



**Reproductive cycle of the population of European clam,
Ruditapes decussatus, from Lagoa de Óbidos, Leiria,
Portugal.**

Daniela Teresa Sebastião Machado

[2015]



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Dissertação para a Obtenção do Grau de Mestre em Aquacultura

Dissertação de Mestrado realizada sob a orientação da Especialista Teresa Baptista e da Doutora Domitília Matias.

[2015]

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Título: Reproductive cycle of the population of European clam, *Ruditapes decussatus*, from Lagoa de Óbidos, Leiria, Portugal

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Resumo

Em Portugal, a amêijoa-boia (*Ruditapes decussatus*) representa um importante recurso a nível comercial, sendo necessárias mais áreas para aumentar a sua produção sustentável. A Lagoa de Óbidos é um forte candidato a local de cultivo, contudo a biologia reprodutiva da população presente nesta área ainda não está descrita. Através da monitorização da temperatura da água, clorofila *a* e matéria orgânica particulada, assim como, da determinação dos estádios de desenvolvimento gonadal, visualizados em preparações histológicas, do índice gonadal, do índice de condição e da composição bioquímica (proteínas, glicogénico e lípidos totais) de indivíduos recolhidos na Lagoa de Óbidos, pretendeu-se caracterizar o ciclo reprodutivo da espécie *R. decussatus*. Este estudo foi efetuado ao longo de 10 meses de amostragem (outubro 2014 a julho 2015). O ciclo reprodutivo da população de *R. decussatus* da Lagoa de Óbidos apresenta uma ciclicidade anual, que compreende o início do ciclo gametogénico no final do inverno (em janeiro de 2015 para fêmeas e em fevereiro de 2015 para os machos), o estágio de maturação na primavera (maio de 2015), seguido pelo período de desova, que começa no final da primavera/início do verão e se estende, possivelmente, até início do outono e um subsequente período de repouso sexual durante o inverno (novembro 2014 - dezembro de 2014). Durante o período de estudo, o índice gonadal seguiu o mesmo padrão do desenvolvimento gonadal. O índice condição apresentou variações sazonais que estão relacionadas com a disponibilidade de alimento (clorofila *a*) na área de estudo. Os resultados de ambos os ciclos de armazenamento e utilização de nutrientes mostraram que esta população segue uma estratégia intermédia (entre a oportunista e a conservadora), que lhe permite uma rápida recuperação após o esforço reprodutivo, muito provavelmente devido à grande disponibilidade de alimento na Lagoa de Óbidos. Este estudo poderá ajudar a melhorar a gestão sustentável desta população, sendo também importante para o desenvolvimento futuro do cultivo desta espécie.

Palavras-chave: Amêijoa-boia; *Ruditapes decussatus*; ciclo reprodutivo; Lagoa de Óbidos; índice de condição; composição bioquímica.

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Abstract

In Portugal, the European clam (*Ruditapes decussatus*) is an important commercial resource, and therefore, in order to increase their exploration, more production areas need to be created. Lagoa de Óbidos is a strong candidate as a cultivation area. However, the reproductive biology of this population has not been described yet. Through monitoring the sea surface temperature, chlorophyll *a* and particulate organic matter and by the determination of gonadal development stages, visualized in histological preparations, gonadal index, condition index and biochemical composition (protein, glycogen and total lipids) was intended to characterize the reproductive cycle of the species *R. decussatus*, during 10 months of sampling (October 2014 to July 2015). The reproductive cycle of *R. decussatus* of Lagoa de Óbidos population followed an annual cyclicity that comprised an onset of the gametogenic cycle in late winter (January 2015 for females and February 2015 for males), a ripe stage in spring (May 2015) followed by spawning that began in end of spring/early summer that possibly extended until early autumn and a subsequent period of sexual rest during the winter (November 2014 – December 2014). During the study period the gonadal index followed the same pattern as the gonadal development. Condition index showed seasonal variations which are related to food availability (chlorophyll *a*). The results of both cycle of nutrients stored and nutrients utilization showed that this population exhibited an intermediate strategy (between opportunistic and conservative) that allows a rapid recovery after the reproductive effort, most likely due to the wide availability of food in the Lagoa de Óbidos. This study can help improve a sustainable management of this wild stock and is important for future aquaculture development of this species.

Keywords: European clam; *Ruditapes decussatus*; reproductive cycle; Lagoa de Óbidos; condition index; biochemical composition

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List of Acronyms

AFDW – Ash free dry weight

ANOVA – Analyses of variance between groups method

Chl a – Chlorophyll a

CI – Condition index

DGRM – Direcção-Geral de Recursos Naturais, Segurança e Serviços Marítimos

FAO – Food and agriculture organization

GI – Gonadal index

SST – Sea water temperature

PCA – Principal component analysis

POM – Particulate organic matter

r – Correlation coefficient

SD – Standard deviation

SST – Sea water temperature

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1. Introduction

In last decades, there was a steady increase in world production of bivalves, coming from fisheries and aquaculture (Helm & Bourne, 2006), contributing to this outlook, the knowledge obtained from research related to biology, life cycle and reproduction of these organisms.

Bivalve aquaculture in coastal areas has been an important source of food and economic activity in many countries (Cardoso *et al.*, 2013). Bivalve molluscs, namely, oysters, mussels, clams and scallops, constitute an important part of world aquaculture production, being the second group most produced (FAO, 2014).

In Portugal, clam production represented 47% of total production of bivalve production in 2014 (DGRM, 2015), being the European clam, *Ruditapes decussatus*, central to aquaculture revenue. *R. decussatus*, is one of the most appreciated species by consumers, with a high commercial value (Matias *et al.*, 2009). The most productive areas of this species are located in the Ria Formosa and the Ria de Aveiro, where the clams grow in based-land cultures in the intertidal zone and where reproductive cycles of this species are well known (Matias *et al.*, 2013). However, during the last two decades, production of this species suffered a considerable decrease mostly due to recruitment failures, excessive pressure on the capture of juveniles on natural banks, severe mortality and introduction of non-native species such as *Ruditapes philippinarum*. To address this situation, artificial spawning and larval rearing programs could provide an alternative source of spat.

The reproduction of various bivalve species has been intensively studied in the last decades, mainly in commercial species, since research on this subject is essential for the development of aquaculture and fisheries management (Joaquim *et al.*, 2008a; Guerra *et al.*, 2011; Matias *et al.*; 2013). Reproduction studies of bivalve molluscs allow understand their life history and problems related to its regulation and conservation (Quayle, 1943). According to Coe (1943), bivalve molluscs have a wide variety of reproductive strategies and reproductive studies in this group of organisms can bring important knowledge about their sexuality. In addition, knowledge of the highest reproductive activity periods is essential to establish fisheries management plans, as well as for restocking of natural stocks (Galvão *et al.*, 2006). Information about the reproductive cycles of bivalves also allows to know the best period of seeds collectors placement for its cultivation in

aquaculture, as well as for the establishment of its production in hatchery (Galvão *et al.*, 2006; Joaquim *et al.*, 2008a). In this way, to be able to establish and improve rearing programs for *R. decussatus* and create effective stock management programs in natural environment populations, a detailed knowledge of the reproductive cycle and spawning periods is crucial (Matias *et al.*, 2013).

The European clam, *R. decussatus* (Linnaeus, 1758) is a bivalve mollusc of the family Veneridae, native to the European Atlantic and Mediterranean coastal waters, from Norway to Somalia, along the Iberian Peninsula, into the Mediterranean Sea and northwest Africa (Parache, 1982). In Portugal, this species has present populations at Ria de Aveiro, Lagoa de Óbidos, estuaries of Tejo, Sado and Arado rivers, Ria de Alvor, Ria Formosa (Vilela, 1950) and in Lagoa da Fajã de Santo Cristo (São Jorge Island, Açores) (Jordaens *et al.*, 2000). It's an eurythermal and euryhaline species inhabiting sheltered coastal areas, such tidal flats, lagoons and estuaries, living buried in the sediment, usually in sandy substrates of medium and fine gravimetry and in muddy substrates, at a maximum depth of 10 to 12 cm, depending on its size. Consistency of the soil, population density, physiological state of individuals, as well as size of siphons are factors that limit their vertical distribution, while distribution to the surface is influenced by tidal boundary lines (Vilela, 1950; Guelorget *et al.*, 1980). Relatively to its external morphology, the specimen's presents an equivalve, elongated and convex shell with an oval shape that can be roughly rectangular too, traversed by radial concentric striations (Banha, 1984). Colour is variable, individuals may be whitish, yellow, orange, light or dark brown, uniform, with stripes or with several marks variable in colour and number (De Valence & Peyre, 1990), being this connected to the nature of the substrate. Internally, the valves have a clear contour of the adductor muscles and the pallial line (Poppe & Goto, 1991), existing also, in this line, an indentation (pallial sinus) which is due to the retractor muscles of siphons. Regarding internal morphology, the European clam is constituted by the following structures: mantle, siphons, gills, labial palps, adductor muscle and visceral mass (Vilela, 1950). The two siphons are separated throughout its length and the distal extremity of them is pigmented (Parache, 1982). Visceral mass is divided in two parts, the visceral mass itself, with the circulatory, digestive, nervous and reproductive systems (Vilela, 1950; Grassé, 1960), and foot, which allows locomotion. It's a filter feeder capable of ingesting different particles suspended in water (eg. bacteria, protists, phytoplankton, invertebrate eggs and larvae) (Parache, 1982). Sexual maturity, which is dependent on size rather than age and geographic distribution (Ojea *et al.*, 2004), is reached when the clams are about 20 mm, being this a gonochoric species with external fertilization in the

water column, females produce oocytes and males produce spermatozoids (Vilela, 1950; Camacho, 1980), although, according to some authors, can present traces of juvenile hermaphroditism, which usually disappear before reaching the functional status of the gonads (Lucas, 1968; Delgado & Pérez-Camacho, 2002).

The reproductive system of bivalve forms a diffuse structure, which occupies the connective tissues and that disappears almost completely in rest period. In the gonads, gametes are formed and their formation in males (spermatogenesis) and females (oogenesis) occurs in gonadal follicles where there are a number of typical cells of each stage of the process which leading to the production of mature spermatozoids and oocytes, emitted during spawning. The gonads form an acinous structure that once developed normally involves the digestive gland and the rest of the organs, filling the free spaces between them. It is organized on a dendritic form composed by a gonoduct, genital ducts and numerous smaller ducts, which form a network of follicles. Gametogenesis is the process by which the formation of gametes occurs; it begins through precursor cells that give rise to gonocytes. These are undifferentiated stem cells which can also be named by gonial stem cells, primordial germ cells, primordial germ cells or protogonias, constituting the first stage of gonadal development. They are found in the peripheral areas of gonadal ducts, attached to the connective tissue and doesn't distinguish between males and females. When multiply actively through successive divisions these originate another cell type, the first differentiated cells, known as primary gonias (primary spermatogonias in males and primary ovogonias in females) which are structured in tubular follicles giving rise to two different processes depending on sex: spermatogenesis and oogenesis (Joaquim *et al.*, 2008b; Guerra *et al.*, 2011). In males, primary spermatogonias, form one or two concentric layers lining the wall of the follicle. These, after suffering the process of mitosis, are converted into definitive spermatogonias, which distinguished in primary spermatocytes that become detached from the wall of the follicle, remaining in a continuous layer. These undergo meiosis, giving rise to secondary spermatocytes, which stay within an inner layer, and later to spermatids. Spermatids differentiate in order to become mature spermatozoids, which are located in the center of the follicle, with the flagellum pointed to the lumen and the head pointed to the wall. In females, the primary ovogonia are attached to follicular wall, as spermatogonia in spermatogenesis process. While some of them remain at rest to a posterior development during the reproductive cycle, others divide by mitosis give rise to secondary ovogonias. Then, some of these secondary ovogonia undergo meiosis give rise to oocytes, while others remain at rest. Fully-formed oocytes begin to grow, and continue to do so until the

end of oogenesis, being its development divided into two phases, previtellogenesis and vitellogenesis. During previtellogenesis, growth is slower, oocytes increase in size and suffer the first meiotic division, the nucleus and the cytoplasm reappears and the cytoplasm increases its volume. During vitellogenesis, when the oocytes become mature, the chromatin becomes blurred, the nucleus increases its size and reserves are accumulated in the cytoplasm, leading to a considerable increase in egg size. Throughout the process of oogenesis, when oocytes are small, they are attached to the wall of the follicle, when they increase in size they are joined by a peduncle and finally when they are mature, they appear floating in the lumen (free oocytes) (Bayne, 1976; Joaquim *et al.*, 2008b; Guerra *et al.*, 2011).

Seed (1976) defined reproductive cycle as "the entire cycle of events from activation of the gonad, through gametogenesis to spawning and subsequent recession of the gonad", differentiating two distinct periods during the cycle, a reproductive period and a rest period. Thus, the bivalve gametogenic cycle is constituted by a succession of steps that goes from emission of gametes and spawning to the next stage of the development of the gonads. In the process of reproduction, the gametic cycles may be annual, semi-annual, or continuous, that is, one or two reproduction periods per year with a resting period, or continuous spawns throughout the year. These cycles are determined by the interaction between endogenous and exogenous factors, being reproduction process a genetically controlled response to the environment (Sastry, 1979). Together, the exogenous factors (such as temperature, food, age and size, tides, pathology and photoperiod) and endogenous factors (genetic and hormonal activity) are responsible for the development of the reproductive system of bivalves and determine the timing and magnitude of spawning, being the most importance factors temperature and quantity and quality of available food (Gabbott, 1976; Bayne & Newell, 1983; Pérez-Camacho *et al.*, 2003; Joaquim *et al.*, 2011). Therefore, bivalves are subject to an annual cycle of accumulation and use of energy reserves associated with gametogenesis (Albentosa *et al.*, 2007), which is mostly regulated by temperature and food availability (Joaquim *et al.*, 2011; Matias *et al.*, 2013). These external factors typically vary from year to year, giving rise to between-year variation in the timing of the reproductive cycle.

In temperate latitudes, nutrient availability typically shows a high seasonal variability and bivalve molluscs respond to this variability in different ways. In some species, the peak period of gamete production coincides with the nutrient availability peak, in others, nutrients are stored in different body organs and gamete production takes place during

low-nutrient periods, however, many species follow intermediate strategies, using both stored and recently assimilated nutrients. This triggers changes in each biochemical component and show that these are closely linked to the state of sexual maturity (Sastry, 1979), can be classified bivalve species as either conservative or opportunist, based on the relationship between gonad development and the accumulation and utilization of nutrients (Bayne, 1976). In conservative category, gametogenesis takes place at the expense of previously acquired reserves (Zandee *et al.*, 1980, Bayne *et al.*, 1982) and in opportunist category, gametogenesis occurs when there is an abundance of food in the environment, and sexual maturing follows the accumulation of nutrients (Pérez-Camacho *et al.*, 2003). Actually, a close relationship between gonadal development cycles and energy storage and utilization cycles (= biochemical cycles) have been documented by several authors in a variable number of bivalve species (Gabbot & Bayne, 1973; Barber & Blake, 1981; Lowe *et al.*, 1982; Fernández Castro & Vido de Mattio, 1987; Gouilletquer *et al.*, 1988; Le Pennec *et al.*, 1991; Massapina *et al.*, 1999; Pérez-Camacho *et al.*, 2003; Ojea *et al.*, 2004; Joaquim *et al.*, 2011; Matias *et al.*, 2013). So, accumulation of reserves, allocation of stored energy and the importance of each gross biochemical component to the reproductive process under different nutritional conditions play a role in the adapt strategies to different areas of a given species (Goodman, 1979; Pérez-Camacho *et al.*, 2003), indeed, there is evidence that responses to different conditions vary between different geographical populations, even at the same latitude, of the same species could strongly differ in terms of their fecundity levels and biochemical compositions (Shafee & Daoudi, 1991; Trigui-El-Menif *et al.*, 1995; Iglesias *et al.*, 1996; Avendaño & Le Pennec, 1997; Matias *et al.*, 2013).

In marine bivalves, reserves accumulate in the form of protein, glycogen and lipid substrates and are utilized in gametogenic synthesis when metabolic demand is high (Giese, 1969; Bayne, 1976; Mathieu & Lubet, 1993; Dridi *et al.*, 2007). Proteins are the most abundant biochemical component in tissues; these 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 & Lucas, 1984; Galap *et al.*, 1997). Carbohydrates are assumed to constitute the most important bio-energy reserve in bivalve molluscs and, because of their hydrosolubility, are available for immediate use, having two major biological functions, as a long-term energy storage and as structural elements (Robledo *et al.*, 1995), being glycogen the most prominent carbohydrate for supplying energy demands (Fernández Castro & Vido de Mattio, 1987) and reproductive cycle (Newell & Bayne, 1980; Pazos *et*

al., 2005), representing well the nutritional condition of bivalves (Uzaki *et al.*, 2003). Generally, glycogen reserves are used during gametogenic processes when lipids are not available (Serdar & Lök, 2009). Lipids represent an important reserve due to their high caloric content (Serdar & Lök, 2009), playing an important role in the gamete formation, being the main reserve of oocytes (Matias *et al.*, 2009, 2011).

