychaetes could be considered as a source of these compounds, the GAGs quantity is roughly one half of green mussel and it is considerably higher than some other benthonic marine invertebrates as blue mussel or scallops (Sicuro et al., 2010). At present, commercially available GAGs are expensive and the extraction of GAGs from *S. spallanzanii* increases the economic interest in its meal utilisation. Finally, a GAGs enriched diet is also

interesting for farmed fish with partially cartilaginous skeleton, as sturgeon, that have high requirement of cartilage precursors in their diet (Daprà et al., 2009; Palmegiano et al., 2005) considering that Italy is the first European country for sturgeon farming.

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REFERENCES

REFERENCES

Alkanani T, Parrish C.C., Thompson R.J., and McKenzie C.H., 2007. Role of fatty acids in cultured mussels, *Mytilus edulis*, grown in Notre Dame Bay, Newfoundland. J. Exp. Mar. Biol. Ecol. 348: 33-45.

Allen Davis D., Jirsa D., Arnold C. R. 1995. Evaluation of soybean proteins as replacements for menhaden fish meal in practical diets for the red drum *Sciaenops ocellatus*. Journal of the World Aquaculture Society, Vol. 26, 1, pp. 48–58

Anderson J.S., Lall S.P., Anderson D.A., McNiven M.A. 1995. Availability of amino acids from various fish meals fed to Atlantic salmon (*Salmo salar*). Aquaculture 138, 1-4, 291-301

AOAC. (1997). Official methods of analysis (16th ed). Arlington, Virginia, USA: Association of Official Analytical chemists.

Bakke-Mckellep, A.M., 1999. Intestinal nutrient absorption in Atlantic salmon (*Salmo salar* L.) and pathophysiological response of the intestinal mucosa to dietary soybean meal. PhD Thesis. Norwegian School of Veterinary Science, Oslo, Norway. ISBN 82-7725-059-2

McGoogan B.B. & Gatlin D.M. III. T., 2000. Changes in immune and enzyme histochemical phenotypes of cells in the intestinal mucosa of Atlantic salmon, *Salmo salar* L., with soybean meal-induced enteritis. J. Fish Dis. 23,115-127

Beukema, J. J., de Bruin, W. 1977. Seasonal changes in dry weight and chemical composition of the soft parts of the tellinid bivalve *Macoma balthica* in the Dutch Wadden Sea. Neth. J. Sea Res. 11: 42-55

Bitter T., Muir, H.M.. 1962. A modified uronic acid carbazole reaction.. Analytical Biochemistry, 4:330– 334. 24.

Botsford, L.W., Castilla, J.C., Peterson, C.H., 1997. The management of fisheries and marine ecosystems. Science 277, 509–515.

Bucolo G. and David H. 1973. Quantitative determination of serum triglycerides by the use of enzymes. *Clinical Chemistry* 19, pp.476-482

Budge, S.M. and Parrish, C.C. 2003. FA determination in cold water marine samples. *Lipids* 38, 781–791.

Cautadella S., and Bronzi P. 2001. Acquacoltura responsabile verso le produzioni acquatiche del terzo millennio pp. 683

Cappelletti R, Del Rosso M, Vannucchi S, Cella C, Fibbi G, Chiarugi VP. 1980. Involvement of modulation of cell coat glycosaminoglycans in guinea pig peritoneal macrophage activity. *Journal of the Reticuloendothelial Society* ,27: 383-391.

Cavallarini L., Antoniazzi A., Borreani G., Tabacco E. (2005). Effects of wilting and mechanical conditioning on proteolysis in sainfoin (*Onobrychis viciifolia* Scop.) wilted herbage and silage. *Journal of the Science of Food and Agriculture*, 85: 831-838.

Choi, S.-M., Wang, X., Park, G.-J., Lim, S.-R., Kim, K.-W., Bai, S. C. and Shin, I.-S. 2004. Dietary dehulled soybean meal as a replacement for fish meal in fingerling and growing olive flounder *Paralichthys olivaceus* (Temminck et Schlegel). *Aquaculture Research*, 35: 410–418.

