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The reproductive cycle of the European clam *Ruditapes decussatus* (L., 1758) in two Portuguese populations: Implications for management and aquaculture programs

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Abstract:

The reproductive cycle, as well as its nutrient storage and utilization, of two populations of *Ruditapes decussatus* from the main production areas of this species: Ria de Aveiro and Ria Formosa Lagoon (Portugal) were studied here over a 24 month period (May 2010–April 2012). The reproductive cycle of both populations of *R. decussatus* followed an annual cyclicality that comprised a ripe stage in spring followed by a spawning period that began in late spring and extended throughout summer until early autumn. This extended and continuous spawning period may be an advantageous strategy for this species by ensuring a continuous supply of gametes. Moreover, *R. decussatus* can adopt different reproductive strategies depending on the geographical origin. The results of both cycle of nutrients stored and nutrients utilization showed that clams of both populations present a high reproductive effort that almost depletes its energy reserves. Nevertheless, while Ria de Aveiro population retrieves them immediately after spawning, the same is not verified in clams from Ria Formosa Lagoon with their consequent debilitation. Also, based on the glycogen pattern it was possible to infer that the Ria Aveiro population is an opportunistic one, while the Ria Formosa Lagoon population exhibited an intermediate strategy. However, both populations could be considered as viable broodstock for intensive hatchery production of juveniles and the observed extended spawning periods presents interesting implications for the implementation of profitable aquaculture. Moreover, this species presented a great capacity for gonadal regeneration, which coupled with its high gonadal development rate would provide larvae during most of the year without extensive and expensive broodstock conditioning.

Highlights

► Characterize the reproductive cycle of two Portuguese populations of R. decussatus ► Describe the patterns of nutrient stored and utilization during gametogenic cycle ► The species showed a long spawning period that is useful to aquaculture production. ► R decussatus can adopt different reproductive strategies ► Contribute to establish a successful hatchery-based production of R. decussatus

Keywords: European clam ; *Ruditapes decussatus* ; Reproductive cycle ; Portuguese populations ; Biochemical composition ; Seasonal variations

1. Introduction

The European clam *Ruditapes decussatus* is widely distributed along the coastal and estuarine areas of Europe and Northern Africa and represents an important resource income due to its high commercial value (Matias et al., 2009). *R. decussatus* is extensively produced and harvested in Portugal, where clam farming represents an important economical sector. This species is central to aquaculture's revenue, indeed in 2009, the national annual production reported reached 2 metric tons (representing 27% of the total seafood cultured in Portugal) (DGPA, 2011). The main production areas of this species are the Ria de Aveiro (40º42′N; 08º40′W) and the Ria Formosa Lagoon (37º01′N; 07º49′W). In these production areas, clams are reared in plots in the intertidal zone. Clam farming involves seeding juveniles, collected from natural beds, into plots maintained in tidal flats and harvesting commercial size animals. The culture of *R. decussatus* in Ria Formosa Lagoon represents 90% of the national production and it is central to the socioeconomic framework. However, during the last two decades, the European clam production has suffered an important decrease due to several constraints, namely recruitment failures and excessive pressure on the capture of juveniles on natural banks and severe clam mortalities.

To address this situation, artificial spawning and larval rearing programs could provide an alternative source of spat.

To be able to establish and improve rearing programs for *R. decussatus*, a detailed knowledge of the species reproductive cycle and spawning periods is crucial. Effectively, the differences in gonadal cycles and conditioning optima in different populations have to be considered in hatchery operations (Devauchelle and Mingant, 1991; Lannan et al., 1980). There is also evidence that responses also vary between different geographical populations of the same species, as has been found for *Mytilus galloprovincialis* (Iglesias et al., 1996) and *Argopecten purpuratus* (Avendaño and Le Pennec, 1997). In the case of the European clam, in natural conditions, it has been reported that the ecotype decussatus living in different areas, even at the same latitude, could strongly differ in terms of their fecundity levels and biochemical compositions (Shaffee and Daoudi 1991; Trigui-El-Menif et al. 1995).

A relationship between the reproductive cycle and energy storage and utilization cycles has also already been reported by several authors for a wide variety of bivalves (*e.g.* Barber and Blake, 1981; Fernández-Castro and Vido-de-Mattio, 1987; Joaquim et al., 2011; Massapina et al., 1999; Pérez-Camacho et al., 2003; Ojea et al., 2004). The energy storage and utilization cycles translate into a seasonal pattern of biochemical composition that can vary according to species and geographical origin (Albentosa et al., 2007; Matias et al., 2009). Energy reserves are of considerable importance in reproduction and seasonal energy storage and utilization in bivalves are closely correlated to environmental conditions and the annual gametogenic cycles (*e.g.* Delgado et al., 2004; Holland, 1978; Ojea et al., 2004; Tlili et al., 2012). Food and temperature are the main factors that regulate the timing and rate of energy storage in bivalves (Joaquim et al., 2011). The effect of these variables is complex and depends specifically on acquisition and expenditure of energy (Pérez-Camacho et al., 2003). The most common model consists of an accumulation of energy during the periods where food is abundant. This energy is then used for the gametogenic synthesis and latter released during the spawning process (Albentosa et al., 2007). Proteins are mainly used in structural functions and represent an energy reserve in adult bivalves, particularly during gametogenesis and in situations of low glycogen levels, or severe energy equilibrium (Beninger and Lucas, 1984). Carbohydrates are assumed to constitute the most important bio-energy reserve in bivalve molluscs and, because of their hydro-solubility, are available for immediate use; being glycogen the main component for supplying energy demands (Fernández-Castro and Vito-de-Mattio, 1987) and reproductive cycle (*e.g.* Newell and Bayne 1980; Pazos et al., 2005). Lipids, due to their large calorific contributions per structural unit, account for a greater proportion of the energy reserves in bivalves than carbohydrates or proteins (Ojea et al., 2004). They play an important role in the gamete formation and are the main reserve of oocytes and bivalve larvae (Matias et al., 2009; 2011).

Although previous works have studied the natural reproduction of *R. decussatus* and its biochemical composition (Benninger and Lucas, 1984; Chryssanthakopoulou and kasparis, 2005; Hamida et al., 2004; Laruelle et al., 1994; Ojea et al., 2004; Rodríguez-Moscoso and Arnaiz, 1998; Serdar and Lök, 2009; Shafee and Daoudi, 1991; Trigui-El-Menif et al., 1995; Xie and Burnell, 1994), in Portuguese populations of this species only the gametogenic cycle has been determined in former studies of Vilela (1950) and Pacheco et al. (1989). Therefore, the present study aims to characterize the reproductive cycle of two populations of *R. decussatus* from the main production areas of this species: Ria de Aveiro and Ria Formosa Lagoon, and also include patterns of nutrient storage and utilization. This information would be essential for the establishment of a successful hatchery-based production.

