SEASONAL VARIATIONS IN GROSS BIOCHEMICAL COMPOSITION, PERCENTAGE EDIBILITY AND CONDITION INDEX OF THE CLAM *RUDITAPES DECUSSATUS* CULTIVATED IN THE RIA FORMOSA (SOUTH PORTUGAL)

Short running title: Seasonal biochemical composition in *R decussatus*

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ABSTRACT

The grooved carpet shell clam, *Ruditapes decussatus* (L. 1758), is one of the most popular and profitable molluscs exploited in rearing plots in the Mediterranean. However, annual catch has been declining steadily since the early nineties. In order to understand the seasonality of its nutritional value, thus providing an improved basis for economical valuation of the resource, gross biochemical composition, percentage edibility and condition index were investigated during a year with monthly periodicity in a commercially exploited population of the clam *Ruditapes decussatus* in the Ria Formosa, a temperate mesotidal coastal lagoon located in the south of Portugal. Our results show that total and non-protein nitrogen co-varied during the year, resulting in a protein content that peaked in the warmest months. Although complementary in summer, carbohydrate and lipid contents showed irregular annual trends. The observed seasonality was comparable to that shown by studies elsewhere at similar latitudes, and are underpinned by the reproductive cycle of the species. Our results show the clams to be at their prime nutritional value at the beginning of summer, when protein content peaks.

KEY WORDS: Biochemical composition, condition index, percentage edibility, Ria Formosa, *Ruditapes decussatus*, seasonal variations

INTRODUCTION

The grooved carpet shell clam, *Ruditapes decussatus* (L. 1758), is one of the most popular and profitable molluscs of lagoon and coastal sites in the Mediterranean, having been used as a food source for centuries. Between 1996 and 2008, official statistics suggested an average pooled catch of 4 metric tons in Portugal, Spain, France, Ireland and Tunisia (FAO 2010). In Portugal, the harvesting of bivalves in particular *R. decussatus* is central to aquaculture revenue. In 2007, the national annual production reported for this species reached 2 metric tons – representing 27% of total seafood cultured in Portugal –, of which approximately 90% originate in the Ria Formosa (INE 2007). Here, clams are grown ("farmed") in plots exploited by clam farmers, locally known as "mariscadores", usually organized in professional associations. Clam farming involves seeding juveniles collected from natural beds into plots maintained in tidal flats and harvesting commercial size animals (i.e. >20 mm). The culture of *R.* de*cussatus* in the Ria Formosa is central to the local socioeconomic framework involving, directly or indirectly, more than 4500 people (INE 2007). In 1996, there were 1587 licensed clam farming plots within the intertidal area of the Ria Formosa, covering a total of 0.47×10^{6} m^2 (Cachola 1996), approximately 1% of the total intertidal area of the lagoon. More recently, the ICNB (2004) confirmed the existence of 1290 plots in the Ria Formosa, a decrease in numbers but a ten-fold increase in the occupied intertidal area (about 4.76×10^{6} m^2). In addition to the official catch figures, widespread illegal and largely opportunistic fishing and harvesting by elements foreign to the local associative system most probably doubles the official production estimates (António Labóia, VIVMAR, 2007, personal communication).

Percentage edibility (i.e. meat content/yield), physiological condition and biochemical composition of bivalves vary seasonally with latitude and are strongly related to water temperature, food availability and the gametogenic cycle (Beninger & Lucas 1984, Karakoltsidis et al. 1995, Okumus & Stirling 1998, Orban et al. 2002, Delgado et al. 2004, Ojea et al. 2004, Orban et al. 2006). Proteins, lipids, carbohydrates and minerals are major contributors to the nutritional value and organoleptic properties of clams (Orban et al. 2006), and justify the very high demand for this product in national and international markets. In *R. decussatus*, both stored and recently assimilated nutrients are used for gametogenesis (Pérez-Camacho et al. 2003), characteristic of an intermediate strategy between opportunistic and conservative lifestyles (Rodríguez-Moscoso & Arnaiz 1998). During the reproductive cycle of this species, gametogenesis extends from the end of winter and spring; spawning occurs all through the summer months and a resting period in autumn and early winter (Rodríguez-Moscoso & Arnaiz 1998).

Percentage edibility or the condition index (CI) have long been used for biological and commercial purposes (Venkataraman & Chari 1951, Baird 1958). These are closely related to the gametogenic and nutrient reserve storage-consumption cycles, and thus to meat quality (Gabbott 1975). In industrial settings, CI has been adopted in international trade as a standard criterion to select the best product. It is also recognized as a useful biomarker reflecting the ability of bivalves to withstand adverse natural and/or anthropogenic stress (Mann 1978, Bressan & Marin 1985, Fernandez-Castro & de Vido de Mattio 1987). Hence, the CI may be considered a measure of “fatness” and “marketability” of a commercially exploited species and, together with proximate biochemical composition, is probably the most practical and simple method of monitoring gametogenic activity (Okumus & Stirling 1998).

In order to understand the seasonality of *R. decussatus* nutritional value, thus providing an improved basis for its economical valuation, this study aimed to investigate the changes in grooved carpet shell clam gross biochemical composition in the Ria Formosa (southern Portugal). Moisture, ash, protein, total and non-protein nitrogen, carbohydrates and lipid contents, as well as the percentage edibility and condition index were assessed with monthly periodicity for one year.

MATERIALS AND METHODS

Environmental data, sampling and processing

The study was carried out between January and December 2006. Monthly average air temperature and precipitation recorded at a meteorological station (Faro International Airport) in the Ria Formosa were used to assess seasonality in climatic conditions. Samples of *R. decussatus* clams (ca. 1 kg, about 140 individuals) were obtained directly from a farmer's plot belonging to VIVMAR association on a monthly basis. This assured that all tested biological material was of commercial value and was harvested from the same area of the Ria Formosa. Immediately after harvest, the samples were transported to the laboratory in a refrigerated box, washed and placed in pre-filtered (Whatman GF/C) sea water for 3 to 4 h in order to purge pseudo faeces and stomach content. Thirty individuals were then randomly selected for biometric measurements and for the determination of the percentage edibility and condition index. The remaining clams were stored at –20 ºC for later biochemical composition analysis.

