



*Reproductive strategy evaluation of mussel *Mytilus galloprovincialis* produced in longlines in two areas of Algarve coast*

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[2017]



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Dissertação para obtenção do Grau de Mestre em Aquacultura

Dissertação de Mestrado realizada sob a orientação da Especialista Teresa Baptista e da Doutora Sandra Joaquim.

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Título: Reproductive strategy evaluation of mussel *Mytilus galloprovincialis* produced in longlines in two areas of Algarve coast

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Escola Superior de Turismo e Tecnologia do Mar – Peniche
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Resumo

Nos últimos anos, a criação de zonas de produção aquícola na costa portuguesa, tem permitido a implementação de empresas *offshore* para a produção de mexilhão (*Mytilus galloprovincialis*) sobretudo na costa Algarvia, nomeadamente em Sagres, Lagos e Olhão. O conhecimento da performance reprodutiva das espécies de mexilhão, para além de permitir efetuar uma caracterização biológica, é também essencial para permitir a maximização da produção aquícola, a gestão da fixação de pós-larvas, bem como a gestão da colocação do produto no mercado. Assim, este estudo visa avaliar o ciclo reprodutivo de *M. galloprovincialis* produzido em duas áreas *offshore* na costa Algarvia, Olhão e Lagos, através do estudo histológico do desenvolvimento gonadal, composição bioquímica (proteínas, glicogénio e lípidos totais) e índice de condição, durante o período de 19 meses, de março de 2014 a setembro de 2015. Para além destes parâmetros, também foram monitorizados a temperatura da água e a clorofila *a* para avaliar o efeito do ambiente no ciclo reprodutivo. A atividade reprodutiva dos mexilhões provenientes dos dois locais não segue um ciclo sazonal. O início da gametogénese parece ocorrer durante janeiro/fevereiro para uma pequena percentagem de mexilhões. Ambas as populações apresentaram maior esforço reprodutivo no final da primavera de 2014, onde foi observada a maior percentagem de indivíduos maduros. Não foi observado um período específico de desova, os mexilhões desovaram durante todos meses de amostragem, no entanto o período de desova mais intensa foi observado durante o verão. Os mexilhões estiveram sucessiva e continuamente em recuperação gonadal e em desova durante quase todo o período de estudo. Os valores do índice de condição atingidos foram muito mais baixos em 2015 comparativamente com os do mesmo período de 2014, uma vez que os mexilhões não atingiram o mesmo esforço reprodutivo como em 2014. O ciclo reprodutivo de *M. galloprovincialis* produzidos nestes dois locais da costa algarvia sofreu uma variabilidade de ano para ano durante o período experimental, onde revelaram uma condição significativamente melhor em 2014 do que em 2015. É amplamente aceite que essas diferenças podem ser correlacionadas com mudanças nas condições ambientais, no entanto, neste estudo, a temperatura e a clorofila *a* não permitiram explicá-las. Os resultados de ambos os ciclos de armazenamento e utilização de nutrientes mostraram que as populações seguem uma estratégia conservativa, que lhes permite uma rápida recuperação gonadal após a desova, muito provavelmente devido à constante disponibilidade de alimento nos dois locais. O conhecimento alcançado neste

estudo pode ser um importante contributo para a rentabilização das empresas, uma vez que estas informações irão permitir uma melhor gestão da produção.

Palavras-chave: *Mytilus galloprovincialis*; Olhão; Lagos; ciclo reprodutivo; índice de condição; composição bioquímica.

Abstract

In recent years, the development of aquaculture production areas on the Portuguese coast, has allowed the implementation of offshore companies for mussel (*Mytilus galloprovincialis*) production especially on the Algarve coast, namely in Sagres, Lagos and Olhão. The knowledge of the reproductive performance of the species, behind biological characterization, will be an essential help to increase farm productions, adjusting the placement timings for larval recruitment maximization, according to the marketing needs. This study aimed to evaluate the reproductive cycle of *M. galloprovincialis* produced in two offshore areas along the Algarve coast, Olhão and Lagos, through the histological study of gonadal development, biochemical composition (proteins, glycogen and total lipids), and condition index. Also, the monitoring of water temperature and chlorophyll *a* was recorded to evaluate the effect of the environment on the reproductive cycle. This study was carried out during a period of 19 months, from March 2014 to September 2015. The mussels reproductive activity from those two sites does not follow a seasonal cycle. The beginning of gametogenesis seems to occur during January/February for a small percentage of mussels. Both populations presented higher reproductive effort at the end of the spring 2014, where a higher percentage of mature individuals were observed. No specific spawning period was observed, indeed mussels spawn during all study months, however the most intensive spawning period was registered during summer. Mussels were successively and continuously in gonadal recovery and spawning in almost all study period. The condition index values were much lower in 2015 than in the same seasonal period of 2014 and mussels did not reach the same reproductive effort as in 2014. The reproductive cycle of *M. galloprovincialis* produced in these two sites of the Algarve coast suffered an year to year variability during the experimental period, where revealed a significantly better condition in 2014 than in 2015. It is widely accepted that these differences can be correlated with environmental conditions changes, however, in our study, temperature and chlorophyll *a* do not allowed to explain them. The results of both storage and nutrient utilization cycles showed that populations followed a conservative strategy, which permitted a rapid gonadal recovery after spawning, probably due to the constant food availability in both sites. The knowledge reached in this study can be an important contribution to the farms profitability, since this information will allow a better production management.

Keywords: *Mytilus galloprovincialis*; Olhão; Lagos; reproductive cycle; condition index; biochemical composition.

Index

Agradecimientos	v
Resumo	vii
Abstract	ix
List of figures	xiii
List of tables	xv
List of acronyms	xvii
1. Introduction	1
2. Materials and methods	7
3. Results	15
4. Discussion	27
References	33
Attachments	41

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List of Figures

Figure 1.1. Geographical distribution of *Mytilus galloprovincialis* (www.aquamaps.org, version of Aug. 2013. Web accessed 15 Dec. 2016).

Figure 1.2. Life cycle of *Mytilus spp.* (adapted from www.cornishshellfish.co.uk).

Figure 2.1. Location of the sample areas of *Mytilus galloprovincialis*: Olhão – APPAA - Armona Pilot Aquaculture Production Area and Lagos.

Figure 2.2. Gametogenic stages of female *Mytilus galloprovincialis*. (A) Sexual rest; (B) Early growth stage; (C) Late growth stage, Oo – Oocytes at different maturity stages, Po – pedunculated oocyte; (D) Ripe; (E) Spawning; (F) Regeneration, R – formation of new oocytes; (G) Late spawning, Art – oocytes in atresia. Scale bar: 500 μm in D and E; 200 μm in A, B, F and G, 100 μm in C.

Figure 2.3. Gametogenic stages of male *Mytilus galloprovincialis*. (A) Sexual rest; (B) Early growth stage, Sg - Spermatogonia; (C) Late growth stage, Sp – Spermatozoid; (D) Ripe; (E) Spawning; (F) Regeneration, R – formation of new spermatozoids; (G) Late spawning. Scale bar: 500 μm in C and F; 200 μm in A, D, E and G; 100 μm in B.

Figure 3.1. Monthly values of sea surface temperature (SST) in Olhão and Lagos (mean \pm SD) during the sampling period (March 2014 to September 2015). (*statistically significant differences, $P < 0.001$, found between sites).

Figure 3.2. Monthly values of chlorophyll *a* in Olhão and Lagos during the sampling period (March 2014 to September 2015).

Figure 3.3. Monthly variation in gonadal development of *Mytilus galloprovincialis* obtained in Olhão during the sampling period (March 2014 to September 2015).

Figure 3.4. Monthly variation in gonadal development of *Mytilus galloprovincialis* obtained in Lagos during the sampling period (March 2014 to September 2015).

Figure 3.5. Monthly variation in gonadal index (GI) (mean, $n = 10$) of *Mytilus galloprovincialis* from Olhão and Lagos during the sampling period (March 2014 to September 2015).

Figure 3.6. Monthly variation in condition index (CI) (mean \pm SD, $n = 10$) of *Mytilus galloprovincialis* from Olhão and Lagos during the sampling period (March 2014 to September 2015). (*statistically significant differences, $P < 0.05$ found between populations; ^{a b} between 2014 and 2015 in Olhão and Lagos, $P < 0.001$).

Figure 3.7. Principal component analysis (PCA) on the parameters used to characterize the reproductive cycle of *Mytilus galloprovincialis* from two sampling places: a) Olhão and b) Lagos. Each vector represents one of the parameters analyzed (SST - Sea surface temperature; Chlo *a* - chlorophyll *a*; GI- Gonadal index; Prot- Proteins; Glyc- Glycogen; Tlip- Total lipids; Ten- Total energy) and each point represents the sampling month.

List of Tables

Table 2.1. Reproductive scale for *Mytilus galloprovincialis* according to Lubet (1959).

Table 3.1. Results of Pearson (r) and Spearman (s) correlation between studied parameters (r , correlation coefficient; P - P value; n.c. - no correlation was found).

Table 3.2. Mean values (\pm SD) of proteins, total lipids, glycogen ($\mu\text{g mg}^{-1}$ AFDW) and total energy (KJmg^{-1} AFDW) of *Mytilus galloprovincialis* from Olhão and Lagos during the sampling period (March 2014 to September 2015). (*statistically significant differences, $P < 0.05$ found between populations; ^{a, b} between 2014 and 2015 in Olhão and Lagos, $P < 0.001$; ** insufficient number of sampling individuals for biochemical contents evaluation).

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List of Acronyms

ADG – Adipogranular cells

AFDW – Ash free dry weight

ANOVA – Analyses of variance between groups method

Chlo *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

r – Correlation coefficient

SD – Standard deviation

SST – Sea water temperature

VC – Vesicular cells

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1. Introduction

According to FAO (2016), the culture of molluscs represented 15.9% of world's aquaculture production in 2014. Bivalve molluscs, especially oysters, clams, scallops and mussels, constitute an important part of the world aquaculture production, being the second group most produced, with mussels reaching approximately 15%¹. European mussel production has stagnated in the last decade due to a reach of the full carrying capacity in traditional locations (Smaal, 2002). This led to an increase in imports by Europe up to nearly 40% of EU production in 2010 (189,700 tons; FAO, 2014) and a loss in revenues for the EU trade balance. Indeed, in addition to the gastronomic interests, mussel culture has been subject of another attention, due to the presence of bioactive compounds in their meat, which have positive effects on human health (Grienke *et al.*, 2014).

Bivalves accounted for 45.0% of the total Portuguese aquaculture production in 2014, being clams as the most relevant species (2,251 tons), followed by mussels (1,547 tons) and oysters (1,085 tons) (DGRM, 2016). Aquaculture production technology has developed, and the implementation of aquaculture offshore areas are now being regarded as new grounds for production of traditional species. Portugal has 2,130 km² of offshore area with potential for the culture of *Mytilus galloprovincialis* due to its very attractive hydrographic conditions (Kapetsky *et al.*, 2013). In recent years, the establishment of aquaculture production areas in Portugal coast, given the opportunity to the establishment of offshore companies for mussel production, particularly on the Algarve coast. Presently there are three operating near Sagres, one in Lagos, and one in Olhão, who have been focusing on this specie production, leading to an increase on its production in Portugal, reaching at 380 tons in 2012 (DGRM, 2015), and almost duplicated in 2013 (DGRM, 2016). The Algarve coastal waters are among the most productive of the Iberian Peninsula due to their geo-location between the Mediterranean Sea, North Africa and the eastern Atlantic and due to the high diversity of resources and generally calm ocean conditions (Stelzenmüller *et al.*, 2013). Mussels are commonly cultivated on artificial structures, essentially in longlines to facilitate company operations.

¹Source: <http://www.fao.org/figis/servlet/TabSelector>, accessed in 01/03/2017.

The environmental background conditions can define the production potential of a mussel offshore company with longlines systems, being determinant to scale the production according to the environmental factors.

Longlines are relatively simple constructions comprised by two or more parallel lines at sea surface and usually oriented parallelly to the dominating current directions. Specifically, the interactions among type and abundance of phytoplankton, temperature, salinity, and local hydrodynamics (currents and waves) are expected to modulate the growth rates and the condition index (Azpeitia *et al.*, 2016). Nevertheless, there are differences inherent to each local (physical-chemical parameters) along the coast which can influence the reproductive cycle of the species.

Bivalve's reproduction, mainly in commercial species, has been intensively studied in the last decades, being this research essential for the development of aquaculture and fisheries management (Joaquim *et al.*, 2008a; Guerra *et al.*, 2011; Matias *et al.*, 2013). They have a wide variety of reproductive strategies (Coe, 1943), and the knowledge of the highest reproductive activity periods is essential to establish fisheries/aquacultures management plans (Galvão *et al.*, 2006). In addition, the well understanding of the bivalve's reproductive cycles allow to determine the best period of seeds collector's placement for its cultivation in aquaculture systems and for the establishment of its production in hatchery (Galvão *et al.*, 2006; Joaquim *et al.*, 2008a).

The Mediterranean mussel *M. galloprovincialis* (Lamarck, 1819) is a warm water mollusc of the family Mytilidae, firstly detected on the west coast of South Africa in the late 1970's (Bownes and McQuaid, 2006; Picker and Griffiths, 2011), that spreads from south-west Great Britain, Atlantic French, Spanish, Portuguese and Morocco coasts and the Mediterranean Sea (Tirado and Macías, 2006). Globally it is found on temperate sheltered and exposed rocky shores but is generally absent from heavily silted or sandy areas (Figure 1.1).

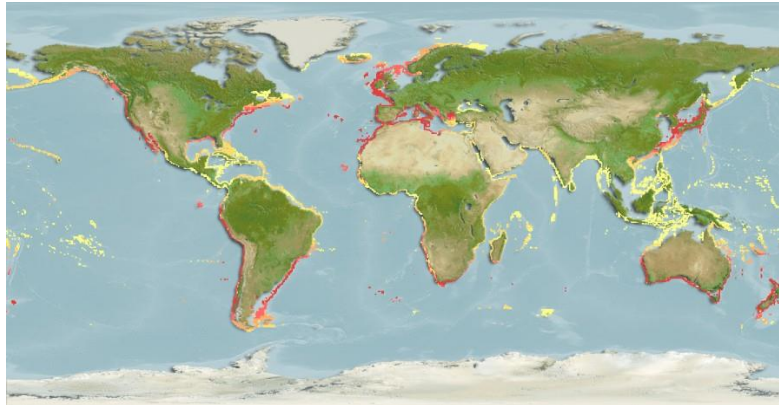


Figure 1.1. Geographical distribution of *Mytilus galloprovincialis* (www.aquamaps.org, version of Aug. 2013. Web accessed 15 Dec. 2016).