Condition index is used for biological purposes (Baird, 1958), since this is closely related to the gametogenic and nutrient reserve storage-consumption cycles, being also recognized as a useful biomarker reflecting the ability of bivalves to withstand adverse natural stress (as the reproduction period) (Mann, 1978; Fernández Castro & de Vido de Mattio, 1987). Thus, condition index, together with monitoring of gametogenic activity and biochemical composition, are all parameters that enable knowledge of the reproductive cycle of a species of bivalve once their seasonal variations are related.

Nevertheless previous works have studied the natural reproduction of *R. decussatus* different Portuguese populations (Vilela, 1950; Pacheco *et al.*, 1989; Matias *et al.*, 2013), the reproductive cycle and its patterns of nutrient storage and utilization can differ according to geographic location of populations (Ojea *et al.*, 2004). Therefore, the present study aims to characterize the reproductive cycle of Lagoa de Óbidos population of *R. decussatus*, where the reproductive biology of this species is still unknown. Although, the commercial exploitation of this population is low (Leite *et al.*, 2004), *R. decussatus* could constitute a potential candidate for a large production in Lagoa de Óbidos. Also, information obtained would be essential for the establishment of a successful hatchery-based production.

2. Materials and methods

2.1. Sample collection

Forty adult specimens of *R. decussatus* (length = 40.20 ± 1.74 mm, weight = 15.42 ± 1.10 g) were monthly collected during a period of ten months (October 2014–July 2015) in Lagoa de Óbidos ($39^{\circ}23'51.2''\text{N}$; $9^{\circ}12'57.1''\text{W}$) (Figure 2.1) and also water samples (4 L) were taken to evaluate the chlorophyll *a* and particulate organic matter (POM) concentrations. The sea surface temperature (SST) was monitored *in situ* using a multiparameter probe. Samples (organisms and water) were transported to the laboratory in an isothermal container.

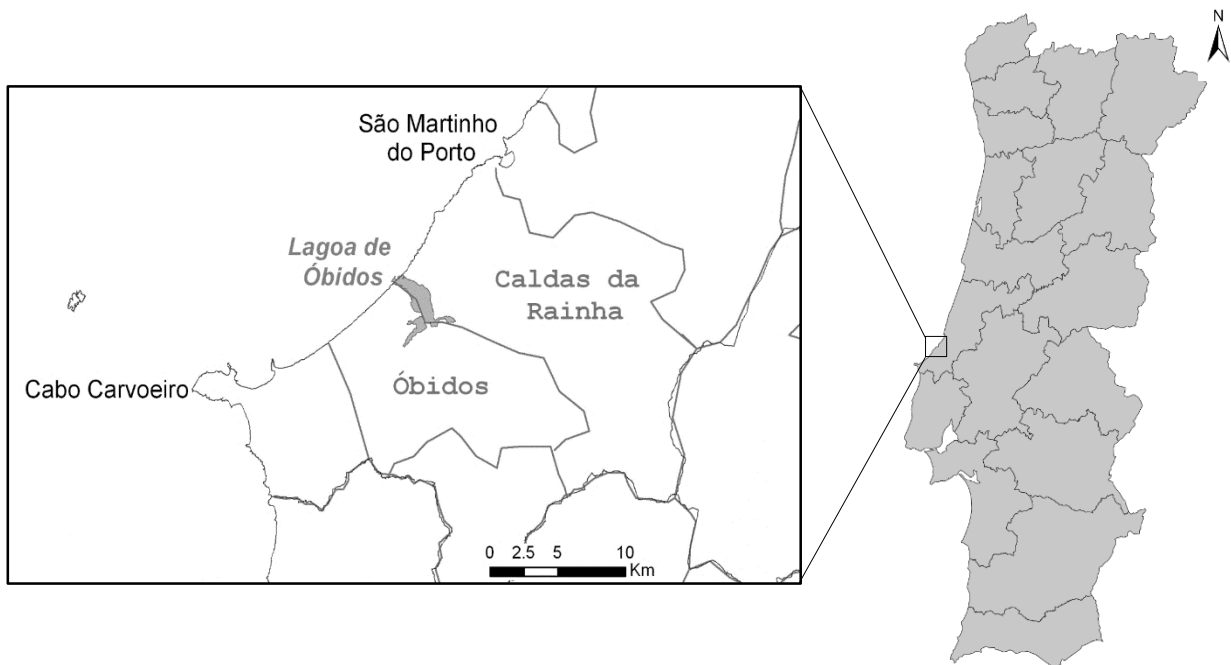


Figure 2.1 – Location of Lagoa de Óbidos where *Ruditapes decussatus* individuals were collected.

Lagoa de Óbidos is an interior lagoon located in the western region of Portugal. This is a small and shallow coastal system with a wet surface area variable, approximate 6.0 km^2 on average, a maximum length and width of 4.5 km and 1800 m, respectively, that is connected to the sea by a narrow inlet (on the order of 100 m), which undergoes severe migration on monthly time scales (Oliveira *et al.*, 2006). Two main regions, with distinct morphological and sedimentary characteristics, can be identified in the lagoon: the lower lagoon, with several sand banks and channels with strong velocities, and the upper lagoon, characterized by low velocities and muddy bottom sediments (Freitas, 1989; Oliveira *et al.*, 2006). The upper lagoon (where this study was carried) comprises a large,

shallow basin, with two elongated bays (the Braço da Barrosa and the Braço do Bom Sucesso) and a small embayment on the southern margin (Poça das Ferrarias) (Oliveira *et al.*, 2006). The average depth is small, on the order of 2-3 m on the average sea level and the regime of tides is semi-diurnal (twice-daily tides), with high amplitude (mesotidal), tidal ranges vary between 0.5 and 4.0 m, depending upon location and tidal phase (Malhadas *et al.*, 2009).

2.2. Analytical analyses

2.2.1. Water analysis (Chlorophyll *a* and Particulate organic matter)

Chlorophyll *a* concentration was determined using the spectrophotometric method proposed by Jeffrey & Lorenzen (1980). Water samples (about 1 L, in duplicate) were filtered through a Whatman GF/C glass paper filter. Then, chlorophyll *a* was extracted, adding 10 mL of 90% acetone (C₃H₆O) and placed over 24 hours at 4°C. Subsequently, samples were centrifuged at 4000 rpm for 10 minutes. For the correction of phaeopigments after a first reading of absorbance at 665nm and 750nm, samples were acidified with diluted hydrochloric acid (HCl) and the absorbance read again at the same wavelengths. The content of chlorophyll *a* was calculated according to Lorenzen equation (1967):

$$Chl\ a\ (mg\ m^{-3}) = \frac{A \times K \times [(665_0 - 750_0) - (665_a - 750_a)] \times v}{V \times L}$$

Where,

A – absorption coefficient of chlorophyll *a* = 11,

K – factor to equate the reduction in absorbance to initial chlorophyll concentration = 2.43,

665₀ – absorbance at 665nm before acidification,

750₀ – absorbance at 750nm before acidification,

665_a – absorbance at 665nm after acidification,

750_a – absorbance at 750nm after acidification,

v – volume of acetone used for extraction = 10 mL,

V – liters of water filtered = 1 L,

L – path length of cuvette = 1 cm.

Particulate organic matter (POM) was determined using a gravimetric method (Strickland & Parsons, 1972). Water samples (about 1 L, in duplicate) were filtered through a Whatman GF/C glass paper filter, previously ashed for 2 hours at 450°C and weighed. Total particulate matter was first determined after drying the filter at 80°C for 24 hours, filters were then ashed at 450°C for 24 hours and POM was determined as the loss in weight due to ashing (Jones & Iwama, 1991), according to the formula:

$$\text{POM (mg l}^{-1}\text{)} = \frac{\text{Weight of total matter (g)} - \text{Weight of ashes (g)}}{\text{Volume of water filtered (l)}} \times 1000$$

2.2.2. Laboratory analysis (Gametogenic stage, Condition index and Biochemical composition)

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

2.2.2.1. Histology

Twenty individuals (ten males and ten females, when possible the distinction) from each month sample were examined histologically to determine the gametogenic stages in both sexes. The visceral mass was separated from siphons and gills and fixed in Davidson solution for 48 hours, 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 eosin. The histologically prepared slides were examined using a microscope at 40x magnification and for each individual was assigned a stage which represented the gonadal state. Then, clam reproductive maturity was categorized into six stages using a scale according to Delgado & Pérez-Camacho (2005) and adapted by Matias *et al.* (2013) (Table 2.1 and Figures 2.2 and 2.3). 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.

Table 2.1 – Reproductive scale for *Ruditapes decussatus* according to Delgado & Pérez-Camacho (2005) and adapted by Matias *et al.* (2013).

| 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, respectively. 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 a large part of the visceral mass. The presence of muscular and connective tissue is reduced. At the end of this stage, characterized by intense cellular growth in females, the oocyte protrudes from the center of the lumen, remaining attached to the all 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 spermatozooids. |
| 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 spermatozooids. |
| 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 broken. There are many empty spaces between and within the follicles. |
| Spent (Phase VI) | Abundant interfollicular connective tissue. Occasional residual sperm or oocytes resent. |

A mean gonadal index (GI) was also calculated using the method proposed by Seed (1976):

$$GI = \frac{(\sum \text{Ind. each stage} \times \text{Stage ranking})}{\text{Total ind. each month}}$$

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 and spent = 1. The GI ranged from 0 (all individuals in the sample are in rest stage) to 5 (all individuals are in ripe stage).

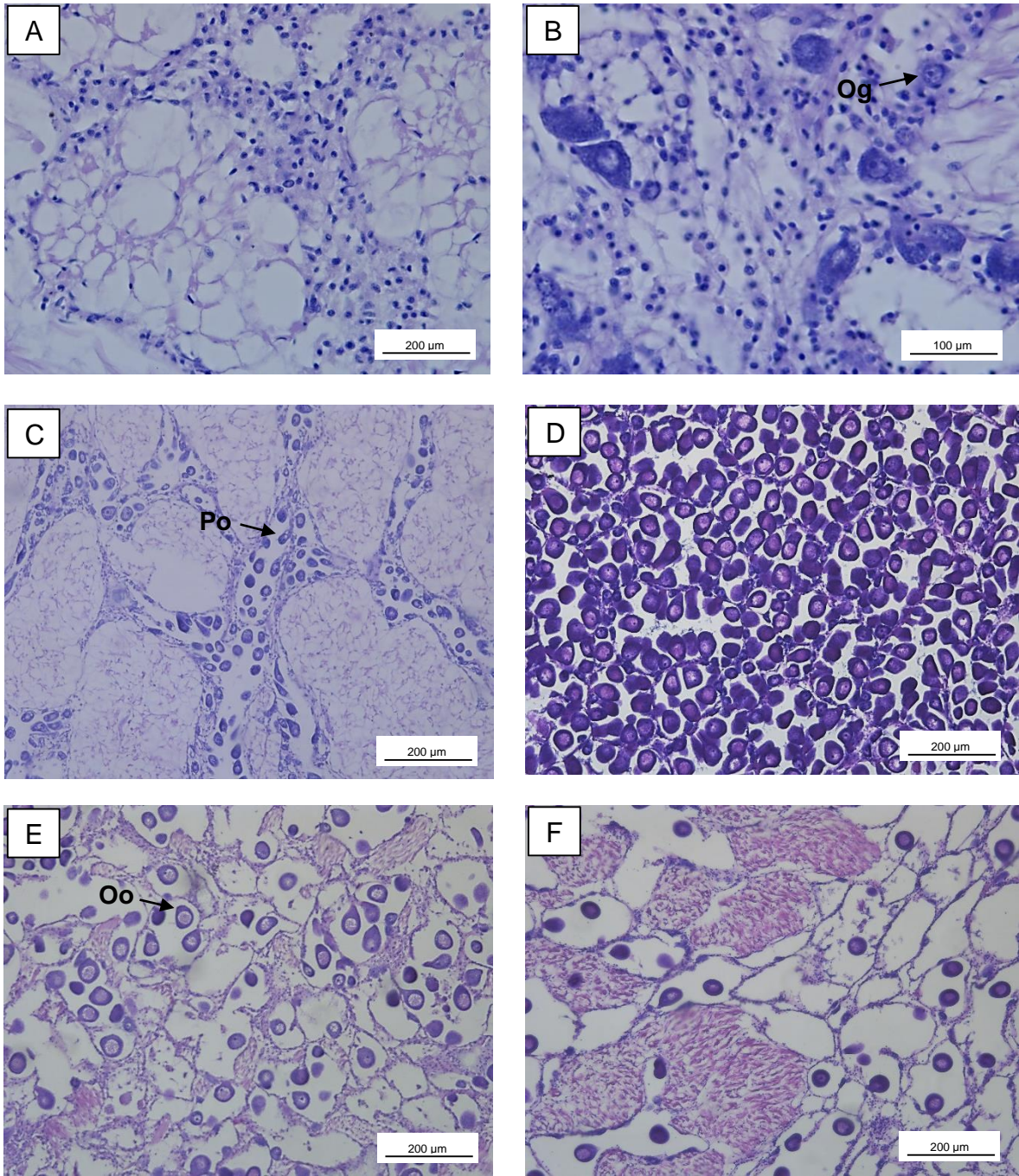


Figure 2.2 – Photomicrographs showing development stages of *Ruditapes decussatus* female gonad. A – Sexual rest; B – Initiation of gametogenesis, Og – Ovogonia; C – Advanced gametogenesis, Po – Pedunculated oocyte; D – Ripe; E – Partially spawned, Oo – Oocyte; F – Spent.

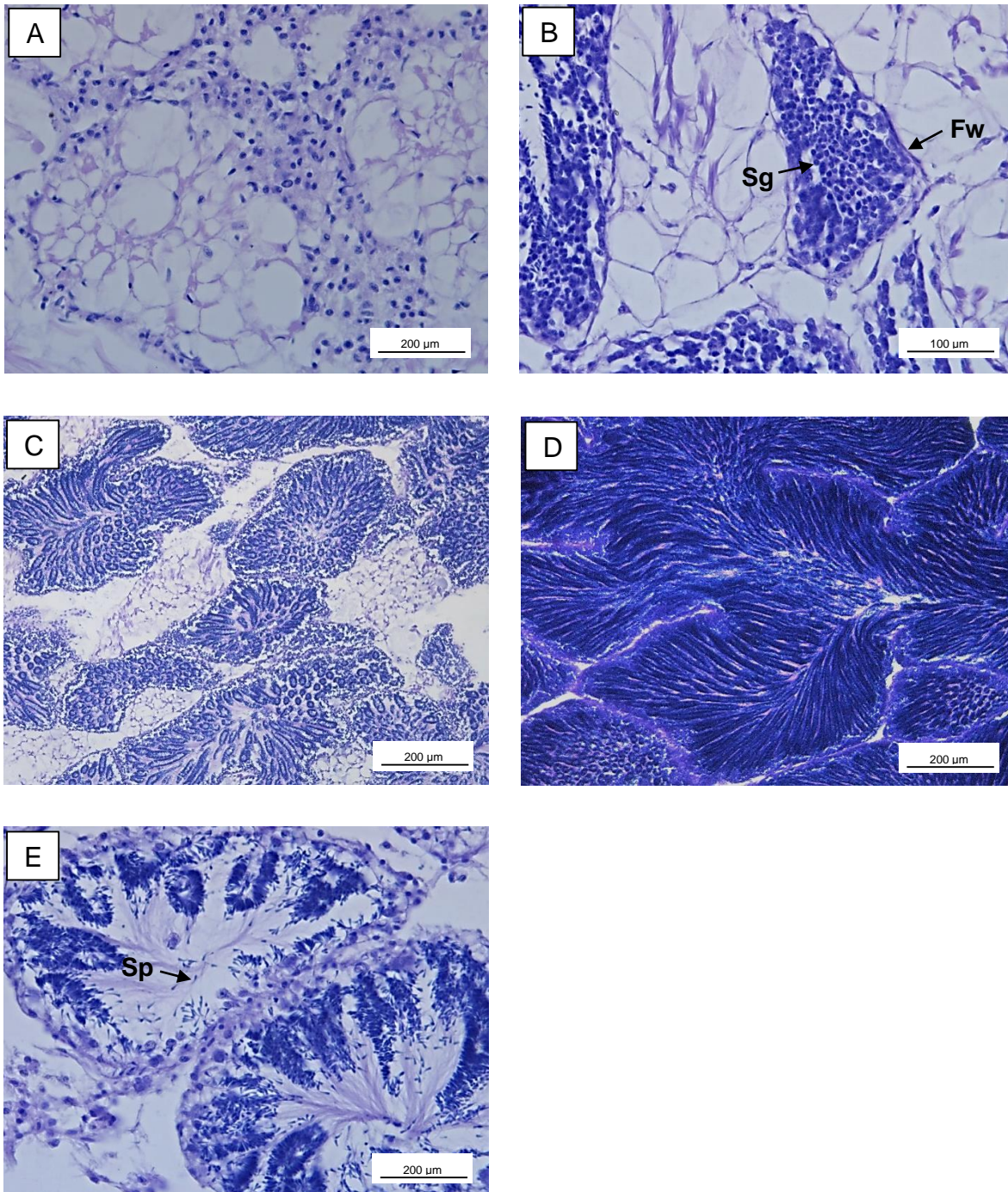


Figure 2.3 – Photomicrographs showing development stages of *Ruditapes decussatus* male gonad. A – Sexual rest; B – Initiation of gametogenesis, Sg – Spermatogonia, Fw – Follicle wall; C – Advanced gametogenesis; D – Ripe; E – Partially spawned, Sp - Spermatozoid.

