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# Reproductive cycle and follicle cleaning process of *Mytilus galloprovincialis* (Mollusca: Bivalvia) from a polluted coastal site in Algeria

Yamina Leila Rouabhi[a,b], Philippe Grosjean[a], Zitouni Boutiba[b], Omar Rouane Hacene[b] and Jonathan Richir[c,d]

[a]Numerical Ecology of Aquatic Systems, Complexys Institute, University of Mons, Mons, Belgium; [b]Laboratory Network for Environmental Monitoring (LRSE), Department of Biology, University of Oran 1, Oran, Algeria; [c]Chemical Oceanography Unit, FOCUS UR, University of Liège, Liège, Belgium; [d]Laboratory of Oceanology, FOCUS UR, University of Liège, Liège, Belgium

**ABSTRACT**

This work focused on the reproductive cycle and cleaning process of follicles in *Mytilus galloprovincialis* and aimed to extend knowledge of the reproductive cycles of Mytilidae. Biometric and histological measurements were taken monthly over 12 months from mussels at a polluted site, the port of Oran in Algeria. Environmental parameters were monitored concomitantly. *Mytilus galloprovincialis* reproduced throughout the year, with a main spawning period between November and February and a second between March and June. Several follicle cleaning processes were observed throughout the reproductive cycle. They occurred under two circumstances. First, in the absence of reserve tissues, mature gametes were degraded. This happened when spawning was about to end and corresponded to the last stage of reproduction. Second, atresia, gamete degeneration and a cessation of spawning occurred whatever the stage of the gonad development and whatever the environmental parameter values. These disturbances of reproduction may have resulted from pollution in the port of Oran and increased when temperature exceeded critical thresholds for gametogenesis. To conclude, gamete degeneration and spawning cessation because of coastal pollution and global warming could threaten *M. galloprovincialis* recruitment, and ultimately the shellfish economy, and could distort biomonitoring strategies using mussels.

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Mytilidae; gonad histology; gamete degeneration; environment; pollution

## Introduction

Mussels are among the best-adapted organisms to environmental changes. However, they show a high capacity for bioaccumulation of different types of chemicals (e.g. trace metals, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs)) (Sasikumar et al. 2006; Pan and Wang 2012). Several authors consider mussels, especially the genus *Mytilus*, among the most appropriate organisms to biomonitor the marine environment (Casas and Bacher 2006; Sasikumar et al. 2006; Pan and Wang 2012). As a result, biomonitoring programs of passive and active environmental quality assessment (e.g. Mussel Watch USA, OSPAR, RNO France) have used mussels all over the world (e.g. Andral et al. 2004; Rainbow et al. 2004) providing a large database on marine biomonitoring.

The mussel *Mytilus galloprovincialis* Lamarck, 1819 has a worldwide distribution and may have several spawning periods that depend on the interaction between different environmental factors that vary between geographic areas (Villalba 1995; Casas and Bacher 2006). Abiotic parameters that influence mussel reproduction the most are temperature, food availability and environmental quality (Suárez et al. 2005; Casas and Bacher 2006). Under the current conditions of climate change and increasing marine pollution, the biology of these organisms is greatly affected (OSPAR 2010). To understand the impact of environmental changes on the biology of mussels, condition indices and histopathological analyses can be used. These give indications on the condition and health status of animals (Grant and Tyler 1983; Lucas and Beninger 1985).

The physiological process causing the cessation or slowing down of reproduction is known as atresia: gamete degeneration or follicle cleaning process (Sunila 1987; Motavkine 1989; Suárez et al. 2005). Reproductive failure, the degeneration of male and female gametes, and the deformation of oocytes can not only affect mussel recruitment (Beninger 2017) but also distort the interpretation of results from environmental monitoring based on the use of mussels. Before undertaking a biomonitoring study, the biology of the

**CONTACT** Yamina Leila Rouabhi rouabhi-leila@hotmail.fr Numerical Ecology of Aquatic Systems, Complexys Institute, University of Mons, Pentagone 3D08, 6, Avenue du Champ de Mars, 7000, Mons, Belgium Jonathan Richir jonathan.richir@uliege.be Chemical Oceanography Unit, FOCUS UR, University of Liège, Liège, Belgium

target organism and its response to changes of the surrounding environment must be studied in depth. Several passive monitoring programs have used wild *M. galloprovincialis* as bioindicator species in environmental quality assessment surveys along the Algerian coast, in particular in the area of Oran, the second largest city in Algeria (Taleb et al. 2007, 2009; Rouane-Hacene et al. 2015; Benali et al. 2015; Guendouzi et al. 2018). However, to the best of our knowledge, nobody has studied the reproductive biology of this mussel species at this locality, which is an essential prerequisite to their proper use as a bioindicator species.

According to Beninger (2017) atresia and the environmental factors that drive this phenomenon remain poorly understood in *Mytilus* and the majority of other bivalves. Most studies of atresia have been done *in vitro* and were short-term studies (Ortiz-Zarragoitia and Cajaraville 2006; Múgica et al. 2015; Smolarz et al. 2017). In these studies, mussels underwent important stress over short periods of time and under low contrasting environmental conditions. Such conditions happen only rarely or never in natural environments. In addition, the work performed in natural environments has mainly been on farmed mussels from shellfish farms where contaminants are found in low concentrations. This minimizes the value of these studies. Therefore, the aim of the present work is to document for the first time the reproductive biology and atresia of wild *M. galloprovincialis* from the west coast of Algeria in a degraded environment, where the temperature often exceeds levels likely to affect physiological processes including reproduction. Specific objectives are: (i) to describe the reproductive cycle of wild *M. galloprovincialis* in west Algeria, (ii) to describe the follicle cleaning process and atresia in Mytilidae, (iii) to investigate *in situ* factors that may interfere with reproduction and (iv) to study correlational relationships between environmental parameters and mussel biological parameters.