Results from a study conducted by Steffani and Branch (2003) shows that greatest recruitment and growth rates are achieved at exposed sites in comparison to sites sheltered from direct wave action or at sites exposed to extreme wave action. It has been suggested that this better growth rates can be attributed to greater food supply, however, opportunities for attachment may diminish at extremely exposed sites (Branch and Steffani, 2004). This species is semi-sessile epibenthic and can tolerate wide variation in salinity, desiccation, temperature and oxygen concentration, characteristics that result in the ability to occupy a large variety of micro-habitats. They can live stocked to a secure substrate, which include; rocks, stones, gravel, shingle and dead shells, making them an ideal species to be produced in ropes. The bathymetric range of distribution covers mostly the littoral to sub littoral zones (<99 m) of oceanic and polyhaline to mesohaline estuarine environments. The life cycle can be divided into the free swimming larval phase and the largely sedentary juvenile and adult phase (Figure 2.2). They are filter feeders, filtering seawater through the gills. On average, the shell's length is approximately 5 cm, but it can grow up to 12 cm (Picker and Griffiths, 2011). They exhibit a rapid growth and can attain 7 cm at first year at favorable sites (Picker and Griffiths, 2011). Mussels are dioecious, though rare instances of hermaphroditism have been reported. The gonads extend throughout the body and are cream colored in males and orange in females. Millions of gametes are released during spawning events with fertilized eggs developing into free swimming planktotrophic larvae capable of dispersing large distances (Picker and Griffiths, 2011). The planktonic life varies from 2-4 weeks depending on temperature, food supply and availability of suitable settlement substratum. Reproduction takes place more than once each year with annual reproductive output (Van Erkom *et al.*, 1991).

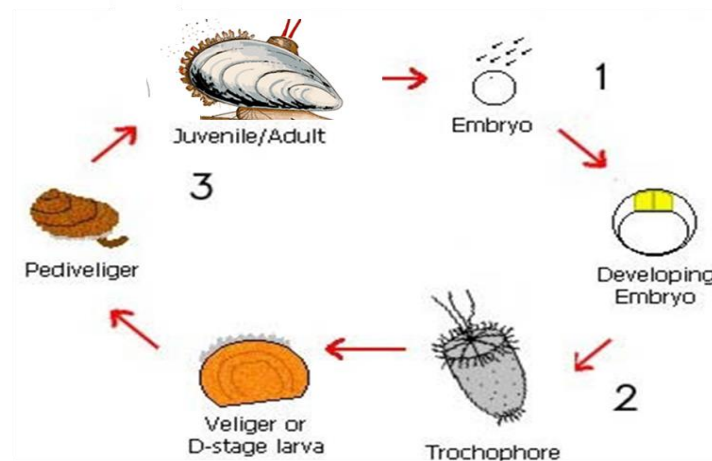


Figure 1.2. Life cycle of *Mytilus* spp. (adapted from www.cornishshellfish.co.uk).

According to Kennedy *et al.*, (1996), the gonadal development depends on the synergetic effect of both internal and external factors. Specific endogenous rhythms synchronized by external factors, such as temperature, nutrition, animal health and others, regulate the reproductive cycle (Robert and Gerard, 1999; Chávez-Villalba *et al.*, 2002; Joaquim *et al.*, 2008a), being food the most important (Utting and Millican, 1997; Marshall *et al.*, 2010). 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 gonia stem 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 does not 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 ovogenesis (Joaquim *et al.*, 2008b; Guerra *et al.*, 2011). Reproduction is a process that affects the physiological condition of bivalves, having a direct influence on the quality of the final product. Thus, the reproductive cycle of *Mytilus* spp., although it is characteristic of this species, timing and duration, are determined by the interaction between endogenous and exogenous factors, varying with the geographical area and different annual environmental conditions (Rodhouse *et al.*, 1984, Seed and Suchanek, 1992, Villalba, 1995). A variation on the reproductive responses according to the different geographical populations of *M. galloprovincialis* has been found by Iglesias *et al.* (1996).

In species of the genus *Mytilus*, the anatomic proximity of the genital papilla to the mantle tissue is used for the expansion of the gonadal structure and the gametogenic development that takes place mainly in this tissue. Mantle tissue is defined as a laminar anatomic space, of variable thickness, surrounded by an epithelium, comprising different types of cells subject to fluctuations in the population following a seasonal rhythm. Mantle basically constitutes reserve tissue formed by vesicular cells (VC) where glycogen is stored and adipogranular cells (ADG) that accumulate protein granules, including lipids and small amounts of glycogen (Pipe, 1987; Peek *et al.*, 1989). The gonad is comprised of different germinal cells, gonoducts, and auxiliary cells, and the circulatory system formed by hemolymph, hemocytes, sinuses, veins, and channels. The mantle tissue of *Mytilus* has two interrelated physiologic functions: accumulation of reserve substances in the VC and ADG cells and development of the gonad that invades the mantle, proliferating at the expense of the reserve tissue. An inverse seasonal development has been described between the reserve tissue cycle, especially glycogen and the gametogenic cycle (Bayne *et al.*, 1982; Lowe *et al.*, 1982), and their control by some endogenous factors, especially neuroendocrine factors (Mathieu *et al.*, 1991; Danton *et al.*, 1996). During gonadal maturation, marine bivalves showed changes in biochemical composition related to energy storage and utilization for gamete production (Barber and Blake, 1981; Ruiz *et al.*, 1992; Ojea *et al.*, 2004). Energy reserves assume a considerable importance in reproduction and in seasonal energy storage and utilization in bivalves, who are closely correlated to environmental conditions and to the annual gametogenic cycles (Delgado *et al.*, 2004; Ojea *et al.*, 2004; Tlili *et al.*, 2012). Indeed, Joaquim *et al.* (2011) showed that food and temperature are the main factors that regulate the timing and rate of energy storage in bivalves. Glycogen and proteins are mostly used in reproductive organ formation and gamete proliferation, while lipids are very important energy reserves in the oocytes, assuring the viability of larvae (Whyte *et al.*, 1991; Napolitano *et al.*, 1992; Leonardos and Lucas, 2000).

This study aimed to evaluate the reproductive cycle of *M. galloprovincialis* produced in two offshore areas along the Algarve coast, Olhão and Lagos, through the histological study of gonadal development, biochemical composition (proteins, glycogen and total lipids), and condition index (CI). This analysis allows to characterize the reproductive strategy of the two populations, according to the hydrographic conditions of each sampling site. The knowledge of the reproductive performance of the species will be essential for help to

increase farm productions, adjusting the placement timings for a maximization of larval recruitment, and the marketing needs. Maximum marked value for mussels is obtained when they are in advanced gametogenesis.

2. Materials and methods

2.1. Sample collection

Mussels *M. galloprovincialis* with size of 72 ± 9.5 mm from two longline production areas in Algarve coast, Olhão - APPAA (Armona Pilot Aquaculture Production Area) ($36^{\circ}01,007'N$; $07^{\circ}44,499'W$) and Lagos ($37^{\circ}04,200'N$; $8^{\circ}42,800'W$) (Figure 3) were monthly collected during 19 months (March 2014 - September 2015) for histological study of gonadal development, determination of condition index and biochemical composition (proteins, glycogen and total lipids). After collection, samples were immediately processed for histological analyses and stored at $-20^{\circ}C$ for the remaining determinations. APPAA is located at the Algarve coast of Portugal, near Olhão, and inlet of Ria Formosa estuary at approximately 2 miles from the coast of the island of Armona. The water depth in this area is between 20 and 30 meters and tidal range is around 3.5 meters. The dominant current flows from the west to the east since south-western winds are dominant. Mussels sampled in Lagos were collected in a semi-submerged longline production area with about 400 hectares, at 6 to 8 meters under the sea, located at 1 mile from the coast between the Port of Lagos and Sagres. The two areas are distant from each other in about 46 miles, and are subject to a variety of phenomena (temperature, depth, abundance and food availability, among others) inherent to the geographical location.

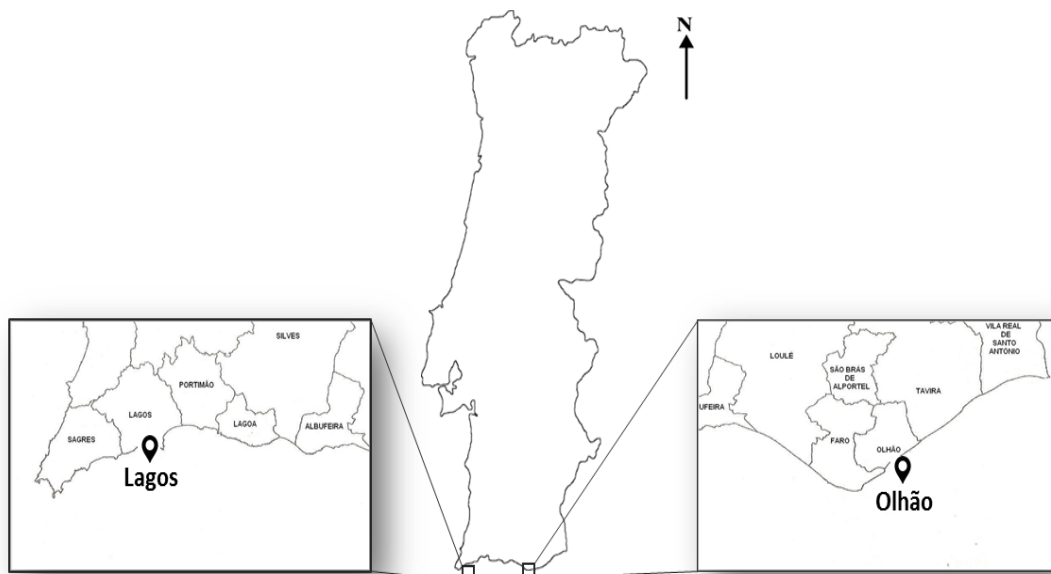


Figure 2.1. Location of the sample areas of *Mytilus galloprovincialis*: Olhão – APPAA - Armona Pilot Aquaculture Production Area and Lagos.

2.2. Analytical analyses

2.2.1. Sea surface temperature (SST) and chlorophyll *a* (Chlo *a*)

The sea surface temperature (SST) and chlorophyll *a* (Chlo) *a* data were obtained through monthly measurements. The SST data from Lagos were provided by a mussel producer company obtained with a multiparameter probe HI 929829 - v1.0.13. In Olhão this environmental parameter was obtained through the www.tunipex.eu/information_oceanic.php site. Chlo *a* data was derived from satellite remote sensing data, collected from the Giovanni online data system (<http://disc.sci.gsfc.nasa.gov/giovanni/overview/index.html>) (Acker and Leptoukh, 2007), for both sampled areas.

2.3. Laboratory analysis

The laboratory analyses were performed for standard animals with a length of 72 ± 9.5 mm, to avoid the influence of growth, and thus minimize bias from increasing somatic weight and focus on the accumulation or loss of organic matter associated with reproduction (Beninger and Lucas, 1984).

2.3.1. Histology

Ten individuals of each sex from each sampling site (Olhão and Lagos) were monthly sampled and examined histologically to determine their gametogenic stages. Each mussel was carefully opened and visceral mass was separated from gills and siphons and then fixed in Davidson solution for 24 hours. After fixation, tissues were washed in running water and stored in 70% alcohol. Subsequently, samples suffered a process of dehydration with serial dilutions of alcohol (ETOH) and finally impregnated in paraffin. Sections ranging between 5 and 7 μ m thick were performed with an automatic rotation microtome and stained with hematoxylin-eosin (Martoja and Martoja, 1967), and mounted in synthetic resin (DPX). The histologically prepared slides were examined using a microscope at 40x and 100x magnification. Mussel reproductive maturity was categorized into seven stages using the scale proposed by Lubet (1959) (Table 2.1 and Figures 2.2 and 2.3). When more than one

development stage occurred simultaneously within a single individual, the assignment of the stage criteria decision was based upon the condition of the dominant stage.

Table 2.1. Reproductive scale for *Mytilus galloprovincialis* according to Lubet (1959).

Stage	Histologic description
<i>Sexual rest</i> (stage 0)	Period characterized by rapid regeneration of reserve tissues; absence of gonadal tissue.
<i>Early growth stage</i> (stage I)	Regeneration of the reserve tissue; proliferation of oogonia and spermatogonia (initial states) with formation of new follicles; abundance of reserve tissue.
<i>Late growth stage</i> (stage II)	Presence of spermatogonia, spermatocytes and spermatids arranged in radial columns in males; presence of oogonia and oocytes at different maturity stages attached to the follicular wall (peduncle) in females.
<i>Ripe</i> (stage III A)	The number and size of follicles increases with the decrease of the reserve tissue. This stage occurs before spawning when follicles are filled with mature gametes.
<i>Spawning</i> (stage III B)	The discharged of gametes is checked by the presence of sperm and mature oocytes in gonoducts and by the break of follicular walls.
<i>Regeneration</i> (stage III C)	Regeneration of the germ cells; Formation of new gametes along the follicular walls.
<i>Late spawning</i> (stage III D)	Occurrence of the discharge of some of last gametes produced in stage IIIB and the produced in stage III C, wherein occurs some follicular degradation (atresia in females) and reserve tissue regeneration.

A mean gonadal index (GI) was calculated according the method proposed by Seed (1976), with adaptations to the used reproductive scale:

$$GI = \frac{\sum \text{ind. each stage} \times \text{stage ranking}}{\text{total ind. each month}}$$

For each gonadal stage was assigned a numerical ranking as follows: sexual rest = 0, early growth stage = 3, late growth stage = 4, ripe = 5, spawning and late spawning = 1, regeneration = 2. The GI ranged from 0 (when all individuals in the sample are in sexual rest) to 5 (when all individuals are in ripe stage).