2.2.2.2. Condition index

The dry meat and shell weight of ten clams, from each month sample, were determined after oven drying at 80°C for 24 hours. The 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 & Mann (1975):

$$CI = \frac{\text{Ash free dry weight (AFDW) of meat (g)}}{\text{Dry shell weight (g)}} \times 100$$

2.2.2.3. Biochemical composition

The meat of ten clams from each month sample was frozen and stored at -20°C for biochemical analyses. For each individual, protein was determined using the modified Lowry method (Shakir *et al.*, 1994), glycogen content was determined from dried (80°C for 24 hours) homogenate using the anthrone reagent (Viles & 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 & Weinstein, 1966). Duplicate determinations were performed in all analyses 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 & De Bruin, 1979), 33 KJ g⁻¹ (Beninger & Lucas, 1984) and 17.2 KJ g⁻¹ (Paine, 1971), respectively.

2.3. Statistical analysis

Seasonal variations in condition index, biochemical composition and gonadal index were analyzed by one-way analysis of variance (ANOVA) or Kruskal–Wallis non-parametric test on ranks whenever the assumptions of ANOVA failed (namely data normality and homogeneity of variances). Multiple pairwise comparisons were performed using the post-hoc parametric Tukey HSD in order to detect significant differences between monthly consecutive samples. The Pearson correlation coefficient was used to determine the degree of association between parameters (SST, Chl *a*, POM, GI, CI, proteins, glycogen, total lipids and total energy). Results were considered significant at *p*-value < 0.05. Where applicable, results are presented as mean ± standard-deviation (SD). All calculations were performed with IBM SPSS Statistics 22.

A principal component analysis (PCA) was performed to evaluate distribution patterns based on the SST, Chl *a*, POM, GI, CI, proteins, glycogen, total lipids, total energy and the sampling months. PCA is used to reduce the dimensionality of a data set, while retaining as much of the original information (variability) as possible (Vega *et al.*, 1998; Helena *et al.*, 2000). The most important principal components (PC1 and PC2) are calculated by linear combination of original variables and they adequately represent the original data. The positions of original variables in the principal component plot relevantly represent their interrelations. Thus, if the variables are in the opposite position, then the given variables are negatively correlated. However, if the variables are very closely located, their interrelation is strong and positive. Hence, graphical representation of the objects investigated in the plot is very useful in detecting their possible association (Bednárová *et al.*, 2013). Moreover, in the principal component biplot, simultaneously representing the objects and the variables, it is possible to detect those variables which are associated with the group formed from closely located objects and in this way the mutual relationships among the objects and variables can be discovered. Canoco for Windows 4.5 (ter Braak & Smilauer, 1998) software was used to perform graphs.

3. Results

3.1. Sea surface temperature, Chlorophyll a and Particulate organic matter

The evolution of monthly sea surface temperature (SST) during the sampling period in Lagoa de Óbidos (Figure 3.1) showed that values ranged between 11.5°C in January and 26°C in May and June, following a seasonal variation.

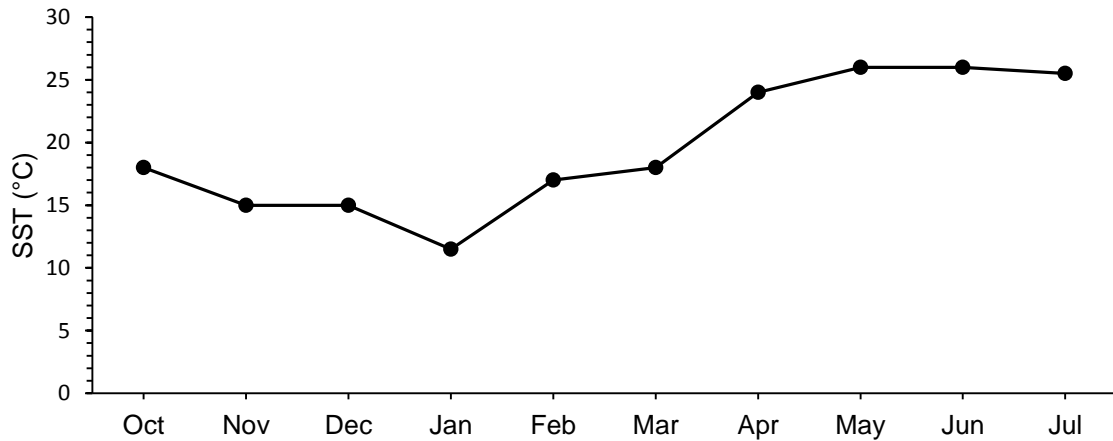


Figure 3.1 – Monthly values of sea surface temperature (SST) in Lagoa de Óbidos from October 2014 to July 2015.

The evolution of the chlorophyll a during the sampling period in Lagoa de Óbidos (Figure 3.2) showed that monthly values ranged between $0.94 \pm 0.19 \text{ mg m}^{-3}$ in February and $73.64 \pm 0.57 \text{ mg m}^{-3}$ in April, observing a phytoplanktonic bloom in spring (April).

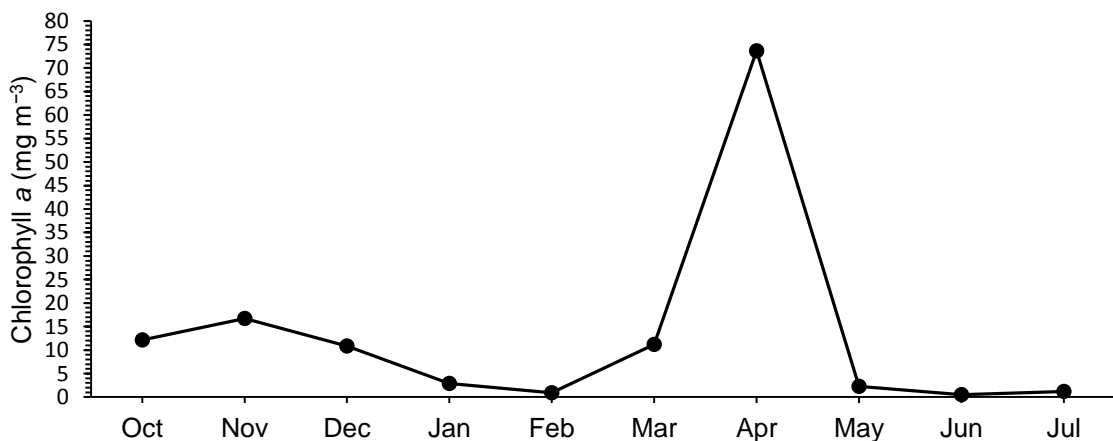


Figure 3.2 – Monthly values of chlorophyll a (mean \pm SD, n=2) in Lagoa de Óbidos from October 2014 to July 2015.

The evolution of the particulate organic matter (POM) during the sampling period in Lagoa de Óbidos (Figure 3.3) showed that monthly values ranged between $2.45 \pm 0.21 \text{ mg L}^{-1}$ in May and $23.78 \pm 0.03 \text{ mg L}^{-1}$ in November, being the second higher value of $8.85 \pm 0.07 \text{ mg L}^{-1}$ in April. The abnormal value registered in November was possibly associated with the climacteric conditions of agitation observed at sampling time that raised sediment from the bottom.

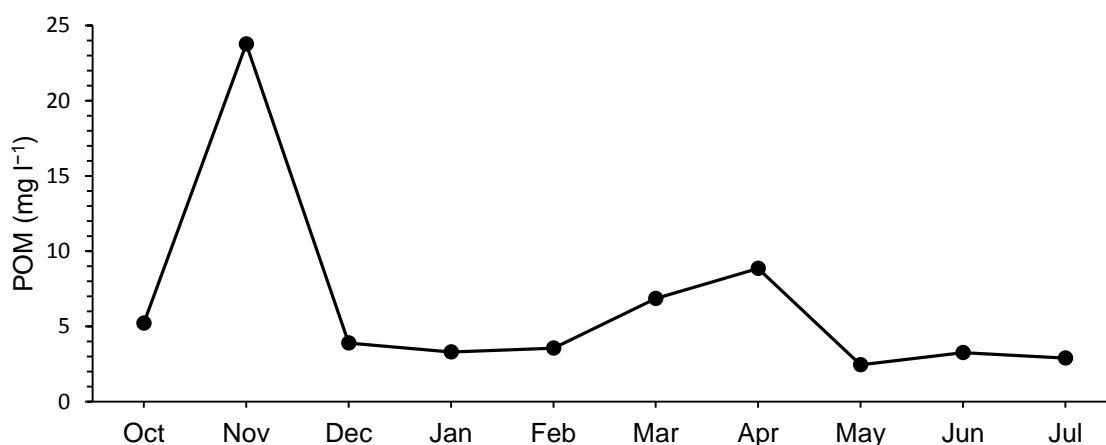


Figure 3.3 – Monthly values of particulate organic matter (POM) (mean \pm SD, n=2) in Lagoa de Óbidos from October 2014 to July 2015.

No correlations were observed between SST and chlorophyll *a*, SST and POM and chlorophyll *a* and POM (Table 3.3).

3.2. Gametogenic cycle

During the study no hermaphrodite individuals were observed in Lagoa de Óbidos, being sexes clearly separated. Both sexes showed synchronism in gonadal development, although males seem slightly delayed compared to females, particularly during winter months. The reproductive cycle of *R. decussatus* was characterized by a seasonal pattern (Figure 3.4). In the beginning of the study (October 2014), no males were observed being in period of sexual rest (phase I, males GI = 0) (Figure 3.4 and Table 3.1). Females in the same month, consisted in 10 % partially spawned (phase V), 60 % spent (phase VI) and 30 % individuals were in sexual rest (females GI = 0.8) (Figure 3.4 and Table 3.1), showing the population a mean GI value of 0.4 (Figure 3.5). In November and December the majority of individuals are in period of sexual rest, which coincided with the lowest population mean GI values (November = 0.2 and December = 0.1). The onset of the gametogenic cycle occurred in January for females and in February for males, which coincided with an increase of SST, chlorophyll *a* and POM and consequently with the

increase of the population mean GI, although no correlations were found between these parameters (Table 3.3). After this, an intensification on the gonad development was verified until the month of April 2015, where all individuals (males and females) were in advanced gametogenesis (phase III), which coincided with the highest values of chlorophyll *a* and POM (when excluded the November value). During May, 50 % of males and 100 % of females reach stage IV (ripe), reaching the population at this point its peak of reproductive effort, represented by the highest GI values (mean GI = 4.75, males GI = 4.5 and females GI = 5) (Figures 3.4, 3.5 and Table 3.1). Spawning occurred during the last two months (late spring/early summer) of the study, being males in July 100 % partially spawned (phase V) and 10 % of females ripe (phase IV), 50 % partially spawned (phase V) and 40 % spent (phase VI), which coincided with the decrease of chlorophyll *a*, POM and population mean GI, although no correlations were found between these parameters as mentioned above (Table 3.3). During the study period the gonadal index followed the same pattern as the gonadal development (Figures 3.4 and 3.5).

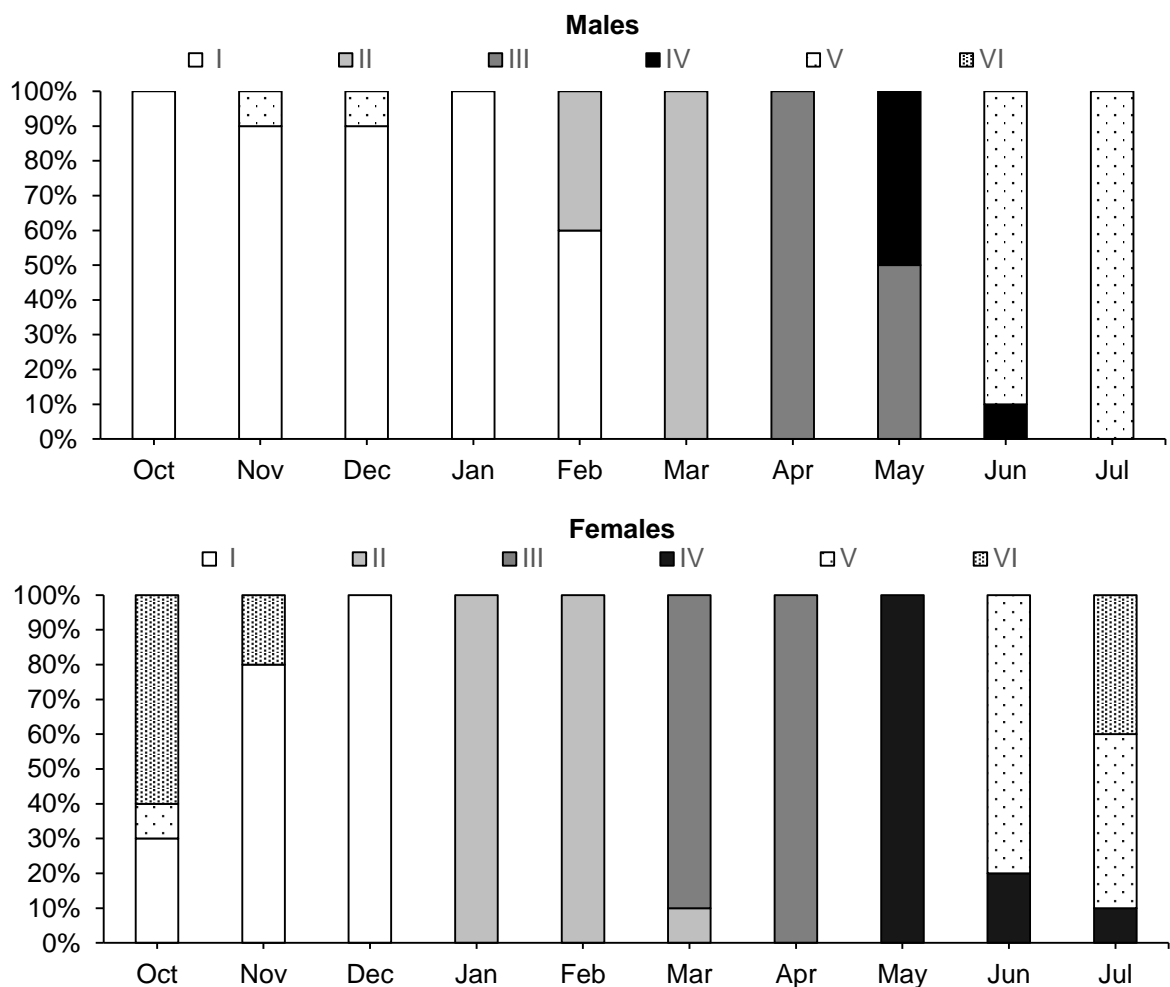


Figure 3.4 – Monthly variations in gonadal development of *Ruditapes decussatus* population from Lagoa de Óbidos, during October 2014 to July 2015. Males (top) and females (bottom).