2. Materials and Methods

2.1. Sample collection

Generally, samples of *R. decussatus* were hand-collected, monthly, at the same location in Ria de Aveiro and Ria Formosa Lagoon, during 24 months (May 2010 – April 2012). Both areas are shallow water mesotidal lagoons with semidiurnal tidal regimes that constitute the major hydrodynamic forces (Dias et al., 2000; Nobre et al., 2005) (Figure 1). These lagoons, that distance 500 km from each other, have several channels and a large intertidal area covered by sand, muddy sand-flats, and salt marshes (Falcão and Vale, 1990; Picado et al., 2009). Ria Formosa has an extension of 55 km and a maximum width of 6 km (Newton and Mudge, 2003). The lagoon is separated from the Atlantic Ocean by several barrier islands and two peninsulas. The tidal range varies from 1.35 m on neap tides to 3 m on spring tides, and the coefficient of renovation of the lagoon is 3.2 in a spring tide and 1.0 in a neap tide. The freshwater inputs are almost negligible and salinity remains close to 36 all year long (Águas, 1986; Falcão and Vale, 1990). The Ria de Aveiro is 45 km long and 10 km wide, being connected to the Atlantic Ocean by only a narrow channel (Picado et al., 2009), and the tidal amplitude is 0.6 m in neap tides and 3.2 m in spring tides (Dias et al., 2000). This lagoon has an important freshwater input coming from the Vouga and the Antuã rivers (Dias et al., 2000; Moreira et al., 1993) and salinity ranged between 31 and 36. These two ecosystems are currently used for clam production and fish aquaculture ponds.

Monthly data on sea surface temperature (SST) and chlorophyll during the study period were derived from satellite remote sensing data, collected from the Giovanni online data system (MODIS-Aqua 4 km, monthly processed data, available at http://disc.sci.gsfc.nasa.gov/giovanni/overview/index.html), developed and maintained by the NASA Goddard Environmental Sciences Data and Information Services Center - GESDISC (Acker and Leptoukh, 2007).

2.2. Laboratory analysis

In the laboratory, clams were placed in 0.45 μm-filtered seawater at 20 °C for 24 h to purge their stomachs before histological, condition index and biochemical analyses.

2.2.1. Histology

Ten individuals of each sex from each monthly sample and each population were examined histologically to determine the gametogenic stages in both sexes. The visceral mass was separated from siphons and gills and fixed in Davison solution for 48 h, then transferred to 70 % ethyl alcohol (ETOH) for storage. Tissues from these samples were dehydrated with serial dilutions of alcohol and embedded in paraffin. Thick sections (6–8 μm) were cut on a microtome and stained with haematoxylin and eosin. The histologically prepared slides were examined using a microscope at 40× magnification and each specimen was assigned to a stage which represented the gonadal state. Clam reproductive maturity was categorized into six stages using a scale development based on Delgado and Pérez-Camacho (2005) (Table 1). When more than one developmental stage occurred simultaneously within a single individual, the assignment of a stage criteria decision was based upon the condition of the majority of the section.

A mean gonadal index (GI) was calculated using the method proposed by Seed (1976): GI = [($\sum$ ind. each stage X stage ranking) / total ind. each month].

For each of the stages a numerical ranking was assigned as follows: Period of sexual rest (0); initiation of gametogenesis (3); advanced gametogenesis (4); ripe (5); partially spawned (2); spent (1).

The GI ranged from 0 (all individuals in the sample are in rest stage) to 5 (all individuals are in ripe stage).

2.2.2. Condition index

The dry meat and shell weight of 10 clams, from each monthly sample and from each population, were determined after oven drying at 80 °C for 24 h. Meat samples were then ashed at 450 °C in a muffle furnace, ash weight determined, and organic matter weight calculated as the ash free dry meat weight (AFDW). The condition index (CI) was calculated according to Walne and Mann (1975): [ash free dry weight (AFDW) of meat (g)/dry shell weight (g)]*100.

2.2.3. Biochemical composition

The meat of ten clams from each monthly sample of the two populations was frozen and stored at −20 °C for biochemical analyses. For each specimen, protein was determined using the modified Lowry method (Shakir et al., 1994), glycogen content was determined from dried (80 °C for 24 h) homogenate using the anthrone reagent (Viles and Silverman, 1949) and total lipids were extracted from fresh homogenized material in chloroform/methanol (Folch et al., 1957) and estimated spectrophotometrically after charring with concentrated sulphuric acid (Marsh and Weinstein, 1966). Duplicate determinations were performed in all cases and values are expressed as a percentage of AFDW. Caloric content of proteins, lipids and carbohydrates in tissues was calculated using the factors 17.9 KJ g^{-1} (Beukema and De Bruin, 1979), 33 KJ g^{-1} (Beninger and Lucas, 1984) and 17.2 KJ g^{-1} (Paine, 1971), respectively.

2.3. Statistics

Seasonal variations in condition index, biochemical composition and histological parameters were analyzed by one-way ANOVA or Kruskal–Wallis ANOVA on ranks whenever the assumptions of analysis of variance (ANOVA) failed. Percentage data were arcsine transformed to normalize variance (Sokal and Rohlf, 1981). Multiple pairwise comparisons were performed using the post-hoc parametric Tukey test or the non-parametric Dunn’s test in order to detect significant differences between monthly consecutive samples. The Pearson correlation coefficient was used to determine the degree of association between parameters. Results were considered significant at $P<0.05$. The statistical analyses were performed using the SIGMASTAT 3.11 statistical package.

3. Results

3.1. Temperature and chlorophyll

The evolution of the monthly SST and chlorophyll during the experimental period in Ria de Aveiro and Ria Formosa Lagoon are presented in Figure 2. Ria de Aveiro presented lower temperature values than Ria Formosa Lagoon (around less 2.95±1.31ºC). A seasonal cycle in SST was observed in the two geographical locations studied, and the monthly means ranged between 19.49 ºC in September 2010 and 12.99 ºC in February 2012 for Ria de Aveiro and 24.01 ºC in August 2010 and 15.03 ºC in February 2012 in Ria Formosa Lagoon. The evolution of the chlorophyll during the experimental period showed seasonal variations in geographical locations with several phytoplanktonic blooms in spring for Ria Formosa Lagoon and in spring/summer and autumn in Ria de Aveiro. Ria Formosa Lagoon presented lower values than Ria de Aveiro (around less 3.49±3.37 mg m^{-3}) and the average of chlorophyll was 4.12±3.19 mg m^{-3} and 0.63±0.60 mg m^{-3} for Ria de Aveiro and Ria Formosa Lagoon, respectively.