Biometric parameters, percentage edibility and condition index

Individual clams were weighted (± 0.1 mg) and their maximum length measured using a precision caliper (to 0.05 mm). Clams were manually shucked by cutting the adductor muscle with a knife, and the meat was pressed with blotting paper to remove excess moisture before weighting. The meat and shells were subsequently dried at 105 ºC for 24 h and weighted again.

Percentage edibility (PE) was calculated as PE = (MWW/TW) × 100, where MWW is meat wet weight (g) and TW is the total clam weight including the shell (g) (Venkataraman & Chari 1951, Mohite et al. 2009). Condition index (CI) was calculated as CI = (MDW/SDW) × 1000, where MDW is meat dry weight (g) and SDW is the shell dry weight (g), following Lucas and Beninger (1985) and Orban et al. (2006).

Biochemical analyses

Moisture and ash contents of clams were determined for thirty individuals using the AOAC (2005) methods ref 950.46 and 938.08, respectively. Total nitrogen (bulk protein content) was determined by the Kjeldahl method (Ref 955.04, AOAC 2005). This was also used to determine non-protein nitrogen content after precipitation of proteins with 10% (w/v) trichloroacetic acid. Net protein content was calculated hence as the difference between total nitrogen and non-protein nitrogen multiplied by 6.25, the conversion factor used for meat and meat products (Pearson 1973). Carbohydrate and total lipids were determined according to Dubois et al. (1956) and Bligh and Dyer (1959), respectively. The biochemical analyses were carried out on individually on nine randomly selected individuals.

Statistics

Initially, one-way analysis of covariance (ANCOVA) was used to test if putative seasonal variations (Month) in the biological traits (response variables) co-varied with individual size (length). Since no significant effects of length on biochemical composition were found (Table 1), one-way analysis of variance (ANOVA) and the

Tukey Honestly Significant Difference (HSD) test were carried out to uncover any significant seasonal changes in biochemical composition, condition or edibility. The lipid content values were log-transformed to correct for (severe) non-normality. In addition, relationships between monthly data on biochemical composition, condition and edibility were investigated using Spearman rank correlation analysis. All statistical procedures were carried out at the 0.05 level of significance using R (R Development Core Team 2007).

RESULTS

Monthly average air temperature and precipitation for the Ria Formosa are illustrated in Fig. 1. Higher temperatures were observed in summer (25 ºC in July and August) and the lowest in winter (<9º C). Monthly precipitation records showed an extreme value in November with 252 mm, much higher than the range observed throughout the rest of the year (15 to 40 mm).

Biochemical composition (Fig. 2), percentage edibility and condition index (Fig. 3) varied with the season (Table 2). Average monthly moisture contents were significantly higher ($p<0.05$) during autumn and winter (85.9% in March and 86.9% in November) than in June (82.6%). On the other hand, ash, which indicates the inorganic compounds content, was significantly higher ($p<0.05$) during January and February (3.4 and 3.6%) when compared to the rest of the year. Between March and August a second period of intermediate values (ca. 3.1%) was observed. From August to November, the clams had the lowest values of ash content (about 2.8%).

The carbohydrate content varied widely from 0.4% in January and July to to 2.6% in September. Average log-transformed lipid content showed no significant seasonal variation in spite of the large variability on a monthly basis. The lowest lipid content was measured in April (0.07%) and the lowest total nitrogen content was measured between October and November (ca. 1.2%). In contrast, total nitrogen content was significantly higher in June, August and September (ca. 1.5%). In spite of null values of non-protein nitrogen found in May and July, the total and non-protein nitrogen contents evidenced complementary seasonal trends. The resultant protein content was significantly higher ($p<0.05$) during early summer, averaging 8.5% from May to July, in contrast to winter values ranging from 5.9% in December to 6.7% in January.

Percentage edibility of clams was significantly lower ($p<0.05$) in January and February (~24%) when compared to the April/June or October/December trimesters (30-32%). On the other hand, the condition index of the clams was significantly higher ($p<0.05$) from April to June (98.6 to 107.2) than from July to December, when intermediate values of 83.4 – 88.2 were recorded. Clams showed the lowest condition indices (<77.2) in January and March.

Few pair wise correlation coefficients (Table 3) were judged to be significant ($p<0.05$). Temperature and/or precipitation were associated with ash, total nitrogen and/or protein content and condition index. Moreover, condition index and percentage edibility were intercorrelated and associated with protein content and non-protein nitrogen contents.

DISCUSSION

Temperature and food availability have been considered the main factors conditioning the growth and hence production in bivalves. The effect of these variables is complex and depends on species-specific acquisition and expenditure of energy in the natural environment (Bayne & Newell 1983). In temperate tidal lagoons with considerable open-sea water exchanges and high primary production (*e.g.* Sufa Lagoon in Turkey or the Ria Formosa in Portugal), the food supply is not considered a limiting factor for growth and reproduction of bivalves (Serdar & Lök 2009), which feed mainly on microalgae (Delgado & Pérez-Camacho 2005). Consequently, temperature is the key factor controlling the reproductive cycle of *R. decussatus* (Urrutia et al. 1999, Delgado & Pérez-Camacho 2007, Matias et al. 2009), influencing seasonal biochemical composition and nutritional conditions (Gözler & Tarkan 2000, Pérez-Camacho et al. 2003, Fernández-Reiriz et al. 2007).