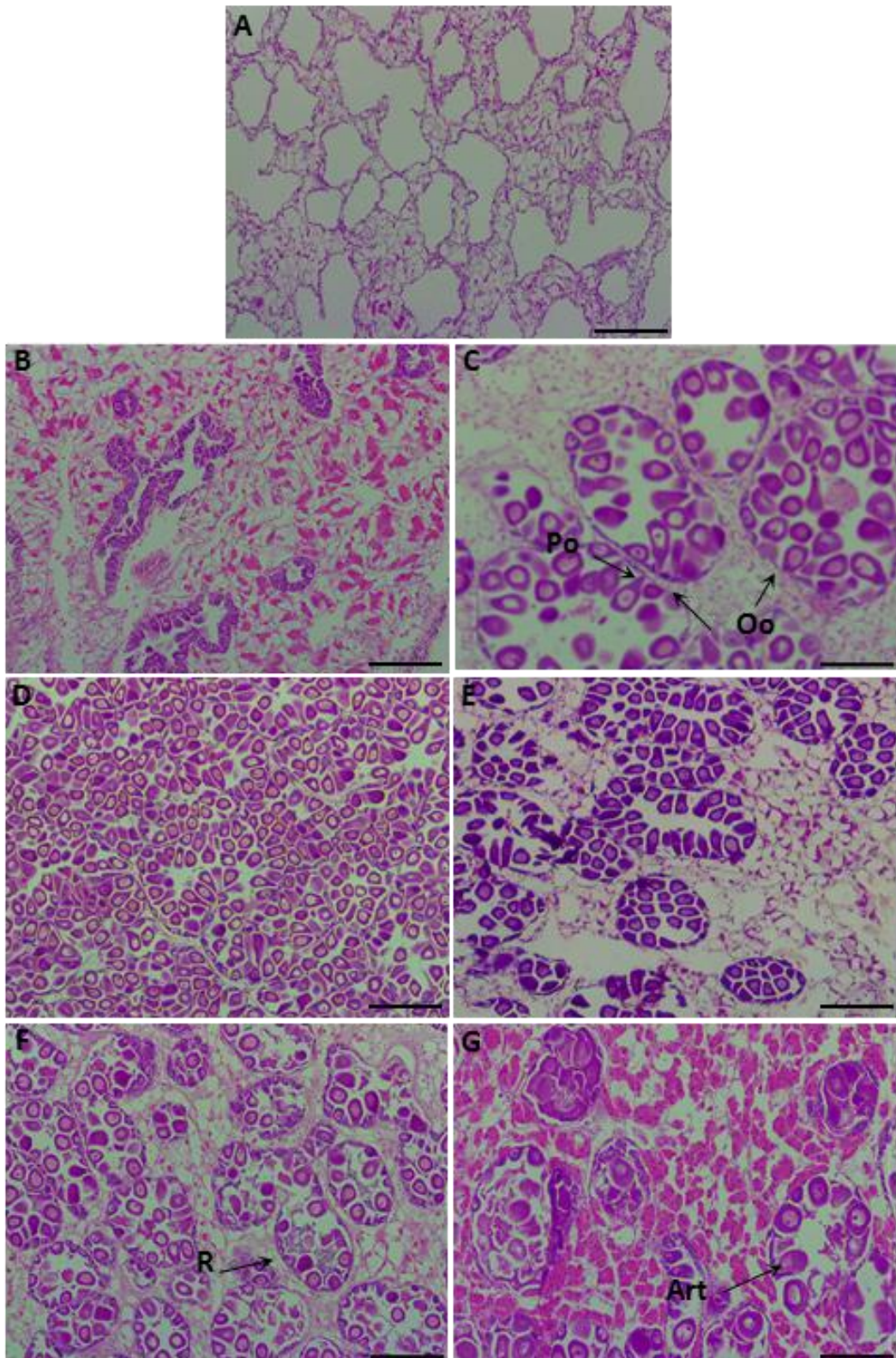


Figure 2.2. Gametogenic stages of female *Mytilus galloprovincialis*. (A) Sexual rest; (B) Early growth stage; (C) Late growth stage, Oo – oocytes at different maturity stages, Po – pedunculated oocyte; (D) Ripe; (E) Spawning; (F) Regeneration, R – formation of new oocytes; (G) Late spawning, Art – oocytes in atresia. Scale bar: 500 µm in D and E; 200 µm in A, B, F and G, 100 µm in C.

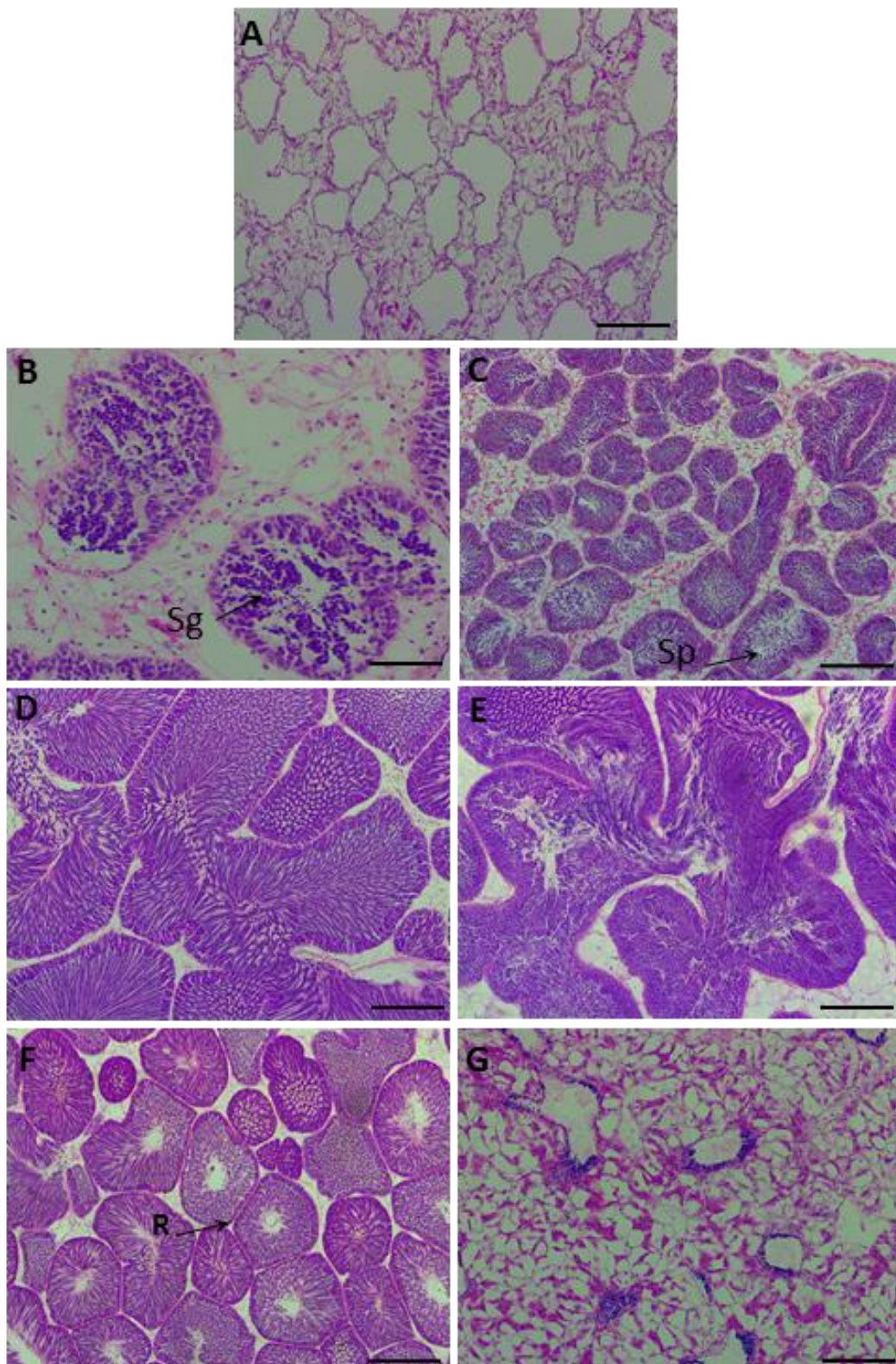


Figure 2.3. Gametogenic stages of male *Mytilus galloprovincialis*. (A) Sexual rest; (B) Early growth stage, Sg - spermatogonia; (C) Late growth stage, Sp – spermatozoid; (D) Ripe; (E) Spawning; (F) Regeneration, R – formation of new spermatozoids; (G) Late spawning. Scale bar: 500 μm in C and F; 200 μm in A, D, E and G; 100 μm in B.

2.3.2. Condition index

Dry soft tissues and shell weights of 10 mussels from each month sample, from both places, were determined after oven drying at 80°C for 24 h. Soft tissue samples were then ashed at 450°C in a muffle furnace, the ash weight was determined and the organic matter weight calculated as the ash-free dry soft-tissue weight (AFDW). The condition index (CI) was calculated according to Walne and Mann (1975):

$$CI = \frac{\text{dry soft tissues weight (mg)} - \text{ash (mg)}}{\text{dry shell weight (mg)}} \times 100$$

2.3.3. Biochemical composition

Ten mussels from each month sample from both places were unfrozen, carefully opened and the entire soft body removed from the shell. The soft tissue was homogenized in an ice bath and aliquots of each individual were used for proteins, total lipids and glycogen determination. Protein was determined using the modified Lowry method (Shakir *et al.*, 1994), glycogen content was determined from dried (80°C for 24 h) homogenate using the anthrone reagent (Viles and Silverman, 1949) and total lipids were extracted from fresh homogenized material in chloroform/methanol (Folch *et al.*, 1957) and estimated spectrophotometrically after charring with concentrated sulphuric acid (Marsh and Weinstein, 1966). Duplicate determinations were performed in all cases and values were expressed as a percentage of AFDW. The caloric content of protein, lipid and carbohydrates in tissues was calculated using the factors 17.9 kJ g⁻¹ (Beukema and De Bruin, 1979), 33 kJ g⁻¹ (Beninger and Lucas, 1984) and 17.2 kJ g⁻¹ (Paine, 1971), respectively.

2.4. 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 analysis of variance (ANOVA) failed (namely data normality and homogeneity of variances). Percentage data were arcsine transformed to

normalize variance (Sokal and Rohlf, 1981). Multiple pairwise comparisons were performed using the post-hoc parametric Tukey HSD test or the non-parametric Dunn's test to detect significant differences between monthly consecutive samples. The Pearson and Spearman correlation coefficient was used to determine the degree of association between parameters. Results were considered significant at $P < 0.05$. Data were performed using the SIGMASTAT 3.11 statistical package. A principal component analysis (PCA) was performed to evaluate distribution patterns on the SST, Chlo *a*, 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. Positions of original variables in the principal component plot relevantly represent their interrelations. If variables are very closely located, their interrelation is strong and positive, if they are on the opposite position, the given variables are negatively correlated. Consequently, graphical representation of the objects in the plot is useful to detect possible association between those objects (Bednářová *et al.*, 2013). Furthermore, in the principal component biplot, the simultaneously representation of the objects and the variables, makes possible to detect those variables which are associated with the group formed for closely located objects and in this way, the mutual relationships among objects and variables can be discovered. Graphs were performed using the XLSTAT 2016 statistical program.

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3. Results

3.1. Sea surface temperature and chlorophyll *a*

The evolution of monthly SST and Chlo *a* data during the sampling period in both studied sites are presented in Figures 3.1 and 3.2, respectively. A seasonal cycle in SST was observed in the two geographical locations, and the monthly means ranged between $13.7\pm 0.1^{\circ}\text{C}$ and $21.7\pm 1.4^{\circ}\text{C}$ in Olhão and $13.1\pm 0.7^{\circ}\text{C}$ and $20.6\pm 1.4^{\circ}\text{C}$ in Lagos. The lowest values of SST were registered in February 2015 for both locations, while the highest values were registered in July and June 2015, in Olhão and Lagos, respectively. Statistical significant differences were observed between sites (ANOVA, $P < 0.001$), namely in September 2014, July, August and September 2015.

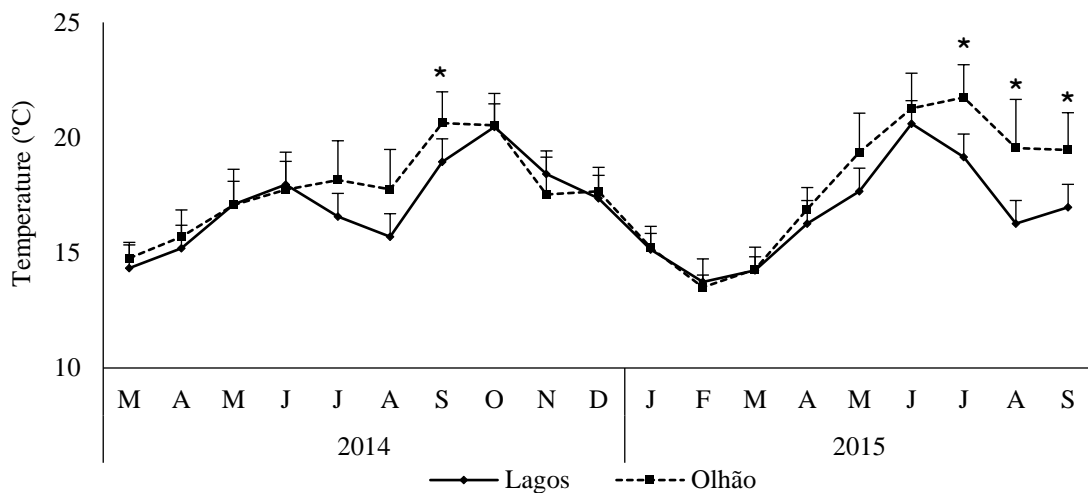


Figure 3.1. Monthly values of sea surface temperature (SST) in Olhão and Lagos (mean±SD) during the sampling period (March 2014 to September 2015). (*statistically significant differences, $P < 0.001$, found between sites).

Chlo *a* ranged between 0.39 mg m^{-3} (June 2015) and 2.1 mg m^{-3} (August 2014) in Olhão. In general, in Lagos Chlo *a* reached higher values than in Olhão, ranging between 0.4 mg m^{-3} (June 2015) and 4.3 mg m^{-3} (March 2014). Statistical significant differences were observed between sampling sites (ANOVA, $P = 0.01$), namely during 2014. In Lagos, it was observed several phytoplankton peaks during the sampling period ($4.3\pm 1.1 \text{ mg m}^{-3}$ in March 2014; $3.5\pm 5 \text{ mg m}^{-3}$ in August 2014; $3\pm 0.3 \text{ mg m}^{-3}$ in November 2014; $3.6\pm 1.2 \text{ mg m}^{-3}$ in March 2015 and $2.9\pm 0.02 \text{ mg m}^{-3}$ in April 2015) all coincident with lower SST values, thus giving

rise to a negative correlation between these parameters ($r_{\text{Spearman}} = -0.50$, $P < 0.05$) (Table 3.1). In Olhão, the lowest Chlo *a* value was registered in the precedent month of the highest SST.