3.2. Gametogenic cycle

The sexes were clearly separated and no hermaphrodites were found. Both sexes showed synchronism in gonadal development. The reproductive cycle of *R. decussatus* was characterized by a seasonal pattern in both populations (Figure 3), however, no significant correlations were found between SST and GI and in chlorophyll and GI in both populations (Table 2). The onset of the gametogenic cycle occurred in February in 2011 for males and females of both populations, in March in 2012 for males and females of Ria de Aveiro population and in April and March in 2012 for males and females of Ria Formosa Lagoon population, respectively. Despite the fact that no correlations were observed between SST and GI and chlorophyll and GI in both populations, the onset of the gametogenic cycle coincided with the increase of SST and with phytoplanktonic blooms. The development of gametes intensified quickly during the following month. The two populations reached its peak of reproductive effort between May and June, represented by the highest values of GI (Ria de Aveiro: females=4.4 in May and males=3.5 in June; Ria Formosa Lagoon: females=4.2 in May and males=3 in June) (Figure 4). Spawning began in late spring for both populations; in 2010, generally in June (except for males of Ria Formosa Lagoon population that began in May) and in March and May 2011 for Ria Formosa Lagoon and Ria Aveiro populations, respectively. Spawning of *R. decussatus* intensified during summer as SST increased, and continued until early autumn in both populations. Nevertheless, during this period and in spite of the seasonal pattern, *R. decussatus* did not show a continuous gonad development, after spawning clams did not progress to an inactive stage. Indeed, in the microscopic examinations of the gonadal tissues, all clams showed simultaneous spawning and recovery of the gonad. So, we considered this stage of the reproductive cycle of *R. decussatus* as partially-spawned (stage V – Table 1).This phenomenon occurred in all studied years and for both males and females of the two populations. In October the majority of clams had already spawned and was inactive and remained in this stage during approximately six months (which coincided with the decrease of SST and chlorophyll) until the next onset of gametogenesis. The gonadal index followed the same pattern as the gonadal development; no significant differences in GI were found between populations or between sexes (ANOVA, P>0.05).

3.3. Condition index

Condition index exhibited statistically significant differences between populations (K–W., H=7.683, d.f.=1, P=0.006), especially when clams were in the inactive stage, between September and October 2010 and August and November 2011. These differences between populations were also relevant between May and July 2010 (ANOVA, F=26.61, d.f.=7, P<0.001). In Ria de Aveiro population, CI was positively correlated with SST (Pearson, r=0.74, P<0.001), however no correlation was observed between these two parameters in Ria Formosa Lagoon. No correlations were observed between CI and chlorophyll for both populations. In 2010, the CI of the Ria de Aveiro population generally trended upwards until September following SST increase (with an exception in July, with a GI contribution) and phytoplanktonic bloom (August), when the highest value (11.96±1.38) of the sampling period was registered (Figure 5), coinciding with the end of the reproductive cycle of the species. In the following month the CI decreased coinciding with the end of spawning. However, no relationship was observed between GI and CI for the Ria de Aveiro population (Table 2). In the Ria Formosa Lagoon population the CI in 2010 remained high and relatively stable in the first three months of sampling when the majority of clams were in late activity and ripe stage, decreasing sharply from July with the evolution of spawning and consequent rest period. This decline was also coincident with the lowest values of chlorophyll. The lowest CI value (4.00±0.80) of the sampling period was registered in October 2010 in the Ria Formosa Lagoon population. In 2011, Ria de Aveiro population showed two peaks in the CI, the first one in May and another one in October. In contrast, Ria Formosa Lagoon population only showed one peak in May/June which correspond to the maximum GI value of this year achieved with the contribution of the ripe stage of the most clams. The decline of Ria Formosa population CI coincided with the rest period of clams. The CI of the Ria Formosa Lagoon population was positively correlated with the GI (Pearson, r=0.87, P<0.001) (Table 2). Condition index of both populations increased slightly from February to April 2012, following the trend of 2011 (Figure 4).

3.4. Biochemical composition

Proteins were the predominant dry tissue constituent of the clams followed by total lipids and glycogen (Table 3). The highest protein content values were recorded in

May 2010 (531.7±180.0 μg mg^{-1} AFDW) and October 2010 (520.8±123.5 μg mg^{-1} AFDW) and the lowest in September 2010 (128.3±32.6 μg mg^{-1} AFDW) and February 2011 (142.2±23.6 μg mg^{-1} AFDW) for Ria de Aveiro and Ria Formosa Lagoon populations, respectively. Significant differences were found between populations (ANOVA, F=4.04, d.f.=1, P=0.045), especially in July, August and September 2010 and February 2011. Glycogen content between clams of the two populations showed significant differences (ANOVA, d.f.=1, F=58.89 P<0.001), especially when clams were inactive in terms of reproduction (Period of sexual rest). In this period, the reserves of glycogen were considerably higher in the Ria de Aveiro clams than in the ones from Ria Formosa. This content generally decreased in both populations until August 2010. In September 2010, the highest (53.7±22.9 μg mg^{-1} AFDW) and the lowest (7.2±2.4 μg mg^{-1} AFDW) values were recorded for Ria de Aveiro and Ria Formosa Lagoon populations, respectively. After that, the glycogen contents showed opposing trends until February 2011, when the values of the two populations approached. The lowest glycogen values of clams from Ria de Aveiro (9.1±5.8 μg mg^{-1} AFDW) were observed in June 2011. Glycogen was positively correlated with CI (Pearson, r=0.79, P<0.001) and GI (Pearson, r=0.60, P=0.01) in Ria Formosa Lagoon population (Table 2). The lowest (Ria de Aveiro: 35.0±9.8 μg mg^{-1} AFDW; Ria Formosa: 27.2±7.3 μg mg^{-1} AFDW) and the highest (Ria de Aveiro: 118.1±20.5 μg mg^{-1} AFDW; Ria Formosa: 112.1±15.1 μg mg^{-1} AFDW) total lipid values were reached in October 2010 and June 2010 and April 2012 and July 2011, respectively. No significant differences were found between populations for total lipids (ANOVA, P>0.05). Total lipids of clams from Ria de Aveiro population were negatively correlated with proteins (Pearson, r=-0.52, P<0.039). Proteins contributed the most to the total energy content (Ria de Aveiro: Pearson, r=0.87, P<0.001; Ria Formosa Lagoon: Pearson, r=0.91, P<0.001) (Table 2). Significant differences were observed between populations for total energy (K-W., H=3.899, d.f.=1, P<0.05), especially in July and August 2010 and February 2011 and 2012.

Discussion

The reproductive activity of bivalves, which includes a sequence of events from gametogenesis to spawning, is controlled by the interaction between endogenous (Enríquez-Díaz et al., 2009; Normand et al., 2008) and exogenous factors, mainly food availability and temperature. Moreover, temperature is closely linked to the geographical locations affecting indirectly the availability of food and/or consequently the timing and duration of the reproductive cycle and number of spawning *per* year. For *R. decussatus* a different number of annual spawns has been described within Europe (da Costa et al., 2012).