Seasonal biochemical composition followed the changes in percentage edibility and condition index, which indirectly reflect *R. decussatus*' reproductive phases (Gözler & Tarkan 2000, Mohite et al. 2009). From January until June, the rising temperatures induce gametogenesis (Delgado & Pérez-Camacho 2007) resulting in an increase in percentage edibility and condition index. During this period, *R. decussatus* accumulated and used carbohydrates, lipids, proteins and minerals presumably for gonad development. The sudden decrease in all these parameters occurred between June and July most probably coincided with spawning and the phase may have lasted until September, when the species entered the resting phase.

Our results compare well with studies on the seasonality of clam physiology at other latitudes, including the Galician Rias in NW Spain (Ojea et al. 2004), the Lagoon of Venice in Italy (Marin et al. 2003), Sufa and Çardak lagoons in Turkey (Gözler & Tarkan 2000, Serdar & Lök 2009) and Atlantic coast of Morocco (Shafee & Daoudi 1991), despite the differences in methodology. Overall, carbohydrates and lipids show complementary trends, with carbohydrates (and moisture levels) reaching minima in summer when lipid and protein content peaked. In addition, all previously mentioned study sites sustain similar seasonal trends of condition indices, with small latitudinal changes. The southernmost the location, the sooner gametogenesis, ripping and spawning occurs (Meneghetti et al. 2004), with spawning starting in August in NW Spain (Peréz-Camacho et al. 2003), in June-July in south Portugal and in May in Morocco (Shafee & Daoudi 1991).

In the Ria Formosa, total nitrogen, non-protein nitrogen and thus protein contents show that the nitrogen metabolism in *R. decussatus* varies on a monthly basis. However, the lower non-protein nitrogen registered in the summer, suggests that the majority of the clam nitrogen metabolism is being channelled to the spawning process during that period (Marin et al. 2003). Although the glycogen content is the parameter most often linked to the seasonal variation in clam carbohydrate levels, direct determination of total carbohydrates not only allows the evaluation of the same type of seasonal changes, but also adds all the other types of mono- and polysaccharides involved in the clam's life cycle, thus becoming a more integrative parameter. In fact, the seasonal variation of glycogen and carbohydrates is strongly correlated, with the former being responsible for approximately 50% of the variance of the latter (Robert et al. 1993, Serdar & Lök 2009). In autumn and early winter, *R. decussatus* accumulates glycogen prior to gametogenesis, before it is used as an energy source for gonad development, in anticipation of the spawning period taking place in summer (Ojea et al. 2004).

Lipids are the biomolecules that are more influenced by the clams annual reproductive cycle because of their relationship with gonad maturation. The large variability of lipid content evidenced by the standard deviations may be related to the differential gender-related sexual dynamics of this species (Pérez-Camacho et al. 2003). The link between the lipid and carbohydrate contents may be thus rooted on the assumption that many lipids accumulated by clams are sourced from glycogen reserves

(Marin et al. 2003). This metabolic relationship underpins the opposite trends observed between the seasonal variations of these two parameters observed in this study. This was particularly evident in July and August. During the remainder of the year, the clams attempt to accumulate lipidic reserves through food ingestion in preparation for the next reproductive cycle (Marin et al. 2003).

Seasonal variations on the nutritional value can also be accessed, in a more global approach, using meat yield related indices. In the Ria Formosa, the physiological condition of *R. decussatus* is higher between April and June, and lower during the rest of the year. Taking into account the physiological condition of the species and its nutritional value, in terms of protein content, the best period of the year to consume *R. decussatus* would be summer. However, this same period is understood to be the worst to do so from a toxicological point of view, because of the increased risk of poisoning by shellfish toxins (Vale & Sampayo 2002). In summer, consumers should exercise particular care when buying these bivalves, always making sure to acquire depurated and certified products (Vale et al. 2008). On the other hand, the demand for *R. decussatus* and consequently its market value are also very high during Christmas season. The product's "health threat" is not an issue at that time of the year (Vale & Sampayo 2003) but its physiological condition is at its worst leading to a discrepancy between the nutritional value and the demand for this product, similarly to the case of *R. philippinarum* in the Lagoon of Venice in Italy (Marin et al. 2003).

CONCLUSIONS

The clam *R. decussatus* is an important natural resource and food product in the Ria Formosa and its exploitation sustains an significant part of the local economy. The analysis of *R. decussatus* percentage edibility and condition indices allowed inference of its reproductive cycle: gametogenesis started in January; spawning took place from June to September, and the resting stage occurred between October and December. The high seasonal variability observed in the biochemical composition of this species was most probably due to the reproductive cycle and showed typical features of the life history of bivalve molluscs at temperate latitudes. Similarly to mariculture populations in Galicia (Spain), Lagoon of Venice (Italy), Turkey and Morocco, the peak in nutritional value is observed during the summer, whilst the slump occurs during winter. Curiously, these two periods coincide with the peaks of major commercial demand.

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LITERATURE CITED

AOAC. 2005. Official methods of analysis. 18th edition, Maryland, USA, Association of Official Analytical Chemists.

Baird, R. H. 1958. Measurement of condition in mussels and oysters. J. Cons. Perm. Inter. Explor. Mer 23:249-257.

Bayne, B. L. & R. C. Newell. 1983. Physiological energetic of marine mollusks. In: A. S. M. Saleudin & K. M. Wilbur, editors. The Mollusca, Academic Press, New York. pp. 407-515.

Beninger, P. G. & A. Lucas. 1984. Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L. (Jeffreys) and *Tapes philippinarum* (Adam & Reeves). J. Exp. Mar. Biol. Ecol. 79:19-37.

Bligh, E. G. & W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Bressan, M. & M. G. Marin. 1985. Seasonal variations in biochemical composition and condition index of cultured mussels (*Mytillus galloprovincialis*) in the Lagoon of Venice (North Adriatic). Aquaculture 48:13-21.