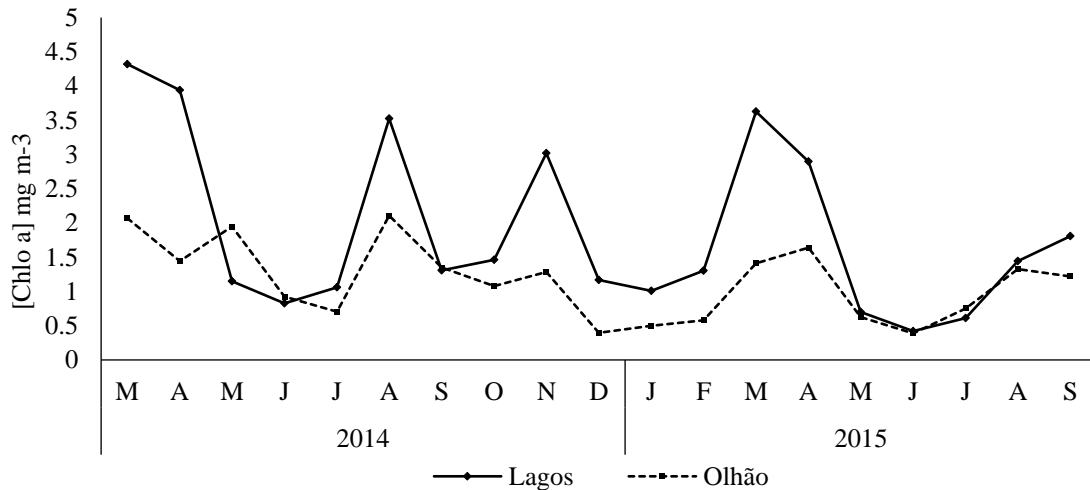


Figure 3.2. Monthly values of chlorophyll *a* in Olhão and Lagos during the sampling period (March 2014 to September 2015).

3.2. Gametogenic cycle

Both population has sexes clearly separated and no hermaphrodites were found. The gonadal development (Figures 3.3 and 3.4) followed the same pattern as the gonadal index (GI) (Figure 3.5), and no significant differences in GI were found between populations or between sexes (ANOVA, $P > 0.05$), although males seem slight delayed compared with females, particularly during spring of 2014. In this way, the results of this parameter are presented for both sexes together (Figure 3.5). It was not observed a period were both males and females, were all in sexual rest, explaining why GI never reach 0. Indeed, individuals from both populations were observed in sexual rest (stage 0) few times during this study and in a small percentage (Olhão: October 2014: 17% females and 20% males; December 2014: 25% females, 14% males; August 2015: 14% females, 7% males; September 2015: 64% females and 44% males; Lagos: February: 11% females and 8% males; August 2015: 10% females and 9% males; September 2015: 22% females and 15% males), except in Olhão in September, where the majority of individuals reached this stage, determining the lowest reproductive period. The two populations reached its peak of reproductive effort in May 2014, represented by the highest values of GI (Olhão: mean GI= 3.4; Lagos: mean GI = 4.6)

(Figure 3.3, 3.4 and 3.5). Other GI peaks were observed, in February, April and July 2015 in Olhão and in July 2014 and February 2015 in Lagos, but all with lower intensity (Olhão: mean GI February = 2.65, mean GI April = 2.15, mean GI July = 1.8; Lagos: mean GI July = 3.2; mean GI February = 2.15). Spawning occurred during almost all sampling period, in both populations, however, with more intensity from July to December 2014, in Olhão and from August 2014 to January 2015, in Lagos, coinciding with the SST increase. This spawning represents the discharge of the mature gametes, nevertheless, during this study, *M. galloprovincialis* did not show a continuous gonad development, and after this spawning period (stage III B - Table 2.1) mussels did not progress to an inactive stage. Indeed, the microscopic examinations of the gonadal tissues, revealed that after stage III B, mussels showed regeneration of the germ cells where occurred the formation of new gametes. This reproductive stage of *M. galloprovincialis* was considered as regeneration stage (stage III C - Table 2.1), and was registered mainly during June 2014, and from February to June 2015, in both populations, coinciding with the end of spring when was registered a *Chlo a* increase. After this stage, other spawning episodes occurred, however in a lower intensity, where gametes produced in stage III C were discharged while occurred the degradation of gametes which were not released in stage III B. This stage was called late spawning (stage III D - Table 2.1), and occurred in both studied years and for both males and females of the two populations. In fact, mussels showed a lower reproductive activity in 2015, compared to the same period of 2014. No correlations were found between SST and GI and either between GI and *Chlo a* in both populations (Table 3.1).

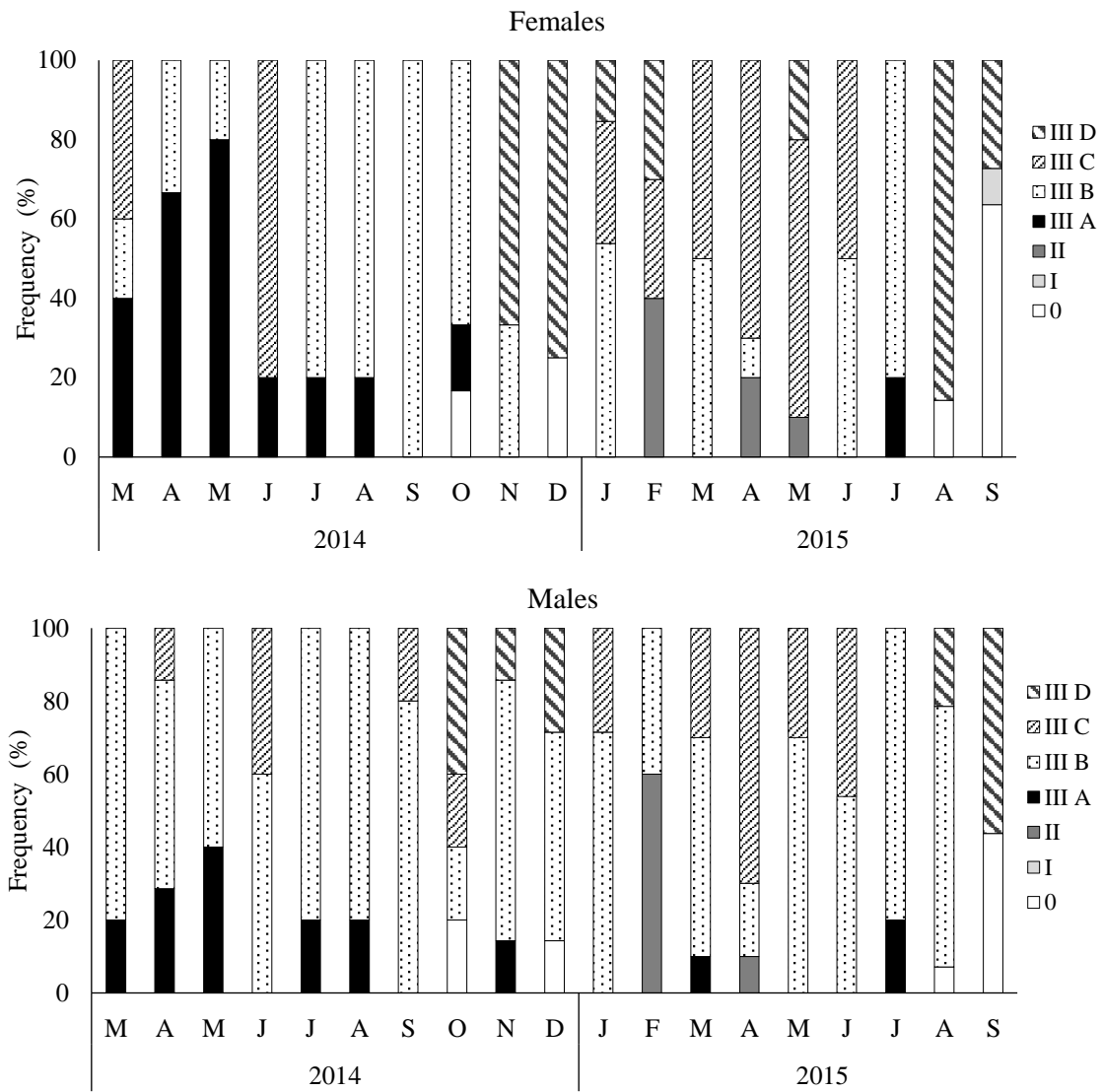


Figure 3.3. Monthly variation in gonadal development of *Mytilus galloprovincialis* obtained in Olhão during the sampling period (March 2014 to September 2015).

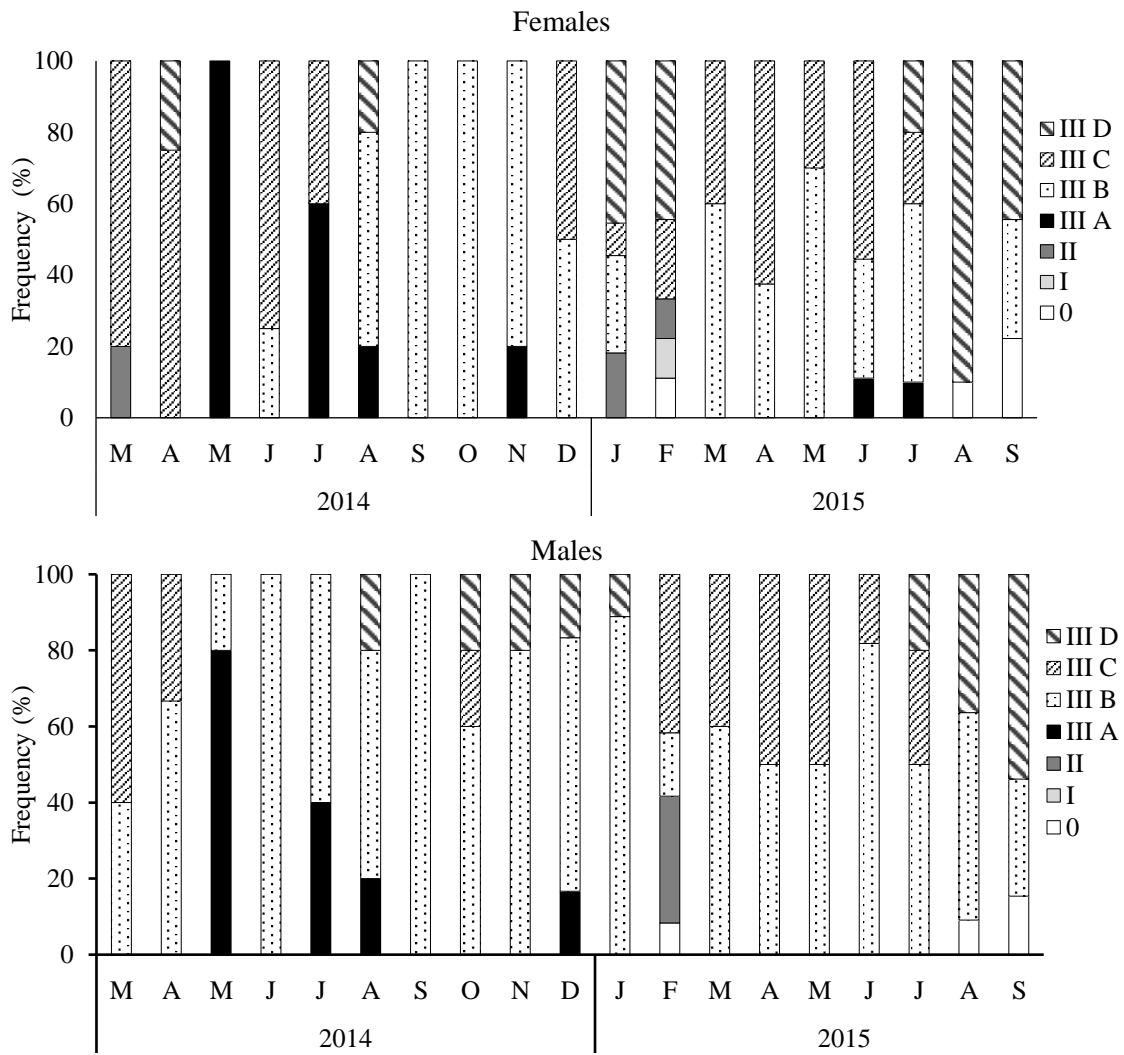


Figure 3.4. Monthly variation in gonadal development of *Mytilus galloprovincialis* obtained in Lagos during the sampling period (March 2014 to September 2015).

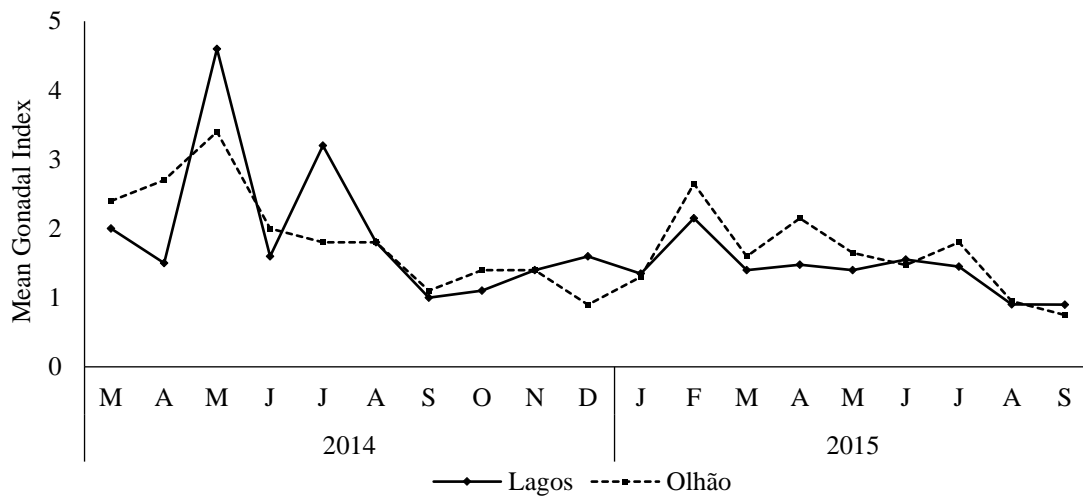


Figure 3.5. Monthly variation in gonadal index (GI) (mean, n= 10) of *Mytilus galloprovincialis* from Olhão and Lagos during the sampling period (March 2014 to September 2015).