Daprà F., Gai F., Palmegiano G.B., Sicuro B, Falzone M., Cabiale K., Galloni M. 2009. Siberian sturgeon (*Acipenser baeri*, Brandt JF 1869) gut: anatomic description. *International Aquatic Research* 1: 45-60

Davis, JP., Wilson, JG. 1983. Seasonal changes in tissue weight and biochemical composition of the bivalve *Nucula turgida* in Dublin Bay with reference to gametogenesis. *Neth. J. Sea Res.* 17: 84-95

De Moreno JEA., Moreno V.J., Brenner R.R., 1976. Lipid metabolism of the yellow clam, *Mesodesma mactroides*: 2-polyunsaturated fatty acid metabolism. *Lipids* 11, 561– 566.

de Oliveira A.M.B.M.S., Cyrino J.E.P. 2004. Attractants in plant protein-based diets for the carnivorous largemouth bass *Micropterus salmoides*. *Scientia Agricola*, 61, 3, pp. 326-331

De Silva S.S., Anderson T.A. 1995. Fish nutrition in aquaculture. Chapman & Hall Ed, London, UK, pp 309

Dias J., Gomes E.F. , Kaushik S. 1997. Improvement of feed intake through supplementation with an attractant mix in European seabass fed plant-protein rich diets. Aquat. Living Resour. 10 pp 385-389

Dobenecker, B., Beetz, Y. & Kienzle, E. 2002. A placebo-controlled double-blind study on the effect of nutraceuticals (chondroitin sulfate and mussel extract) in dogs with joint diseases as perceived by their owners. American Society for Nutritional Sciences. J. Nutr. 132:1690

Espe M., A. Lemme, Petri A., El-Mowafi A. 2007. Assessment of lysine requirement for maximal protein accretion in Atlantic salmon using plant protein diets. Aquaculture 263: 168–178

FAO. 2010. The State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department, Rome, pp 472

FAO-FIGIS 2004 Fisheries Global Information System (FIGIS)

Folch, J., Less, M. and Stone Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226, 497–508.

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factor present in plant-derived alternate fish feed ingredients and their effect in fish. Aquaculture, 199, 197-227.

Freites L., Labarta U., Fernández-Reiriz M.J. 2002a. Evolution of fatty acid profiles of subtidal and rocky shore mussel seed (*Mytilus galloprovincialis*, Lmk), Influence of environmental parameters, Journal of Experimental Marine Biology and Ecology 268:185-204

Freites L., Fernández-ReirizMJ.,Labarta U. 2002b. Fatty acid profiles of *Mytilus galloprovincialis* (Lmk) mussel of subtidal and rocky shore origin. Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology 132, 2: 453-461

Fuentes A., Fernández-Segovia I., Escriche I., Serra J.A. 2009. Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis* Lmk.) from different Spanish origins. Food Chemistry 112: 295–302

García-Alonso J. Müller C.T. ,Hardege J.D. 2008. Influence of food regimes and seasonality on fatty acid composition in the ragworm. *Aquatic Biology* Vol. 4: 7–13

Garcia, S., and de Leiva Moreno, I., 2000. Trends in World Fisheries and their Resources. The State of World Fisheries and Aquaculture. FAO, Rome.

Gatlin D.M., Barrows F.T., Brown P., Dabrowski K., Gaylord G., Hardy R., Herman E., Hu G., Krogh A., Nelson R., Overturf K., Rust M., Sealey W., Skonberg D., Souza E.S., Stone D., Wilson R., Wurtele E. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research*, 2007, 38, 551- 579

Giangrande A., Licciano M., Pagliara P., Gambi MC. 2000 Gametogenesis and larval development in *Sabella spallanzanii* (Polychaeta: Sabellidae) from the Mediterranean Sea. *Mar Biol* 136:847–862

Giangrande, A., Cavallo, A., Licciano, M., Mola, E., Pierri, C., Trianni, L., 2004. Utilization of the filter feeder *Sabella spallanzanii* as bioremediator in aquaculture. *Aquaculture International* 13, 129–136.

Gonzalez M, Caride B, Lamas A, Taboada C 2001. Nutritional value of the marine invertebrates *Anemonia viridis* and *Haliotis tuberculata* and effects on serum cholesterol concentration in rats open star. *J Nutr Biochem*.12(9):512-517.

Guillaume J., Kaushik S., Bergot P., Metailler R. 2001. Nutrition and Feeding of Fish and Crustaceans. Ed. Springer-Verlag, New York, USA, pp. 403

Gutnikov G. 1995. Fatty acid profiles of lipid samples *J. Chromatogr. B. Biomed Appl.*15,671(1-2), 71-89

Harrison KE (1991) Crustacean reproduction nutrition. *Crustac Nutr Newsl* 7:62–70

Hites R.A., Foran J.A., Carpenter D.O., Hamilton M.C., Knuth B.A., Schwager S.J. 2004. Global Assessment of Organic Contaminants in Farmed Salmon. *Science* vol. 303, 223-229

Karakoltsidis P.A., Zotos A., Constantinides S.M. 1995. Composition of the commercially important Mediterranean Finfish, Crustaceans, and Molluscs, *Journal of Food Composition and Analysis* 8:258-273

Kikuchi K. 1999. Use of defatted soybean meal as a substitute for fish meal in diets Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* 179, 3–11.