Cachola, R. 1996. Viveiros de amêijoa boa *Ruditapes decussatus* da região algarvia. Instituto de Investigação das Pescas e do Mar, Lisboa.

Delgado, M. & A. Pérez-Camacho. 2005. Histological study of the gonadal development of *Ruditapes decussatus* (L.) (Mollusca: Bivalvia) and its relationship with available food. Sci. Mar. 69:87-97.

Delgado, M. & A. Pérez-Camacho. 2007. Comparative study of gonadal development of *Ruditapes philippinarum* (Adams and Reeve) and *Ruditapes decussatus* (L.) (Mollusca: Bivalvia): Influence of temperature. Sci. Mar. 71:471-484.

Delgado, M., A. Pérez-Camacho, U. Labarta & M. J. Fernández-Reiriz. 2004. The role of lipids in the gonadal development of the clam *Ruditapes decussatus* (L.). Aquaculture 241:395-411.

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

FAO. 2010. Species Fact Sheets: *Ruditapes decussatus* (Linnaeus, 1758). http://www.fao.org/fishery/species/3542/en. Cited 24 May 2010

Fernandez-Castro, N. & N. de Vido de Mattio. 1987. Biochemical composition index and energy value of *Ostrea puelchanan* (D'Orbigny): relationships with the reproductive cycle. J. Exp. Mar. Biol. Ecol. 108:113-126.

Fernández-Reiriz, M. J., A. Pérez-Camacho, M. Delgado & U. Labarta. 2007. Dynamics of biochemical components, lipid classes and energy values on gonadal development of

R. philippinarum associated with the temperature and ingestion rate. Comp. Biochem. Physiol. A 147:1053-1059.

Gabbott, P. A. 1975. Storage cycles in marine bivalve mollusks: A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In: Barnes H (ed) Proceedings of the 9th Eur Mar Biol Symp. Aberdeen University Press, Aberdeen. pp. 191-211.

Gözler, A. M., A. N. Tarkan. 2000. Reproductive biology of *Ruditapes decussatus* (Linnaeus, 1758) in Çardak Lagoon, Dardanelles Strait. Turk. J. Mar. Sci. 6:175-198.

ICNB. 2004. Programa de ordenamento do Parque Natural da Ria Formosa. Relatórios, Volume I. Estudos de Caracterização. Instituto de Conservação da Natureza e Biodiversidade, Lisboa. 655 pp.

INE. 2007. Estatística da pesca 2006. Instituto Nacional de Estatística, Lisboa. 97 pp.

Karakoltsidis, P. A., A. Zotos & S. M. Constantinides. 1995. Composition of the commercially important Mediterranean finfish, crustaceans and molluscs. J. Food Compos. Anal. 8:258-273.

Lucas, A. & P. G. Beninger. 1985. The use of physiological condition indices in marine bivalve aquaculture. Aquaculture 44:187-200.

Mann, R. 1978. A comparison of morphometric, biochemical and physiological indexes of condition in marine bivalve molluscs. In: J. H. Thorpe & J. W. Gibbons, editors. Energy and Environmental Stress in Aquatic System, Technical Information Center, U.S. Department of Energy, Oak Ridge, TN. pp 484-497.

Marin, M. G., V. Moschino, M. Deppieri & L. Lucchetta. 2003. Variations in gross biochemical composition, energy value and condition index of *T. philippinarum* from the lagoon of Venice. Aquaculture 219:859-871.

Matias, D., S. Joaquim, A. Leitão & C. Massapina. 2009. Effect of geographic origin, temperature and timing of broodstock collection on conditioning, spawning success and larval viability of *Ruditapes decussatus* (Linné, 1758). Aquacult. Int. 17:257-271.

Meneghetti, F., V. Moschino & L. Da Ros. 2004. Gametogenic cycle and variations in oocyte size of *Tapes philippinarum* from the Lagoon of Venice. Aquaculture 240:473-488.

Mohite, S. A. & A. S. Mohite & H. Singh. 2009. On condition index and percentage edibility of the shortneck clam *Paphia malabarica* (Chemintz) from estuarine regions of Ratnagiri, west coast of India. Aquacult. Res. 40:69-73.

Ojea, J., A. J. Pazos, D. Martínez, S. Novoa, J. L. Sánchez & M. Abad. 2004. Seasonal variation in weight and biochemical compostion of the tissues of *Ruditapes decussatus* in relation to the gametogenic cycle. Aquaculture 238:451-468.

Okumus, I. & H. P. Stirling. 1998. Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. Aquaculture 159:249-261

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

Orban, E., G. Di Lena, T. Nevigato, I. Casini, R. Caproni, G. Santorini & G. Giulini. 2006. Nutritional and commercial quality of the striped venus clam, *Chamelea gallina*, from the Adriatic sea. Food Chem. 101:1063-1070.

Pearson, D. 1973. Laboratory techniques in food analysis. Butterworths and Co., London. 315 pp.

Pérez-Camacho, A., M. Delgado, M. J. Fernández-Reiriz & U. Labarta. 2003. Energy balance, gonad development and biochemical composition in the clam *Ruditapes decussatus*. Mar. Ecol. Prog. Ser. 258:133-145.

R Development Core Team. 2007. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org.

Robert, R., G. Trut & J. L. Laborde. 1993. Growth, reproduction and gross biochemical composition of the Manila clam *Ruditapes philippinarum* in the Bay of Arcachon, France. Mar. Biol. 116:291-299.

Rodríguez-Moscoso, E. & R. Arnaiz. 1998. Gametogenesis and energy storage in a population of the grooved carpet-shell clam, *Tapes decussatus* (Linné, 1787), in northwest Spain. Aquaculture 162:125-139.

Serdar, S. & A. Lök. 2009. Gametogenic cycle and biochemical composition of the transplanted carpet shell clam *Tapes decussatus*, Linnaeus 1758 in Sufa (Homa) Lagoon, Izmir, Turkey. Aquaculture 293:81-88.