3.3. Condition index

No statistical significant differences were found in condition index (CI) between males and females of both populations, so the results are presented for both sexes, simultaneously (Figure 3.6). CI followed the same pattern in both sampling sites, statistically significant differences were observed between populations (ANOVA, $P < 0.001$) and in each population between years (K-W., $P < 0.001$) (Figure 3.6). A higher condition index was evident in 2014 compared to 2015, in both populations. During 2014, CI of both populations generally trended upwards until September, reaching at this month the highest values of the sampling period (Olhão: 17.8 ± 0.9 ; Lagos: 21.3 ± 1.6), coinciding with the highest SST values in this sampling year. In the followings months, CI decrease sharply until January and February 2015 in Lagos and Olhão, respectively, following the SST decrease, reaching at these months the lowest CI values (Olhão: 6.2 ± 1.4 ; Lagos: 6.2 ± 1.6), being coincident, in Lagos, with lower SST during all study. After that, this parameter showed lower variability since March until July, in Olhão and until the end of the sampling period, in Lagos. However, it was registered, in Olhão, a slight increase in March and between April and July, for both populations. A positive correlation was observed between CI and SST only in Lagos ($r_{\text{Spearman}} = 0.67$, $p < 0.005$). In spite of no correlations were found between CI and GI, it was observed that CI was higher during the spawning period. No correlations were found between CI and *Chlo a* in any population.

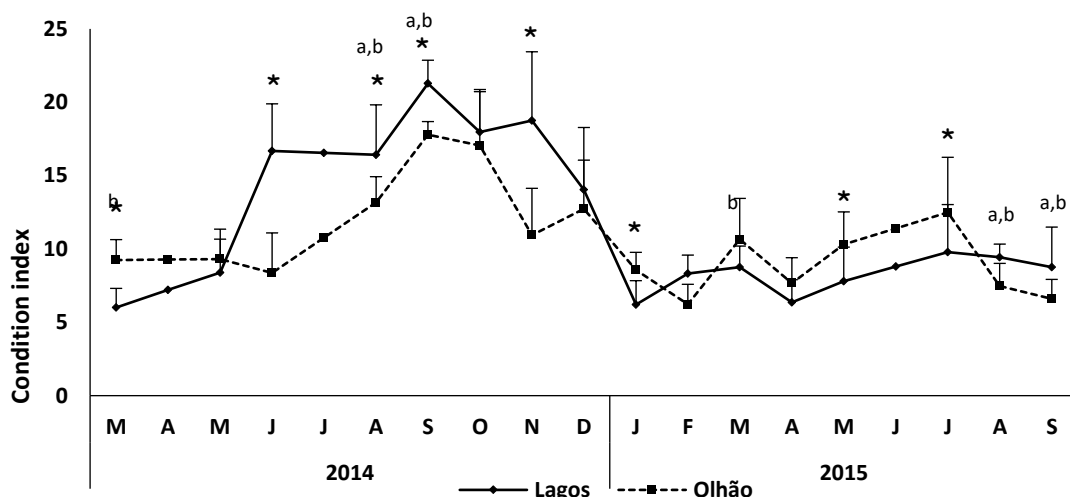


Figure 3.6. Monthly variation in condition index (CI) (mean \pm SD, n= 10) of *Mytilus galloprovincialis* from Olhão and Lagos during the sampling period (March 2014 to September 2015). (*statistically significant differences, $P < 0.05$ found between populations; ^{a, b} between 2014 and 2015 in Olhão and Lagos, $P < 0.001$).

3.4. Biochemical composition

Proteins were the predominant dry tissue constituent of the mussels followed by total lipids and glycogen (Table 3.2). The highest protein content values were recorded in Olhão at March 2015 ($442.9 \pm 76.7 \mu\text{g mg}^{-1}$ AFDW) and in Lagos at August 2015 ($438.5 \pm 72.1 \mu\text{g mg}^{-1}$ AFDW) and the lowest in March 2014 (Olhão: $183.9 \pm 38.6 \mu\text{g mg}^{-1}$ AFDW) and August 2014 (Lagos: $220.1 \pm 50.3 \mu\text{g mg}^{-1}$ AFDW). Significant statistical differences were found between populations, (ANOVA, $P < 0.001$), especially in March, June, September, November and December 2014 and in March, April and September 2015, and in each population between years (Olhão: ANOVA, $P < 0.001$; Lagos: K-W., $P < 0.001$) (Table 3.2). In general, this content showed an increasing trend during this study in both populations and no seasonal variations were observed. Glycogen is the biochemical component with major fluctuations over the sample period and significant statistical differences were observed between populations, namely in May, August, November, December 2014 and in February, April, July and August 2015 (ANOVA, $P < 0.001$) (Table 3.2) and between sampling years in Olhão (ANOVA, $P < 0.001$). Glycogen content showed lower values since the beginning of this study until August 2014. An increase of this content was observed after this month, in both places, reaching in September and November 2014 the highest values, Lagos ($92.6 \pm 32.8 \mu\text{g mg}^{-1}$ AFDW) and Olhão ($111 \pm 36.5 \mu\text{g mg}^{-1}$ AFDW), respectively,

coinciding with the SST increase verified in this months, however only in Lagos a positive correlation was verified between these two parameters ($r_{\text{Spearman}} = 0.56$, $P < 0.05$). In the followings months, this content suffered a sharply decrease in both populations until April 2015, coinciding with CI decrease, revealing a positively correlation between glycogen and CI in in both sampling sites (Olhão: $r_{\text{Pearson}} = 0.62$, $P < 0.01$; Lagos: $r_{\text{Spearman}} = 0.62$, $P < 0.05$) (Table 3.1). The lowest glycogen values were reached in August 2014 in Olhão ($10.0 \pm 5.5 \mu\text{g mg}^{-1}$ AFDW) and in March 2014 in Lagos ($12.6 \pm 9.1 \mu\text{g mg}^{-1}$ AFDW), coinciding with the highest reproductive effort of both populations, however, only in Lagos a negative correlation was found between GI and glycogen ($r_{\text{Spearman}} = -0.64$, $P < 0.05$). The highest total lipid values were registered in January 2015, (Olhão: $129.9 \pm 63.1 \mu\text{g mg}^{-1}$ AFDW; Lagos: $141.6 \pm 61.9 \mu\text{g mg}^{-1}$ AFDW), while the lowest were detected in September ($50.4 \pm 8.4 \mu\text{g mg}^{-1}$ AFDW) and May 2015 ($63.1 \pm 18.7 \mu\text{g mg}^{-1}$ AFDW), in Olhão and Lagos respectively (Table 3.2). Significant statistical differences (ANOVA, $P < 0.001$) were found between populations in this content only in March 2014 and July and September 2015 (Table 3.2). Indeed, such as glycogen, total lipids presented some pronounced fluctuations over the sampling period, highlighting the sharply increase verified in January 2015 in both populations, however, no statistical significant differences were obtained for total lipids between sampling years, and no correlations were found between this content and any other parameter analyzed, except a positive correlation between total lipids and total energy in Olhão ($r_{\text{Pearson}} = 0.5$, $P < 0.05$) (Table 3.1). Total energy reached the highest values in January 2015 in both populations (Olhão: 11.9 KJ mg AFDW ; Lagos: 12.4 KJ mg AFDW), while the lowers values were obtained in March and June 2014, (5.8 KJ mg AFDW , 7.9 KJ mg AFDW) in Olhão and Lagos, respectively. Significant statistical differences in total energy were observed between populations (ANOVA, $P < 0.05$) and in each population between sampling years (ANOVA, $P < 0.001$). Proteins contributed the most to the total energy content in both populations, followed by total lipids in Olhão, which explains the positive correlations found between total energy and proteins in both populations (Olhão: $r_{\text{Pearson}} = 0.76$, $P < 0.001$; Lagos: $r_{\text{Pearson}} = 0.83$, $P < 0.001$), and between total energy and total lipids in Olhão as previously mentioned (Table 3.1).

Table 3.1. Results of Pearson (p) and Spearman (s) correlation between studied parameters (r, correlation coefficient; P - P value; n.c. - no correlation was found).

	<i>Olhão</i>							<i>Lagos</i>						
	<i>SST</i>	<i>GI</i>	<i>CI</i>	<i>Proteins</i>	<i>Glycogen</i>	<i>Total lipids</i>	<i>Total energy</i>	<i>SST</i>	<i>GI</i>	<i>CI</i>	<i>Proteins</i>	<i>Glycogen</i>	<i>Total lipids</i>	<i>Total energy</i>
<i>Chlo a</i>	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	$r_s = -0.50,$ $P < 0.05$	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
<i>SST</i>		n.c.	n.c.	n.c.	n.c.	n.c.	n.c.		n.c.	$r_s = 0.67,$ $P < 0.05$	n.c.	$r_p = 0.56,$ $P < 0.05$	n.c.	n.c.
<i>GI</i>			n.c.	n.c.	n.c.	n.c.	n.c.			n.c.	n.c.	$r_s = -0.64,$ $P < 0.05$	n.c.	$r_s = -0.59,$ $P < 0.05$
<i>CI</i>				n.c.	$r_p = 0.62,$ $P < 0.05$	n.c.	n.c.				n.c.	$r_s = 0.62,$ $P < 0.05$	n.c.	n.c.
<i>Proteins</i>					n.c.	n.c.	$r_p = 0.76,$ $P < 0.001$					n.c.	n.c.	$r_s = 0.82,$ $P < 0.001$
<i>Glycogen</i>						n.c.	n.c.						n.c.	$r_s = 0.50,$ $P < 0.05$
<i>Total lipids</i>							$r_p = 0.50,$ $P < 0.05$							n.c.

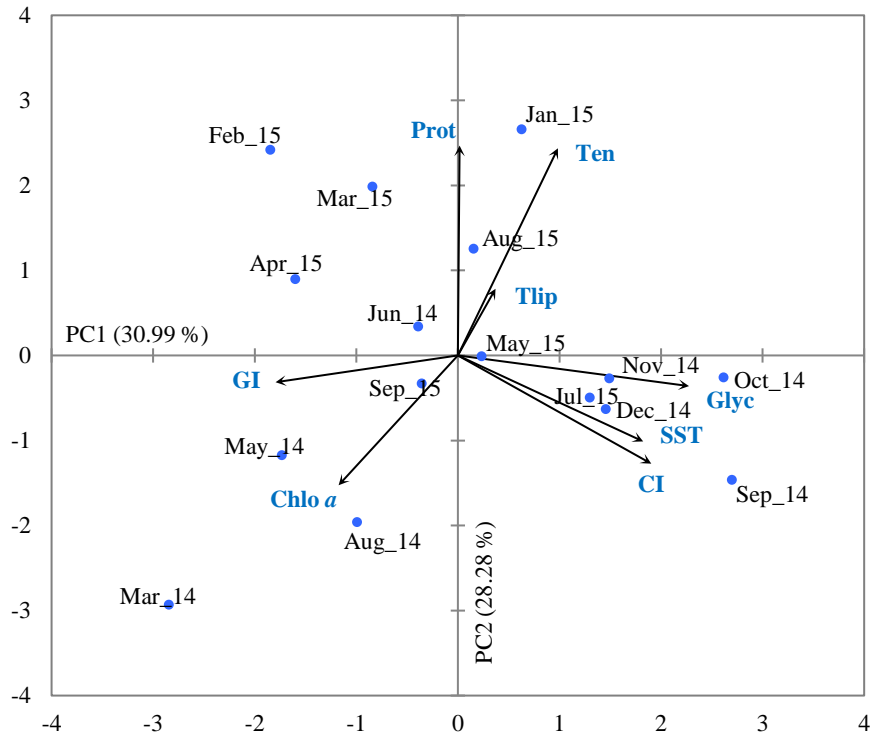
Table 3.2. Mean values (\pm SD) of proteins, total lipids, glycogen ($\mu\text{g mg}^{-1}$ AFDW) and total energy (KJmg^{-1} AFDW) of *Mytilus galloprovincialis* from Olhão and Lagos during the sampling period (March 2014 to September 2015). (*statistically significant differences, $P < 0.05$ found between populations; ^{a, b} between 2014 and 2015 in Olhão and Lagos, $P < 0.001$; ** insufficient number of sampling individuals for biochemical contents evaluation).

Year	Month	Olhão				Lagos			
		Protein ($\mu\text{g mg}^{-1}$ AFDW)	Glycogen ($\mu\text{g mg}^{-1}$ AFDW)	Total lipids ($\mu\text{g mg}^{-1}$ AFDW)	Total energy (kJ mg^{-1} AFDW)	Protein ($\mu\text{g mg}^{-1}$ AFDW)	Glycogen ($\mu\text{g mg}^{-1}$ AFDW)	Total lipids ($\mu\text{g mg}^{-1}$ AFDW)	Total energy (kJ mg^{-1} AFDW)
2014	Mar	183.9 \pm 38.6 ^{*a}	18.6 \pm 11.5	60.4 \pm 17.6 [*]	5.8 ^a	306.5 \pm 78.1 ^{*b}	12.6 \pm 9.1	89.8 \pm 24.9 [*]	8.9 ^b
	**Apr	---	---	---	---	---	---	---	---
	May	221.9 \pm 33.4 ^a	38.4 \pm 26.6 [*]	104.9 \pm 46.2	8.4 ^a	285.3 \pm 77.2 ^b	12.8 \pm 5.5 [*]	81.8 \pm 24.3	8.2 ^b
	Jun	309.5 \pm 64.6 [*]	42.9 \pm 25.8	88.2 \pm 49.1	9.4	239.2 \pm 82.2 [*]	43.1 \pm 23.4	82.3 \pm 42.2	7.9
	**Jul	---	---	---	---	---	---	---	---
	Aug	215.6 \pm 77.3 ^a	10.0 \pm 5.5 ^{*a}	110.6 \pm 60.5	7.9 ^{*a}	220.1 \pm 50.3 ^b	20.9 \pm 14.5 [*]	111.3 \pm 56.9	8.2 [*]
	Sep	240.6 \pm 70.9 ^{*a}	88.7 \pm 34.9 ^a	106.8 \pm 53.9	9.6 [*]	317.8 \pm 91.0 ^{*b}	92.6 \pm 32.8	99.1 \pm 45.2	10.8 [*]
	Oct	334.3 \pm 99.9	88.8 \pm 48.5	87.1 \pm 42.4	10.6 [*]	353.6 \pm 95.7	72.9 \pm 20.9	94.5 \pm 40.8	10.9 [*]
	Nov	260.1 \pm 57.4 [*]	111.0 \pm 36.5 [*]	100.4 \pm 40.5	10.1	370.7 \pm 135.1 [*]	55.3 \pm 23.1 [*]	88.0 \pm 43.1	10.7
	Dec	260.3 \pm 122.6 [*]	72.9 \pm 33.8 [*]	68.4 \pm 17.6	8.3	420.9 \pm 133.9 [*]	39.9 \pm 32.4 [*]	70.9 \pm 34.9	10.8
2015	Jan	355.5 \pm 89.7	54.4 \pm 30.5	129.9 \pm 63.1	11.9 [*]	361.6 \pm 108.6	52.8 \pm 21.5	141.6 \pm 61.9	12.4 [*]
	Feb	381.7 \pm 118.7	19.1 \pm 15.6 [*]	100.1 \pm 52.6	10.7	424.8 \pm 79.8	30.4 \pm 15.0 [*]	112.8 \pm 75.2	12.1
	Mar	442.9 \pm 76.7 ^{*a}	21.8 \pm 7.5	82.4 \pm 25.0	11.3 ^a	364.4 \pm 68.9 ^{*b}	23.8 \pm 6.4	82.8 \pm 33.8	9.9 ^b
	Apr	393.7 \pm 48.5 [*]	13.0 \pm 8.3 [*]	78.7 \pm 14.9	10.0	316.6 \pm 27.7 [*]	21.9 \pm 6.6 [*]	85.1 \pm 41.2	9.1 ^b
	May	322.1 \pm 59.3 ^a	34.0 \pm 20.2	70.2 \pm 32.4	8.9 ^{*a}	336.9 \pm 65.4 ^b	43.8 \pm 23.8	63.1 \pm 18.7	9.0 [*]
	**Jun	---	---	---	---	---	---	---	---
	Jul	323.4 \pm 75.1	58.6 \pm 29.7 [*]	67.8 \pm 56.7 [*]	9.2	325.4 \pm 48.4	31.1 \pm 16.7 [*]	80.4 \pm 17.4 [*]	9.2
	Aug	399.8 \pm 67.9 ^a	27.7 \pm 13.9 ^{*a}	83.2 \pm 28.2	10.6 ^a	438.5 \pm 72.1 ^b	49.5 \pm 18.5 [*]	82.5 \pm 20.8	11.7 ^b
	Sep	332.8 \pm 54.8 ^{*a}	25.9 \pm 10.4 ^a	50.4 \pm 8.4	8.2 [*]	403.4 \pm 90.9 ^{*b}	23.6 \pm 17.3	94.3 \pm 34.5 [*]	10.9 [*]