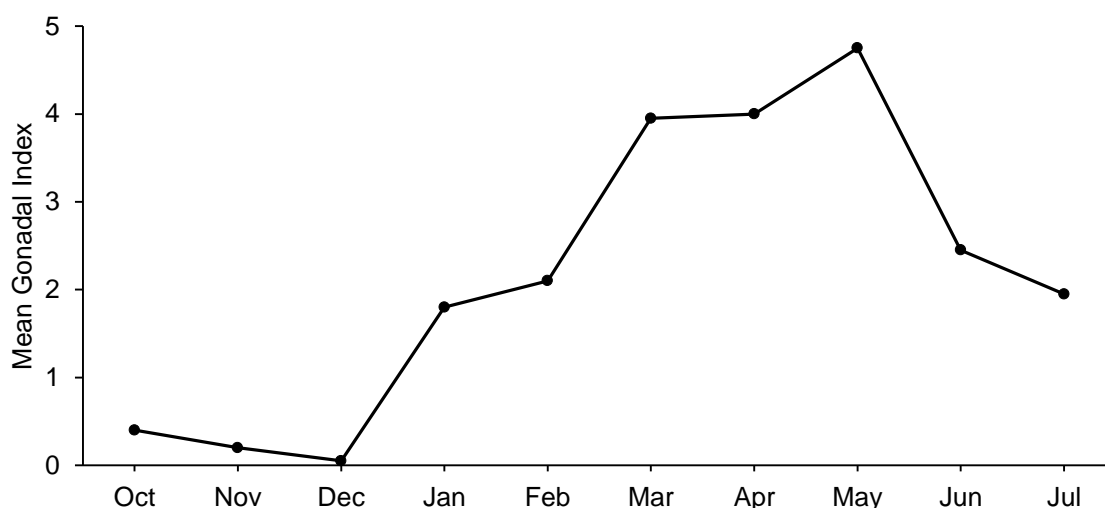


Figure 3.5 – Monthly variations in gonadal index (GI) (mean, n = 20) of *Ruditapes decussatus* population from Lagoa de Óbidos, during October 2014 to July 2015.

Table 3.1 – Monthly variations in gonadal index (GI) (mean, n = 10) of *Ruditapes decussatus* males and females from Lagoa de Óbidos, during October 2014 to July 2015.

| GI | October | November | December | January | February | March | April | May | June | July |
|----------------|---------|----------|----------|---------|----------|-------|-------|-----|------|------|
| Males | 0 | 0.2 | 0.2 | 0 | 1.2 | 3 | 4 | 4.5 | 2.3 | 2 |
| Females | 0.8 | 0.2 | 0 | 3 | 3 | 3.9 | 4 | 5 | 2.6 | 1.9 |

3.3. Condition index

Condition index values during the sampling period in Lagoa de Óbidos (Figure 3.6) ranged between 5.76 ± 1.09 in February and 14.61 ± 1.50 in April, these lowest and highest values, respectively, correspond to the lowest and highest values of chlorophyll *a* in the same months, being indeed these two parameters positively correlated ($r_{\text{Pearson}} = 0.682$, $p\text{-value} < 0.05$) (Table 3.3). No correlations were found between CI and SST and CI and POM, however, the CI generally trended upwards until April following SST and POM increase when the highest value of CI was registered, coinciding with the phase III (advanced gametogenesis) of the gonadal development of the population (Figure 3.4). In the following month (May) the CI value decreased as chlorophyll *a* and POM values. During the last two months of the study, the CI and the population mean GI continue to decrease as spawning occurs, being the CI of the population positively correlated with the GI ($r_{\text{Pearson}} = 0.752$, $p\text{-value} < 0.05$) (Table 3.3). Condition index exhibited statistically significant differences between sample months (ANOVA, $p\text{-value} < 0.05$) showing seasonal variations. Autumn months (October and November) exhibited statistically

significant differences (Tukey HSD, p -value < 0.05, see attachment 1) when compared to the spring months (March, April and May) and October exhibit statistically significant differences (Tukey HSD, p -value < 0.05, see attachment 1) when compared to February (winter month). Winter months (December, January and February), also showed statistically significant differences (Tukey HSD, p -value < 0.05, see attachment 1) when compared to spring months. Regarding to spring months, those revealed statistically significant differences (Tukey, HSD, p -value < 0.05, see attachment 1), in addition to the months of autumn and winter, with the months of summer (June and July). June (summer month) show statistically significant differences (Tukey HSD, p -value < 0.05, see attachment 1) when compared with February (winter month).

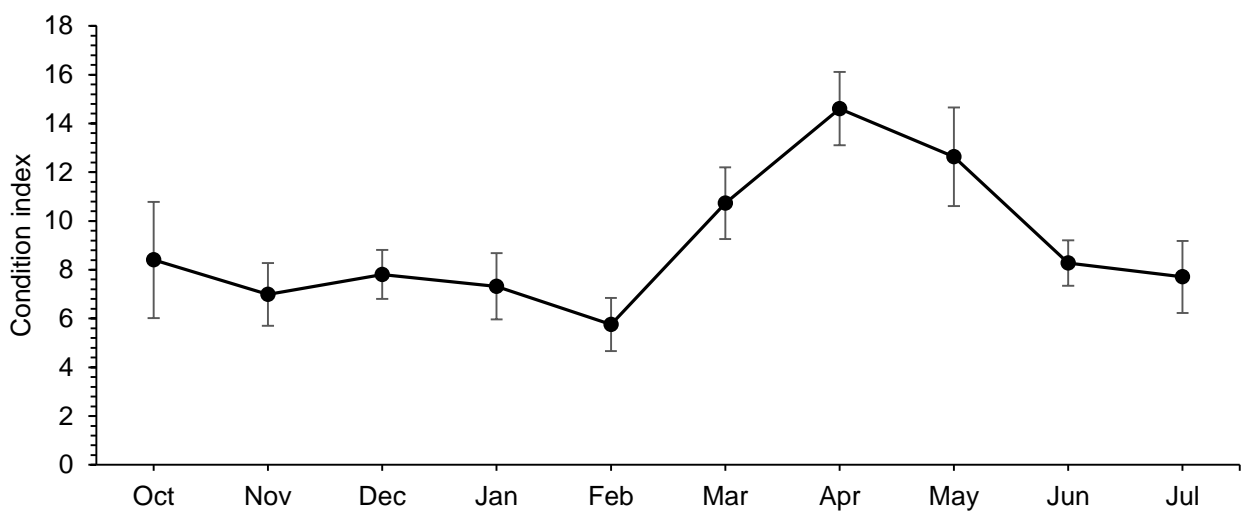


Figure 3.6 – Condition index (mean±SD, n=10) of *Ruditapes decussatus* population from Lagoa de Óbidos, during October 2014 to July 2015.

3.4. Biochemical composition

Proteins were the predominant dry tissue constituent of clams followed by glycogen and total lipids (Table 3.2). The highest protein values were observed in July ($370.77 \pm 29.15 \mu\text{g mg}^{-1}$ AFDW) and the lowest in April ($237.02 \pm 28.38 \mu\text{g mg}^{-1}$ AFDW). Proteins contributed the most to the total energy content which explains the positive correlation found between these parameters ($r_{\text{Pearson}} = 0.786$, p -value < 0.05) (Table 3.3), negative correlations were verified between proteins and CI ($r_{\text{Pearson}} = -0.645$, p -value < 0.05) and proteins and glycogen ($r_{\text{Pearson}} = -0.734$, p -value < 0.05) (Table 3.3). Although, protein values exhibited statistically significant differences (ANOVA, p -value < 0.05) between sample months, no seasonal variations were observed, protein content varies independently of the season. The higher glycogen content was observed in April

(169.05±15.62 µg mg⁻¹ AFDW) coinciding with the phytoplankton bloom (highest value of chlorophyll *a* observed), decreasing after that, during spawning, until the last month of the study and the lowest value was registered in October (92.03±32.94 µg mg⁻¹ AFDW). Beyond the negative correlation between glycogen and proteins mentioned above, positive correlations between glycogen and chlorophyll *a* ($r_{\text{Pearson}} = 0.755$, $p\text{-value} < 0.05$) and between glycogen and CI ($r_{\text{Pearson}} = 0.694$, $p\text{-value} < 0.05$) were found. Additionally, statistically significant differences (Kruskal-Wallis, $p\text{-value} < 0.05$) were found in this biochemical content when compared sampling months. Although, once again no seasonal variations were observed, however, it should be mentioned that all samples months show statistically significant differences when compared with April, where the highest value of glycogen was observed and when the clams were in advanced gametogenesis (phase III) (Tukey HSD, $p\text{-value} < 0.05$, see attachment 1) (Figure 3.4). Concerning the total lipids, the highest value was observed in July (77.56±22.60 µg mg⁻¹ AFDW), coinciding with the female gonadal development phases of ripeness and spawning period. The lowest value was observed in October (34.60±5.32 µg mg⁻¹ AFDW), when the majority of the clams were in period of sexual rest and some females partially spawned and spent (Figure 3.4), although no correlations were found between total lipids and GI. Actually, no correlations were found between total lipids and other parameter analyzed, except a positive correlation between total lipids and total energy ($r_{\text{Pearson}} = 0.697$, $p\text{-value} < 0.05$) (Table 3.3). Once more, statistically significant differences (Kruskal-Wallis, $p\text{-value} < 0.05$) between sample months were found, although not seem to exist seasonal variations. Indeed total lipid values do not present pronounced fluctuations such as protein and glycogen values. Even so, July showed statistically significant differences when compared with all other months. Total energy reached the higher value in July (10.85 kJ mg⁻¹ AFDW) and the lowest in March (7.80 kJ mg⁻¹ AFDW). Only positive correlations between total energy and proteins and total energy and total lipids were found as already mentioned. Statistically significant differences (ANOVA, $p\text{-value} < 0.05$) between sample months were found, but these do not coincide with seasonal variations.

Table 3.2 – Mean values (\pm SD) of proteins, glycogen, total lipids ($\mu\text{g mg}^{-1}$ AFDW) and total energy (kJ mg^{-1} AFDW) of *Ruditapes decussatus* during the sampling period.

| Month | Proteins | Glycogen | Total Lipids | Total Energy |
|----------|-------------------------------|-------------------------------|-------------------------------|-----------------------------|
| | ($\mu\text{g mg}^{-1}$ AFDW) | ($\mu\text{g mg}^{-1}$ AFDW) | ($\mu\text{g mg}^{-1}$ AFDW) | (kJ mg^{-1} AFDW) |
| October | 345.74 \pm 35.74 | 92.03 \pm 32.94 | 34.60 \pm 5.32 | 8.91 |
| November | 258.70 \pm 40.01 | 122.98 \pm 29.77 | 42.89 \pm 10.66 | 8.16 |
| December | 362.24 \pm 30.81 | 98.06 \pm 31.54 | 51.25 \pm 9.69 | 9.86 |
| January | 289.05 \pm 33.67 | 132.85 \pm 42.52 | 47.06 \pm 7.96 | 9.01 |
| February | 323.26 \pm 75.86 | 93.37 \pm 31.32 | 44.76 \pm 7.48 | 8.87 |
| March | 247.53 \pm 27.02 | 108.15 \pm 22.80 | 45.87 \pm 6.85 | 7.80 |
| April | 237.02 \pm 28.38 | 169.05 \pm 15.62 | 58.06 \pm 10.86 | 9.07 |
| May | 247.08 \pm 31.16 | 125.48 \pm 30.46 | 52.32 \pm 15.32 | 8.31 |
| June | 328.14 \pm 29.65 | 115.55 \pm 35.33 | 49.08 \pm 16.70 | 9.48 |
| July | 370.77 \pm 29.15 | 95.94 \pm 18.59 | 77.56 \pm 22.60 | 10.85 |

Table 3.3 – Results of Pearson correlation between studied parameters (r, correlation coefficient; *p-value*; n.c., no correlation was found).

| | SST | Chlorophyll a | POM | GI | CI | Proteins | Glycogen | Total Lipids | Total Energy |
|---------------|-----|---------------|------|------|--|---|---|--------------|--|
| SST | | n.c. | n.c. | n.c. | n.c. | n.c. | n.c. | n.c. | n.c. |
| Chlorophyll a | | | n.c. | n.c. | $r = 0.682$ $p\text{-value} < 0.05$ | n.c. | $r = 0.755$ $p\text{-value} < 0.05$ | n.c. | n.c. |
| POM | | | | n.c. | n.c. | n.c. | n.c. | n.c. | n.c. |
| GI | | | | | $r = 0.752$ $p\text{-value} < 0.05$ | n.c. | n.c. | n.c. | n.c. |
| CI | | | | | | $r = -0.645$ $p\text{-value} < 0.05$ | $r = 0.694$ $p\text{-value} < 0.05$ | n.c. | n.c. |
| Proteins | | | | | | | $r = -0.734$ $p\text{-value} < 0.05$ | n.c. | $r = 0.786$ $p\text{-value} < 0.05$ |
| Glycogen | | | | | | | | n.c. | n.c. |
| Total Lipids | | | | | | | | | $r = 0.697$ $p\text{-value} < 0.05$ |

3.5. Principal component analysis (PCA)

The results obtained from PCA were in accordance with correlations identified by Pearson's coefficient. Moreover this analysis showed some additional relationships which complement the pattern already observed. The PCA results led us to two principal components (PC1 and PC2) that together accounted for 70.4 % of the overall variability of the data (Figure 3.7). The PCA biplot illustrates that POM and chlorophyll *a* are positively correlated, as well as glycogen content, CI, GI and SST between themselves. Positive correlations are also found between chlorophyll *a* and glycogen content, chlorophyll *a* and CI, total energy and proteins content and total energy and total lipids content (angle formed between the vectors is less than 90°). In contrast, proteins content are negatively correlated with CI and with glycogen content (angle formed between the vectors is higher than 90°). Null correlations between chlorophyll *a* and GI and between glycogen content and total lipids content were observed (angle formed between the vectors is close to 90°). A seasonal variation of parameters is also possible to observe. In general, the months of autumn and winter show lower values in the parameters analyzed than the spring and summer months, which showed higher values, highlighting the exception of November regarding POM, due to the climatic agitation that raised sediment from the bottom which influenced the values.

4. Discussion

The reproductive cycle of bivalves is influenced by environmental conditions, particularly by the availability and quality of food and temperature (Chávez-Villalba *et al.*, 2003; Matias *et al.*, 2013). Temperature assumes a more significant role because is closely associated with geographic location, affecting indirectly the availability of food and thus many studies reported the importance of geographical locations in defining and controlling gametogenesis and spawning (Holland & Chew, 1974; Beninger & Lucas, 1984; Rodríguez-Moscoso *et al.*, 1992; Xie & Burnell, 1994; Laruelle *et al.*, 1994; Gribben *et al.*, 2004; Meneghetti *et al.*, 2004; Matias *et al.*, 2013). In Lagoa de Óbidos, sea surface temperature, chlorophyll *a* and particulate organic matter showed the expected seasonal variation, typical from temperate climates. Temperature decreased from the first month of sampling until reaching the lowest values in the winter months, starting increase in the spring months, reached the highest values in the summer. Chlorophyll *a* and POM values, that illustrate the availability of food, showed a phytoplanktonic bloom in the spring, namely in April. Clearly, these environmental parameters influence gametogenesis of *R. decussatus*, despite no correlations were found between these parameters and gonadal index. The sequence of gametogenic stages showed that the reproductive cycle of *R. decussatus* follows a seasonal cycle, which is in agreement with several authors for bivalve species (Xie & Burnell, 1994; Joaquim *et al.*, 2008a). *R. decussatus* population in Lagoa de Óbidos presents a ripe stage in the spring followed by spawning that began in end of spring/early summer that possibly extended until early autumn, since in October, female individuals were found in phase V and VI of gonadal development. This accords with the reproductive cycle of other population of this species already described in Galicia (Spain) (Ojea *et al.*, 2004) and in two populations of Portugal localized in Ria de Aveiro e Ria Formosa Lagoons in a study performed by Matias *et al.* (2013). Even though other authors have shown the occurrence of two major periods of spawning, in spring and in summer or early autumn in different populations of this species (Shafee & Daoudi, 1991; Chryssanthakopoulou & Kaspiris, 2005), including populations of Ria Formosa Lagoon (Vilela, 1950). These differences between studies are explained by influence of geographical location and therefore by the environmental factors (precisely, by temperature and availability of food) (da Costa *et al.*, 2012; Matias *et al.*, 2013). Also the onset of the gametogenic cycle seems to be associate with the increase of SST in mid-winter (for females) and in late winter (for males), these results were similar with the previous ones by Shaffe & Daoudi (1991) and Chryssanthakopoulou & Kaspiris (2005), although in this study were not verified more than one onset of gametogenesis.

Chlorophyll *a* and POM don't seem to be associate with the onset of the gametogenic cycle, but it should be noted that in the months prior to the onset of gametogenesis chlorophyll *a* and POM values were higher, this leads to believe that the clams take advantage of the food availability during this period to use the accumulated reserves later when the gametogenic cycle begins. In relation to the period of reproductive rest, according to Matias *et al.* (2013), the populations of Ria Formosa and the Ria de Aveiro Lagoons have a long period of sexual rest that was extended by a period of approximately six months, during autumn and winter, in Lagoa de Óbidos population, this period seems to be shorter, possibly due to the higher availability of food observed in the months of autumn and early winter, that consequently conduce to start early the gametogenesis. Synchronism in gonadal development between males and females is essential to the reproductive success of the species since sperm and oocytes are expelled into the water column simultaneously during the spawning, increasing the probability of fertilization (Joaquim *et al.*, 2011; Matias *et al.*, 2013). In this study, although the males seem slightly delayed compared to females, particularly in winter months, both sexes showed synchronism in gonadal development in the rest of the study period such as reported for this species by Ojea *et al.* (2004) and Matias *et al.* (2013).