Shafee, M. S. & M. Daoudi. 1991. Gametogenisis and spawning in the carpet-shell clam, *Ruditapes decussatus* (L.) (Molluca: Bivalvia), from the Atlantic coast of Morocco. Aquacult Fish. Manage. 22:203-216.

Urrutia, M. B., I. Ibarrola, J. I. P. Iglesias & E. Navarro. 1999. Energetics of growth and reproduction in a high-tidal population of the clam *Ruditapes decussatus* from Urdaibai Estuary (Basque Country, N. Spain). J. Sea Res. 42:35-48.

Vale, P. & M. Sampayo. 2002. Esterification of DSP toxins by Portuguese bivalves from the Northwest coast determined by LC-MS – a widespread phenomenon. Toxicon 40:33-42.

Vale, P. & M. Sampayo. 2003. Seasonality of diarrhetic shellfish poising at a coastal lagoon in Portugal: rainfall patterns and folk wisdom. Toxicon 41:187-197.

Vale, P., M. J. Botelho, S. M. Rodrigues, S. S. Gomes & M. Sampayo. 2008. Two decades of marine biotoxin monitoring in bivalves from Portugal (1986-2006): A review of exposure assessment. Harmful Algae 7:11-25.

Venkataraman, R. & S. D. T. Chari. 1951. Studies on oysters and clams – biochemical variations. Indian J. Med. Res. 39:533-541.

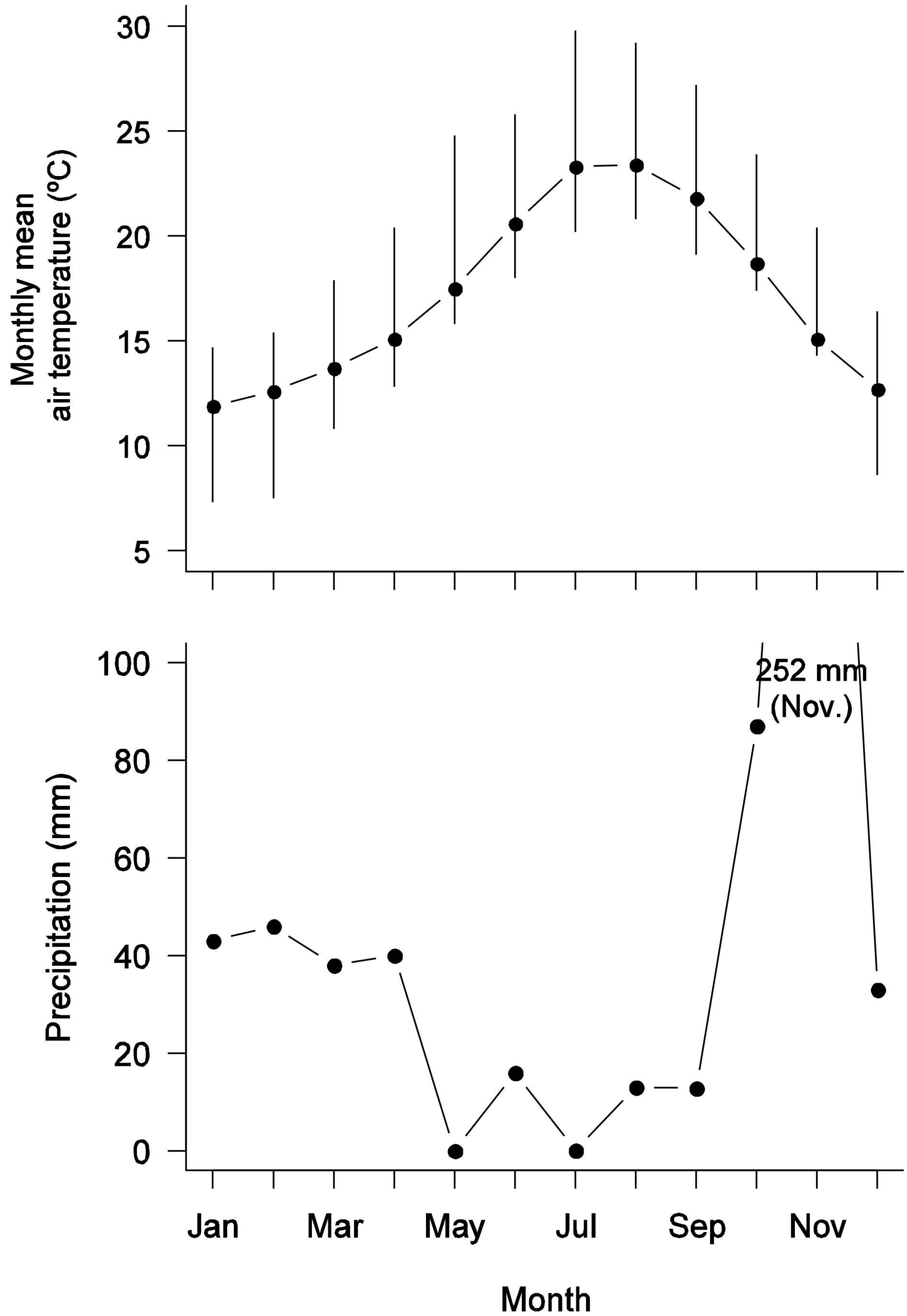

Figure 1. Average monthly air temperature (left) and monthly precipitation recorded at a meteorological station in the Ria Formosa (Faro International Airport) (right).