Kikuchi, K. and Furuta, T. 2009. Use of Defatted Soybean Meal and Blue Mussel Meat as Substitute for Fish Meal in the Diet of Tiger Puffer, *Takifugu rubripes*. *Journal of the World Aquaculture Society*, 40: 472–482.

King I., Childs MT., Dorsett C., Ostrander JG., Monsen ER. 1990. Shellfish: proximate composition, minerals, fatty acids, and sterols. *J Am Diet Assoc.* 90(5):677-85

Kitson R.E., Mellon M.G. 1944. Colorimetric determination of phosphorus as molybdivanadophosphoric acid. *Industrial and Engineering Chemistry, Analytical Edition*. Easton, Pa. 16, pp. 379

Klingensmith, SJ. and Stillway, WL. 1982. Lipid composition of selected tissues of hardshell clam *Mercenaria mercenaria*. *Comparative Biochemistry and Physiology*, 71: 111-112.

Kolkovski S.. Digestive enzymes in fish larvae and juveniles—implications and applications to formulated diets 2001. *Aquaculture* 200, 1-2, pp 181-201

Kolkovski S., Czesny S., Dabrowski K. 2000. Use of krill hydrolysate as a feed attractant for fish larvae and juveniles. *Journal of the World Aquaculture Society* 31, 1: 81–88

Krogdahl, Å., Bakke-McKellep, A.M., Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquacult. Nutr.* 9 (6), 361-371.

Kromhout D, Bosschieter EB, de Lezenne Coulander C.1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N. Engl. J. Med.* 9, 312 (19), 1205-9

Lebel, L., Tri, N.H., Saengnoee, A., Pasong, S., Buatama, U., Thoa, L.K., 2002. Industrial transformation and shrimp aquaculture in Thailand and Vietnam: pathways to ecological, social and economic sustainability? *Ambio* 31 (4), 311–323.

Lomovasky B.J., Malanga G., Calvo J. 2004. Seasonal changes in biochemical composition of the clam, *Eurhomalea exalbida* (bivalvia: veneridae), from the beagle channel, Argentina.: *Journal of Shellfisheries Research* 23:1 p.81(7)

Luis O.J. and Passos A.M. 1995. Seasonal changes in lipid content and composition of the polychaete *Nereis (Hediste) diversicolor*. *Comp Biochem Physiol B* 111:579–586

Mahan L.K., Escott-Stump S. 2000. Krause's food, nutrition, & diet therapy

Martinez, G. 1991. Seasonal variation in biochemical composition of three size classes of the Chilean scallop *Argopecten purpuratus* Lamarck, 1819, *Veliger* 34:335-343.

McGoogan B.B. & Gatlin D.M. III. 1997. Effects of replacing fish meal with soybean meal in diets for red drum *Sciaenops ocellatus* and potential for palatability enhancement. *Journal of the World Aquaculture Society* 28, 374–385.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2001. Effect of aquaculture on world fish supplies. *Nature* 405, 1017-1024.

Nechev J., Stefanov K., Popov S. 2006. Effect of cobalt ions on lipid and sterol metabolism in the marine invertebrates *Mytilus galloprovincialis* and *Actinia equina*. *Comp. Biochem. Physiol.*, 144 (A): 112-118.

Olive P.J.W. (1999) Polychaete aquaculture and polychaete science: a mutual synergism. *Hydrobiologia* 402:175–183

Olive, P.J.W., Craig, S., Cowin, P.B.D., Islam, M.D., Rutherford, G., 2002. The culture of Polychaeta as a contribution to sustainable production of aquafeeds. *Aquaculture Europe 2001. Book of Abstracts. European Aquaculture Society Special Publication*, pp 23 - 26

Orban E., Di Lena G., Casini I., Gambelli L., Marzetti A., Caproni R. 2002. Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian sites, *Food Chem.*, 77:57-65

Otles, S. and Sengor, G. 2005. Effect of Various Technological Processes on the Fatty Acid Composition of Mussel (*Mytilus galloprovincialis*, L.)," *International Journal of Food Engineering* Vol. 1: 3,5.

Palmegiano, G.B., Agradi, E., Forneris, G., Gai, F., Gasco, L., Rigamonti, E., Sicuro, B., Zoccarato, I., 2005. Spirulina as a nutrient source in diets for growing sturgeon (*Acipenser baeri*). *Aquacult. Res.* 36, 188-195.

Primavera, J.H., 2000. Development and conservation of Philippine mangroves: institutional issues. *Ecological Economics* 35, 91–106.