Seawater temperature and chlorophyll patterns, in this study, were common for both studied lagoons and were typical from temperate climates. In both lagoons, temperature increased during spring, stabilized in summer, decreased during autumn and remained low in winter. Also both lagoons are characterized with several phytoplanktonic blooms in spring/summer. Nevertheless, Ria de Aveiro was distinguished by relatively lower SST values and higher chlorophyll values than Ria Formosa Lagoon.

Despite no significant correlation was found between SST and the gonadal index, the sequence of gametogenic stages showed that the reproductive cycle of these two populations of *R. decussatus* follow a seasonal cycle, as has been previously reported for this and several other bivalve species (*e.g.* Albentosa et al., 2007; Gabbott, 1976; Xie and Burnell 1994). The reproductive cycle of *R. decussatus* comprised a ripe stage in spring followed by spawning that began in late spring and extended during summer until early autumn in both populations. A similar reproductive cycle was described in the studies by Breber (1980) and Ojea et al. (2004) for a population of *R. decussatus* from Galicia (Spain) and from Venice (Italy), respectively.

However, other authors have shown the occurrence of two major periods of spawning, in spring and then in summer or early autumn in different populations of this species (Morocco - Shafee and Daoudi, 1991; France - Borsa and Millet, 1992; Lauruelle et al., 1994; Greece – Chryssanthakopoulou and Kaspiris, 2005) including in populations from the Ria Formosa Lagoon (Pacheco et al., 1989; Vilela, 1950). The differences observed between studies have been frequently associated to the influence of the geographical location and consequently by the inherent environmental factors (*vide* da Costa et al., 2012). Nevertheless, in our study, no significant differences were observed between the reproductive cycles of the two geographically distinct populations studied.

Beside the fact that the seasonal pattern presented a spawning period well-defined in time, clams of the two studied populations exhibited an advantageous reproductive strategy for the species ensuring a consistent supply of gametes during the whole spawning period. Indeed, histological analyses showed simultaneously gonias, maturing gametocytes and variable proportions of fully matured gametes in the same individual, both in males and females. This high capacity for gonadal regeneration had also been previously observed by our team in *Venerupis senegalensis* from Ria de Aveiro (Joaquim et al., 2011). Nevertheless, and despite the intra-individual asynchrony, the maintenance of a synchronized gonadal development observed between males and females ensures the reproductive success of the species since sperm and oocytes will be expelled into the water column simultaneously during the spawning period, augmenting the probability of fertilization. This synchronism had previously been reported by Laruelle et al. (1994) and Ojea et al. (2004) for this species. Both populations showed a long reproductive rest phase that was extended by a period of approximately six months, during autumn and winter.

The onset of the gametogenic cycle with the proliferation of gonias seems to associate with the rise of SST and chlorophyll in late winter/early spring for both populations and the development of gametes intensified quickly until the attainment of the ripe stage late spring. These results were generally consistent with the previous findings by Chryssanthakopoulou and Kaspiris (2005), Shafee and Daoudi (1991) and Vilela (1950 in other populations of this species, although, as previously mentioned, these authors reported another onset of gametogenesis in July/August.

Condition index is generally considered to reflect the reproductive activity of bivalves (Fernández-Castro and Vido de Mattio, 1987; Massapina et al., 1999; Ojea et al., 2004). The positive correlation between these two parameters has been observed in several bivalve species from the Portuguese coast (e.g. Gaspar and Monteiro, 1988; Joaquim et al., 2011; Moura et al., 2008). In this study the CI exhibited significant differences between populations, especially when clams were in the inactive stage (Period of sexual rest), between September and October 2010 and August and November 2011. Although, in the general no significant correlations between CI and chlorophyll were observed in both areas, this fact seems to be associated with the greater food availability in the Ria de Aveiro comparatively with the Ria Formosa lagoon. Despite the fact that a positive correlation between CI and SST was observed in Ria de Aveiro, the CI did not reflect the reproductive cycle of this population, since no significant relationship was found between these parameters. In 2010, the CI of this population followed the SST increase and generally trended upwards until September. After that, the CI decreased, coinciding with the end of the spawning period and SST decrease. In 2011, Ria de Aveiro population showed two peaks in CI that accompanying the SST trend; the first one in May when the majority of the clams were ripe and another one in October, however, in this last one the rest stage was the more frequent stage observed. In contrast, no significant positive correlation was found between CI and SST in the Ria Formosa Lagoon population, however, in this population, the CI was positively correlated with the GI. In 2010, CI remained high when the majority of clams were in advanced gametogenesis and ripe stages, decreasing sharply with partial spawning and consequent rest period. In 2011, Ria Formosa Lagoon population only showed one peak of CI in May/June which corresponded to the maximum GI value of that year achieved with the contribution of the ripe stage of most of the clams. The reduction of the Ria Formosa Lagoon CI coincided with the rest period of clams.

In previous studies it has been reported that the CI is highly influenced by the energy storage and exploitation strategy of bivalve species (Delgado and Pérez-Camacho, 2005; Joaquim et al., 2008; 2011). Clams from Ria de Aveiro seem to have the ability to recover quickly the reserves after spawning when the SST and food availability (chlorophyll) are still high. These reserves seem to be harnessed to maintain their physiological state during winter. The same was not verified in the Ria Formosa Lagoon population. Clams lost their reserves with the intensification of the spawning event and were only able to recover them, slowly during winter, and more rapidly with the next SST increase. This fact is probably associated to the low levels of chlorophyll observed in Ria Formosa Lagoon, especially in the Summer/Autumn months. The major energetic effort suffered by clams during spawning leads then to their debility which might be at the origin of episodes of severe mortalities in the Ria Formosa Lagoon population after the reproductive period. Several factors can contribute to this difference in the energy storage and exploitation strategy between the two studied populations. da Costa et al. (2012) reports that another environmental variable that affects the energetic strategy of bivalves is immersion time. In our study, the subtidal clams from Ria de Aveiro showed higher CI values than the intertidal clams of Ria Formosa Lagoon during the rest period.

Several studies on bivalves have shown that sexual maturity is related to energy supply from previously stored reserves or the ingestion of available food and consequently is closely linked with the biochemical composition (Pérez-Camacho et al., 2003; Sastry, 1979). The reproductive cycle translates a seasonal pattern of biochemical composition that can vary among populations and species (Albentosa et al., 2007). The relative amounts of proteins (Ria de Aveiro - 128 to 532 µg mg^{-1} AFDW; Ria Formosa Lagoon – 142 to 520 µg mg^{-1} AFDW), glycogen (Ria de Aveiro - 9 to 54 μg mg^{-1} AFDW; Ria Formosa Lagoon - 7 to 45 μg mg^{-1} AFDW) measured in *R. decussatus* were lower, in term of the proportions, to those previously described in the literature for this species, however the amount of total lipids (Ria de Aveiro - 35 to 118 μg mg^{-1} AFDW; Ria Formosa Lagoon – 27 to 112 μg mg^{-1} AFDW) was higher (*e.g.* Aníbal et al., 2011; Ojea et al., 2004).