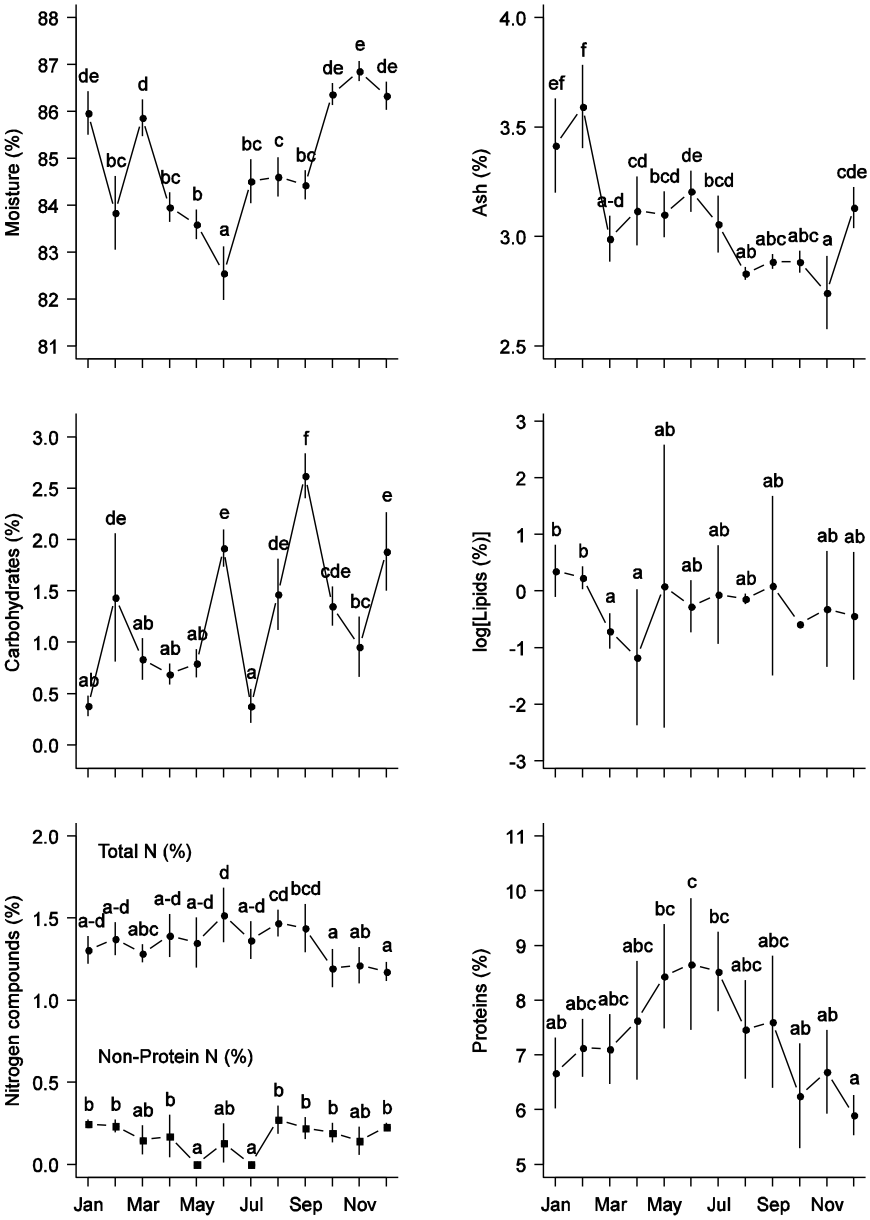

Figure 2. Monthly variations (means ± 95% confidence intervals) of moisture (%), ash content (%), carbohydrates content (%), log(lipids content) (%), nitrogen compounds' content (%) and protein content (%) in *R. decussatus* from the Ria Formosa. Values not sharing the same superscript(s) are significantly different (*p*<0.05).