3.4. Principal component analysis (PCA)

The results obtained from PCA's were in accordance with correlations identified by Spearman's (Lagos) and Pearson's (Olhão) coefficients. This PCA's analysis, provided some additional relationships which complement data previously observed. The PCA's results led us to two principal components (PC1 and PC2), that together accounted for 59.3% (Olhão) (Figure 3.7a) and 65.7% (Lagos) (Figure 3.7b), of the overall variability of the data.). In Olhão, the PCA biplot illustrates that CI, SST and glycogen (Glyc) content are positively correlated between themselves. Positive correlations are also found between total energy (Ten) and proteins (Prot), total energy (Ten) and total lipids (Tlip). In contrast, Chlo *a* are negatively correlated with total energy (Ten) and GI with SST. In Lagos, PCA biplot illustrate the same positive correlations observed in Olhão PCA. Also, glycogen (Glyc) content correlated positively with total energy (Ten). Negative correlations were found between SST and Chlo *a*, GI and proteins (Prot), as well as between GI and glycogen content (Glyc) and GI and total energy (Ten). No seasonal variation was found between the study parameters.

a)



b)

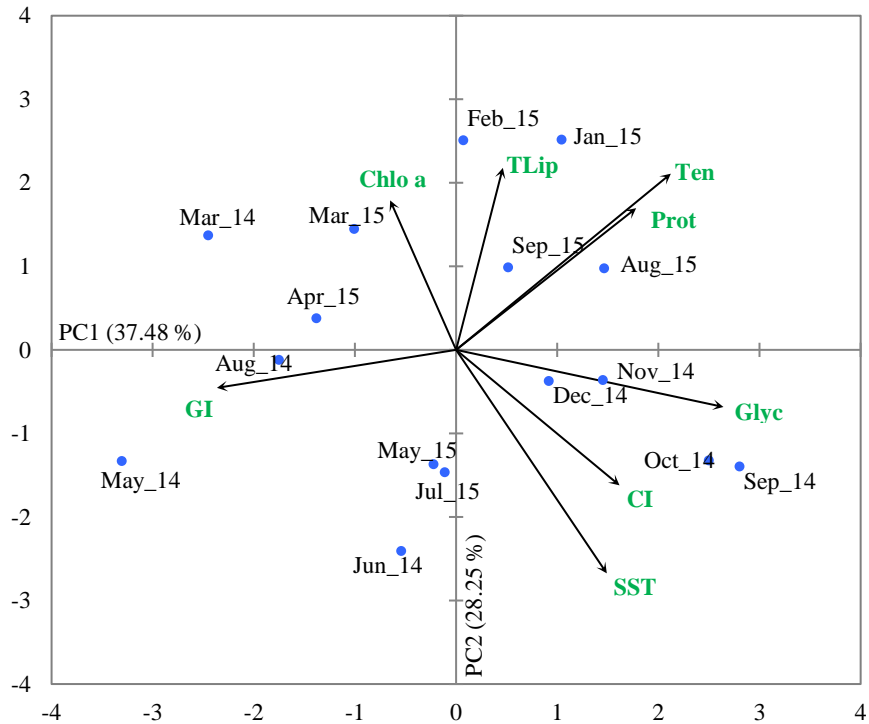


Figure 3.7. Principal component analysis (PCA) on the parameters used to characterize the reproductive cycle of *Mytilus galloprovincialis* from two sampling places: a) Olhão and b) Lagos. Each vector represents one of the parameters analyzed (SST - Sea surface temperature; Chlo a - chlorophyll a; GI- Gonadal index; Prot- Proteins; Glyc- Glycogen; Tlip- Total lipids; Ten- Total energy) and each point represents the sampling month.

4. Discussion

The reproductive activity of bivalves is controlled by the interaction of endogenous (Normand *et al.*, 2008; Enríquez-Díaz *et al.*, 2009) and exogenous factors, mainly quality and food availability and temperature (Chávez-Vilalba *et al.*, 2003, Matias *et al.*, 2013). Geographical locations are closely related with temperature, who indirectly affect the availability of food, and/or consequently the timing and duration of the reproductive cycle and number of spawning per year. Indeed, Seed (1976) and Sastry (1979) related that water temperature and food availability are among the main exogenous factors controlling the gonadal cycle of mussels. Wide differences have been recorded in the temporal pattern of gonad development of *M. galloprovincialis* in different geographical areas (Seed, 1976; Lubet *et al.*, 1981; Da Ros *et al.*, 1985). In our study, seawater temperature follows the same pattern in both sampling places. It was expected that SST variation was similar to the typical pattern of temperate climate, however, in spite temperature increased during spring in both sites, in summer a slightly decrease was registered until August, which is more accentuated in Lagos. SST increased again until mid-autumn, revealing after that a decrease that extends until the end of the winter where was registered the lowest values in both sampling sites. Several phytoplanktonic blooms are observed during all study in both places but in Lagos these peaks are much more pronounced, reaching far higher values. The Algarve coast, more particular the western part, is subject to seasonal and occasional phenomena of upwelling (Loureiro *et al.*, 2005) bringing to surface waters rich in nutrients biologically regenerated in depth (Roff and Zacharias, 2011), contributing to the increase in primary production and providing food for filter-organisms, such as mussels, who can explain this accentuated chlorophyll peaks in Lagos.

In this study, some synchronism was revealed in the sequence of gametogenic stages of *M. galloprovincialis* from both sampling sites, with a lower reproductive activity in 2015 than in the same period of 2014. In spite of the observed differences in SST and Chlo *a* between sampling sites, those were not significantly reflected in the reproductive cycle of mussels, since no relationship was found between these environmental parameters and GI. Orban *et al.* (2002) observed the occurrence of the year-to-year variability of the climatic conditions, and consequently of the phytoplankton populating the sea water and of mussels gametogenic cycle in *M. galloprovincialis* harvested in two Italian sites. Nevertheless, in this study, the lower reproductive activity of mussels found in 2015, were

not the reflection of the studied environmental parameters, since no significant differences were found between years.

In our study, the reproductive activity does not follow a seasonal cycle. The beginning of gametogenesis seems to occur during January/February for a small percentage of mussels from both studied places, with the proliferation of gonias and formation of new follicles, coinciding with the onset of the higher values of *Chlo a* and SST. Both populations presented their higher reproductive effort in late spring 2014, where was observed the highest percentage of individuals in ripe stage. During 2014, only in Lagos, another reproductive peak was observed during summer, in July, while in Olhão the percentage of mussels who reach this stage was insignificant. In 2015 there was not a ripe stage season, and the percentage of individuals in this stage was very low. There was not observed a specific spawning period for the two populations. Mussels were in spawning during all sampling months, however there was observed an intensive spawning period, during summer. This was observed in both sampling sites, and the beginning of spawning coincide with the onset of SST. During this period, both populations have the capacity to regenerate their gonads, being constant and successively in spawning and in gonadal recovering. Indeed, mussels did not reveal a continuous gonadal development, and after a first spawning, gonads did not progress to an inactive stage, were all individuals were in sexual rest, in fact they showed a regeneration of the germ cells where occur the formation of new gametes. Mussels are successively in gonadal recovering and spawning during almost all the studied period. The spawning intensity decrease during autumn in both populations, coinciding with SST decrease. Effectively, both populations never reached the full rest. There were few months in which individuals were in sexual rest, and in a small percentage, except in September 2015 when the majority of mussels from Olhão were in this stage. The inexistence of a specific inactive phase of gonadal development of a *M. galloprovincialis* population was also observed by Bhaby *et al.* (2014). In the Mediterranean and in the Atlantic coast of Spain, gametogenesis of this species starts during autumn and early winter, followed by ripe and spawning seasons during late spring and summer (Villalba 1995, Cáceres-Martínez and Figueras 1998, Bodin *et al.*, 2004). Similarly, in Pacific coast of Mexico, the reproductive season occurs from autumn to early spring (Cáceres-Martínez, 2004). Also, Okaniwa *et al.* (2010) have described only a spawning period for *M. galloprovincialis* in Tokyo Bay. Few authors have defined such a prolonged spawning season for this species, although Schurink and Griffiths (1991)

showed that in South Africa, *M. galloprovincialis* spawned over two extended periods, during summer and winter. Bhabu (2015), revealed that in Cape Beddouza in Morocco, *M. galloprovincialis* were characterized by a synchronous gametogenesis broadly overlapping reproductive cycle, with gametogenesis taking place over the entire year.

The maintenance of a synchronized gonadal development observed between males and females ensures the reproductive success of the species since sperm and oocytes will be expelled into the water simultaneously increasing the probability of fertilization. In both study areas, gametes are released into the water column during almost all study period, being this an advantageous reproductive strategy for the species. Indeed, histological analyses showed variable proportions of fully matured gametes and restoration of germ cells with the formation of new gametes. This high capacity for gonadal regeneration had also been previously observed in *Ruditapes decussatus* (Matias *et al.*, 2013) and in *Venerupis senegalensis* (Joaquim *et al.*, 2011).

Reproductive activity of bivalves is generally reflected by condition index (Fernández-Castro and Vido-de-Mattio, 1987; Massapina *et al.*, 1999; Ojea *et al.*, 2004). A positive correlation between these two parameters has been observed in several bivalve species from the Portuguese coast (Gaspar and Monteiro, 1998; Joaquim *et al.*, 2011; Moura *et al.*, 2008). It is widely assumed that mussels CI varied as a function of food availability and reflected the different stages of the reproductive cycle (Pérez-Camacho *et al.*, 1995; Stirling and Okumus, 1995; Cheshuk *et al.* 2003). However, in this study, no correlations were found between CI and GI in any of the two sampling places and in spite of CI follows the same pattern than SST in both sampling places, only in Lagos a positive correlation was found between these two parameters. In 2014, CI of both populations followed the SST increase until May and still to upward until September, although the observed SST decrease from June until August. The highest CI was reached in September 2014, coinciding with highest SST and with a phytoplankton bloom registered in the previous month. After that, CI decreased until late winter. The values of CI found for mussels in 2014 from both sampling sites were similar to those found by Orban *et al.* (2002). In 2015, a slightly CI increase is verified with the SST and *Chlo a* onset, however CI values reached were much lower than in the same period of 2014 since mussels have not achieved the ripeness stage such as in 2014.

Several studies have reported that CI is highly influenced by the energy storage and exploitation strategy of bivalve species (Delgado and Pérez-Camacho, 2005; Joaquim *et al.*, 2011). In other hand, bivalves' sexual maturity is related to energy supply from previously stored reserves or the ingestion of available food and consequently is closely linked with the biochemical composition (Sastry, 1979; Pérez Camacho *et al.*, 2003). During gonadal maturation, marine bivalves showed changes in biochemical composition related to energy storage and utilization, for gamete production (Barber and Blake, 1981, 1985; Ruiz *et al.*, 1992; Ojea *et al.*, 2004). In general, energy is stored prior to gametogenesis in the form of glycogen, proteins and lipids in the mantle and the digestive gland. Glycogen and proteins are used in reproductive organ formation and gamete proliferation, while lipids are very important energy reserves in the oocytes, assuring the viability of larvae (Whyte *et al.*, 1991; Napolitano *et al.*, 1992; Leonardos and Lucas, 2000). Several authors have reported maximum glycogen content immediately preceding, and during gamete proliferation (Kopp *et al.*, 2005; Dridi *et al.*, 2007; Pronker *et al.*, 2008).