Several authors have suggested that, in bivalves, somatic proteins are used as an energy reserve in situations of nutritional stress and energy imbalance or during gonadal maturation (*e.g.*Gabbott and Bayne, 1973; Liu et al., 2008). Moreover, it has also been suggested that some species use proteins as a source of energy maintenance when carbohydrate reserves have already been depleted (Albentosa et al., 2007; da Costa et al., 2012; Joaquim et al., 2011). In this study, in 2010 the sudden decrease in protein content in both populations after spawning, when glycogen reserves were also depleted, suggests that proteins were indeed used for maintenance of the species; however, the same was not true for 2011 where no regular seasonal trend was observed. In this study, no correlation was found between proteins and glycogen and GI and significant differences were found between both populations protein content.

Glycogen is the main energy reserve in adult bivalves. In *R. decussatus* it can be an energy source for growth and at the same time stored in specific cells as an energetic reserve for the gametogenesis and gonadal development (Rodríguez et al., 1993). In this study, differences were observed between the two populations concerning glycogen content, although the rapid gonadal development and spawning process forced a striking consumption of the glycogen in both populations, after that, higher and lower values were recorded for Ria de Aveiro and Ria Formosa Lagoon, respectively. During the rest period the glycogen content of both populations also showed opposite trends. Some bivalve species store glycogen when food is abundant and gametogenesis takes place when there is low food availability, thus allowing the first spawning release when seawater temperature increases (conservative strategy), and there are others species in which the gamete production occurs from spring onwards, coupled with the first phytoplanktonic blooms (opportunistic strategy) (da Costa et al., 2012). Ojea et al. (2004) and Urrutia et al. (1999) in studies with Spanish populations (Galician and Basque Country) have considered *R. decussatus* as a conservative species however Aníbal et al. (2011) concluded that *R. decussatus* from the Ria Formosa Lagoon exhibited an intermediate strategy. Our results corroborate this last thesis. In fact, although glycogen was positively correlated with GI, which is typical from an opportunistic species, the Ria Formosa Lagoon population stored this reserve during the winter and after that both stored and recently assimilated glycogen content was used for gametogenesis. In Ria de Aveiro population, despite the fact that clams have used the after spawning stored glycogen reserves during autumn and winter, which is typical from a conservative species, gametogenesis was not started and no significant correlations were found between glycogen content and GI. The onset of gamete production was only verified in early spring, associated with the first phytoplanktonic bloom, which is typical of an opportunistic strategy. These differences in the reproductive strategy between geographically distinct populations of the same species were also reported by Cerviño-Otero (2011) and da Costa et al. (2012). As previously mentioned the two populations also differed in the amount of glycogen stored, especially when clams were inactive in terms of reproduction. In fact, reserves were stored as glycogen soon after the spawning period in Ria de Aveiro population which is coincident with the phytoplanktonic bloom observed in autumn, however the same was not verified in the Ria Formosa Lagoon population. Consequently, in rest stage, the reserves of glycogen were considerably higher in Ria de Aveiro clams then in Ria Formosa Lagoon one's, corroborating the CI results. A significant positive correlation between glycogen content and CI was found in Ria Formosa Lagoon population.

Several authors (*e.g.* Beninger and Lucas, 1984; Mouneyrac et al., 2008; Ojea et al., 2004) have reported that lipid seasonal variations are inversely related with glycogen, due to the conversion of glycogen to lipids, biosynthesized during the formation of gametes (Gabbott, 1975). In the present study, total lipids increased with the onset of gametogenesis. After that and during the reproductive period, an erratic variation of total lipids occurred in both populations that could be related with the successive gamete production and release, typical of a partial spawning species. Total lipids also peaked immediately after spawning. These results suggest that, more than a consequence of gametogenesis, the total lipids content also reflects the energy accumulation process and its consumption during bivalve somatic development, as has been previously reported by other authors (Albentosa et al., 2007; Joaquim et al., 2008, 2011).

The negative correlation found between proteins and total lipids in clams from Ria de Aveiro population reinforce the idea that proteins can be used as an energy reserve in stress situations. Moreover, in our study, protein was the constituent that most contributed to the total energy of *R. decussatus*.

In conclusion, the results of this study show that *R. decussatus* has a partial spawning period, during which occurs successive and simultaneous production of gametes and spawning. Moreover, it was also demonstrated that this species can adopt different energy storage depending on the geographic origin. Clams of both populations show a high reproductive effort that almost depletes its energy reserves; however, while the Ria de Aveiro population retrieves them immediately after spawning, the same is not verified in clams from the Ria Formosa Lagoon which leads to their consequent debilitation, probably due to the different levels of food availability in the two lagoons.

Both populations presented however viable broodstock for intensive hatchery production of juveniles and the extended spawning period of both *R. decussatus* populations has interesting implications for the implementation of profitable aquaculture. Moreover, the high gonadal regeneration capacity presented by this species coupled with its high gonadal development rate would provide larvae during most of the year without the need of performing extensive and expensive broodstock conditioning.

We believe that the results of our study could then be useful to *R. decussatus* hatchery production programs. These programs should take into consideration that: the extended natural spawning period implies that manipulation of broodstock in terms of conditioning, which should be initiated in early winter, would be relatively easy; the great capacity for gonadal regeneration, coupled with its high gonadal development rate would provide larvae over much of the year without extensive and expensive broodstock conditioning; the Ria de Aveiro population are the most suitable broodstock for hatchery production due to their reproductive strategy. Moreover the global information obtained in this study on the gametogenic cycle and consequent energy storage will also allow the determination of the optimal reproductive time for artificial spawning induction for aquaculture production of this species.

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Figures legends

Figure 1 – Ria de Aveiro and Ria Formosa Lagoon location.

Figure 2 – Monthly mean values of sea surface temperature (SST) (A) and chlorophyll in Ria de Aveiro and Ria Formosa Laggon from May 2010 to April 2012.

Figure 3 – Monthly variations in gonadal development of *Ruditapes decussatus* populations from Ria de Aveiro and Ria Formosa lagoon, during May 2010 to April 2012. Males (top) and Females (bottom).

Figure 4 – Monthly variations in gonad index (GI) (mean, n= 20) of *Ruditapes decussatus* populations from Ria de Aveiro and Ria Formosa Lagoon, during May 2010 to April 2012.