Condition index is generally considered to reflect reproductive activity. In this study, this was also observed, CI increased during gametogenesis and decreased during spawning, being actually this parameter (positively) correlated with the GI, what has already been detected in several species of bivalves from the Portuguese coast (Gaspar & Monteiro, 1998; Gaspar & Monteiro, 1999; Gaspar *et al.*, 1999; Moura *et al.*, 2008; Joaquim *et al.*, 2011). A significant positive correlation between CI and chlorophyll *a* was found too, which reinforce the fact that food availability contributes to the higher physiological condition of clams (the higher value of CI was observed in April of 2015 when was observed the higher values of chlorophyll *a* also), which reflect the reproductive activity. This parameter also exhibited statistically significant differences (ANOVA, *p-value* < 0.05) between sample months, showing seasonal variations, during the winter when the clams are in sexual rest and the CI values are low and during the spring when the clams are in gametogenesis and the CI values are high, decreasing after that, in the summer, when spawning occurs, reinforcing once again that CI reflect reproductive activity. Furthermore, according with previous studies (Delgado & Pérez-Camacho, 2005; Joaquim *et al.*, 2011), CI is highly influenced by the energy storage and exploitation strategy of bivalve species. In Lagoa de Óbidos population, although some summer and autumn months of 2015 are not included in this study, in the year of 2014 clams seem recover the

reserves after spawning, when the SST and chlorophyll *a* values remain high (months of October and November), being, possibly, these reserves used to maintain their physiological state during winter and started the gametogenesis early as mentioned before. A similar strategy was reported for Ria de Aveiro Lagoon population, as regards to maintain physiological state during winter, by Matias *et al.* (2013), not occurring the same for Ria Formosa Lagoon, which leads to severe mortalities in this population after the reproductive effort.

In bivalves, exist a close relationship between reproductive cycle and energy storage and utilization cycles (Barber & Blake, 1981; Fernández Castro & Vido de Mattio, 1987; Massapina *et al.*, 1999; Pérez-Camacho *et al.*, 2003; Ojea *et al.*, 2004; Joaquim *et al.*, 2011) that is controlled by temperature and food availability, that regulate mainly the timing and rate of energy storage (Joaquim *et al.*, 2011), being the effect of these variables complex and dependent from acquisition and expenditure of energy (Pérez-Camacho *et al.*, 2003). These energy storage and utilization cycles translate into a seasonal pattern of biochemical composition (in the form of proteins, glycogen and lipids) that can vary according to species and geographical location of the populations (Albentosa *et al.*, 2007; Matias *et al.*, 2009). Generally, occurs an accumulation of energy prior to gametogenesis, during the periods where food is abundant, posteriorly, this energy is used for the gametogenic synthesis, when metabolic demand is high (Mathieu & Lubet, 1993) and later released during the spawning process (Albentosa *et al.*, 2007). Proteins are used as an energy in situation of nutritional stress and energy imbalance or during gonadal maturation (Gabbott & Bayne, 1973; Liu *et al.*, 2008). Additionally, 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; Joaquim *et al.*, 2011). In this study, a negative correlation found between proteins and CI, confirms the fact that this reserves are used in situations of physiological stress, such as nutritional stress and energy imbalance, suggesting the negative correlation observed between proteins and glycogen that *R. decussatus* canalize proteins as a source of energy for maintenance when glycogen reserves are low. The positive correlation found between the proteins and the total energy were observed due to the fact that proteins were the predominant dry tissue constituent of the clams. The same result was detected for the populations of this species from Ria de Aveiro and Ria Formosa Lagoons (Matias *et al.*, 2013), although in these populations the relative amounts of proteins reached higher values (Ria de Aveiro: $531.7 \pm 80.0 \mu\text{g mg}^{-1}$ AFDW; Ria Formosa: $520.8 \pm 123.5 \mu\text{g mg}^{-1}$ AFDW) than in Lagoa de Óbidos population ($370.77 \pm 29.15 \mu\text{g mg}^{-1}$ AFDW),

notwithstanding that the lowest value of this content was higher in Lagoa de Óbidos population ($237.02 \pm 28.38 \mu\text{g mg}^{-1}$ AFDW) than in the other two populations (Ria de Aveiro: $128.3 \pm 32.6 \mu\text{g mg}^{-1}$ AFDW; Ria Formosa: $142.2 \pm 23.6 \mu\text{g mg}^{-1}$ AFDW). When compared these results with a study performed by Ojea *et al.* (2004), in terms of proportions, the *R. decussatus* Lagoa de Óbidos population also showed a lower relative amount of proteins. Glycogen is considered the main reserve in adult bivalves (Joaquim *et al.*, 2008a; Joaquim *et al.*, 2011; Matias *et al.*, 2013) that can be used simultaneously an energy source for growth and be stored in specific cells as an energy reserve during the vitellogenic process (Marin *et al.*, 2003). In the present study, the relative amount of glycogen (92.03 ± 32.94 to $169.05 \pm 15.62 \mu\text{g mg}^{-1}$ AFDW) was significantly higher than to those previously described by Matias *et al.* (2013) (Ria de Aveiro: 9.1 ± 5.8 to $53.7 \pm 22.9 \mu\text{g mg}^{-1}$ AFDW; Ria Formosa: 7.2 ± 2.4 to $45.0 \pm 9.3 \mu\text{g mg}^{-1}$ AFDW), being the results of glycogen content of *R. decussatus* Lagoa de Óbidos population, more similar with those described by Ojea *et al.* (2004), in terms of proportions, in a population from Galicia, Spain. During the first half of the study period (October to February), glycogen values trended to increase and subsequently decrease (in the following month), verifying, in the second half of the study, the highest increase of the values from February until April, followed by the highest decrease of the values until July. This evolution of glycogen content suggests that during the months of sexual rest the clams intersperse between proteins and glycogen as sources of energy maintenance, changing this pattern after the onset of the gametogenic cycle with a high increase in glycogen content, when the temperature and chlorophyll *a* (having been actually found a positive correlation between glycogen and chlorophyll *a*) starts to increase too, that leads (after April, when individuals are in phase III of the gametogenic development) to a striking consumption of the glycogen coincident with a rapid gonadal development and spawning process. Thus, we can consider that population of *R. decussatus* from Lagoa de Óbidos is not a conservative or an opportunistic species, exhibiting an intermediate strategy, since, after spawning, this population stored glycogen reserves during autumn and winter, which is typical from a conservative species, using after that both stored and recently assimilated glycogen content for gametogenesis. Ojea *et al.* (2004) have considered this species as a conservative species, however Matias *et al.* (2013) conclude that *R. decussatus* from Ria Formosa Lagoon exhibited an intermediate strategy, being *R. decussatus* Lagoa de Óbidos population, similarly to that population. In both populations (Ria Formosa Lagoon and Lagoa de Óbidos) it was observed that glycogen content was positively correlated with CI. Nevertheless, Lagoa de Óbidos population seems recover the reserves after the spawning, like a strategy reported for Ria de Aveiro Lagoon population, in the same study.

Lipids are formed due to the conversion of glycogen to lipids, biosynthesized during the formation of gametes (Gabbott, 1975), being these the main reserves of oocytes. The relative amount of total lipids (34.60 ± 5.32 to $77.56 \pm 22.60 \mu\text{g mg}^{-1}$ AFDW) observed in the study population was similar than to those previously described by Matias *et al.* (2013) (Ria de Aveiro: 35.0 ± 9.8 to $118.1 \pm 20.5 \mu\text{g mg}^{-1}$ AFDW; Ria Formosa: 27.2 ± 7.3 to $112.1 \pm 15.1 \mu\text{g mg}^{-1}$ AFDW), and by Ojea *et al.* (2004), in terms of proportions. Although, several authors have reported a negative relationship between glycogen and total lipids contents (Beninger & Lucas, 1984; Ojea *et al.*, 2004; Mouneyrac *et al.*, 2008), in this study only a positive correlation between total lipids and total energy was found. However, generally speaking, total lipids values were higher in the months after individuals have reached the phase III (advanced gametogenesis) in April, while glycogen content decrease, suggesting that glycogen is canalized to gametes formation, especially in females. The statistically significant differences (Kruskal-Wallis, $p\text{-value} < 0.05$) found in the month of July 2015 when compared with all other months (exist a clear peak in this month), lead to believe 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.*, 2008a, 2011; Matias *et al.*, 2013), being this fact reinforced by the positive correlation found between total lipids content and total energy. The erratic variation followed by total lipids content after the onset of gametogenesis could be related with the successive and simultaneous gamete production and release of them, typical of a partial spawning species as reported by Matias *et al.*, (2013).

The characterization of the reproductive cycle of *Ruditapes decussatus* population from Lagoa de Óbidos provides a useful knowledge about the biology of this species. *R. decussatus* Lagoa de Óbidos population show an intermediate strategy of reproduction (between opportunistic and conservative), adopting an energy storage medium that allows a rapid recovery after the reproductive effort, most likely due to the wide availability of food in the Lagoa de Óbidos. The obtained results can help improve a sustainable management of this wild stock and is important for future aquaculture development of this species, mainly in terms of optimal reproductive time for artificial spawning induction in aquaculture, since this population can provide a suitable broodstock for intensive hatchery production of juveniles, which eliminates the problem of seed demand, allowing also, restocking actions based on aquaculture production.

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Attachments

➤ Attachment 1

Table 1 – Multiple comparisons of condition index using Tukey HSD test.

| Multiple Comparisons | | | | | | |
|-------------------------------------|-----------|-----------------------|------------|--------|-------------------------|-------------|
| Dependent Variable: Condition Index | | | | | | |
| Tukey HSD | | | | | | |
| (I) Month | (J) Month | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | Lower Bound | Upper Bound |
| January | February | 1,5655819 | 0,6771852 | 0,3920 | -0,631496 | 3,76266 |
| | March | -3,4063939* | 0,6771852 | 0,0000 | -5,603472 | -1,209316 |
| | April | -7,2852878* | 0,6771852 | 0,0000 | -9,482366 | -5,08821 |
| | May | -5,3135740* | 0,6771852 | 0,0000 | -7,510652 | -3,116496 |
| | June | -0,9559472 | 0,6771852 | 0,9200 | -3,153025 | 1,241131 |
| | July | -0,3836381 | 0,6771852 | 1,0000 | -2,580716 | 1,81344 |
| | October | -1,0801699 | 0,6771852 | 0,8470 | -3,277248 | 1,116908 |
| | November | 0,3327458 | 0,6771852 | 1,0000 | -1,864332 | 2,529824 |
| | December | -0,486093 | 0,6771852 | 0,9990 | -2,683171 | 1,710985 |
| February | January | -1,5655819 | 0,6771852 | 0,3920 | -3,76266 | 0,631496 |
| | March | -4,9719758* | 0,6771852 | 0,0000 | -7,169054 | -2,774898 |
| | April | -8,8508696* | 0,6771852 | 0,0000 | -11,047948 | -6,653792 |
| | May | -6,8791559* | 0,6771852 | 0,0000 | -9,076234 | -4,682078 |
| | June | -2,5215291* | 0,6771852 | 0,0120 | -4,718607 | -0,324451 |
| | July | -1,94922 | 0,6771852 | 0,1270 | -4,146298 | 0,247858 |
| | October | -2,6457518* | 0,6771852 | 0,0070 | -4,84283 | -0,448674 |
| | November | -1,232836 | 0,6771852 | 0,7210 | -3,429914 | 0,964242 |
| | December | -2,0516748 | 0,6771852 | 0,0880 | -4,248753 | 0,145403 |
| March | January | 3,4063939* | 0,6771852 | 0,0000 | 1,209316 | 5,603472 |
| | February | 4,9719758* | 0,6771852 | 0,0000 | 2,774898 | 7,169054 |
| | April | -3,8788939* | 0,6771852 | 0,0000 | -6,075972 | -1,681816 |
| | May | -1,9071801 | 0,6771852 | 0,1470 | -4,104258 | 0,289898 |
| | June | 2,4504467* | 0,6771852 | 0,0170 | 0,253369 | 4,647525 |
| | July | 3,0227558* | 0,6771852 | 0,0010 | 0,825678 | 5,219834 |
| | October | 2,3262240* | 0,6771852 | 0,0290 | 0,129146 | 4,523302 |
| | November | 3,7391397* | 0,6771852 | 0,0000 | 1,542062 | 5,936218 |
| | December | 2,9203009* | 0,6771852 | 0,0020 | 0,723223 | 5,117379 |
| April | January | 7,2852878* | 0,6771852 | 0,0000 | 5,08821 | 9,482366 |
| | February | 8,8508696* | 0,6771852 | 0,0000 | 6,653792 | 11,047948 |
| | March | 3,8788939* | 0,6771852 | 0,0000 | 1,681816 | 6,075972 |
| | May | 1,9717138 | 0,6771852 | 0,1180 | -0,225364 | 4,168792 |
| | June | 6,3293406* | 0,6771852 | 0,0000 | 4,132263 | 8,526419 |
| | July | 6,9016497* | 0,6771852 | 0,0000 | 4,704572 | 9,098728 |
| | October | 6,2051179* | 0,6771852 | 0,0000 | 4,00804 | 8,402196 |

| | | | | | | |
|----------|----------|-------------|-----------|--------|-----------|-----------|
| | November | 7,6180336* | 0,6771852 | 0,0000 | 5,420956 | 9,815112 |
| | December | 6,7991948* | 0,6771852 | 0,0000 | 4,602117 | 8,996273 |
| May | January | 5,3135740* | 0,6771852 | 0,0000 | 3,116496 | 7,510652 |
| | February | 6,8791559* | 0,6771852 | 0,0000 | 4,682078 | 9,076234 |
| | March | 1,9071801 | 0,6771852 | 0,1470 | -0,289898 | 4,104258 |
| | April | -1,9717138 | 0,6771852 | 0,1180 | -4,168792 | 0,225364 |
| | June | 4,3576268* | 0,6771852 | 0,0000 | 2,160549 | 6,554705 |
| | July | 4,9299359* | 0,6771852 | 0,0000 | 2,732858 | 7,127014 |
| | October | 4,2334041* | 0,6771852 | 0,0000 | 2,036326 | 6,430482 |
| | November | 5,6463199* | 0,6771852 | 0,0000 | 3,449242 | 7,843398 |
| | December | 4,8274811* | 0,6771852 | 0,0000 | 2,630403 | 7,024559 |
| June | January | 0,9559472 | 0,6771852 | 0,9200 | -1,241131 | 3,153025 |
| | February | 2,5215291* | 0,6771852 | 0,0120 | 0,324451 | 4,718607 |
| | March | -2,4504467* | 0,6771852 | 0,0170 | -4,647525 | -0,253369 |
| | April | -6,3293406* | 0,6771852 | 0,0000 | -8,526419 | -4,132263 |
| | May | -4,3576268* | 0,6771852 | 0,0000 | -6,554705 | -2,160549 |
| | July | 0,5723091 | 0,6771852 | 0,9980 | -1,624769 | 2,769387 |
| | October | -0,1242227 | 0,6771852 | 1,0000 | -2,321301 | 2,072855 |
| | November | 1,2886931 | 0,6771852 | 0,6670 | -0,908385 | 3,485771 |
| | December | 0,4698543 | 0,6771852 | 0,9990 | -1,727224 | 2,666932 |
| July | January | 0,3836381 | 0,6771852 | 1,0000 | -1,81344 | 2,580716 |
| | February | 1,94922 | 0,6771852 | 0,1270 | -0,247858 | 4,146298 |
| | March | -3,0227558* | 0,6771852 | 0,0010 | -5,219834 | -0,825678 |
| | April | -6,9016497* | 0,6771852 | 0,0000 | -9,098728 | -4,704572 |
| | May | -4,9299359* | 0,6771852 | 0,0000 | -7,127014 | -2,732858 |
| | June | -0,5723091 | 0,6771852 | 0,9980 | -2,769387 | 1,624769 |
| | October | -0,6965318 | 0,6771852 | 0,9900 | -2,89361 | 1,500546 |
| | November | 0,7163839 | 0,6771852 | 0,9870 | -1,480694 | 2,913462 |
| | December | -0,1024548 | 0,6771852 | 1,0000 | -2,299533 | 2,094623 |
| October | January | 1,0801699 | 0,6771852 | 0,8470 | -1,116908 | 3,277248 |
| | February | 2,6457518* | 0,6771852 | 0,0070 | 0,448674 | 4,84283 |
| | March | -2,3262240* | 0,6771852 | 0,0290 | -4,523302 | -0,129146 |
| | April | -6,2051179* | 0,6771852 | 0,0000 | -8,402196 | -4,00804 |
| | May | -4,2334041* | 0,6771852 | 0,0000 | -6,430482 | -2,036326 |
| | June | 0,1242227 | 0,6771852 | 1,0000 | -2,072855 | 2,321301 |
| | July | 0,6965318 | 0,6771852 | 0,9900 | -1,500546 | 2,89361 |
| | November | 1,4129157 | 0,6771852 | 0,5420 | -0,784162 | 3,609994 |
| | December | 0,5940769 | 0,6771852 | 0,9970 | -1,603001 | 2,791155 |
| November | January | -0,3327458 | 0,6771852 | 1,0000 | -2,529824 | 1,864332 |
| | February | 1,232836 | 0,6771852 | 0,7210 | -0,964242 | 3,429914 |
| | March | -3,7391397* | 0,6771852 | 0,0000 | -5,936218 | -1,542062 |
| | April | -7,6180336* | 0,6771852 | 0,0000 | -9,815112 | -5,420956 |
| | May | -5,6463199* | 0,6771852 | 0,0000 | -7,843398 | -3,449242 |
| | June | -1,2886931 | 0,6771852 | 0,6670 | -3,485771 | 0,908385 |