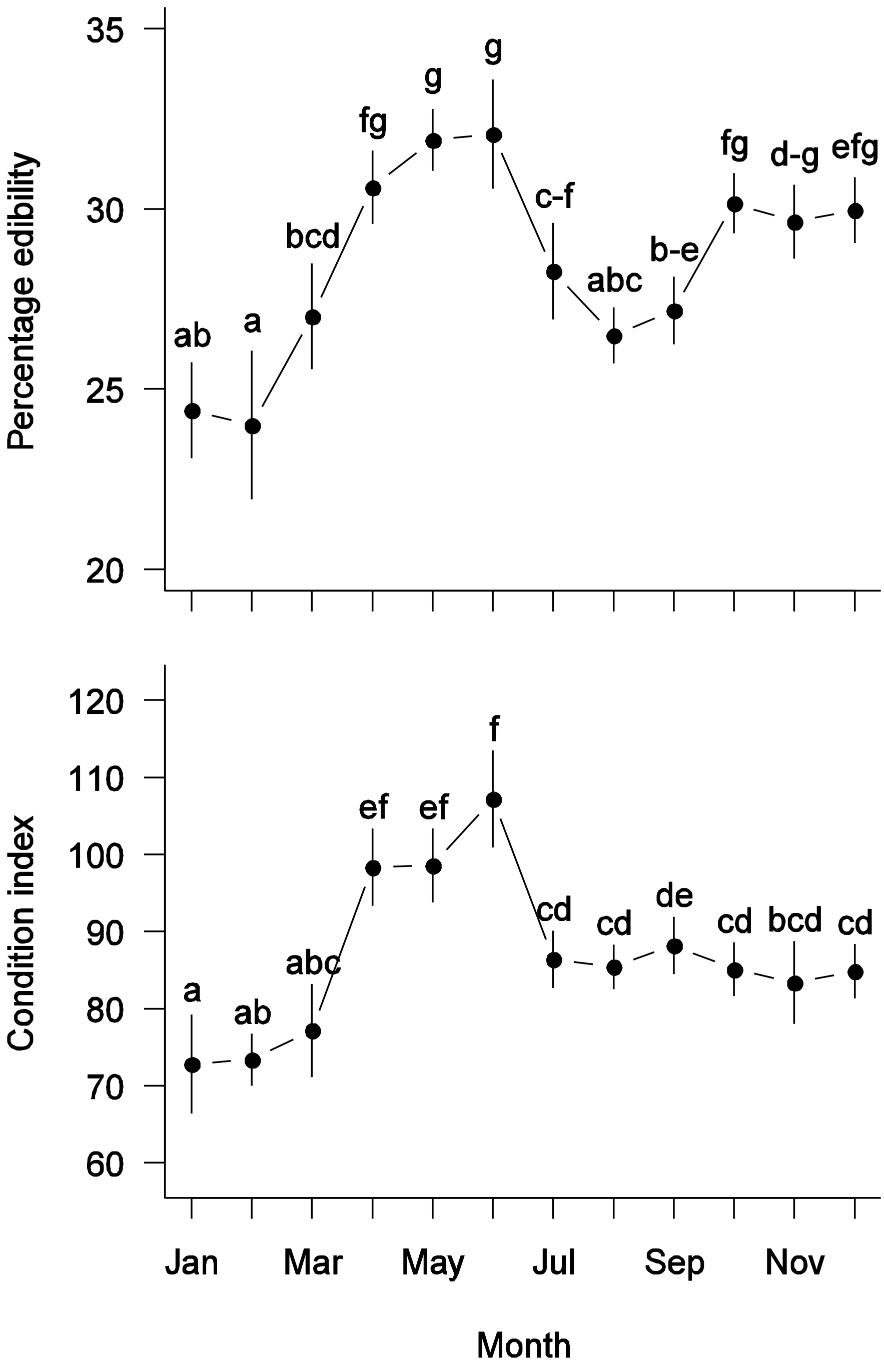

Figure 3. Seasonal variations (means ± 95% confidence intervals) in the percentage edibility and condition index of *R. decussatus* from the Ria Formosa. Values not sharing the same superscript(s) are significantly different (*p*<0.05).

TABLE 1.
Results of Analyses of covariance (ANCOVAs) per parameter studied in *R. decussates* from the Ria Formosa (south Portugal).

Parameter		df	SS	MS	F	p-value
Moisture	Month	11	558.61	50.78	42.91	$<10^{-6}$
	Length	1	0.18	0.18	0.15	0.6945
	Month x Length	11	16.74	1.52	1.29	0.2308
	Residuals	335	396.44	1.18		
Ash	Month	11	19.70	1.79	15.11	$<10^{-6}$
	Length	1	0.07	0.07	0.57	0.4526
	Month x Length	11	1.05	0.10	0.81	0.6317
	Residuals	336	39.82	0.12		
Carbohydrates	Month	11	45.98	4.18	30.14	$<10^{-6}$
	Length	1	0.25	0.25	1.78	0.1857
	Month x Length	11	0.93	0.09	0.61	0.8153
	Residuals	83	11.51	0.14		
$\log_{10}$(Lipids)	Month	11	10.88	0.99	4.31	0.0010
	Length	1	0.47	0.47	2.06	0.1625
	Month x Length	10	2.33	0.23	1.01	0.4569
	Residuals	27	6.20	0.23		
Total N	Month	11	1.18	0.11	5.20	$<10^{-5}$
	Length	1	0.00	0.00	0.15	0.7021
	Month x Length	11	0.34	0.03	1.51	0.1435
	Residuals	81	1.67	0.02		
Non-protein N	Month	11	0.77	0.07	8.04	$<10^{-6}$
	Length	1	0.00	0.00	0.03	0.8742
	Month x Length	11	0.15	0.01	1.57	0.1234
	Residuals	82	0.72	0.01		
Proteins	Month	11	76.64	6.97	5.76	$<10^{-6}$
	Length	1	0.30	0.30	0.25	0.6211
	Month x Length	11	16.44	1.49	1.24	0.2777
	Residuals	80	96.73	1.21		
Condition index	Month	11	35064	3188	19.88	$<10^{-6}$
	Length	1	19	19	0.12	0.7284
	Month x Length	11	1562	142	0.89	0.5549
	Residuals	335	53717	160		
Percentage edibility	Month	11	2451.8	222.9	20.96	$<10^{-6}$
	Length	1	24.7	24.7	2.32	0.1285
	Month x Length	11	102.9	9.4	0.88	0.5605
	Residuals	335	3563.1	10.6		

df – degrees of freedom; SS – sum of squares; MS – mean squares.

TABLE 2.

Biochemical composition, condition index and percentage edibility of *R. decussatus* from Ria Formosa: seasonal variation (mean ± standard deviation) for monthly samples of n individuals. Parameters are in % except condition index.