Figure 4 – Condition index (mean ± SD) of *Ruditapes decussatus* populations from Ria de Aveiro and Ria Formosa lagoon, during May 2010 to April 2012. (*statistically significant differences, $P<0.05$ found between populations).

Figure 1

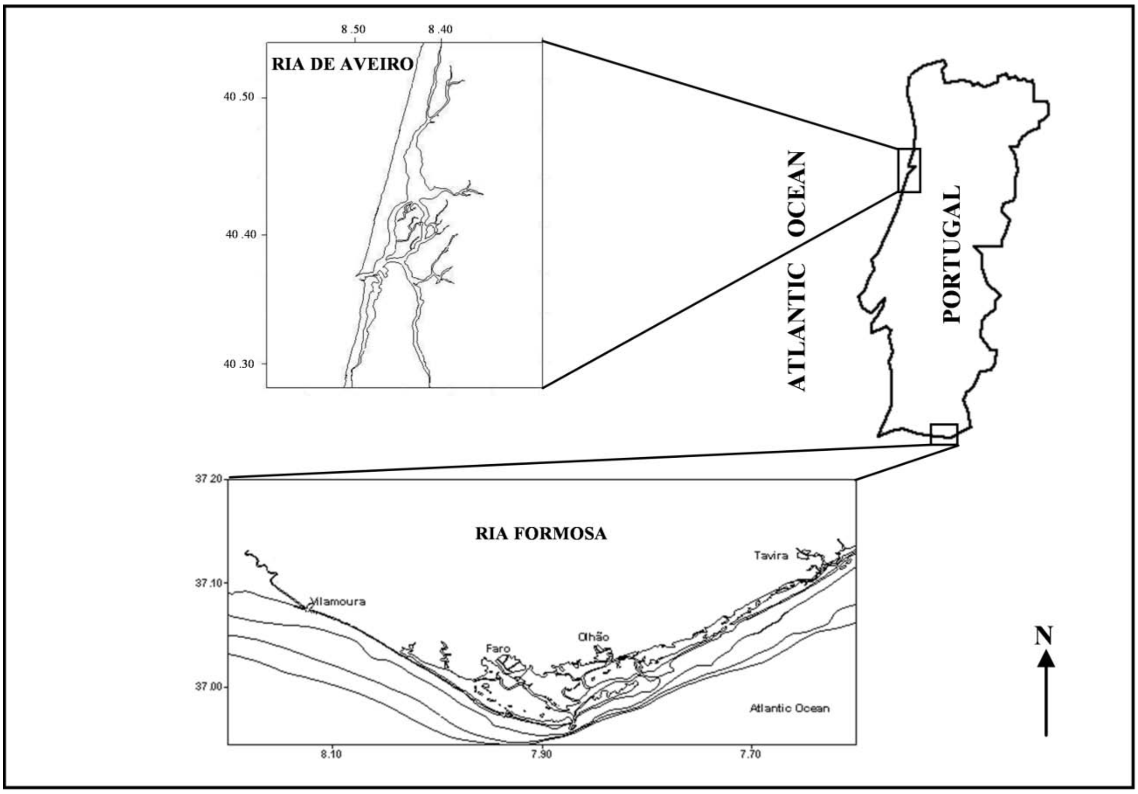
8.50
8.40
RIA DE AVEIRO
40.50
40.40
40.30
ATLANTIC OCEAN
PORTUGAL
37.20
RIA FORMOSA
Tavira
37.10
Vilamoura
Faro
Olhão
37.00
Atlantic Ocean
8.10
7.90
7.70
N

Figure 2

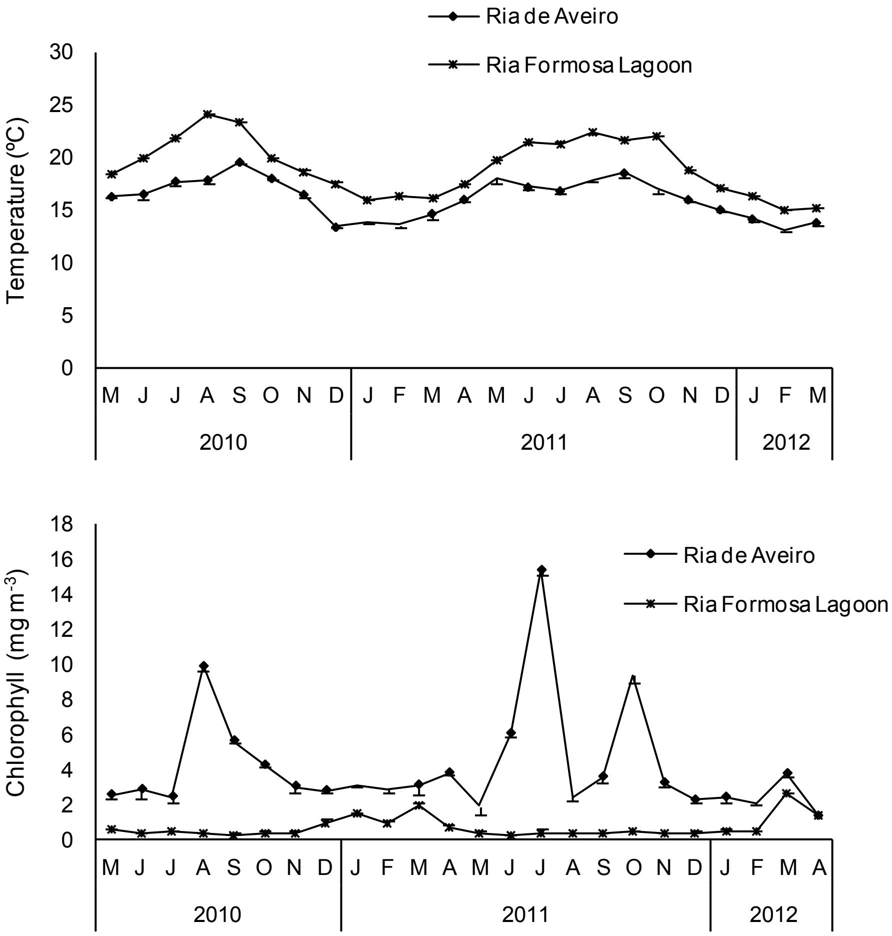
Ria de Aveiro
Ria Formosa Lagoon
Temperature (ºC)
30
25
20
15
10
5
0
M J J A S O N D J F M A M J J A S O N D J F M
2010
2011
2012
Ria de Aveiro
Ria Formosa Lagoon
Chlorophyll (mg m^{-3})
18
16
14
12
10
8
6
4
2
0
M J J A S O N D J F M A M J J A S O N D J F M A
2010
2011
2012