Parrish C.C., Yang Z., Lau A., Thompson R.J. 1996. Lipid composition of *Yoldia hyperborea* (Protobranchia), *Nephtys ciliata* (Nephtyidae) and *Artacama proboscidea* (Terebellidae) living at sub-zero temperatures. *Comparative Biochemistry and Physiology B: Biochemistry and Molecular Biology* 114, 1, pp. 59-67

Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. *Lipids in Aquatic Ecosystems*, Arts, M.T., Brett, M.T. and Kainz, M.J. (eds). Springer, Dordrecht. . 377: pp309-326

Pollero, R.J., Re M.E., Brenner R.R., 1979. Seasonal changes of the lipids of the mollusc *Chlamys tehuelcha*. *Comp. Biochem. Physiol.* 64A:257-263.

Pocock D. M. E., Marsden J. R., Hamilton J. C. 1971. Lipids in an intertidal polychaete and their relation to maturation of the worm. *Comparative Biochemistry and Physiology*, 39 (A): 683-697

Quinlan K.P. and Desesa M.A. 1955 Spectrophotometric determination of Phosphorus as molybdovanadophosphoric acid. *Analytical Chemistry* 27, 1626-1629.

Salze G., McLean E., Battle P.R., Schwarz M.C., Craig S.R. 2010. Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, *Rachycentron canadum*. *Aquaculture* 298: 294–299

Sanchez-Machado D.I., Lopez-Cervantes J., Lopez-Hernandez J. and P.Paseiro-Losada, 2004. Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chem.*, 85: 439-444.

Sicuro B., Mioletti Silvia, Abete Cesarina, Amedeo S., Panini Elsa, Forneris. G. 2010. Potential utilization of farmed freshwater mussels (*Anodonta anatina* and *Unio mancus*) in Italy. *Cuban Journal of Agriculture*, in press

Shankar R., Murthy H.S., Pavadi P., Thanuja K. 2008. Effect of betaine as a feed attractant on growth, survival, and feed utilization in fingerlings of the Indian major carp, *Labeo rohita*. *Israeli Journal of aquaculture – Bamidgeh*, 60, 2, pp. 95 - 99

Shimizu C., Ibrahim A., Tokoru T. & Shirakawa Y. 1990. Feeding stimulation in sea bream (*Pagrus major*) fed diets supplemented with Antarctic krill meals. *Aquaculture* 89, 43–53.

Stabili L., Licciano M., Giangrande A., Fanelli G., Cavallo R.A. 2006. *Sabella spallanzanii* filter-feeding on bacterial community: Ecological implications and applications. *Marine Environmental Research* 61: 74–92

Stabili L., Schirosi R., Licciano M., Mola E., Giangrande A 2010. Bioremediation of bacteria in aquaculture waste using the polychaete *Sabella spallanzanii*. *New Biotechnology* 27(6): 774-781.

Tiril S.U., Alagil F., Yagci F.B., Aral O. 2008. Effects of betaine supplementation in plant protein based diets on feed intake and growth performance in rainbow trout (*Oncorhynchus mykiss*). *Israeli Journal of aquaculture – Bamidgeh*, 60, 1, pp 57 -64

Trinder P. 1969. Simple turbidometric method for the determination of serum cholesterol. *Annal of Clinical Biochemistry* 6, p.165

Vernocchi P., Maffei M., Lanciotti R., Suzzi G., Gardini F. 2007. Characterization of mediterranean mussel *Mytilus galloprovincialis* harvested in Adriatic Sea (Italy), Food Control 18. 1575-158

Warnau M., Biondo R., Temara A., Bouquegneau J., Jangoux M., Dubois P. 1998. Distribution of heavy metals in the echinoid *Paracentrotus lividus* from the Mediterranean *Posidonia oceanica* ecosystem: seasonal and geographical variations. Journal of Sea Research 39: 267–280

Wenne, R. and Polak, L. 1989. Lipid composition and storage in the tissues of the *Macoma balthica*. Biochemical Systematic and Ecology, 17: 583-587.

Yilmaz E. 2005. The effects of two chemo-attractants and different first feeds on the growth performances of African catfish (*Clarias gariepinus*, Burchell, 1822) at different larval stages. Turkish Journal of Veterinary and Animal Sciences, 29, 2 pp.309-314.

Zandee DI., Kluytmans JH., Zurburg W., Pieter RH. 1980. Seasonal variations in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis. Neth. J. Sea Res. 14: 1–29.

Zöllner N. and Kirsch K. 1962. Determination of the total lipid concentration in serum. Zentralblatt für Gesamte Experimentale Medizin 135, 545-549.

FIGURE LEGENDS

Fig. 1 Map of the Apulian coast showing the location of the sampling site

Fig.2 *Sabella spallanzanii* composition: water content and dried weight (A), organic and inorganic residuals (B), organic components: proteins, lipids and other (C).

Fig.3 Total lipids profile of *Sabella spallanzanii*.