The relative amounts of proteins (Olhão - 184 to 443 $\mu\text{g mg}^{-1}$ AFDW; Lagos - 220 to 439 $\mu\text{g mg}^{-1}$ AFDW) and glycogen (Olhão - 10 to 111 $\mu\text{g mg}^{-1}$ AFDW; Lagos - 13 to 93 $\mu\text{g mg}^{-1}$ AFDW) measured in *M. galloprovincialis* were lower, in term of the proportions, to those previously described in the literature for this specie, however the amount of total lipids (Olhão - 50 to 130 $\mu\text{g mg}^{-1}$ AFDW; Lagos - 63 to 142 $\mu\text{g mg}^{-1}$ AFDW) was similar (Orban *et al.*, 2002; Celik *et al.*, 2014, Oliveira *et al.*, 2015). During all study, glycogen was the biochemical compound who presents the major fluctuations. Orban *et al.* (2002) showed similar fluctuations on glycogen content of *M. galloprovincialis* reared in longlines in two different Italian sites. Usually, glycogen is used as an energy source during gametogenic development, who could explain this variation once both populations are in constant gonadal recovering with constantly formations of gametes, using the stored glycogen reserves. In Lagos, this fact was confirmed statistically by the negative correlation found between glycogen and GI. Glycogen was also correlated with CI, following the same pattern in both populations. Although CI does not reflect the reproductive cycle, this relationship revealed that CI reflect the glycogen reserves accumulation. A sharp increase of this content is verified in September 2014 in both populations and generally decreased during autumn and winter in Lagos. In Olhão, the glycogen still increase until November 2014 and suffered a decline who extends until

April 2015. A positive correlation was found between glycogen and SST in both populations. Several authors have suggested that proteins in bivalves are used as an energy reserve in situations of nutritional stress and energy imbalance or during gonadal maturation (Gabbott and Bayne, 1973; Liu *et al.*, 2008). Moreover, it has also been suggested that some species use proteins as a source of energy maintenance when carbohydrate reserves have already been depleted (Albentosa *et al.*, 2007; Joaquim *et al.*, 2011; da Costa *et al.*, 2012). In this study, proteins content increase during all sampling study, who suggest that mussels resort firstly to glycogen content to ensure their maintenance/growth and for the gametogenesis and gonadal development, revealing that mussels were in good physiological condition. Mussels seem to have the ability to recover quickly the reserves during spawning, when the SST and food availability (chlorophyll) are still high. These reserves seem to be harnessed to maintain their physiological state and to assure the gonadal recovering simultaneously. The higher glycogen value was reached in autumn, when both populations are mainly in spawning.

Analysis of data revealed that mussels store glycogen when food is abundant for further use in gametogenesis thus allowing the spawning release when seawater temperature increase. In fact, glycogen tended to correlate negatively with GI in both sites, which is typical for a conservative species (Joaquim *et al.*, 2011). This relationship was more evident in Lagos where the phytoplanktonic blooms were more pronounced. The several peaks of food abundance in both places allowed the constant and consecutive gonadal regeneration and spawning.

Several authors (Beninger and Lucas 1984; Ojea *et al.*, 2004; Mouneyrac *et al.*, 2008) have reported that lipid seasonal variations are inversely related to glycogen owing to the conversion of glycogen into lipids, biosynthesized during the formation of gametes (Gabbott, 1975). Pazikowska and Szaniawska (1988) showed that the highest lipid content of *M. edulis* (Gulf of Gdansk) was noted during a period of reproduction, who could be associated to the conversion of stored glycogen into lipid material for maturing eggs (Pieters *et al.*, 1979; Zandee *et al.*, 1980). In conservative species, this process is detected by a negative correlation between total lipids and glycogen content, since the reserve accumulation lags behind gametogenesis (Joaquim *et al.*, 2015). In our study, no correlations were found between total lipids and glycogen probably due to the constantly spawning and gonadal recovering periods. Protein was the biochemical constituent that

most contributed to the total energy of *M. galloprovincialis* from both sites, however the negative relationship found between total energy and GI, and glycogen and GI in Lagos, may reflect the importance of glycogen as energetic reserve in the reproductive cycle.

In conclusion, the results of this study show that mussels of both sampling sites have an extended spawning period during which occurs successive spawning and gonadal recovering with a decrease intensity of gametes production during the year. This mussel capacity is an added value for the implanted mussel farms in the studied areas, since the recruitment of the species can assure the longlines colonization for most of the year. Indeed, mussel's quality is assessed by the consumers as a result of gonadal development, the fuller the gonad is, the higher commercial value. In spite the high gonadal development along almost all the year, the condition index and the reproductive cycle of *M. galloprovincialis* produced in these two areas suffered a year-to-year variability, during the experimental period. In fact, mussels showed a significant better condition in 2014, than in 2015. It is widely accepted that these differences can be correlated to changes in environmental conditions, however in our study, temperature and Chlo *a* did not allow explain them. The high incidence of toxic phytoplanktonic blooms reported along the Algarve coast during 2015² could probably have affected the mussel physiological condition and reproductive cycle. Gainey and Shumway (1988) and Larsson *et al.* (2014) observed a reduction in soft body mass increase and gonad build-up of blue mussel, as a consequence of the reduction of filtering capacity in presence of toxic phytoplanktonic cells. The knowledge achieved in this study can be an important contribution to increase farms profitability, since this information will allow a better production management and the adjustment of the marketing needs.

²Source: www.ipma.pt/pt/pecas/bivalves/historico/biotox/index.jsp, accessed in 30/11/2016.

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Attachments

Attachment 1

Poster communication in XV Congresso Nacional Y I Congresso Ibérico de Acuicultura: **Matias, A.M.**, Matias D., Joaquim, S., Moura P., Gaspar, M. B. 2015. Avaliação da estratégia reprodutiva do mexilhão *Mytilus edulis* produzido em *longlines* em duas zonas da costa algarvia. XV Congresso Nacional de Acuicultura e I Congresso Ibérico de Acuicultura 2015, 13 a 16 de outubro, Huelva;

Avaliação da estratégia reprodutiva do mexilhão *Mytilus edulis* produzido em longlines em duas zonas da costa algarvia

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Abstract

The evaluation of the *Mytilus edulis* reproductive strategy, reared in long-lines system, in two areas along the Algarve Coast, (Olhão and Lagos), was performed through the characterization of the condition index and biochemical composition (proteins, total lipids and glycogen). The knowledge of the reproductive performance of this species will contribute to maximize the production of the aquaculture companies. In this study, the preliminary results suggest the existence of a similar reproductive strategy in both areas. The onset of gametogenesis seems to occur in June, reaching a peak in September, which could be associated to the increase of seawater temperature, an important factor in the regulation and energy storage used in the reproductive process in bivalves. An extended spawning period was observed until January.

Resumo

A estratégia reprodutiva da espécie *Mytilus edulis* produzida em sistemas long-lines em duas zonas (Olhão 36°01,007'N e 07° 44,499'W e Lagos 37°04,200'N e 8°42,800'W) da costa algarvia foi avaliada através da caracterização do índice de condição e da composição bioquímica dos indivíduos. O conhecimento da performance reprodutiva da espécie, contribuirá para maximizar a produção das empresas. De uma forma geral, os resultados sugerem que os mexilhões apresentaram uma estratégia reprodutiva semelhante nas duas zonas. Os parâmetros analisados indicaram que o início da gametogénese ocorreu em Junho, atingindo o pico em Setembro, o que poderá estar associado ao aumento da temperatura, que no caso dos bivalves é um dos fatores importantes na regulação e armazenamento de energia utilizada no processo reprodutivo. A espécie apresentou um período alargado de desova que se estendeu até Janeiro.

Avaliação da estratégia reprodutiva do mexilhão *Mytilus edulis* produzido em longlines em duas zonas da costa algarvia

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Figura 1. Zonas de investigação na costa algarvia.

Introdução

O mexilhão é uma espécie com um crescente interesse comercial em Portugal. Nos últimos anos tem-se assistido à implementação de várias empresas em *offshore*, em especial na costa algarvia que, tem vindo a apostar nesta espécie. A reprodução é um processo que afeta a condição fisiológica dos bivalves tendo uma influência direta na qualidade do produto final. O conhecimento da estratégia reprodutiva da espécie permitirá gerir a produção e ajustar os *strategies* de comercialização, por forma a rentabilizar as empresas. Neste estudo avaliou-se, a composição bioquímica e o índice de condição de mexilhões provenientes de duas zonas de produção da costa algarvia (Olhão e Lagos). Esta análise possibilita a avaliação da estratégia reprodutiva da espécie face às condições hidrográficas da região.

Material e métodos

As amostras de *Mytilus edulis* foram colhidas mensalmente, em duas zonas (Olhão e Lagos) da costa algarvia, desde Março de 2014 até Fevereiro de 2015. O índice de condição (IC) foi determinado, em 10 indivíduos, de acordo com Wilce & Mann (1975): [peso seco da carne livre de cinzas (AFDW) (g)/ peso seco da concha (g)]*100. A determinação da composição bioquímica foi obtida para 10 indivíduos/mês, sendo as proteínas determinadas pelo método modificado de Lowry (Shahar et al., 1994), o glicogénio pelo método de antrona (Viles & Silveira, 1949) e os lípidos totais foram extraídos com cloroformio/metanol (Folch et al., 1957) e estimados espectrofotometricamente pelo método de Marsh & Weinstein (1966). Os valores foram expressos em percentagem de AFDW.



Resultados e discussão

De uma forma geral, os resultados obtidos permitiram verificar a existência de sincronismo na variação dos parâmetros analisados nos mexilhões provenientes das duas zonas. No entanto, foram observadas diferenças significativas no IC e na composição bioquímica (ANOVA P < 0.05) (Figura 2). De um modo geral, os mexilhões provenientes de Lagos apresentam uma melhor condição relativamente aos provenientes de Olhão. Este facto poderá estar associado a disponibilidade de alimento, que no caso dos bivalves, juntamente com a temperatura da água do mar, regula a utilização e o armazenamento de energia para o processo reprodutivo. A condição dos indivíduos foi superior no período entre agosto e dezembro em ambos os locais. A grande variabilidade do teor em glicogénio e em lípidos totais durante este período, sugere que a estratégia reprodutiva da espécie, assenta num período alargado de desova no qual ocorre em simultâneo a produção de gametas.

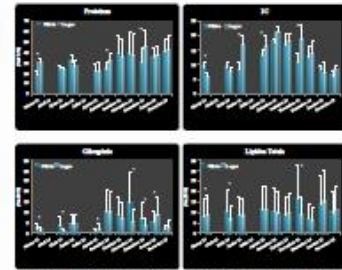


Figura 2. Tendência do índice de condição (IC), proteínas, glicogénio e lípidos totais (valor médio ± desvio padrão, n=10). * dif. estat. significativas (p < 0.05) em duas zonas de produção (Lagos e Olhão).

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Composição bioquímica e Índice de condição do mexilhão *Mytilus galloprovincialis* produzido em mar aberto na costa algarvia

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Figura 1. *Mytilus galloprovincialis*.

O mexilhão *Mytilus galloprovincialis* (Figura 1) é uma espécie com um crescente interesse comercial em Portugal. Nos últimos anos tem-se assistido à implementação de várias empresas em *offshore*, em especial na costa algarvia, que têm vindo a apostar nesta espécie, o que levou a um aumento da sua produção. O processo de reprodução afeta a condição fisiológica dos bivalves tendo uma influência direta na qualidade do produto final. O conhecimento da estratégia reprodutiva da espécie permitirá gerir a produção e ajustar os *timings* de comercialização, contribuindo para maximizar a produção das empresas. Neste sentido, foi feita uma avaliação de dois parâmetros descritores da estratégia reprodutiva em bivalves, a composição bioquímica e o índice de condição (IC), em mexilhões provenientes de duas zonas da costa algarvia (Olhão e Lagos) (Figura 2), produzidos em sistema de *longlines*. Esta análise permite avaliar a estratégia reprodutiva adotada pela espécie face às condições ambientais da região.

Foram colhidas amostras de *M. galloprovincialis* mensalmente, desde Março de 2014 até Fevereiro de 2015, de forma a avaliar o IC e a composição bioquímica (proteínas, glicogénio e lípidos totais). O IC foi determinado em 10 indivíduos/mês de acordo com Walne & Mann (1975): [peso seco da carne livre de cinzas (AFDW)(g)/peso seco da concha (g)]*100 e a composição bioquímica foi obtida para 10 indivíduos/mês, sendo as proteínas determinadas pelo método modificado de Lowry (Shakir et al., 1994), o glicogénio pelo método de antrona (Viles & Silverman, 1949) e os lípidos totais foram extraídos com clorofórmio/metanol (Folch et al., 1957) e estimados espectrofotometricamente pelo método de Marsh & Weinstein (1966). Os valores foram expressos em percentagem de AFDW.



Figura 2. Zonas de amostragem na costa algarvia.

Os resultados obtidos permitiram verificar a existência de sincronismo na variação dos parâmetros analisados nos mexilhões provenientes das duas zonas, apesar das diferenças significativas observadas em alguns meses, no IC e na composição bioquímica (ANOVA $P < 0.05$) (Figura 2). De uma forma geral, os mexilhões provenientes de Lagos apresentaram uma melhor condição relativamente aos provenientes de Olhão, facto que poderá estar associado à disponibilidade de alimento no local, que no caso dos bivalves, juntamente com a temperatura da água do mar, regula a utilização e o armazenamento de energia para o processo reprodutivo. A condição dos indivíduos foi superior no período entre Agosto e Dezembro em ambos os locais. Foi também observada uma grande variabilidade do teor em glicogénio e em lípidos totais durante este período, sugerindo que a estratégia reprodutiva desta espécie assenta num período alargado de desova no qual ocorre em simultâneo a produção de gâmetas.

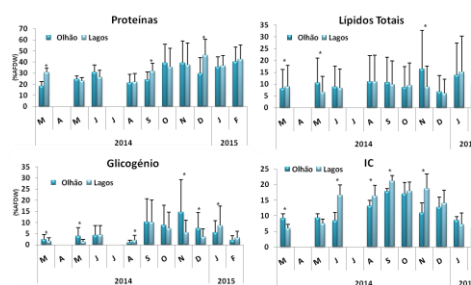


Figura 3. Variação do índice de condição e da composição bioquímica (proteínas, glicogénio e lípidos totais) (média+desvio padrão, n=10; * diferenças significativas ($p < 0.05$)) nas duas zonas de produção (Olhão e Lagos).

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Attachment 2

Participation in task 2 of the final report of the SALBIV project.

2. Estudo da ocorrência, biologia e fisiologia dos moluscos bivalves na zona do Algarve:

2.1 Avaliação da qualidade de mexilhão (*Mytilus galloprovincialis*) em sistema de produção “offshore”

- Desenho experimental

Visando a caracterização da qualidade dos organismos produzidos em sistema “offshore”, em estruturas de produção “long-line”, em dois locais da costa algarvia (Olhão e Porto de Mós), foram analisados dois parâmetros descritores da estratégia reprodutiva em bivalves: a composição bioquímica e o índice de condição de mexilhões (*Mytilus galloprovincialis*). Para tal, foram efetuadas amostragens mensais, no período de março de 2014 a setembro de 2015, na Área Piloto da Armona (APPAA) em Olhão (36°01,007’N; 07°44,499’W) (Figura 1.a) e na concessão “Algarve Offshore Seashells” em Porto de Mós (37°04,200’N; 8°42,800’W) (Figura 1.b).



Figura 1. Localização da zona de amostragem: a) Olhão - Área Piloto de Produção Aquícola da Armona APPA; b) Porto de Mós - "Algarve Offshore Seashells".