| | | | | | | |
|--|----------|-------------|-----------|--------|-----------|-----------|
| | July | -0,7163839 | 0,6771852 | 0,9870 | -2,913462 | 1,480694 |
| | October | -1,4129157 | 0,6771852 | 0,5420 | -3,609994 | 0,784162 |
| | December | -0,8188388 | 0,6771852 | 0,9690 | -3,015917 | 1,378239 |
| December | January | 0,486093 | 0,6771852 | 0,9990 | -1,710985 | 2,683171 |
| | February | 2,0516748 | 0,6771852 | 0,0880 | -0,145403 | 4,248753 |
| | March | -2,9203009* | 0,6771852 | 0,0020 | -5,117379 | -0,723223 |
| | April | -6,7991948* | 0,6771852 | 0,0000 | -8,996273 | -4,602117 |
| | May | -4,8274811* | 0,6771852 | 0,0000 | -7,024559 | -2,630403 |
| | June | -0,4698543 | 0,6771852 | 0,9990 | -2,666932 | 1,727224 |
| | July | 0,1024548 | 0,6771852 | 1,0000 | -2,094623 | 2,299533 |
| | October | -0,5940769 | 0,6771852 | 0,9970 | -2,791155 | 1,603001 |
| | November | 0,8188388 | 0,6771852 | 0,9690 | -1,378239 | 3,015917 |
| *The mean difference is significant at the 0.05 level. | | | | | | |

Table 2 – Multiple comparisons of protein and total energy using Tukey HSD test.

| Multiple Comparisons | | | | | | | |
|----------------------|----------|-----------------------|---------------|------------|-------------------------|--------------|-------------|
| Tukey HSD | | | | | | | |
| Dependent Variable | | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | | |
| | | | | | Lower Bound | Upper Bound | |
| Protein | January | February | -34,2054041 | 12,2121755 | 0,1436564 | -73,3053334 | 4,8945253 |
| | | March | 41,5244168* | 12,2121755 | 0,0275693 | 2,4244875 | 80,6243462 |
| | | April | 52,0350959* | 12,2121755 | 0,0012981 | 12,9351666 | 91,1350253 |
| | | May | 41,9743931* | 12,2121755 | 0,0245642 | 2,8744638 | 81,0743225 |
| | | June | -39,0855037 | 12,2121755 | 0,0501705 | -78,1854331 | 0,0144256 |
| | | July | -81,7147116* | 12,2121755 | 0,0000000 | -120,8146410 | -42,6147823 |
| | | October | -56,6907870* | 12,2121755 | 0,0002709 | -95,7907164 | -17,5908577 |
| | | November | 30,3547820 | 12,2121755 | 0,2830145 | -8,7451474 | 69,4547113 |
| | | December | -74,0085768* | 12,2121755 | 0,0000003 | -113,1085061 | -34,9086474 |
| | February | January | 34,2054041 | 12,2121755 | 0,1436564 | -4,8945253 | 73,3053334 |
| | | March | 75,7298209* | 12,2121755 | 0,0000002 | 36,6298915 | 114,8297503 |
| | | April | 86,2405000* | 12,2121755 | 0,0000000 | 47,1405707 | 125,3404294 |
| | | May | 76,1797972* | 12,2121755 | 0,0000001 | 37,0798678 | 115,2797266 |
| | | June | -4,8800997 | 12,2121755 | 0,9999956 | -43,9800290 | 34,2198297 |
| | | July | -47,5093076* | 12,2121755 | 0,0052839 | -86,6092369 | -8,4093782 |
| | | October | -22,4853829 | 12,2121755 | 0,7080882 | -61,5853123 | 16,6145464 |
| | | November | 64,5601860* | 12,2121755 | 0,0000149 | 25,4602567 | 103,6601154 |
| | | December | -39,8031727* | 12,2121755 | 0,0422685 | -78,9031021 | -0,7032434 |
| | March | January | -41,5244168* | 12,2121755 | 0,0275693 | -80,6243462 | -2,4244875 |
| | | February | -75,7298209* | 12,2121755 | 0,0000002 | -114,8297503 | -36,6298915 |
| | | April | 10,5106791 | 12,2121755 | 0,9973867 | -28,5892503 | 49,6106085 |
| | | May | 0,4499763 | 12,2121755 | 1,0000000 | -38,6499531 | 39,5499057 |
| | | June | -80,6099206* | 12,2121755 | 0,0000000 | -119,7098499 | -41,5099912 |
| | | July | -123,2391285* | 12,2121755 | 0,0000000 | -162,3390578 | -84,1391991 |
| | | October | -98,2152038* | 12,2121755 | 0,0000000 | -137,3151332 | -59,1152745 |
| | | November | -11,1696349 | 12,2121755 | 0,9958507 | -50,2695642 | 27,9302945 |
| | | December | -115,5329936* | 12,2121755 | 0,0000000 | -154,6329230 | -76,4330643 |
| | April | January | -52,0350959* | 12,2121755 | 0,0012981 | -91,1350253 | -12,9351666 |
| | | February | -86,2405000* | 12,2121755 | 0,0000000 | -125,3404294 | -47,1405707 |
| | | March | -10,5106791 | 12,2121755 | 0,9973867 | -49,6106085 | 28,5892503 |
| | | May | -10,0607028 | 12,2121755 | 0,9981370 | -49,1606322 | 29,0392265 |
| | | June | -91,1205997* | 12,2121755 | 0,0000000 | -130,2205291 | -52,0206703 |
| | | July | -133,7498076* | 12,2121755 | 0,0000000 | -172,8497369 | -94,6498782 |
| | | October | -108,7258830* | 12,2121755 | 0,0000000 | -147,8258123 | -69,6259536 |
| | | November | -21,6803140 | 12,2121755 | 0,7498447 | -60,7802433 | 17,4196154 |
| | | December | -126,0436727* | 12,2121755 | 0,0000000 | -165,1436021 | -86,9437434 |
| May | January | -41,9743931* | 12,2121755 | 0,0245642 | -81,0743225 | -2,8744638 | |

| | | | | | | | |
|----------|---------------|---------------|---------------|--------------|--------------|--------------|-------------|
| | | February | -76,1797972* | 12,2121755 | 0,0000001 | -115,2797266 | -37,0798678 |
| | | March | -0,4499763 | 12,2121755 | 1,0000000 | -39,5499057 | 38,6499531 |
| | | April | 10,0607028 | 12,2121755 | 0,9981370 | -29,0392265 | 49,1606322 |
| | | June | -81,0598969* | 12,2121755 | 0,0000000 | -120,1598262 | -41,9599675 |
| | | July | -123,6891048* | 12,2121755 | 0,0000000 | -162,7890341 | -84,5891754 |
| | | October | -98,6651801* | 12,2121755 | 0,0000000 | -137,7651095 | -59,5652508 |
| | | November | -11,6196112 | 12,2121755 | 0,9944275 | -50,7195405 | 27,4803182 |
| | | December | -115,9829699* | 12,2121755 | 0,0000000 | -155,0828993 | -76,8830405 |
| June | January | 39,0855037 | 12,2121755 | 0,0501705 | -0,0144256 | 78,1854331 | |
| | February | 4,8800997 | 12,2121755 | 0,9999956 | -34,2198297 | 43,9800290 | |
| | March | 80,6099206* | 12,2121755 | 0,0000000 | 41,5099912 | 119,7098499 | |
| | April | 91,1205997* | 12,2121755 | 0,0000000 | 52,0206703 | 130,2205291 | |
| | May | 81,0598969* | 12,2121755 | 0,0000000 | 41,9599675 | 120,1598262 | |
| | July | -42,6292079* | 12,2121755 | 0,0207113 | -81,7291372 | -3,5292785 | |
| | October | -17,6052833 | 12,2121755 | 0,9123960 | -56,7052126 | 21,4946461 | |
| | November | 69,4402857* | 12,2121755 | 0,0000021 | 30,3403564 | 108,5402151 | |
| December | -34,9230730 | 12,2121755 | 0,1246716 | -74,0230024 | 4,1768563 | | |
| July | January | 81,7147116* | 12,2121755 | 0,0000000 | 42,6147823 | 120,8146410 | |
| | February | 47,5093076* | 12,2121755 | 0,0052839 | 8,4093782 | 86,6092369 | |
| | March | 123,2391285* | 12,2121755 | 0,0000000 | 84,1391991 | 162,3390578 | |
| | April | 133,7498076* | 12,2121755 | 0,0000000 | 94,6498782 | 172,8497369 | |
| | May | 123,6891048* | 12,2121755 | 0,0000000 | 84,5891754 | 162,7890341 | |
| | June | 42,6292079* | 12,2121755 | 0,0207113 | 3,5292785 | 81,7291372 | |
| | October | 25,0239246 | 12,2121755 | 0,5656362 | -14,0760047 | 64,1238540 | |
| | November | 112,0694936* | 12,2121755 | 0,0000000 | 72,9695642 | 151,1694230 | |
| December | 7,7061349 | 12,2121755 | 0,9997821 | -31,3937945 | 46,8060642 | | |
| October | January | 56,6907870* | 12,2121755 | 0,0002709 | 17,5908577 | 95,7907164 | |
| | February | 22,4853829 | 12,2121755 | 0,7080882 | -16,6145464 | 61,5853123 | |
| | March | 98,2152038* | 12,2121755 | 0,0000000 | 59,1152745 | 137,3151332 | |
| | April | 108,7258830* | 12,2121755 | 0,0000000 | 69,6259536 | 147,8258123 | |
| | May | 98,6651801* | 12,2121755 | 0,0000000 | 59,5652508 | 137,7651095 | |
| | June | 17,6052833 | 12,2121755 | 0,9123960 | -21,4946461 | 56,7052126 | |
| | July | -25,0239246 | 12,2121755 | 0,5656362 | -64,1238540 | 14,0760047 | |
| | November | 87,0455690* | 12,2121755 | 0,0000000 | 47,9456396 | 126,1454983 | |
| December | -17,3177898 | 12,2121755 | 0,9202631 | -56,4177191 | 21,7821396 | | |
| November | January | -30,3547820 | 12,2121755 | 0,2830145 | -69,4547113 | 8,7451474 | |
| | February | -64,5601860* | 12,2121755 | 0,0000149 | -103,6601154 | -25,4602567 | |
| | March | 11,1696349 | 12,2121755 | 0,9958507 | -27,9302945 | 50,2695642 | |
| | April | 21,6803140 | 12,2121755 | 0,7498447 | -17,4196154 | 60,7802433 | |
| | May | 11,6196112 | 12,2121755 | 0,9944275 | -27,4803182 | 50,7195405 | |
| | June | -69,4402857* | 12,2121755 | 0,0000021 | -108,5402151 | -30,3403564 | |
| | July | -112,0694936* | 12,2121755 | 0,0000000 | -151,1694230 | -72,9695642 | |
| | October | -87,0455690* | 12,2121755 | 0,0000000 | -126,1454983 | -47,9456396 | |
| December | -104,3633587* | 12,2121755 | 0,0000000 | -143,4632881 | -65,2634294 | | |

| | | | | | | | |
|--------------|-------------|------------|--------------|------------|------------|-------------|-------------|
| | December | January | 74,0085768* | 12,2121755 | 0,0000003 | 34,9086474 | 113,1085061 |
| | | February | 39,8031727* | 12,2121755 | 0,0422685 | 0,7032434 | 78,9031021 |
| | | March | 115,5329936* | 12,2121755 | 0,0000000 | 76,4330643 | 154,6329230 |
| | | April | 126,0436727* | 12,2121755 | 0,0000000 | 86,9437434 | 165,1436021 |
| | | May | 115,9829699* | 12,2121755 | 0,0000000 | 76,8830405 | 155,0828993 |
| | | June | 34,9230730 | 12,2121755 | 0,1246716 | -4,1768563 | 74,0230024 |
| | | July | -7,7061349 | 12,2121755 | 0,9997821 | -46,8060642 | 31,3937945 |
| | | October | 17,3177898 | 12,2121755 | 0,9202631 | -21,7821396 | 56,4177191 |
| | | November | 104,3633587* | 12,2121755 | 0,0000000 | 65,2634294 | 143,4632881 |
| Total Energy | January | February | 0,1426579 | 0,3085458 | 0,9999843 | -0,8452184 | 1,1305341 |
| | | March | 1,2073242* | 0,3085458 | 0,0048675 | 0,2194480 | 2,1952005 |
| | | April | -0,0542154 | 0,3085458 | 1,0000000 | -1,0420916 | 0,9336609 |
| | | May | 0,7046555 | 0,3085458 | 0,4044348 | -0,2832207 | 1,6925318 |
| | | June | -0,4686053 | 0,3085458 | 0,8831827 | -1,4564815 | 0,5192710 |
| | | July | -1,8342907* | 0,3085458 | 0,0000006 | -2,8221670 | -0,8464145 |
| | | October | 0,0984122 | 0,3085458 | 0,9999994 | -0,8894640 | 1,0862885 |
| | | November | 0,8504920 | 0,3085458 | 0,1594245 | -0,1373842 | 1,8383683 |
| | February | December | -0,8246345 | 0,3085458 | 0,1925533 | -1,8125107 | 0,1632418 |
| | | January | -0,1426579 | 0,3085458 | 0,9999843 | -1,1305341 | 0,8452184 |
| | | March | 1,0646664* | 0,3085458 | 0,0235383 | 0,0767901 | 2,0525426 |
| | | April | -0,1968732 | 0,3085458 | 0,9997612 | -1,1847495 | 0,7910030 |
| | | May | 0,5619977 | 0,3085458 | 0,7208681 | -0,4258786 | 1,5498739 |
| | | June | -0,6112631 | 0,3085458 | 0,6133085 | -1,5991394 | 0,3766131 |
| | | July | -1,9769486* | 0,3085458 | 0,0000001 | -2,9648248 | -0,9890723 |
| | | October | -0,0442456 | 0,3085458 | 1,0000000 | -1,0321219 | 0,9436306 |
| | March | November | 0,7078342 | 0,3085458 | 0,3977341 | -0,2800421 | 1,6957104 |
| | | December | -0,9672923 | 0,3085458 | 0,0604422 | -1,9551686 | 0,0205839 |
| | | January | -1,2073242* | 0,3085458 | 0,0048675 | -2,1952005 | -0,2194480 |
| | | February | -1,0646664* | 0,3085458 | 0,0235383 | -2,0525426 | -0,0767901 |
| | | April | -1,2615396* | 0,3085458 | 0,0025315 | -2,2494158 | -0,2736633 |
| | | May | -0,5026687 | 0,3085458 | 0,8322661 | -1,4905449 | 0,4852076 |
| | | June | -1,6759295* | 0,3085458 | 0,0000075 | -2,6638057 | -0,6880532 |
| | | July | -3,0416149* | 0,3085458 | 0,0000000 | -4,0294912 | -2,0537387 |
| | April | October | -1,1089120* | 0,3085458 | 0,0147807 | -2,0967882 | -0,1210357 |
| | | November | -0,3568322 | 0,3085458 | 0,9777862 | -1,3447084 | 0,6310441 |
| | | December | -2,0319587* | 0,3085458 | 0,0000000 | -3,0198350 | -1,0440824 |
| | | January | 0,0542154 | 0,3085458 | 1,0000000 | -0,9336609 | 1,0420916 |
| February | | 0,1968732 | 0,3085458 | 0,9997612 | -0,7910030 | 1,1847495 | |
| March | | 1,2615396* | 0,3085458 | 0,0025315 | 0,2736633 | 2,2494158 | |
| May | | 0,7588709 | 0,3085458 | 0,2974256 | -0,2290053 | 1,7467472 | |
| June | | -0,4143899 | 0,3085458 | 0,9421577 | -1,4022661 | 0,5734864 | |
| July | -1,7800753* | 0,3085458 | 0,0000014 | -2,7679516 | -0,7921991 | | |
| October | 0,1526276 | 0,3085458 | 0,9999720 | -0,8352486 | 1,1405039 | | |
| November | 0,9047074 | 0,3085458 | 0,1040159 | -0,0831688 | 1,8925837 | | |