## Material and methods

### Study site

Sampling was performed in the port of the city of Oran (35°42 N, 00°37 W) located in the Bay of Oran on the western coastal region of Algeria. The Bay of Oran is 28 km long and forms a rough semicircle between Cape Falcon and Cape East Aiguille. Oran is the second most important city of the country. It has been classified by the PNUE/WHO (2003) reports and several other studies as one of the pollution hotspots in the Mediterranean Sea, especially around its port (Taleb et al. 2007, 2009; Rouane-Hacene et al. 2015; Benali et al. 2015; Guendouzi et al. 2018). Previous studies have reported high to very high levels of contaminants in *M. galloprovincialis* flesh from the port of Oran (e.g. Zinc (Zn) = 542 $\mu g/g_{dw}^{-1}$, Lead (Pb) = 7.8 $\mu g/g_{dw}^{-1}$, PCBs = 98 $ng/g_{dw}^{-1}$, PAHs = 2892 $ng/g_{dw}^{-1}$), levels that are higher than other regions and international norms (Rouane-Hacene et al. 2015; Benali et al. 2015; Rouabhi et al. 2016; Guendouzi et al. 2018).

### Sampling

From September 2013 to August 2014, 30 *M. galloprovincialis* ranging in size from 5 to 6 cm shell length were collected monthly between 1 and 5 m depth in the inner side of the pier of the port of Oran. Sampled mussels transported to the laboratory in an isothermal box were measured (to the nearest 0.1 mm), carefully dissected and weighed (to the nearest 0.01 g) on the same day.

Temperature (°C) and pH were recorded three times a month on different days at a depth of 1 m using a multiparameter probe (HANNA HI 9829). Water samples for total suspended matter (TSM), organic matter (OM) and biological oxygen demand (BOD) were collected once monthly in triplicate the same day as mussel sampling at a depth of 1 m. Parameter recording and sampling were performed between 9 and 11 am (local time). Water samples were stored in opaque bottles in an isothermal box and analyzed in the laboratory within 24 hours.

### Morphometric indices

Of the several morphometric indices published in the scientific literature, only the two most relevant ones chosen using the Escoufier's equivalent vectors method were retained. These indices are the Condition Index (CI) of Walne and Mann (1975) and Lucas and Beninger (1985):
CI = (flesh dry weight/shell dry weight), and the Gonad Index (GI) of Hines (1979):
GI = (mantle wet weight/flesh wet weight).
Weights were determined first on drained wet tissues, then on dry tissues (after 48 hours of drying at 60°C). According to Mikhailov et al. (1996) the mantle is the main site of gonad development and gamete storage until spawning in *M. galloprovincialis*. Because the gonads and the digestive gland are not discrete organs that can be excised and weighed with precision, mantle tissue in the immediate vicinity of the digestive gland was not included in the determination of mantle weight and the calculation of the GI.

### Histology

The mussel sex and gonadal stages were determined from histological sections. The mantle of each individual was dissected, fixed in Davidson's solution, embedded in paraffin wax through a 19-hour routine schedule from 70% ETOH, sections cut to 5-µm thickness, which were then processed and stained with Harris' haematoxylin and eosin and examined by light microscopy (Howard 2004). The gonadal stages were classified according to Lubet (1959): stage 0, sexual rest: accumulation of reserves (carbohydrates, lipids), thickening of the mantle by compressing the follicles, gonoducts and genital product not visible; stage I, multiplication of gonia: the follicles begin to develop; stage II, progression of gametogenesis: follicles more apparent, ovules and spermatozoa still immature; stage IIIa, ripe: gametes reach their maturity and are ready to be ejected; stage IIIb, spawning: total or partial release of gametes; stage IIIc, restoring: restoration of new gonads after spawning; and stage IIId, follicle cleaning process: degradation of residual gametes by haemocytes.

The periods of reserve accumulation and subsequent use for gametogenesis are indicated by qualitative changes observed in the reserve tissues, i.e. the presence or absence and the general appearance of adipogranular cells (ADG) and vesicular connective tissue cells (VCT). The prevalence of atretic oocytes is the percentage of the area occupied by atretic oocytes relative to the total area. Changes in reserve tissues and prevalence of atretic oocytes were determined from the observation of five histological sections per individual (averaged by individual and month).

### Statistics

A chi-square test was used to determine if the sex ratio deviated significantly from 1:1. Differences between sexes whether for total flesh wet weight, gonad wet weight, CI and GI during any sampled month were analyzed with a non-parametric Mann-Whitney test. Temporal variations of indices and tissue weights were analyzed for both sexes with a non-parametric Kruskal-Wallis test followed by a post-hoc Duncan test when the null hypothesis was rejected. Spearman correlation matrix with pairwise deletion of missing data was used to study the dependence between environmental and morphometric variables. The minimum level of significance for all the analyses was 0.05. Statistical analyses were performed in R version 3.4.2 (R Core Team 2017).

## Results

### Environmental parameters

Water temperature in the port of Oran was high at the beginning of the survey in September 2013 and decreased gradually until a minimum of 15.5°C in January 2014 (Figure 1). Temperature then increased gradually until August 2014 to reach a maximum of 25.0°C. The pH remained relatively constant at around 8.0 ± 0.2 except in April and May 2014 when it decreased to below 7.5 ± 0.0 (Figure 1).

OM and BOD were significantly correlated ($r$ = 0.75, $p < 0.001$), and they fluctuated in a similar way throughout the year. They increased from September 2013 to May 2014 when they reached their highest values of 148 ± 4 mg/L for OM and 640 ± 4 mg/L for BOD, then decreased until August 2014 (Figure 1). The lowest values of 39 ± 2 mg/L for OM and 179 ± 10 mg/L for BOD were recorded in September 2013. TSM was very high in September 2013 (63 ± 1 mg/L) and remained stable from November until the end of the sampling period (24 ± 2 mg/L), except in December (31 ± 1 mg/L), March (26 ± 1 mg/L) and May 2014 (38 ± 2 mg/L) (Figure 1).

### Sample description and indices

Of the 360 sampled mussels, 52% were females and 48% males. The sex ratio did not differ significantly from 1:1 for global and monthly samplings ($\chi^2$ = 15.86, $p$ = 0.14). There were no statistically significant differences between sexes on a month by month basis for total flesh wet weight (U = 10,772, $p$ = 0.93), gonad wet weight (U = 9882, $p$ = 0.64), CI (U = 11,606, $p$ = 0.18) and GI (U = 10,377, $p$ = 0.80). This absence of differences between sexes on a month by month basis is illustrated on the notched boxplots of Figure 2. Morphometric indices and tissue weights could therefore be analyzed independently of sex.