Figure 3

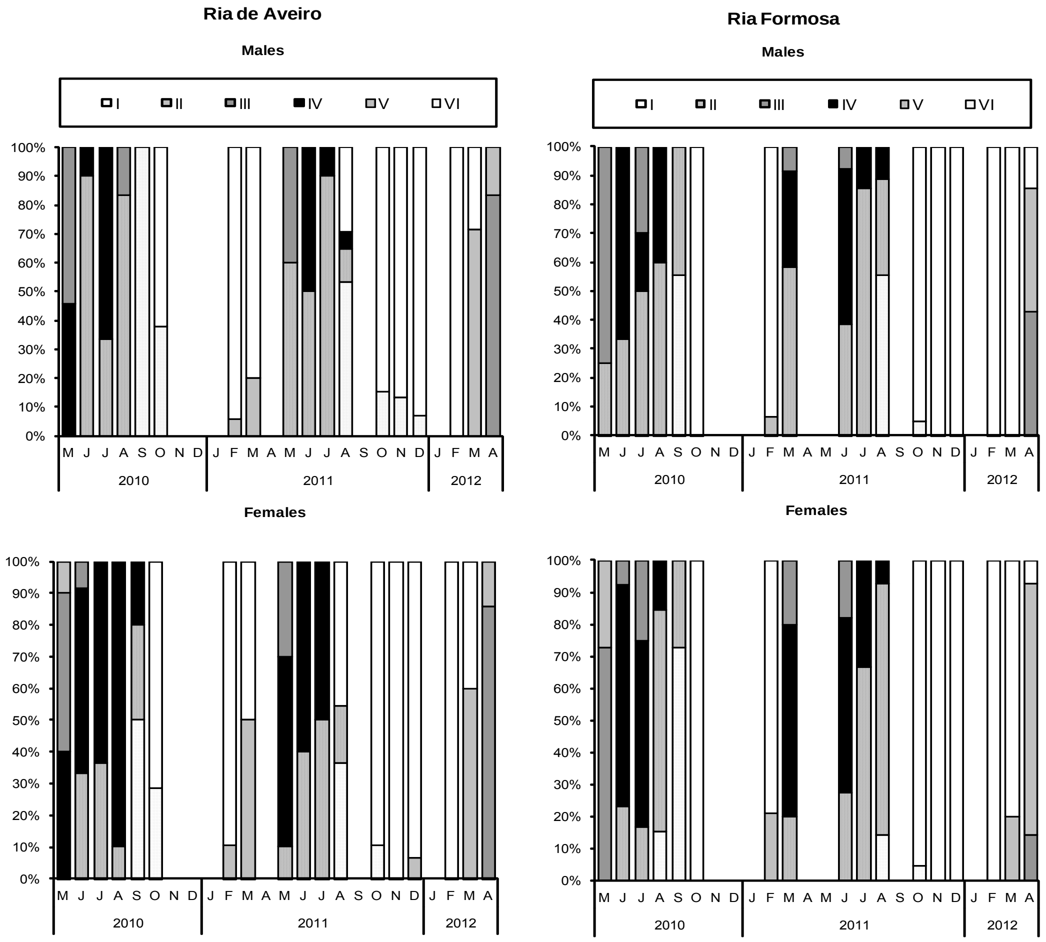
Ria de Aveiro
Males
I
II
III
IV
V
VI
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
M J J A S O N D J F M A M J J A S O N D J F M A
2010
2011
2012
Females
Ria Formosa
Males
Females

Figure 4

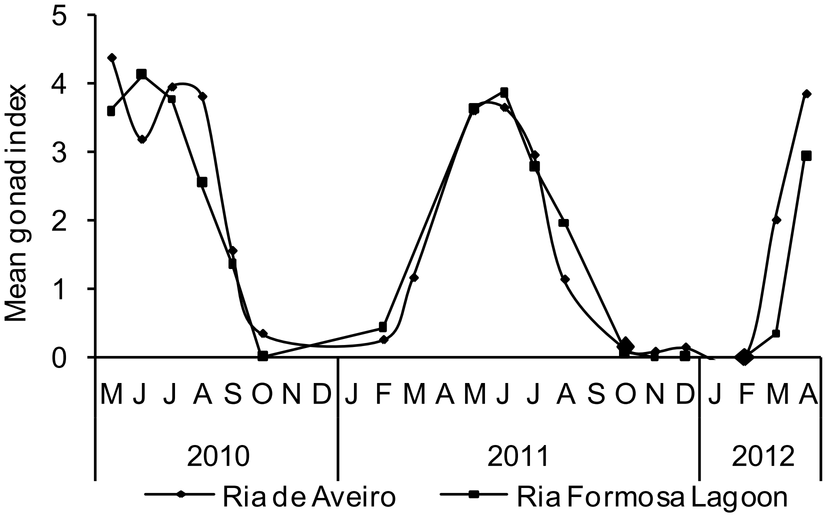
Mean gonad index
5
4
3
2
1
0
M J J A S O N D J F M A M J J A S O N D J F M A
2010
2011
2012
Ria de Aveiro
Ria Formosa Lagoon

Figure 5

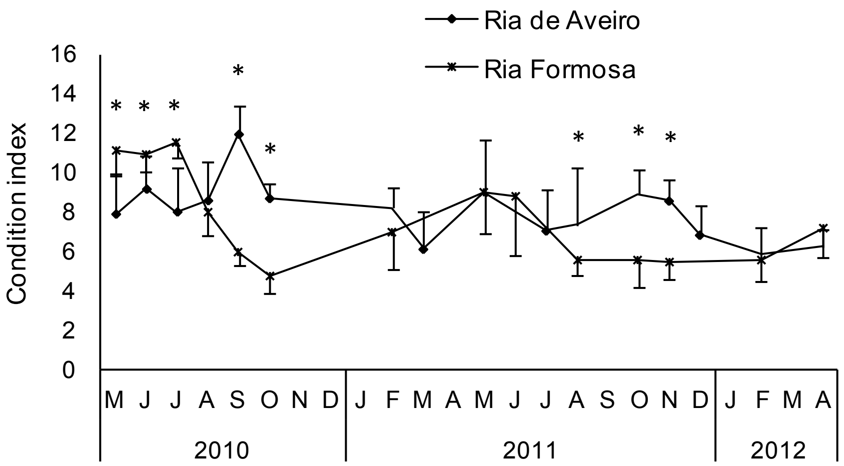
Ria de Aveiro
Ria Formosa
Condition index
0
2
4
6
8
10
12
14
16
*
M J J A S O N D J F M A M J J A S O N D J F M A
2010
2011
2012

Table 1 - Reproductive scale for *Ruditapes decussatus* adapted from Delgado and Pérez-Camacho (2005).