| | | | | | | |
|----------|----------|-------------|-----------|-----------|------------|------------|
| | December | -0,7704191 | 0,3085458 | 0,2768975 | -1,7582954 | 0,2174571 |
| May | January | -0,7046555 | 0,3085458 | 0,4044348 | -1,6925318 | 0,2832207 |
| | February | -0,5619977 | 0,3085458 | 0,7208681 | -1,5498739 | 0,4258786 |
| | March | 0,5026687 | 0,3085458 | 0,8322661 | -0,4852076 | 1,4905449 |
| | April | -0,7588709 | 0,3085458 | 0,2974256 | -1,7467472 | 0,2290053 |
| | June | -1,1732608* | 0,3085458 | 0,0072315 | -2,1611371 | -0,1853845 |
| | July | -2,5389463* | 0,3085458 | 0,0000000 | -3,5268225 | -1,5510700 |
| | October | -0,6062433 | 0,3085458 | 0,6246246 | -1,5941196 | 0,3816330 |
| | November | 0,1458365 | 0,3085458 | 0,9999810 | -0,8420398 | 1,1337127 |
| | December | -1,5292900* | 0,3085458 | 0,0000683 | -2,5171663 | -0,5414138 |
| June | January | 0,4686053 | 0,3085458 | 0,8831827 | -0,5192710 | 1,4564815 |
| | February | 0,6112631 | 0,3085458 | 0,6133085 | -0,3766131 | 1,5991394 |
| | March | 1,6759295* | 0,3085458 | 0,0000075 | 0,6880532 | 2,6638057 |
| | April | 0,4143899 | 0,3085458 | 0,9421577 | -0,5734864 | 1,4022661 |
| | May | 1,1732608* | 0,3085458 | 0,0072315 | 0,1853845 | 2,1611371 |
| | July | -1,3656855* | 0,3085458 | 0,0006677 | -2,3535617 | -0,3778092 |
| | October | 0,5670175 | 0,3085458 | 0,7103742 | -0,4208588 | 1,5548938 |
| | November | 1,3190973* | 0,3085458 | 0,0012267 | 0,3312210 | 2,3069735 |
| | December | -0,3560292 | 0,3085458 | 0,9781206 | -1,3439055 | 0,6318470 |
| July | January | 1,8342907* | 0,3085458 | 0,0000006 | 0,8464145 | 2,8221670 |
| | February | 1,9769486* | 0,3085458 | 0,0000001 | 0,9890723 | 2,9648248 |
| | March | 3,0416149* | 0,3085458 | 0,0000000 | 2,0537387 | 4,0294912 |
| | April | 1,7800753* | 0,3085458 | 0,0000014 | 0,7921991 | 2,7679516 |
| | May | 2,5389463* | 0,3085458 | 0,0000000 | 1,5510700 | 3,5268225 |
| | June | 1,3656855* | 0,3085458 | 0,0006677 | 0,3778092 | 2,3535617 |
| | October | 1,9327030* | 0,3085458 | 0,0000001 | 0,9448267 | 2,9205792 |
| | November | 2,6847828* | 0,3085458 | 0,0000000 | 1,6969065 | 3,6726590 |
| | December | 1,0096562* | 0,3085458 | 0,0406732 | 0,0217800 | 1,9975325 |
| October | January | -0,0984122 | 0,3085458 | 0,9999994 | -1,0862885 | 0,8894640 |
| | February | 0,0442456 | 0,3085458 | 1,0000000 | -0,9436306 | 1,0321219 |
| | March | 1,1089120* | 0,3085458 | 0,0147807 | 0,1210357 | 2,0967882 |
| | April | -0,1526276 | 0,3085458 | 0,9999720 | -1,1405039 | 0,8352486 |
| | May | 0,6062433 | 0,3085458 | 0,6246246 | -0,3816330 | 1,5941196 |
| | June | -0,5670175 | 0,3085458 | 0,7103742 | -1,5548938 | 0,4208588 |
| | July | -1,9327030* | 0,3085458 | 0,0000001 | -2,9205792 | -0,9448267 |
| | November | 0,7520798 | 0,3085458 | 0,3098967 | -0,2357965 | 1,7399560 |
| | December | -0,9230467 | 0,3085458 | 0,0891987 | -1,9109230 | 0,0648295 |
| November | January | -0,8504920 | 0,3085458 | 0,1594245 | -1,8383683 | 0,1373842 |
| | February | -0,7078342 | 0,3085458 | 0,3977341 | -1,6957104 | 0,2800421 |
| | March | 0,3568322 | 0,3085458 | 0,9777862 | -0,6310441 | 1,3447084 |
| | April | -0,9047074 | 0,3085458 | 0,1040159 | -1,8925837 | 0,0831688 |
| | May | -0,1458365 | 0,3085458 | 0,9999810 | -1,1337127 | 0,8420398 |
| | June | -1,3190973* | 0,3085458 | 0,0012267 | -2,3069735 | -0,3312210 |
| | July | -2,6847828* | 0,3085458 | 0,0000000 | -3,6726590 | -1,6969065 |

| | | | | | | | |
|--|----------|----------|-------------|-----------|-----------|------------|------------|
| | | October | -0,7520798 | 0,3085458 | 0,3098967 | -1,7399560 | 0,2357965 |
| | | December | -1,6751265* | 0,3085458 | 0,0000075 | -2,6630028 | -0,6872503 |
| | December | January | 0,8246345 | 0,3085458 | 0,1925533 | -0,1632418 | 1,8125107 |
| | | February | 0,9672923 | 0,3085458 | 0,0604422 | -0,0205839 | 1,9551686 |
| | | March | 2,0319587* | 0,3085458 | 0,0000000 | 1,0440824 | 3,0198350 |
| | | April | 0,7704191 | 0,3085458 | 0,2768975 | -0,2174571 | 1,7582954 |
| | | May | 1,5292900* | 0,3085458 | 0,0000683 | 0,5414138 | 2,5171663 |
| | | June | 0,3560292 | 0,3085458 | 0,9781206 | -0,6318470 | 1,3439055 |
| | | July | -1,0096562* | 0,3085458 | 0,0406732 | -1,9975325 | -0,0217800 |
| | | October | 0,9230467 | 0,3085458 | 0,0891987 | -0,0648295 | 1,9109230 |
| | | November | 1,6751265* | 0,3085458 | 0,0000075 | 0,6872503 | 2,6630028 |
| *The mean difference is significant at the 0.05 level. | | | | | | | |

Table 3 – Multiple comparisons of glycogen and total lipids using Tukey HSD test.

| Multiple Comparisons | | | | | | | |
|----------------------|----------|-------------|-----------------------|------------|-------------|-------------------------|-------------|
| Tukey HSD | | | | | | | |
| Dependent Variable | | | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
| | | | | | | Lower Bound | Upper Bound |
| Glycogen | January | February | 39,4796407* | 9,5087960 | 0,0019870 | 9,0351675 | 69,9241139 |
| | | March | 24,6955985 | 9,5087960 | 0,2263292 | -5,7488748 | 55,1400717 |
| | | April | -36,2079903* | 9,5087960 | 0,0070975 | -66,6524635 | -5,7635171 |
| | | May | 7,3701381 | 9,5087960 | 0,9988460 | -23,0743351 | 37,8146114 |
| | | June | 17,2958641 | 9,5087960 | 0,7224730 | -13,1486092 | 47,7403373 |
| | | July | 36,9085397* | 9,5087960 | 0,0054547 | 6,4640665 | 67,3530129 |
| | | October | 40,8114272* | 9,5087960 | 0,0011477 | 10,3669540 | 71,2559004 |
| | | November | 9,8634753 | 9,5087960 | 0,9895549 | -20,5809979 | 40,3079486 |
| | December | 37,1205078* | 9,5087960 | 0,0050319 | 6,6760346 | 67,5649810 | |
| | February | January | -39,4796407* | 9,5087960 | 0,0019870 | -69,9241139 | -9,0351675 |
| | | March | -14,7840422 | 9,5087960 | 0,8677321 | -45,2285154 | 15,6604310 |
| | | April | -75,6876310* | 9,5087960 | 0,0000000 | -106,1321042 | -45,2431578 |
| | | May | -32,1095026* | 9,5087960 | 0,0296449 | -62,5539758 | -1,6650293 |
| | | June | -22,1837766 | 9,5087960 | 0,3728790 | -52,6282498 | 8,2606966 |
| | | July | -2,5711010 | 9,5087960 | 0,9999999 | -33,0155742 | 27,8733723 |
| | | October | 1,3317865 | 9,5087960 | 1,0000000 | -29,1126867 | 31,7762598 |
| | | November | -29,6161653 | 9,5087960 | 0,0639818 | -60,0606386 | 0,8283079 |
| | December | -2,3591329 | 9,5087960 | 0,9999999 | -32,8036061 | 28,0853403 | |
| | March | January | -24,6955985 | 9,5087960 | 0,2263292 | -55,1400717 | 5,7488748 |
| | | February | 14,7840422 | 9,5087960 | 0,8677321 | -15,6604310 | 45,2285154 |
| | | April | -60,9035888* | 9,5087960 | 0,0000001 | -91,3480620 | -30,4591155 |
| | | May | -17,3254603 | 9,5087960 | 0,7204800 | -47,7699336 | 13,1190129 |
| | | June | -7,3997344 | 9,5087960 | 0,9988088 | -37,8442076 | 23,0447388 |
| | | July | 12,2129413 | 9,5087960 | 0,9560628 | -18,2315320 | 42,6574145 |
| | | October | 16,1158288 | 9,5087960 | 0,7971657 | -14,3286445 | 46,5603020 |
| | | November | -14,8321231 | 9,5087960 | 0,8654718 | -45,2765963 | 15,6123501 |
| | December | 12,4249093 | 9,5087960 | 0,9510958 | -18,0195639 | 42,8693825 | |
| | April | January | 36,2079903* | 9,5087960 | 0,0070975 | 5,7635171 | 66,6524635 |
| | | February | 75,6876310* | 9,5087960 | 0,0000000 | 45,2431578 | 106,1321042 |
| | | March | 60,9035888* | 9,5087960 | 0,0000001 | 30,4591155 | 91,3480620 |
| | | May | 43,5781284* | 9,5087960 | 0,0003482 | 13,1336552 | 74,0226016 |
| | | June | 53,5038544* | 9,5087960 | 0,0000029 | 23,0593811 | 83,9483276 |
| | | July | 73,1165300* | 9,5087960 | 0,0000000 | 42,6720568 | 103,5610032 |
| | | October | 77,0194175* | 9,5087960 | 0,0000000 | 46,5749443 | 107,4638907 |
| | | November | 46,0714656* | 9,5087960 | 0,0001122 | 15,6269924 | 76,5159389 |
| | December | 73,3284981* | 9,5087960 | 0,0000000 | 42,8840248 | 103,7729713 | |
| May | January | -7,3701381 | 9,5087960 | 0,9988460 | -37,8146114 | 23,0743351 | |

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|----------|----------|--------------|--------------|-----------|--------------|-------------|-------------|
| | | February | 32,1095026* | 9,5087960 | 0,0296449 | 1,6650293 | 62,5539758 |
| | | March | 17,3254603 | 9,5087960 | 0,7204800 | -13,1190129 | 47,7699336 |
| | | April | -43,5781284* | 9,5087960 | 0,0003482 | -74,0226016 | -13,1336552 |
| | | June | 9,9257259 | 9,5087960 | 0,9890762 | -20,5187473 | 40,3701992 |
| | | July | 29,5384016 | 9,5087960 | 0,0654492 | -0,9060716 | 59,9828748 |
| | | October | 33,4412891* | 9,5087960 | 0,0190351 | 2,9968159 | 63,8857623 |
| | | November | 2,4933372 | 9,5087960 | 0,9999999 | -27,9511360 | 32,9378104 |
| | | December | 29,7503696 | 9,5087960 | 0,0615145 | -0,6941036 | 60,1948429 |
| June | January | -17,2958641 | 9,5087960 | 0,7224730 | -47,7403373 | 13,1486092 | |
| | February | 22,1837766 | 9,5087960 | 0,3728790 | -8,2606966 | 52,6282498 | |
| | March | 7,3997344 | 9,5087960 | 0,9988088 | -23,0447388 | 37,8442076 | |
| | April | -53,5038544* | 9,5087960 | 0,0000029 | -83,9483276 | -23,0593811 | |
| | May | -9,9257259 | 9,5087960 | 0,9890762 | -40,3701992 | 20,5187473 | |
| | July | 19,6126757 | 9,5087960 | 0,5561465 | -10,8317976 | 50,0571489 | |
| | October | 23,5155632 | 9,5087960 | 0,2899080 | -6,9289101 | 53,9600364 | |
| | November | -7,4323887 | 9,5087960 | 0,9987666 | -37,8768619 | 23,0120845 | |
| | December | 19,8246437 | 9,5087960 | 0,5404669 | -10,6198295 | 50,2691169 | |
| July | January | -36,9085397* | 9,5087960 | 0,0054547 | -67,3530129 | -6,4640665 | |
| | February | 2,5711010 | 9,5087960 | 0,9999999 | -27,8733723 | 33,0155742 | |
| | March | -12,2129413 | 9,5087960 | 0,9560628 | -42,6574145 | 18,2315320 | |
| | April | -73,1165300* | 9,5087960 | 0,0000000 | -103,5610032 | -42,6720568 | |
| | May | -29,5384016 | 9,5087960 | 0,0654492 | -59,9828748 | 0,9060716 | |
| | June | -19,6126757 | 9,5087960 | 0,5561465 | -50,0571489 | 10,8317976 | |
| | October | 3,9028875 | 9,5087960 | 0,9999944 | -26,5415857 | 34,3473607 | |
| | November | -27,0450644 | 9,5087960 | 0,1294720 | -57,4895376 | 3,3994088 | |
| | December | 0,2119680 | 9,5087960 | 1,0000000 | -30,2325052 | 30,6564413 | |
| October | January | -40,8114272* | 9,5087960 | 0,0011477 | -71,2559004 | -10,3669540 | |
| | February | -1,3317865 | 9,5087960 | 1,0000000 | -31,7762598 | 29,1126867 | |
| | March | -16,1158288 | 9,5087960 | 0,7971657 | -46,5603020 | 14,3286445 | |
| | April | -77,0194175* | 9,5087960 | 0,0000000 | -107,4638907 | -46,5749443 | |
| | May | -33,4412891* | 9,5087960 | 0,0190351 | -63,8857623 | -2,9968159 | |
| | June | -23,5155632 | 9,5087960 | 0,2899080 | -53,9600364 | 6,9289101 | |
| | July | -3,9028875 | 9,5087960 | 0,9999944 | -34,3473607 | 26,5415857 | |
| | November | -30,9479519* | 9,5087960 | 0,0428503 | -61,3924251 | -0,5034787 | |
| | December | -3,6909195 | 9,5087960 | 0,9999965 | -34,1353927 | 26,7535538 | |
| November | January | -9,8634753 | 9,5087960 | 0,9895549 | -40,3079486 | 20,5809979 | |
| | February | 29,6161653 | 9,5087960 | 0,0639818 | -0,8283079 | 60,0606386 | |
| | March | 14,8321231 | 9,5087960 | 0,8654718 | -15,6123501 | 45,2765963 | |
| | April | -46,0714656* | 9,5087960 | 0,0001122 | -76,5159389 | -15,6269924 | |
| | May | -2,4933372 | 9,5087960 | 0,9999999 | -32,9378104 | 27,9511360 | |
| | June | 7,4323887 | 9,5087960 | 0,9987666 | -23,0120845 | 37,8768619 | |
| | July | 27,0450644 | 9,5087960 | 0,1294720 | -3,3994088 | 57,4895376 | |
| | October | 30,9479519* | 9,5087960 | 0,0428503 | 0,5034787 | 61,3924251 | |
| | December | 27,2570324 | 9,5087960 | 0,1226026 | -3,1874408 | 57,7015056 | |