There were statistically significant differences in morphometric indices and tissue weights among months. The GI and the CI of mussels showed similar temporal changes (Figure 2) and give an overview of the succession of spawning and restoring periods. The temporal changes of mussel total flesh wet weight and gonad wet weight were very close (Figure 2) and similar to the ones of CI and GI. Flesh and gonad weights were statistically significantly correlated with temperature ($r$ = 0.688 and $r$ = 0.669, respectively, $p < 0.001$) (Table 1). They were high in September 2013 and decreased significantly until

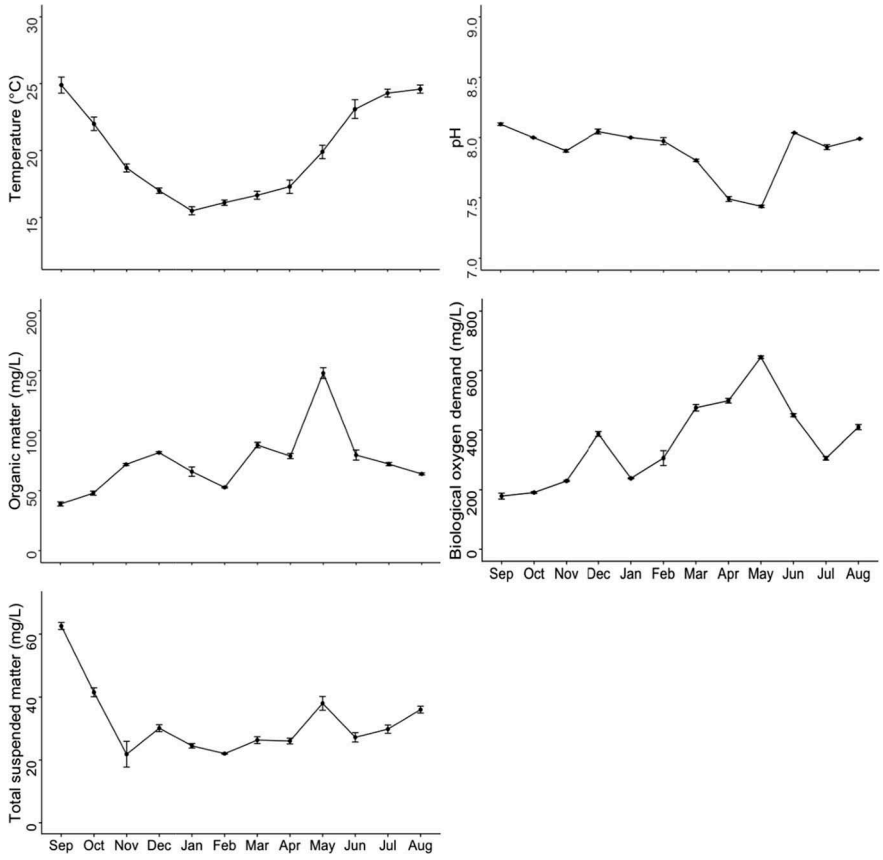


**Figure 1.** Monthly changes of mean environmental parameters (n = 3) in the port of Oran, Algeria. Error bars represent standard deviations.

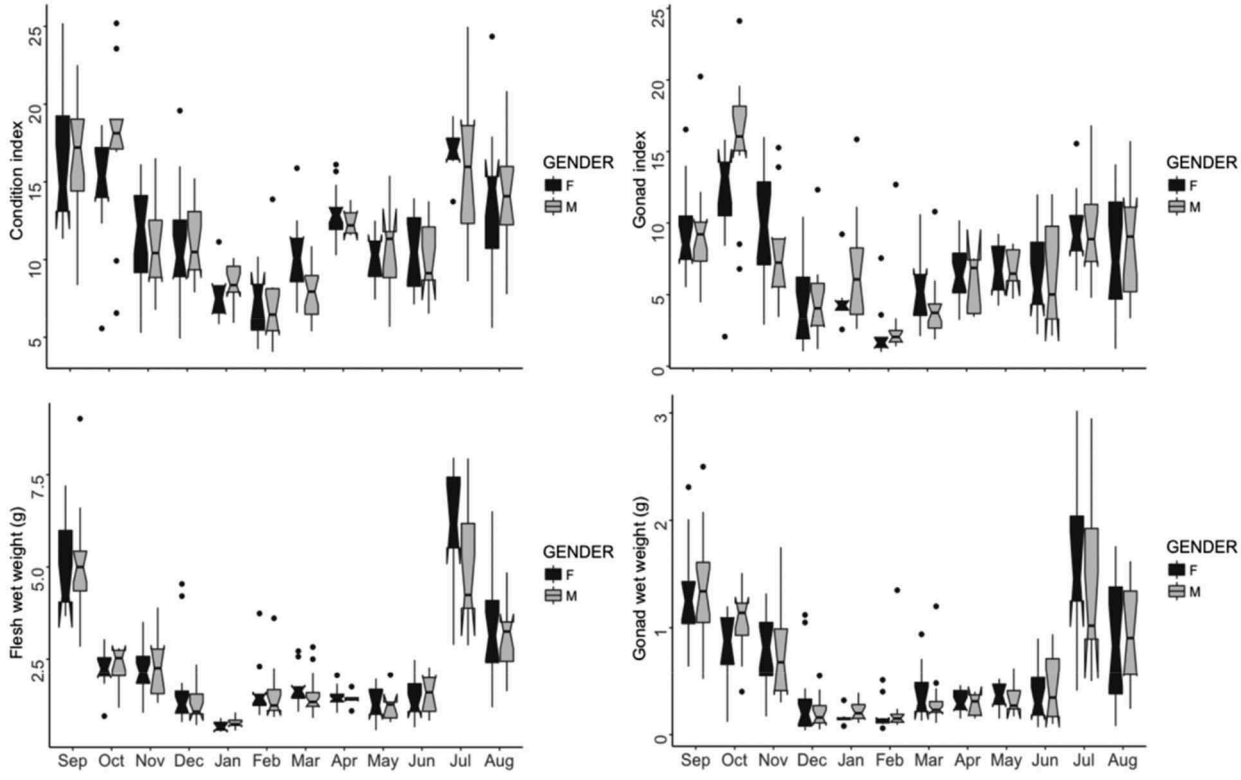


**Figure 2.** Notched boxplots of monthly changes in the port of Oran, Algeria, of *Mytilus galloprovincialis* condition and gonad index (above) values and flesh and gonad wet weights (below) for females (dark grey) and males (light grey).