Stage	Histologic description
Period of sexual rest (phase I)	Gonadal follicles are absent and connective and muscular tissue occupies the entire zone from the digestive gland to foot. There is no evidence of gonadal development and sex determination is not possible.
Initiation of gametogenesis (phase II)	Follicles and gonadal acini begin to appear in females and males. They increase in size, and appear covered with oocytes in the growth phase in the females and with immature gametes (spermatogonia and spermatocytes) in the males.
Advanced gametogenesis (phase III)	The follicles occupy a large part of the visceral mass. The presence of muscular and connective tissue is reduced. At the end of this stage, characterised by intense cellular growth in females, the oocyte protrudes from the centre of the lumen, remaining attached to the wall via the peduncle. The abundance of free oocytes equals those attached to the wall of the follicle. In males, majority of the acini were full of spermatids and
Ripe (phase IV)	Corresponding to the maturity of the majority of gametes. In the mature oocytes the rupture of the peduncle occurs, and the oocytes consequently occupy the follicular interior. In males, the gonadal acini mainly contain spermatozoids.
Partially spawned (phase V)	The gametes are discharged. Depending on the degree of spawning the follicles are more or less empty. The follicle walls are bro-ken. There are many empty spaces between and within the follicles.
Spent (phase VI)	Abundant interfollicular connective tissue. Occasional residual sperm or oocytes resent.

Table 2 - Results of Pearson correlation between studied parameters (*r*, correlation coefficient, *P*, *P* value, n.c., no correlation was found).

	Ria de Aveiro							Ria Formosa Lagoon						
	Temperature (SST)	Gonadal index (GI)	Condition index (CI)	Proteins	Total lipids	Glycogen	Total energy	Temperature (SST)	Gonadal index (GI)	Condition index (CI)	Proteins	Total lipids	Glycogen	Total energy
Chlorophyll	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Temperature (SST)		n.c.	*r* = 0.55 *P* < 0.001	n.c.	n.c.	n.c.	n.c.		n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Gonadal index (GI)			n.c.	n.c.	n.c.	n.c.	n.c.			*r* = 0.87 *P* < 0.001	n.c.	n.c.	*r* = 0.60 *P* = 0.01	n.c.
Condition index (CI)				n.c.	n.c.	n.c.	n.c.				n.c.	n.c.	*r* = 0.78 *P* < 0.001	n.c.
Proteins					*r* = -0.52 *P* < 0.01	n.c.	*r* = 0.87 *P* < 0.001					n.c.	n.c.	*r* = 0.91 *P* < 0.001
Total lipids						n.c.	n.c.						n.c.	n.c.
Glycogen							n.c.							n.c.

Table 3 - Mean values (±sd) of proteins, glycogen, total lipids (µg mg^{-1} AFDW) and total energy (kJ g^{-1} AFDW) of *Ruditapes decussatus* during the experimental period.

		Ria de Aveiro				Ria Formosa Lagoon			
Year	Month	Protein (µg mg^{-1} AFDW)	Glycogen (µg mg^{-1} AFDW)	Total lipids (µg mg^{-1} AFDW)	Total energy (kJ mg^{-1} AFDW)	Protein (µg mg^{-1} AFDW)	Glycogen (µg mg^{-1} AFDW)	Total lipids (µg mg^{-1} AFDW)	Total energy (kJ mg^{-1} AFDW)
2010	May	531.7 ± 180.0	45.0 ± 10.6	46.1 ± 9.7	12.0	406.3 ± 113.6	42.0 ± 14.9	51.6 ± 18.1	9.9
	Jun	482.0 ± 98.0	37.7 ± 12.9	41.9 ± 25.0	10.8	388.5 ± 78.2	45.0 ± 9.3	27.2 ± 7.3	8.7
	Jul	465.4 ± 77.8*	32.0 ± 15.0	38.9 ± 9.6	10.3*	206.2 ± 105.3*	38.7 ± 9.3	37.4 ± 13.1	5.7*
	Aug	349.5 ± 101.0*	18.3 ± 4.2	45.6 ± 10.6	8.2*	157.4 ± 40.0*	28.6 ± 11.5	42.2 ± 8.9	4.8*
	Sep	128.3 ± 32.6*	53.7± 22.9*	53.5 ± 11.7	5.1	274.7 ± 67.5*	7.2 ± 2.4*	40.2 ± 9.1	6.5
	Oct	342.02± 37.2	34.6 ± 12.3*	35.0 ± 9.8	8.0	520.8 ± 123.5	13.3 ± 12.2*	41.3 ± 16.7	11.1
	Nov	--	--	--	--	--	--	--	--
	Dec	--	--	--	--	--	--	--	--
2011	Jan	--	--	--	--	--	--	--	--
	Feb	407.8 ± 69.4*	25.5 ± 7.0	47.3 ± 11.9	9.4*	142.2 ± 23.6*	22.1 ± 7.4	45.6 ± 11.0	4.5*
	Mar	301.2 ± 33.5	48.3 ± 15.6	97.6 ± 17.7	9.7	--	--	--	--
	Apr	--	--	--	--	--	--	--	--
	May	251.5 ± 33.4	33.2 ± 8.2	80.6 ± 18.1	7.9	284.5 ± 24.1	35.4 ± 8.9	93.0 ± 16.3	9.0
	Jun	270.5 ± 40.1	9.1 ± 5.8	113.5 ± 26.1	9.0	283.5 ± 52.0	14.5 ± 8.2	92.1 ± 18.8	8.6
	Jul	276.6 ± 38.2	23.1 ± 5.4	100.2 ± 35.5	8.9	293.2 ± 22.5	18.2 ± 4.1	112.1 ± 15.1	9.5
	Aug	265.4 ± 31.5	39.7 ± 12.8*	66.3 ± 12.2	7.8	349.2 ± 37.5	15.3 ± 2.3*	68.6 ± 12.2	9.0
	Sep	--	--	--	--	--	--	--	--
	Oct	298.7 ± 36.0	50.2 ± 13.4*	69.6 ± 13.4	8.7	393.7 ± 29.7	18.3 ± 4.1*	95.6 ± 14.1	10.8
	Nov	243.0 ± 76.7	50.7 ± 19.7*	62.3 ± 11.5	7.4	248.6 ± 62.7	21.7 ± 4.6*	82.5 ± 10.0	7.8
	Dec	--	--	--	--	--	--	--	--
2012	Jan	--	--	--	--	--	--	--	--
	Fev	297.1 ± 34.1	28.3 ± 23.1	59.4 ± 7.7	7.9*	475.4 ± 38.4	25.0 ± 13.0	90.9 ± 9.6	12.2*
	Mar	--	--	--	--	--	--	--	--
	Apr	235.1 ± 37.9	34.2 ± 12.6	118.1 ± 20.5	9.0	229.2 ± 37.7	35.2 ± 10.5	88.6 ± 15.2	7.9

(*statistically significant differences, *P* <0.05 found between populations)