- Procedimentos analítico

Índice de condição

O índice de condição (IC) foi determinado, mensalmente, em 10 indivíduos provenientes de cada zona de produção, de acordo com o método desenvolvido por Walne & Mann (1975):

$$[\text{peso seco da carne livre de cinzas (AFDW) de carne (g) / peso seco da concha (g)}] \times 100.$$

Para a determinação do IC procedeu-se à remoção da parte edível de cada indivíduo e colocou-se em papel absorvente para retirar o excesso de água. A parte edível e a concha foram colocadas em cadinhos devidamente identificados e pesados. O peso seco da carne e da respetiva concha foi determinado após 24 horas em estufa (ULE 600 Memmert) a 80 °C. O peso das cinzas foi obtido após incineração da parte edível em mufla a 450 °C durante 24 horas. As pesagens foram efetuadas numa balança digital (Mettler AJ100) de precisão às 0,0001g.

Composição bioquímica

A avaliação dos diferentes componentes bioquímicos foi obtida, mensalmente, individualmente em 10 organismos provenientes de cada zona. A parte edível foi homogeneizada com um homogeneizador de tecidos Ultra-Turrax T25, em banho de gelo e armazenadas a - 20 °C, para posterior quantificação dos diferentes componentes bioquímicos (proteínas, lípidos totais e glicogénio). As proteínas foram determinadas através do método de Lowry et al. (1951) modificado (Shakir *et al.*, 1994), após extração com hidróxido de sódio. O glicogénio foi determinado a partir de amostra previamente seca durante 24 horas a 80 °C, utilizando o reagente de antrona (Viles & Silverman, 1949). Os lípidos totais foram extraídos com clorofórmio/metanol (Folch et al., 1957) e determinados de acordo com o método de Marsh & Weinstein (1966). As absorvências foram obtidas a 750, 620 e 375 nm, para as proteínas, glicogénio e lípidos totais respetivamente. Os valores foram expressos em percentagem de AFDW.

- Principais resultados

Índice de condição

O índice de condição reflete, de um modo geral, a condição fisiológica dos bivalves. Na Figura 2 encontra-se representada a variação do IC ao longo do período experimental. Os organismos produzidos na zona de Olhão apresentaram valores de IC que variaram entre 6.23 ± 1.36 e 17.79 ± 0.89 , observando-se os valores mais elevados nos meses compreendidos entre agosto e dezembro de 2014. Relativamente à população da zona de Porto de Mós, os valores de IC variaram entre 6.00 ± 1.31 e 21.28 ± 1.59 e os valores mais elevados foram registados nos meses entre agosto e dezembro. Em ambos os locais, o valor de IC máximo foi atingido no mês de setembro de 2014, o que poderá estar relacionado com o aumento da temperatura da água do mar. Foi observado um decréscimo nos valores de índice de condição em janeiro que se estendeu até abril. Assim, verificou-se que a qualidade dos organismos é nitidamente menor entre os meses de janeiro e abril, em ambos os locais.

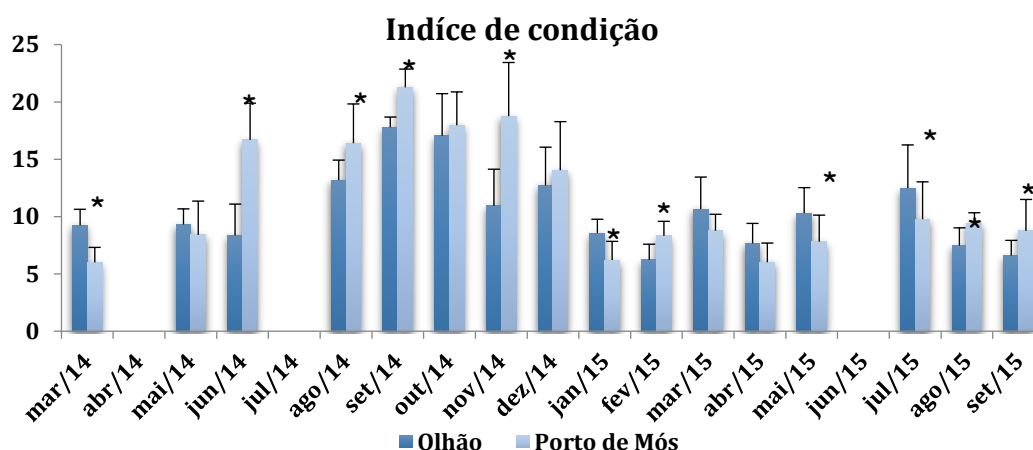


Figura 2. Variação dos valores do índice de condição nos mexilhões (*Mytilus galloprovincialis* média±DP, n=10) provenientes das duas zonas “offshore”, Olhão e Porto de Mós, ao longo do período experimental.

Composição bioquímica

A análise da Figura 3 mostra que os teores em proteínas variaram entre $18.38 \pm 3.86 \text{ mg g}^{-1}$ AFDW e $44.3 \pm 7.67 \text{ mg g}^{-1}$ AFDW na população de Olhão e entre $22.01 \pm 5.03 \text{ mg g}^{-1}$ AFDW e $43.85 \pm 7.21 \text{ mg g}^{-1}$ AFDW, na população de Porto de Mós. Os valores mais elevados deste componente foram alcançados nos meses de março e agosto de 2015, Olhão e Porto de Mós, respetivamente. Observou-se um aumento no teor de proteína a partir de janeiro, de $26.02 \pm 12.25 \text{ mg g}^{-1}$ AFDW para $35.55 \pm 8.96 \text{ mg g}^{-1}$ AFDW em fevereiro e que se estendeu até março ($44,30 \pm 7.67 \text{ mg g}^{-1}$ AFDW), seguido de um ligeiro decréscimo até maio ($32.21 \pm 5.92 \text{ mg g}^{-1}$ AFDW), nos organismos produzidos na zona de Olhão. Nos indivíduos provenientes da zona de Porto de Mós, o aumento no teor em proteína verificou-se a partir do mês de setembro, de $22.00 \pm 5.02 \text{ mg g}^{-1}$ AFDW para $31.78 \pm 9.10 \text{ mg g}^{-1}$ AFDW e que se estendeu até ao mês de dezembro ($42.09 \pm 13.39 \text{ mg g}^{-1}$ AFDW), seguido de um decréscimo até julho ($32.54 \pm 4.83 \text{ mg g}^{-1}$ AFDW). O teor em proteína voltou a subir ligeiramente em agosto em ambas as populações (Olhão - $39.98 \pm 6.79 \text{ mg g}^{-1}$ AFDW; Porto de Mós - $43.85 \pm 4.21 \text{ mg g}^{-1}$ AFDW). Verificou-se a existência de diferenças significativas no conteúdo em proteínas entre os organismos das duas proveniências, nos meses de março, maio, setembro, novembro e dezembro de 2014 e em março, abril e setembro de 2015 (ANOVA, $P < 0.05$).

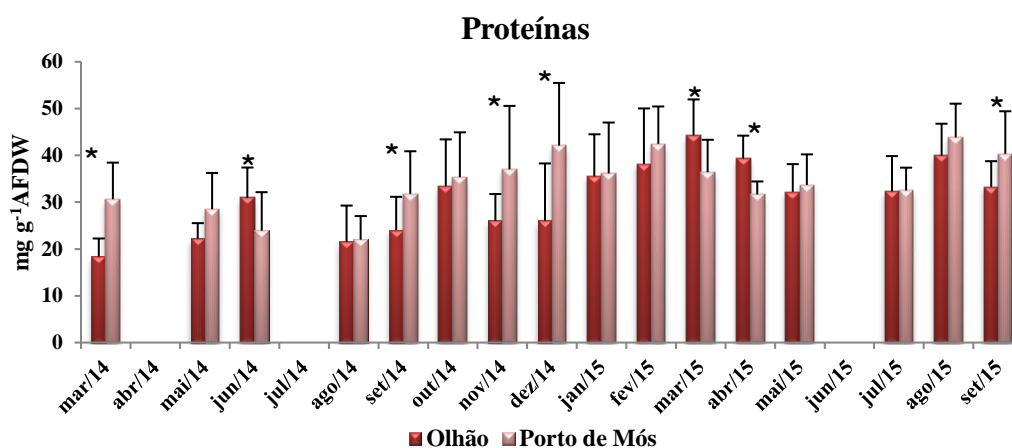


Figura 3. Variação dos valores de proteínas (média±DP) nos mexilhões (*Mytilus galloprovincialis*) provenientes das duas zonas, Olhão e Porto de Mós, ao longo do período experimental.

A análise da Figura 4 mostra que o teor em lípidos totais variou entre $5.04 \pm 0.84 \text{ mg g}^{-1}$ AFDW e $12.99 \pm 6.31 \text{ mg g}^{-1}$ AFDW nos indivíduos provenientes da zona de Olhão e entre $6.31 \pm 1.87 \text{ mg g}^{-1}$ AFDW e $14.16 \pm 6.19 \text{ mg g}^{-1}$ AFDW, nos indivíduos provenientes da zona de Porto de Mós. Os valores mais elevados deste componente foram alcançados no mês de janeiro de 2015 nos organismos provenientes de ambos os locais, seguido de um decréscimo até ao mês de maio (Olhão - $7.01 \pm 3.24 \text{ mg g}^{-1}$ AFDW; Porto de Mós - $6.31 \pm 1.87 \text{ mg g}^{-1}$ AFDW). A variação no teor em lípidos foi semelhante nos organismos provenientes dos dois locais ao longo do período de amostragem, apenas verificando-se a existência de diferenças significativas nos meses de março de 2014, julho e setembro de 2015 (ANOVA, $P < 0.05$).

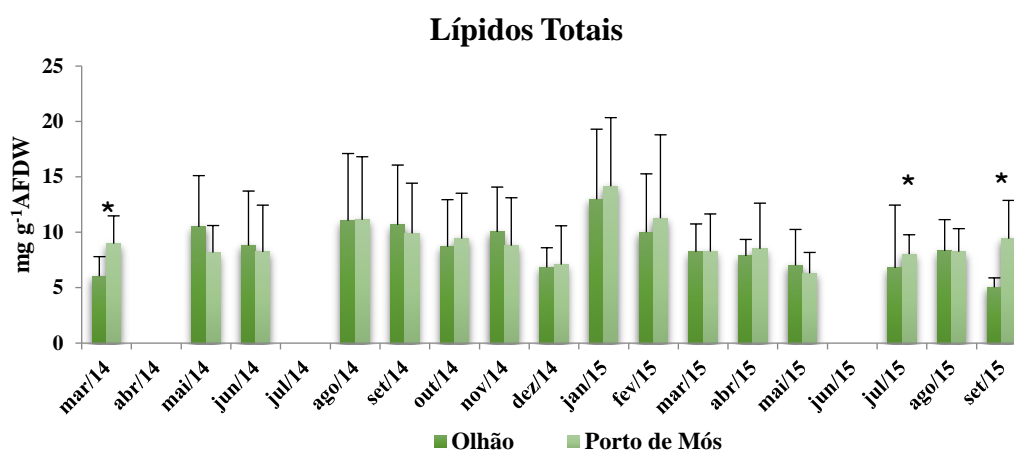


Figura 4. Variação dos valores de lípidos totais (média±DP) nos mexilhões (*Mytilus galloprovincialis*) provenientes das duas zonas, Olhão e Porto de Mós, ao longo do período experimental.

A análise da Figura 5 mostra que a percentagem de glicogénio foi o componente bioquímico que apresentou maiores flutuações ao longo do período de amostragem, alcançando valores entre 1.00 ± 0.55 mg g⁻¹ AFDW e 11.10 ± 3.65 mg g⁻¹ AFDW para os organismos provenientes de Olhão e entre 1.27 ± 0.91 mg g⁻¹ AFDW e 9.26 ± 3.28 mg g⁻¹ AFDW para os organismos provenientes de Porto de Mós. Os valores mais elevados deste componente foram alcançados nos meses de novembro e setembro de 2014 em Olhão e Porto de Mós, respetivamente. Os valores de glicogénio aumentaram bruscamente no mês de setembro de 2014, (Olhão - 1.00 ± 0.55 mg g⁻¹ AFDW para 8.87 ± 3.49 mg g⁻¹ AFDW; Porto de Mós - 2.09 ± 1.45 mg g⁻¹ AFDW para 9.26 ± 3.28 mg g⁻¹ AFDW). O teor de glicogénio aumentou até ao mês novembro de 2014, 11.10 ± 3.65 mg g⁻¹ AFDW, seguido de um decréscimo até fevereiro de 2015, nos indivíduos provenientes da zona de Olhão, enquanto que nos da zona de Porto de Mós, verificou-se um decréscimo logo no mês seguinte que se estendeu até março. A diminuição no teor em glicogénio neste período, coincide com o período em que os organismos apresentam os valores mais elevados de IC, sugerindo que este componente foi gasto no processo de recuperação da gónada. Verificaram-se diferenças significativas no teor em glicogénio entre os organismos provenientes dos dois locais, nos meses de maio, agosto, outubro, novembro e dezembro de 2014 e em fevereiro, abril, julho e agosto de 2015 (ANOVA, P<0.05).

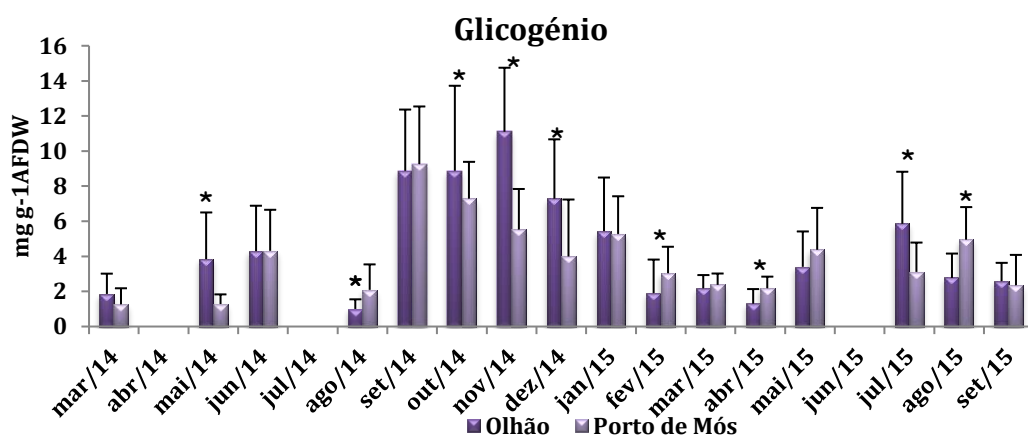


Figura 5. Variação dos valores de glicogénio (média±DP) nos mexilhões (*Mytilus galloprovincialis*) provenientes das duas zonas, Olhão e Porto de Mós, ao longo do período experimental.

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