**Table 1.** Summary of Spearman correlation coefficients between environmental and morphometric variables.

| | Temperature | pH | TSM | OM | BOD |
|---|---|---|---|---|---|
| Condition index | 0.588*** | 0.191* | 0.469*** | −0.311*** | −0.271*** |
| Gonad index | 0.475*** | 0.084 | 0.310*** | −0.320*** | −0.343*** |
| Flesh wet weight | 0.688*** | 0.159 | 0.413*** | −0.445*** | −0.392*** |
| Gonad wet weight | 0.669*** | 0.132 | 0.417*** | −0.421*** | −0.411*** |

TSM: total suspended matter; OM: organic matter; BOD: biological oxygen demand. Stars represent significant correlations between parameters at $p < 0.05$ (*) or $p < 0.001$ (***).

February 2014, which corresponded with the main spawning period. From March 2014, the increase in flesh and gonad weights corresponded with the period of gonad restoration and the spring increase of OM and TSM. Weights reached, like CI and GI, their maximum during summer. Although the weak to modest correlations observed over the whole year between flesh or gonad weights and seawater OM and TSM concentrations (Table 1), the summer peaks following spring peaks of these variables (Figures 1 and 2) indicates the importance of food availability in mussel weight increase after the spawning period when temperatures started to rise.

### *Reproduction, cleaning process and atresia*

Histological sections of gonads showed that the mussels showed reproductive activity throughout the year. The proportional distribution of gonadal developmental stages over the months was very similar between sexes (Figure 3). A few differences were observed, especially for stages I, IIIc and IIId. Gametogenesis (stage I) began in June 2014 after a 3-month period of sexual rest (stage 0) (Figure 4 (c,d)). Male and female follicle maturity (stage IIIa) was reached in October 2013 (Figure 4(e,f)) and was followed by two spawning and gamete restoration periods. The first period of so-called principal spawning (stage IIIb) took place between November 2013 and February 2014, with a large number empty follicles between January and February (Figure 5(a,b)). The first period of gamete restoration started in October 2013 for both sexes, ended in December for females and extended until January 2014 for males (stage IIIc) (Figure 5(c,d)). A second shorter spawning period (stage IIIb) occurred between

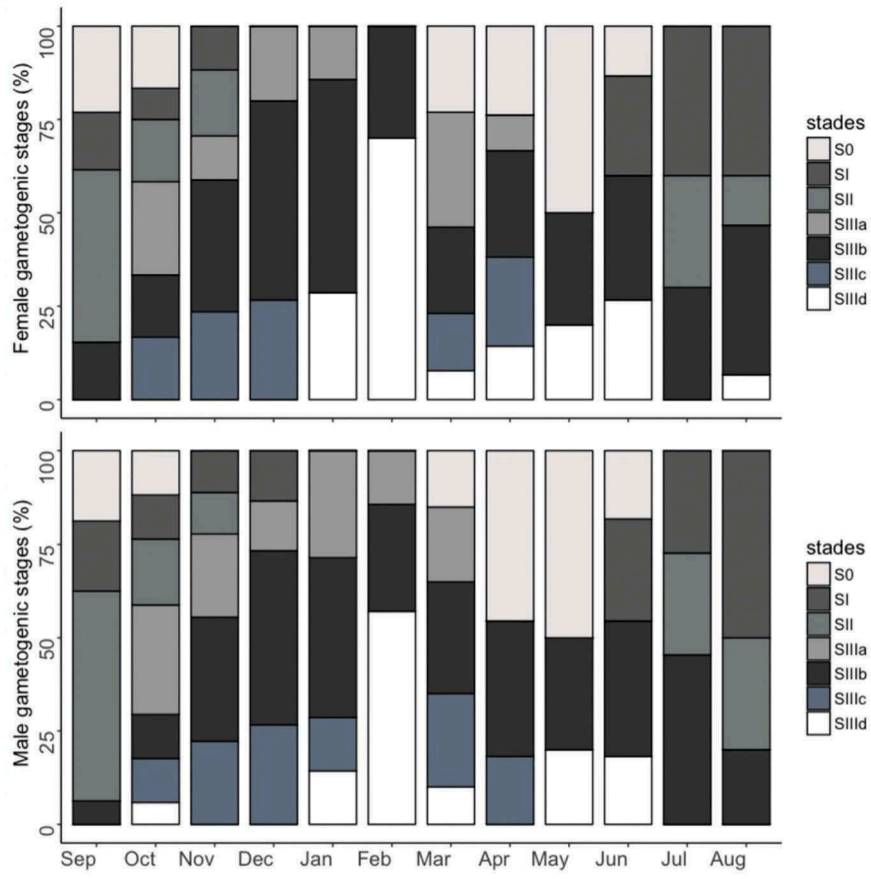


**Figure 3.** Monthly distribution (%) of female (above) and male (below) gametogenic stages in *Mytilus galloprovincialis* from the port of Oran, Algeria. Stage 0: sexual rest; stage I: multiplication of gonia; stage II: progression of gametogenesis; stage IIIa: ripe; stage IIIb: spawning; stage IIIc: restoring; stage IIId: follicle cleaning process.