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|--------------|----------|--------------|--------------|-----------|-------------|--------------|-------------|
| | December | January | -37,1205078* | 9,5087960 | 0,0050319 | -67,5649810 | -6,6760346 |
| | | February | 2,3591329 | 9,5087960 | 0,9999999 | -28,0853403 | 32,8036061 |
| | | March | -12,4249093 | 9,5087960 | 0,9510958 | -42,8693825 | 18,0195639 |
| | | April | -73,3284981* | 9,5087960 | 0,0000000 | -103,7729713 | -42,8840248 |
| | | May | -29,7503696 | 9,5087960 | 0,0615145 | -60,1948429 | 0,6941036 |
| | | June | -19,8246437 | 9,5087960 | 0,5404669 | -50,2691169 | 10,6198295 |
| | | July | -0,2119680 | 9,5087960 | 1,0000000 | -30,6564413 | 30,2325052 |
| | | October | 3,6909195 | 9,5087960 | 0,9999965 | -26,7535538 | 34,1353927 |
| | | November | -27,2570324 | 9,5087960 | 0,1226026 | -57,7015056 | 3,1874408 |
| Total Lipids | January | February | 2,2995386 | 3,9295238 | 0,9998837 | -10,2816843 | 14,8807616 |
| | | March | 1,1900867 | 3,9295238 | 0,9999996 | -11,3911363 | 13,7713096 |
| | | April | -10,9959440 | 3,9295238 | 0,1445572 | -23,5771670 | 1,5852789 |
| | | May | -5,2561353 | 3,9295238 | 0,9435623 | -17,8373583 | 7,3250876 |
| | | June | -2,0140484 | 3,9295238 | 0,9999620 | -14,5952713 | 10,5671746 |
| | | July | -30,4977049* | 3,9295238 | 0,0000000 | -43,0789278 | -17,9164819 |
| | | October | 12,4612360 | 3,9295238 | 0,0545708 | -0,1199870 | 25,0424589 |
| | | November | 4,1663536 | 3,9295238 | 0,9877985 | -8,4148694 | 16,7475765 |
| | December | -4,1925363 | 3,9295238 | 0,9872478 | -16,7737592 | 8,3886867 | |
| | February | January | -2,2995386 | 3,9295238 | 0,9998837 | -14,8807616 | 10,2816843 |
| | | March | -1,1094520 | 3,9295238 | 0,9999998 | -13,6906749 | 11,4717710 |
| | | April | -13,2954827* | 3,9295238 | 0,0290409 | -25,8767056 | -0,7142597 |
| | | May | -7,5556739 | 3,9295238 | 0,6535868 | -20,1368969 | 5,0255490 |
| | | June | -4,3135870 | 3,9295238 | 0,9844431 | -16,8948100 | 8,2676359 |
| | | July | -32,7972435* | 3,9295238 | 0,0000000 | -45,3784664 | -20,2160205 |
| | | October | 10,1616973 | 3,9295238 | 0,2316315 | -2,4195256 | 22,7429203 |
| | | November | 1,8668150 | 3,9295238 | 0,9999802 | -10,7144080 | 14,4480379 |
| | December | -6,4920749 | 3,9295238 | 0,8203761 | -19,0732979 | 6,0891480 | |
| | March | January | -1,1900867 | 3,9295238 | 0,9999996 | -13,7713096 | 11,3911363 |
| | | February | 1,1094520 | 3,9295238 | 0,9999998 | -11,4717710 | 13,6906749 |
| | | April | -12,1860307 | 3,9295238 | 0,0664120 | -24,7672536 | 0,3951923 |
| | | May | -6,4462220 | 3,9295238 | 0,8264688 | -19,0274449 | 6,1350010 |
| | | June | -3,2041350 | 3,9295238 | 0,9982805 | -15,7853580 | 9,3770879 |
| | | July | -31,6877915* | 3,9295238 | 0,0000000 | -44,2690145 | -19,1065686 |
| | | October | 11,2711493 | 3,9295238 | 0,1220553 | -1,3100736 | 23,8523722 |
| | | November | 2,9762669 | 3,9295238 | 0,9990392 | -9,6049560 | 15,5574899 |
| | December | -5,3826229 | 3,9295238 | 0,9348978 | -17,9638459 | 7,1986000 | |
| | April | January | 10,9959440 | 3,9295238 | 0,1445572 | -1,5852789 | 23,5771670 |
| | | February | 13,2954827* | 3,9295238 | 0,0290409 | 0,7142597 | 25,8767056 |
| | | March | 12,1860307 | 3,9295238 | 0,0664120 | -0,3951923 | 24,7672536 |
| | | May | 5,7398087 | 3,9295238 | 0,9056652 | -6,8414142 | 18,3210317 |
| | | June | 8,9818957 | 3,9295238 | 0,4031625 | -3,5993273 | 21,5631186 |
| July | | -19,5017608* | 3,9295238 | 0,0000664 | -32,0829838 | -6,9205379 | |
| October | | 23,4571800* | 3,9295238 | 0,0000005 | 10,8759570 | 36,0384029 | |
| November | | 15,1622976* | 3,9295238 | 0,0059244 | 2,5810747 | 27,7435206 | |

| | | | | | | | |
|--|----------|----------|--------------|-------------|-----------|-------------|-------------|
| | | December | 6,8034078 | 3,9295238 | 0,7762367 | -5,7778152 | 19,3846307 |
| | May | January | 5,2561353 | 3,9295238 | 0,9435623 | -7,3250876 | 17,8373583 |
| | | February | 7,5556739 | 3,9295238 | 0,6535868 | -5,0255490 | 20,1368969 |
| | | March | 6,4462220 | 3,9295238 | 0,8264688 | -6,1350010 | 19,0274449 |
| | | April | -5,7398087 | 3,9295238 | 0,9056652 | -18,3210317 | 6,8414142 |
| | | June | 3,2420869 | 3,9295238 | 0,9981152 | -9,3391360 | 15,8233099 |
| | | July | -25,2415696* | 3,9295238 | 0,0000000 | -37,8227925 | -12,6603466 |
| | | October | 17,7173713* | 3,9295238 | 0,0004750 | 5,1361483 | 30,2985942 |
| | | November | 9,4224889 | 3,9295238 | 0,3330677 | -3,1587340 | 22,0037119 |
| | | December | 1,0635990 | 3,9295238 | 0,9999999 | -11,5176239 | 13,6448220 |
| | | June | January | 2,0140484 | 3,9295238 | 0,9999620 | -10,5671746 |
| | February | | 4,3135870 | 3,9295238 | 0,9844431 | -8,2676359 | 16,8948100 |
| | March | | 3,2041350 | 3,9295238 | 0,9982805 | -9,3770879 | 15,7853580 |
| | April | | -8,9818957 | 3,9295238 | 0,4031625 | -21,5631186 | 3,5993273 |
| | May | | -3,2420869 | 3,9295238 | 0,9981152 | -15,8233099 | 9,3391360 |
| | July | | -28,4836565* | 3,9295238 | 0,0000000 | -41,0648794 | -15,9024335 |
| | October | | 14,4752843* | 3,9295238 | 0,0109261 | 1,8940614 | 27,0565073 |
| | November | | 6,1804020 | 3,9295238 | 0,8595675 | -6,4008210 | 18,7616249 |
| | December | | -2,1784879 | 3,9295238 | 0,9999262 | -14,7597108 | 10,4027350 |
| | July | | January | 30,4977049* | 3,9295238 | 0,0000000 | 17,9164819 |
| | | February | 32,7972435* | 3,9295238 | 0,0000000 | 20,2160205 | 45,3784664 |
| | | March | 31,6877915* | 3,9295238 | 0,0000000 | 19,1065686 | 44,2690145 |
| | | April | 19,5017608* | 3,9295238 | 0,0000664 | 6,9205379 | 32,0829838 |
| | | May | 25,2415696* | 3,9295238 | 0,0000000 | 12,6603466 | 37,8227925 |
| | | June | 28,4836565* | 3,9295238 | 0,0000000 | 15,9024335 | 41,0648794 |
| | | October | 42,9589408* | 3,9295238 | 0,0000000 | 30,3777179 | 55,5401638 |
| | | November | 34,6640585* | 3,9295238 | 0,0000000 | 22,0828355 | 47,2452814 |
| | | December | 26,3051686* | 3,9295238 | 0,0000000 | 13,7239456 | 38,8863915 |
| | | October | January | -12,4612360 | 3,9295238 | 0,0545708 | -25,0424589 |
| | February | | -10,1616973 | 3,9295238 | 0,2316315 | -22,7429203 | 2,4195256 |
| | March | | -11,2711493 | 3,9295238 | 0,1220553 | -23,8523722 | 1,3100736 |
| | April | | -23,4571800* | 3,9295238 | 0,0000005 | -36,0384029 | -10,8759570 |
| | May | | -17,7173713* | 3,9295238 | 0,0004750 | -30,2985942 | -5,1361483 |
| | June | | -14,4752843* | 3,9295238 | 0,0109261 | -27,0565073 | -1,8940614 |
| | July | | -42,9589408* | 3,9295238 | 0,0000000 | -55,5401638 | -30,3777179 |
| | November | | -8,2948824 | 3,9295238 | 0,5221902 | -20,8761053 | 4,2863406 |
| | December | | -16,6537722* | 3,9295238 | 0,0014202 | -29,2349952 | -4,0725493 |
| | November | | January | -4,1663536 | 3,9295238 | 0,9877985 | -16,7475765 |
| | | February | -1,8668150 | 3,9295238 | 0,9999802 | -14,4480379 | 10,7144080 |
| | | March | -2,9762669 | 3,9295238 | 0,9990392 | -15,5574899 | 9,6049560 |
| | | April | -15,1622976* | 3,9295238 | 0,0059244 | -27,7435206 | -2,5810747 |
| | | May | -9,4224889 | 3,9295238 | 0,3330677 | -22,0037119 | 3,1587340 |
| | | June | -6,1804020 | 3,9295238 | 0,8595675 | -18,7616249 | 6,4008210 |
| | | July | -34,6640585* | 3,9295238 | 0,0000000 | -47,2452814 | -22,0828355 |

| | | | | | | | |
|--|----------|----------|--------------|-----------|-----------|-------------|-------------|
| | | October | 8,2948824 | 3,9295238 | 0,5221902 | -4,2863406 | 20,8761053 |
| | | December | -8,3588899 | 3,9295238 | 0,5107938 | -20,9401128 | 4,2223331 |
| | December | January | 4,1925363 | 3,9295238 | 0,9872478 | -8,3886867 | 16,7737592 |
| | | February | 6,4920749 | 3,9295238 | 0,8203761 | -6,0891480 | 19,0732979 |
| | | March | 5,3826229 | 3,9295238 | 0,9348978 | -7,1986000 | 17,9638459 |
| | | April | -6,8034078 | 3,9295238 | 0,7762367 | -19,3846307 | 5,7778152 |
| | | May | -1,0635990 | 3,9295238 | 0,9999999 | -13,6448220 | 11,5176239 |
| | | June | 2,1784879 | 3,9295238 | 0,9999262 | -10,4027350 | 14,7597108 |
| | | July | -26,3051686* | 3,9295238 | 0,0000000 | -38,8863915 | -13,7239456 |
| | | October | 16,6537722* | 3,9295238 | 0,0014202 | 4,0725493 | 29,2349952 |
| | | November | 8,3588899 | 3,9295238 | 0,5107938 | -4,2223331 | 20,9401128 |

*The mean difference is significant at the 0.05 level.

➤ Attachment 2

Poster communication in XV Congresso Nacional Y I Congresso Ibérico de Acuicultura:

- Machado, D.; Anjos, C.; Moura, P.; Pombo, A.; Baptista, T.; Matias, D. (2015). Ciclo reprodutivo da população de amêijoa-boia, *Ruditapes decussatus* da Lagoa de Óbidos, Leiria, Portugal. Actas del XV Congresso Nacional Y I Congresso Ibérico de Acuicultura. Huelva. 200–201.

Ciclo reprodutivo da população de amêijoa-boia, *Ruditapes decussatus* da Lagoa de Óbidos, Leiria, Portugal

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Abstract

In Portugal, the European clam (*Ruditapes decussatus*) is an important commercial resource, and therefore, in order to increase their exploration, more production areas need to be created. Lagoa de Óbidos is a strong candidate as a cultivation area, however, the reproductive biology of this population has not been described yet. Through the determination of gonadal development stages, visualized in histological preparations, we intended to characterize the reproductive cycle of the species *R. decussatus*. During seven months of sampling, it was found that in the months of October, November and December the majority of individuals were in sexual rest period and that in January the individuals showed the first stages of the reproductive cycle (stage II - Initiation gametogenesis), after which it was observed an intensification of development of the gonads until the month of April, where it was possible to identify a stage prior to the maturation (stage III - Advanced gametogenesis). Exogenous factors, such as temperature and food availability, are directly related to how the reproductive cycle of European clam evolves over the sampling period, through successive gametogenic phases.

Resumo

Em Portugal, a amêijoa-boia (*Ruditapes decussatus*) representa um importante recurso a nível comercial, de modo a aumentar a sua exploração, mais áreas de produção desta espécie necessitam de ser criadas. A Lagoa de Óbidos é um forte candidato a local de cultivo, contudo a biologia reprodutiva da população presente nesta área ainda não foi descrita. Através da determinação dos estádios de desenvolvimento gonadal, visualizados em preparações histológicas, pretendeu-se caracterizar o ciclo reprodutivo da espécie *R. decussatus*. Ao longo de sete meses de amostragens, verificou-se que nos meses de outubro, novembro e dezembro a maioria dos indivíduos encontravam-se em período de repouso sexual e a partir de janeiro foram encontrados os primeiros estádios do ciclo reprodutivo (estádio II – iniciação de gametogénese), verificando-se uma intensificação do desenvolvimento da gónada até ao mês de abril, onde foi observado o estágio antecedente à maturação (estádio III – gametogénese avançada). Fatores exógenos, como a temperatura e disponibilidade de alimento, estão diretamente relacionados com a forma como o ciclo reprodutivo da amêijoa-boia evoluiu ao longo dos meses amostrados, apresentando sucessivas fases gametogénicas.

Introdução



Objetivo: Caracterizar o ciclo reprodutivo da população de *R. decussatus* presente na Lagoa de Óbidos.

Materiais e métodos



Figura 1 – Localização geográfica de Lagoa de Óbidos.



Amostragens Mensais
- 20 indivíduos
(10 ♀ + 10 ♂)

Procedimentos Analíticos:

- Histologia

Identificação da sexo e classificação da estado de maturação sexual.

(Figueira e Neves-Correira, 2015)

Resultados e Discussão

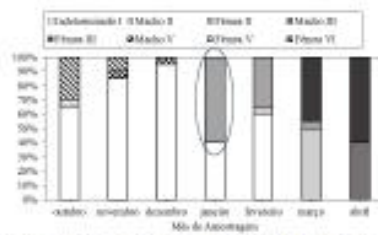
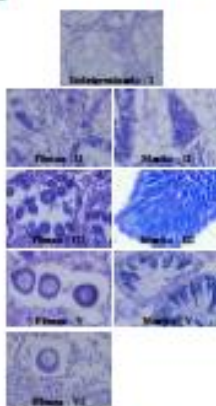


Figura 2 – Variações mensais no desenvolvimento gonadal da população de *Ruditapes decussatus* na Lagoa de Óbidos, durante os meses de outubro de 2014 e abril de 2015.

- Primeiro passo para um sustentável desenvolvimento da exploração comercial desta espécie na Lagoa de Óbidos.
- Potencial utilização da população estudada como reprodutores na produção em maricultura.

A atividade reprodutiva é controlada pelas interações entre os fatores endógenos e exógenos.
(Figueira et al., 2010; Figueira et al., 2012)

Populações da Ria Formosa e Rio de Aveiro
(Ferreira et al., 2010)

≠
População da Lagoa de Óbidos
(Ferreira em estudo II em janeiro – inverno)

Diferenças geográficas = Influência dos fatores exógenos (diferentes condições de temperatura e de disponibilidade de alimento).

Abstract
- Figueira et al. (2010) e Figueira et al. (2012) estudaram a atividade reprodutiva da população de *Ruditapes decussatus* na Ria Formosa e no Rio de Aveiro, Portugal. Os resultados mostraram que a atividade reprodutiva é controlada pelas interações entre os fatores endógenos e exógenos. O presente estudo tem como objetivo caracterizar o ciclo reprodutivo da população de *R. decussatus* presente na Lagoa de Óbidos, durante os meses de outubro de 2014 e abril de 2015. Para isso, foram amostrados 20 indivíduos (10 fêmeas e 10 machos) por mês. Os resultados mostraram que a atividade reprodutiva da população de *R. decussatus* na Lagoa de Óbidos é controlada pelas interações entre os fatores endógenos e exógenos.



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