		Month (2006)											
	n	January	February	March	April	May	June	July	August	September	October	November	December
Moisture	30	85.96 ±0.25 (^{de})	83.84 ±2.10 (^{bc})	85.87 ±1.05 (^{d})	83.96 ±0.84 (^{bc})	83.59 ±0.85 (^{b})	82.55 ±1.54 (^{a})	84.51 ±1.26 (^{bc})	84.61 ±1.21 (^{bc})	84.43 ±0.83 (^{bc})	86.37 ±0.64 (^{de})	86.85 ±0.57 (^{e})	86.33 ±0.81 (^{de})
Ash	30	3.42 ±0.58 (^{ef})	3.59 ±0.51 (^{f})	2.99 ±0.28 ($^{a-d}$)	3.11 ±0.42 (^{cd})	3.10 ±0.28 (^{bcd})	3.21 ±0.25 (^{de})	3.05 ±0.35 (^{bcd})	2.83 ±0.08 (^{ab})	2.89 ±0.09 (^{abc})	2.88 ±0.13 (^{abc})	2.74 ±0.45 (^{a})	3.13 ±0.25 (^{cde})
Carbo-Hydrates	9	0.38 ±0.13 (^{ab})	1.44 ±0.81 (^{de})	0.84 ±0.26 (^{ab})	0.69 ±0.13 (^{ab})	0.79 ±0.18 (^{ab})	1.91 ±0.24 (^{e})	0.38 ±0.21 (^{a})	1.47 ±0.42 (^{de})	2.62 ±0.28 (^{f})	1.35 ±0.25 (^{cde})	0.96 ±0.38 (^{bc})	1.88 ±0.50 (^{e})
Lipids (1)	3	2.25 ±3.57 (^{b})	1.71 ±1.84 (^{b})	0.20 ±2.38 (^{a})	0.07 ±5.71 (^{a})	1.22 ±10.13 (^{ab})	0.53 ±1.53 (^{ab})	0.87 ±2.25 (^{ab})	0.72 ±1.09 (^{ab})	1.23 ±4.36 (^{ab})	0.26 (2) (^{ab})	0.48 ±1.30 (^{ab})	0.36 ±2.85 (^{ab})
Total N	9	1.31 ±0.11 ($^{a-d}$)	1.37 ±0.12 ($^{a-d}$)	1.28 ±0.07 (^{abc})	1.39 ±0.17 ($^{a-d}$)	1.35 ±0.20 ($^{a-d}$)	1.52 ±0.22 (^{d})	1.37 ±0.14 ($^{a-d}$)	1.47 ±0.11 (^{cd})	1.44 ±0.18 (^{bcd})	1.19 ±0.15 (^{a})	1.21 ±0.14 (^{ab})	1.17 ±0.08 (^{a})
Non-protein N	9	0.25 ±0.03 (^{b})	0.23 ±0.05 (^{b})	0.15 ±0.12 (^{ab})	0.17 ±0.17 (^{b})	0.00 ±0.00 (^{a})	0.13 ±0.15 (^{ab})	0.00 ±0.00 (^{a})	0.27 ±0.11 (^{b})	0.22 ±0.09 (^{b})	0.19 ±0.08 (^{b})	0.14 ±0.11 (^{ab})	0.23 ±0.03 (^{b})
Proteins	9	6.67 ±0.77 (^{ab})	7.13 ±0.64 (^{abc})	7.11 ±0.83 (^{abc})	7.63 ±1.41 (^{abc})	8.48 ±1.24 (^{bc})	8.66 ±1.56 (^{c})	8.52 ±0.87 (^{bc})	7.47 ±1.17 (^{abc})	7.60 ±1.44 (^{abc})	6.25 ±1.25 (^{a})	6.69 ±1.00 (^{ab})	5.90 ±0.48 (^{c})
Condition index	30	72.80 ±17.24 (^{de})	73.37 ±8.98 (^{bc})	77.17 ±16.13 (^{d})	98.34 ±13.52 (^{bc})	98.56 ±12.83 (^{b})	107.21 ±16.89 (^{a})	86.40 ±9.92 (^{bc})	85.43 ±7.70 (^{c})	88.19 ±9.98 (^{bc})	85.08 ±9.30 (^{de})	83.37 ±14.31 (^{e})	84.84 ±9.36 (^{de})
Percentage edibility	30	24.4 ±3.6 (^{g})	24.0 ±5.5 (^{fg})	27.0 ±3.9 (^{abc})	30.6 ±2.7 (^{ab})	31.9 ±2.3 ($^{b-e}$)	32.1 ±4.0 ($^{c-f}$)	28.3 ±3.6 (^{g})	26.5 ±2.1 (^{a})	27.2 ±2.5 (^{efg})	30.2 ±2.2 ($^{d-g}$)	29.6 ±2.7 (^{fg})	30.0 ±2.5 (^{bcd})

(1) These values are back-calculated from the log-transformed values used in the analysis. The body mass of at least three individuals had to be pooled to obtain each replicate. (2) Only one replicate available. Within a row, values not sharing the same superscript(s) are significantly different ($p<0.05$).

TABLE 3.

Matrix of Spearman correlation coefficients (*R*) and respective p-values for pair wise correlation analysis (n=12) of monthly mean air temperature and precipitation and biochemical composition, condition index and percentage edibility of *R. decussatus* from Ria Formosa.

Spearman R and p-value	Temperature	Precipitation	Moisture	Ash	Carbohydrates	Log(Lipids)	Total N	Non-Protein N	Protein	Condition index
Precipitation	-0.57									
	0.0543									
Moisture	-0.19	0.55								
	0.5486	0.0625								
Ash	**-0.59**	-0.01	-0.50							
	0.0441	0.9828	0.1006							
Carbohydrates	0.21	-0.06	-0.11	-0.09						
	0.5121	0.8629	0.7292	0.7787						
Log(Lipids)	-0.03	-0.26	-0.29	0.35	0.01					
	0.9225	0.4168	0.3541	0.2652	0.9656					
Total N	0.48	-0.45	**-0.76**	0.14	0.27	0.29				
	0.1114	0.1446	0.0040	0.6646	0.4038	0.3541				
Non-Protein N	-0.32	0.32	0.28	0.13	0.32	0.25	0.03			
	0.3033	0.3126	0.3777	0.6881	0.3126	0.4357	0.9225			
Protein	0.57	**-0.65**	**-0.83**	0.11	-0.08	0.16	**0.78**	-0.56		
	0.0543	0.0220	0.0008	0.7292	0.8122	0.6175	0.0026	0.0562		
Condition index	**0.66**	**-0.64**	**-0.62**	-0.10	0.19	-0.17	0.55	-0.54	**0.76**	
	0.0190	0.0261	0.0332	0.7456	0.5567	0.5868	0.0666	0.0682	0.0040	
Percentage edibility	0.28	-0.21	-0.24	-0.06	0.06	-0.49	-0.01	**-0.66**	0.35	**0.77**
	0.3839	0.5128	0.4433	0.8629	0.8459	0.1063	0.9828	0.0190	0.2652	0.0034