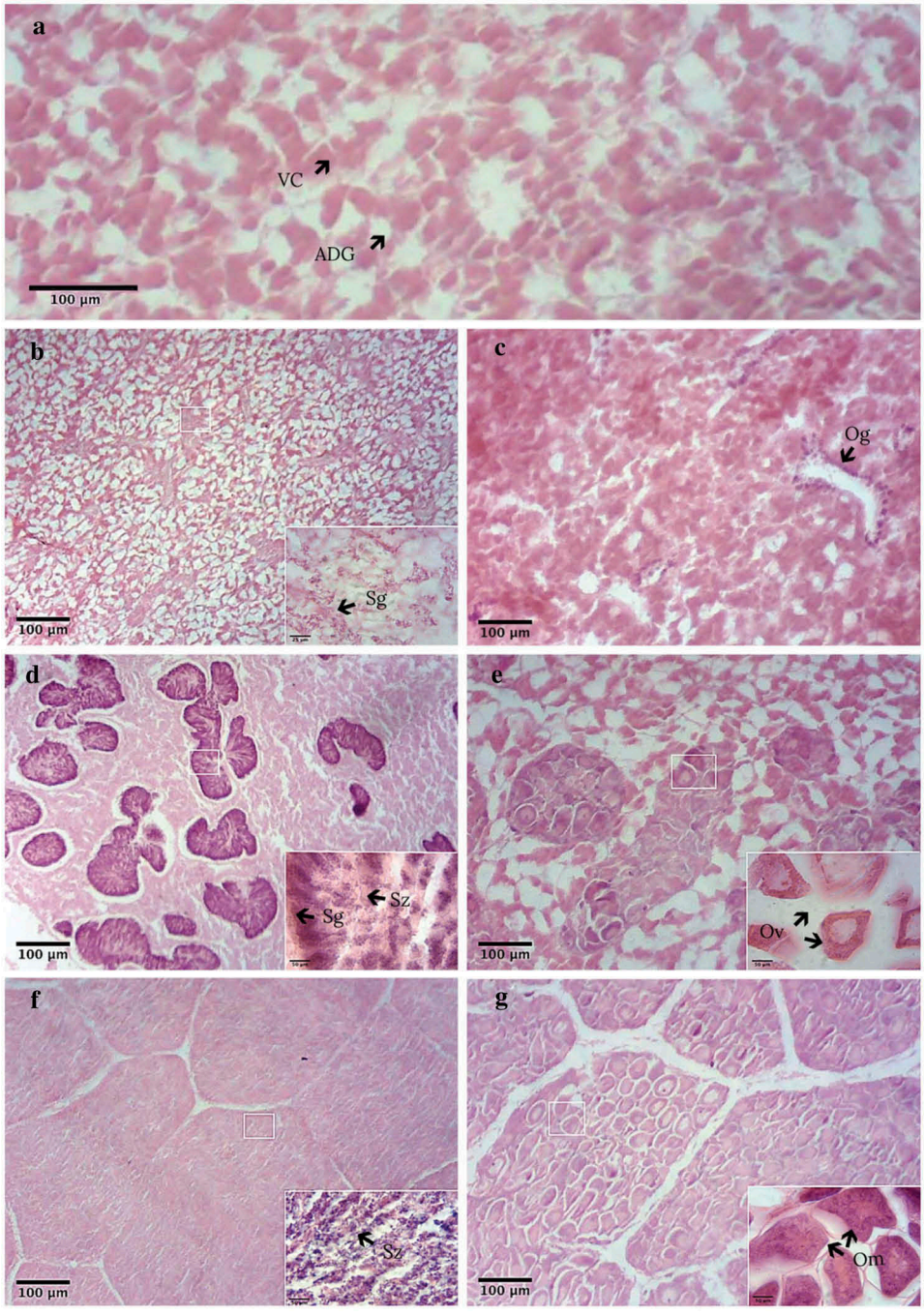


**Figure 4.** Photomicrographs showing the early gametogenic stages in *Mytilus galloprovincialis* from the port of Oran, Algeria. The pictures show: (a) stage 0 (sexual rest); (b) stage I in males (multiplication of gonia); (c) stage I in females (multiplication of gonia); (d) stage II in males (progression of gametogenesis); (e) stage II in females (progression of gametogenesis); (f) stage IIIa in males (ripe); (g) stage IIIa in females (ripe). VC: vesicular cells; ADG: adipogranular cells; Sg: spermatogonia; Og: oogonia; Sz: spermatozoids; Ov: Oocytes; Om: mature oocytes.

March and June 2014 after a second period of gamete restoration (stage IIIc) observed between March and April 2014.

ADG and VCT cells appeared to be largest during spring and summer stage 0. Their size appeared to decrease between summer and autumn as gametogenesis increased. No reserve cells were observed in individuals at stage III of gamete maturation, which occurred mainly from autumn to spring. From October 2013, gonads started being degraded by haemocytes (Figure 6). The number of empty follicles and their degradation appeared to increase as mussels reached the end of the spawning period (Figure 6(c–e)). Gonads did not change shape but seemed to fragment until

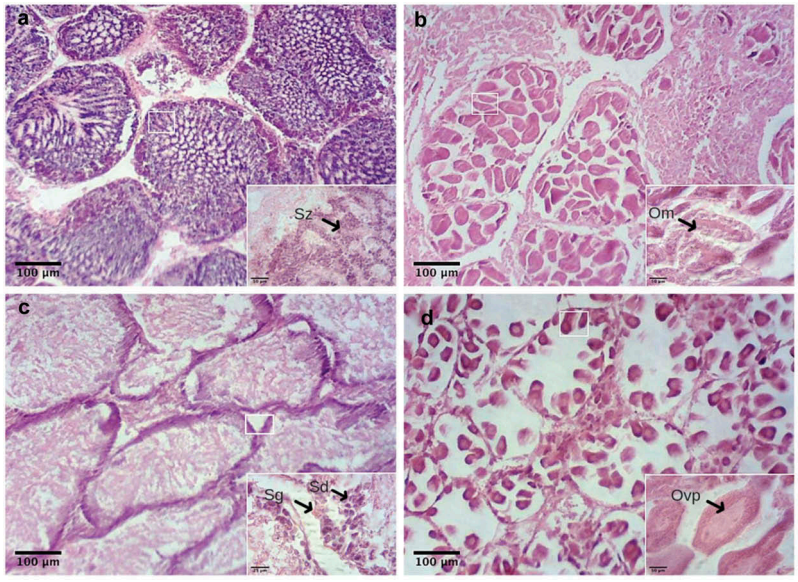


**Figure 5.** Photomicrographs showing the development gametogenic stages in *Mytilus galloprovincialis* from the port of Oran, Algeria. The pictures show: (a) stage IIIb in males (spawning); (b) stage IIIb in females (spawning); (c) stage IIIc in males (restoring); (d) stage IIIc in females (restoring). Sz: spermatozoids; Om: mature oocytes; Sd: spermatids; Sg: spermatogonia; Ovp: vitelogenic-pedunculated oocytes.

disappearance at the end of the spawning period (Figure 6(f–h)).

In parallel with this succession of gametogenic stages, atresia and gamete degeneration were observed in mussels. In September 2013, 20% of females at stage II of reproduction showed gamete degeneration and resorption (Figure 7(a)). In December 2013 and March 2014 this number increased in females with mature gonads (stage III) but without exceeding 30% of individuals (Figure 7(b)). During July 2014, spawning was either slowed down or completely inhibited for a relatively large number of individuals (65%) and nearly 80% follicles were subject to atresia (Figure 7(c)). Inflammation of the mantle was observed in several individuals. This inflammation corresponded to several stages of oocyte degeneration in females and phagocytosis of spermatozoa in the centre of male follicles (Figure 7(g,h)). Female gonads were gradually deforming (Figure 7(c)), they underwent vacuolization (Figure 7(d)) and divided into several small spheres (apoptosis) (Figure 7(e)). During the last phase of atresia, oocyte membranes disaggregated and the cytoplasm spilled into the acinal lumen until complete disappearance of the follicles (Figure 7(f)).

## Discussion

Temperature followed a classic seasonal cycle, with monthly means and annual range of values slightly higher than data previously reported by several authors for the port of Oran (Bouras et al. 2007; Grimes et al. 2010; Remili and Kerfouf 2013). pH was lower in spring. Such a spring reduction is generally related to an increase in biological activities of plankton and other macrophytes and animals (Mollo and Noury 2013). The peaks in May of OM, BOD and TSM coincided with the recorded decrease in pH and may also be related to the spring planktonic proliferation as reported by several studies along the littoral of Oran (Lalami-Taleb 1971; Taleb et al. 2007; Bouras et al. 2007). The other peaks outside the spring period of planktonic proliferation were probably related to intermittent increases of urban discharges from the city of Oran via the two main outlets (Gênet and Fort Lamoune) present on both sides of the port of Oran. Indeed, OM, TSM and BOD concentrations of Oran untreated wastewaters far exceed international norms. According to some authors OM, TSM and BOD are very good indicators of the overall pollution of the port of Oran (Grimes et al. 2010; Remili and Kerfouf 2013; Rouane-Hacene et al. 2015).

A sex ratio of 1:1 has been reported in several studies of wild *M. galloprovincialis* from different regions in the Atlantic and Mediterranean Sea (e.g. Lubet 1959; Villalba 1995; Suárez et al. 2005; Bhaby et al. 2014). During their reproductive cycle, male and female mussels go through different physiological states as highlighted by monthly variations of mean morphometric indices and tissue weights. These parameters can therefore be considered as a simple and effective way to study the reproductive cycle

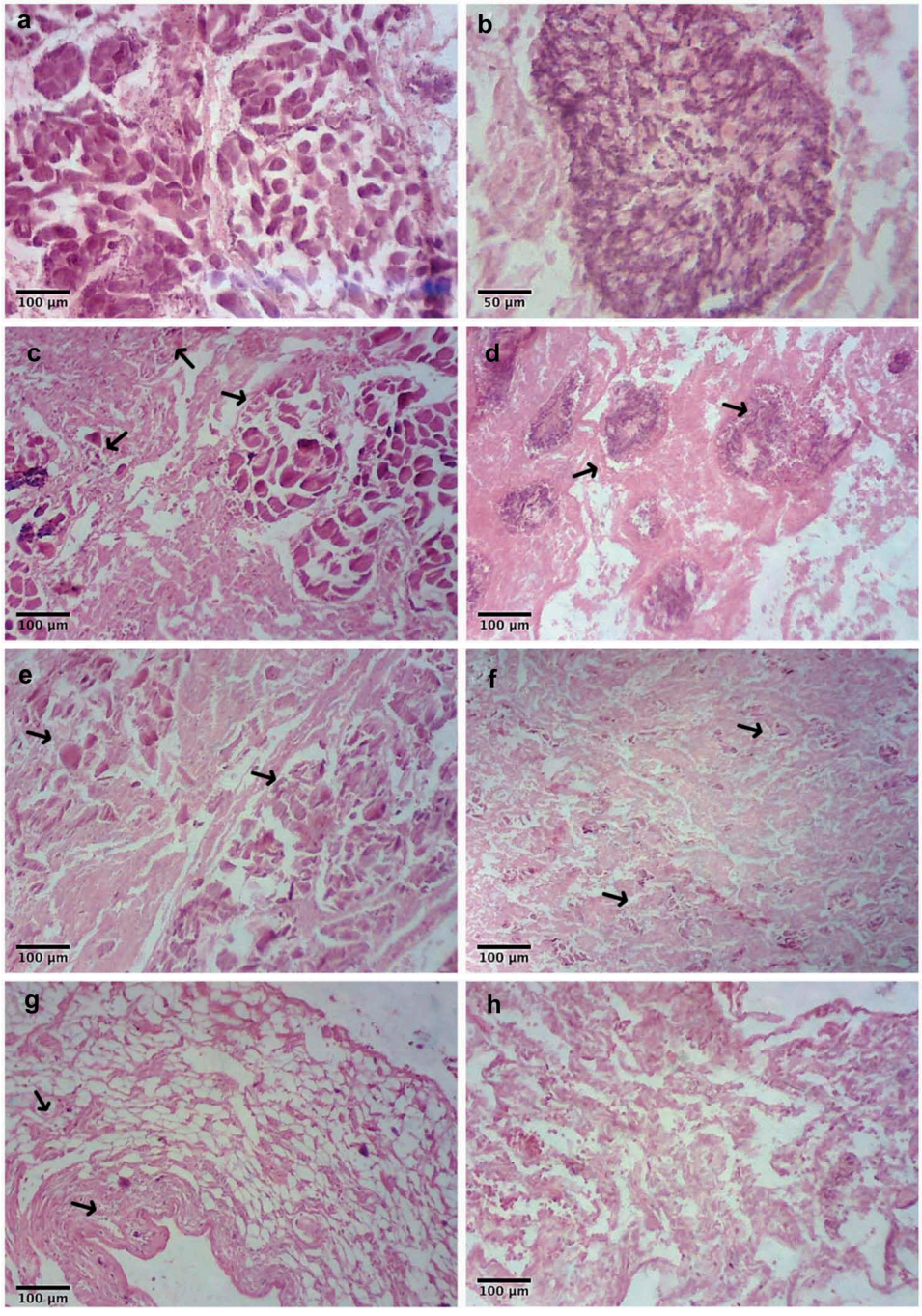


**Figure 6.** Photomicrographs showing the follicle cleaning process (Stage IIId) in *Mytilus galloprovincialis* from the port of Oran, Algeria. The pictures show: (a) aggregation of haemocytes in a female follicle; (b) aggregation of haemocytes in a male follicle; (c) progressive degradation of female gonads during the spawning period (arrows); (d) degradation of male gonads during the spawning period (arrows); (e) increase of gonad degradation as the end of the spawning period approaches; (f, g, h) complete discharge of gonads and advanced state of the follicle cleaning process.

of a species (Grant and Tyler 1983; Lucas and Beninger 1985). The same temporal changes in CI and GI were observed in *M. galloprovincialis* collected seasonally (and not monthly like in this study) in the port of Oran in 2010 (Rouane-Hacene et al. 2015) and between 2011 and 2012 (Benali et al. 2015). Positive statistically significant correlations of indices and weights with temperature and food availability (TSM) suggest that these two parameters are important environmental factors regulating the biological cycle of *M. galloprovincialis* in the western region of Algeria. The effect of seasonality on the biology of any bivalve species is more than expected in temperate areas. Such an observation was reported for different regions and different bivalve species (Suárez et al. 2005, 2007).

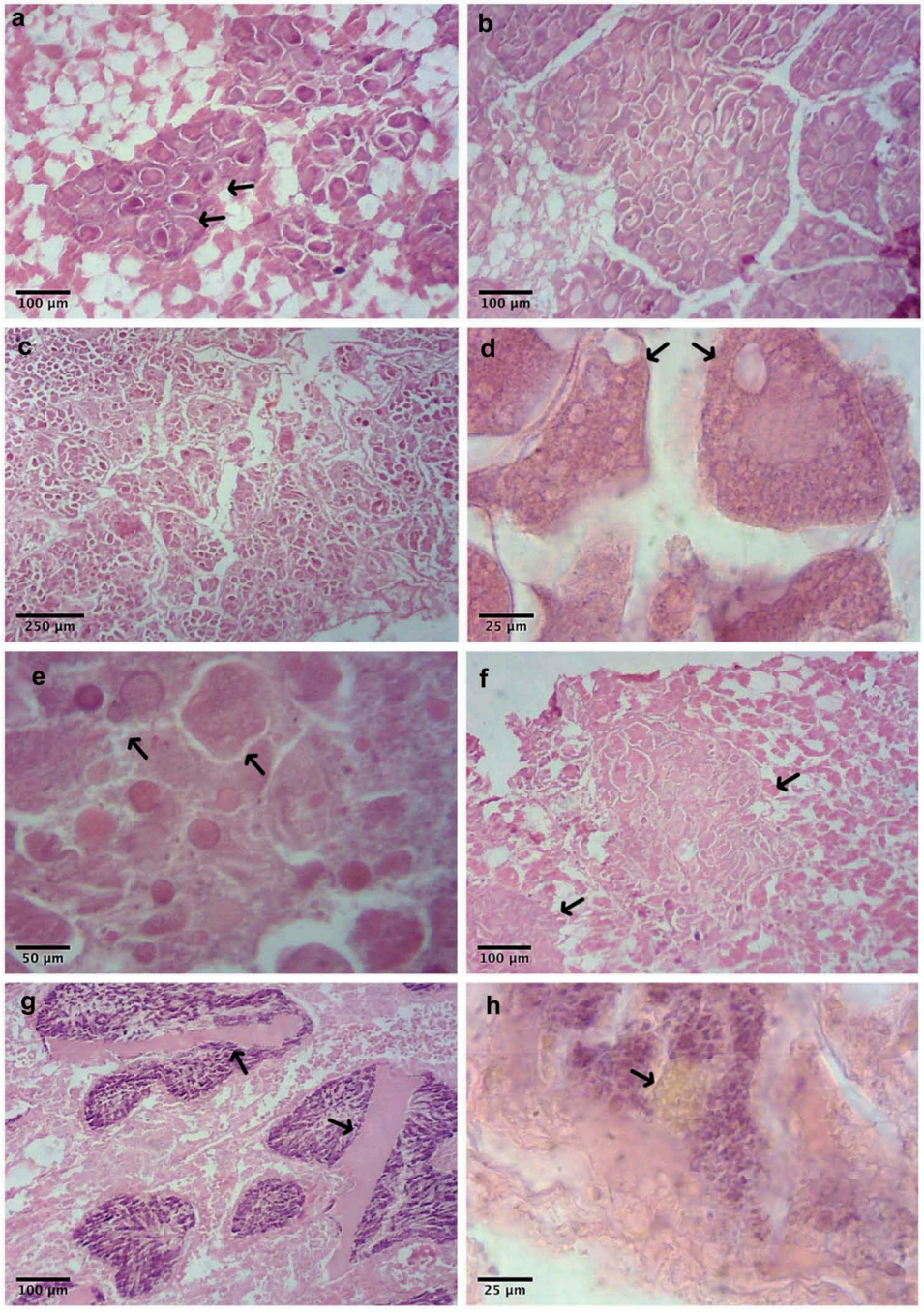


**Figure 7.** Photomicrographs showing the atresia and gonad degeneration in *Mytilus galloprovincialis* from the port of Oran, Algeria. The pictures show: (a) degeneration and resorption of female follicles in stage II (progression of gametogenesis, in September); (b) degeneration and resorption of female follicles in stage IIIa (ripe, in December); (c) degeneration and resorption of female follicles in stage IIIb (spawning, in July); (d) vacuolization of the female gonad, the first step in degeneration (several vacuoles in each ova – arrows); (e) female gonad apoptosis (arrows); (f) advanced state of female follicle atresia; (g) male follicles, where sperm is phagocytosed by haemocytes; (h) concentration of brown cells in the centre of male follicles.

The reproductive cycle of *M. galloprovincialis* from the port of Oran is similar to observations previously reported for mussels from France (Bodin et al. 2004), Spain (Suárez et al. 2005), Tunisia (Banni et al. 2011) and Morocco (Bhaby et al. 2014), with maximum spawning in February and March. The second spawning period was observed later in spring on the Mediterranean coast and in summer on the Atlantic coast. The presence of ADG and VCT cells and the overall appearance of the reserve tissues followed the schemes described by Lubet (1959) and Lowe et al. (1982). Temporal changes of their characteristics further corresponded

to changes in index values and tissue weights. They gave an indication of reserve storage in gonads as lipids and proteins for ADG cells and glycogen in VCT cells (Lowe et al. 1982; Palanivelu et al. 2002).

Several follicle cleaning processes were observed throughout the reproductive cycle. In this study, two reasons for triggering of gamete degeneration and the follicle cleaning process are suggested. At first, spawning was accompanied by the degradation of gametes by haemocytes (Figure 6). Degradation increased as the end of the spawning period was approached. This stage corresponded to the last phase of the reproductive cycle (stage IIId) described by Lubet (1959) and Pipe (1987). Previous studies reported that this cleaning process resulted from the physiological cycle of mussels, which is closely related to changes in environmental parameters including temperature, pollution, and food availability (Lubet and Aloui 1987; Motavkine 1989; Suárez et al. 2005). Our results indicated that this phenomenon could be related to nutrient reserves in the gonads as indicated by the absence of ADG and VCT cells and the decrease in weight and index values. After several spawning and gamete restoration events, mussels would have consumed their nutritive reserves. Indeed, according to Lubet (1959) and Lowe et al. (1982) an inverse seasonal developmental cycle exists between the reserve tissues and gametogenesis, which probably reflects glycogen consumption that feeds the latter. In such a physiological state, mussels were unable to produce new gametes (stage IIIc), so they proceeded to the cleaning stage of the remaining follicles (Stage IIId) and entered a period of sexual dormancy (Stage 0). Conversely, mussels that had not completely spawned but had not completely degraded their gametes continued their reproductive cycle by producing new gametes. Temperature would not influence the follicle cleaning process that follows spawning events since it did not exceed the limit (19°C) tolerated by *M. galloprovincialis* at that time (Lubet and Aloui 1987; Fearman and Moltschaniwskyj 2010).

Some authors have suggested that atresia provides energy through gamete resorption for basal metabolism when the latter slows down (Suárez et al. 2005; Portner and Knust 2007; Azpeitia et al. 2016). Other authors have reported that atresia occurs after thermal stress (Suárez et al. 2007; Múgica et al. 2015; Azpeitia et al. 2016) or in the presence of pollutants (Ortiz-Zarragoitia and Cajaraville 2006; Puy-Azurmendi et al. 2010; Smolarz et al. 2017). Atresia was further observed at different periods of the year, in winter (Suárez et al. 2007; Azpeitia et al. 2016), in spring or in summer (Ortiz-Zarragoitia and Cajaraville 2006; Puy-Azurmendi et al. 2010; Múgica et al. 2015). Atresia events can also occur regularly, e.g. one case over two years at a site far from any industrial or agricultural activities (Bhaby et al. 2014). In the present study, atresia was observed at different periods of the year whatever the stage of gonadal development, presence of mussel reserves, or water temperature. Atresia did not show any relationship with the natural reproductive cycle but coincided with peaks of TSM, OM and BOD. These environmental parameters were considered relevant indicators of the overall pollution status at the port of Oran (Grimes et al. 2010; Remili and Kerfouf 2013; Rouane-Hacene et al. 2015). Pollution could therefore be the cause of atresia in mussels inhabiting this area. Sunila (1987) demonstrated experimentally in an aquarium that degeneration of oocytes and phagocytosis of mature spermatozoa could be induced in mussels contaminated with trace metals. In mussels collected from sites contaminated with trace metals and sulfuric acid, Sunila (1987) even observed the cessation of spawning in *M. edulis*. Spawning inhibition even during exposure to low levels of environmental pollutants can be explained by the disruption of serotonin and dopamine, two molecules responsible for regulating the spawning process (Almeida et al. 2003; Fraser et al. 2014).

The higher number of mussels and higher percentage of follicles affected by atresia and gamete resorption in July suggests that higher summer temperatures could also be responsible for this phenomenon. According to Lubet and Aloui (1987) and Fearman and Moltschaniwskyj (2010), a change of water temperature below or above a certain threshold (about 7°C and 19°C for *M. galloprovincialis*) disturbs gametogenesis and can result in the lysis of gametes. An increase in water temperature can also increase the bioaccumulation of certain contaminants such as metals by improving their solubility (Sokolova and Lannig 2008). Within certain tolerance limits, it can increase biological activity of organisms including filtering (Navarro et al. 2016). Increased filtration rates in mussels can thus lead to higher contaminant absorption (Baines et al. 2006; Mubiana and Blust 2007; Coppola et al. 2018).

A seasonal study of the stability of the lysosomal membrane in mussels from the polluted port of Oran revealed significantly higher labialization of membranes when the temperature was highest (Taleb et al. 2007, 2009). The stability of the lysosomal membrane depended on both the quality and the temperature of the water (Lowe and Fossato 2000; Viarengo et al. 2000; Woo-Geon and Sang-Man 2005). Therefore, atresia could result from the destabilization of the lysosomal membrane of *M. galloprovincialis* oocytes in the polluted port of Oran, even more during hot summer periods.

In conclusion, this study has extended current knowledge of the reproductive cycles of Mytilidae. *Mytilus galloprovincialis* in Western Algeria has a year-long breeding cycle divided into two spawning periods,

one in winter, one in spring. We suggest that reproduction of this mussel is influenced by habitat conditions including the quality of the environment. We hypothesize two reasons for triggering the follicle cleaning process. The first corresponds to the last stage of reproduction (stage IIId) and occurs when nutrient reserves in gonads appear to be lowest. The second occurs after environmental stresses such as rises in water temperature and pollution discharges. It is important to distinguish between these two scenarios because environmental stresses may threaten recruitment and alter the use of mussels as bioindicator organisms. Because studies on the biology of bivalves are most often performed in healthy sites or *in vitro*, working in polluted sites where water temperatures can exceed limits tolerated by Mytilidae for proper functioning of their metabolism could help in the understanding of the effects of coastal pollution and global warming on mussel biology and reproduction.

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## Disclosure statement

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## ORCID

Jonathan Richir http://orcid.org/0000-0001-5890-5724

## Ethical Approval

The collection of wild *Mytilus galloprovincialis* in the port of Oran, Algeria, is not subject to any regulation. All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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