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A Study on the Reproduction Cycle of *Mytilus galloprovincialis* off the Coast of El Jadida (Atlantic Coast, Morocco)

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ABSTRACT

The present work was set as a part of the evaluation of the health status of the Atlantic coastline from the El Jadida region. This study was organized to study the reproduction cycle of the *Mytilus galloprovincialis* mussel, based on the technique of measuring the oocyte diameter and the measurement of biometrics. Thus, about 30 mussels in the size class (4-5) cm were collected in bi-monthly increments from January to December 2019. Two sampling sites were selected: the Jorf-Lasfar site (J) and the Haouzia site (H). The physic-chemical parameters (temperature and pH) were recorded for the water of the study sites.The results obtained indicate that the mean oocyte diameter follows an evolution managed by the reproductive stages of *M. galloprovincialis* from both sites. The minimum oocyte diameter values were 20.5µm and 24.25µm found in mid May, respectively, in J and H. This is the main stage of gamete emission; the secondary emission was noted in August/September and November/December. Variation in the condition index and gonad index supports this study. The reproductive cycle of *M. galloprovincialis* is therefore bisexual. The technique of monitoring oocyte diameter measurement is useful and very practical for studying the reproductive cycle in mussels.

INTRODUCTION

The mussel *Mytilus galloprovincialis* is a marine bivalve with a large geographic distribution. It has the advantage of being readily used in coastal ecosystem, bio-monitoring programs and networks such as "Mussel Watch" **(Goldberg, 1986)** and the National Marine Environmental Quality Observation Network **(Claisse *et al*., 1992)**.

Bivalve reproduction comprises a range of events ranging from gonad development to gamete emission. The first period begins with the initiation of the gametogenesis producing the mature gametes in one or more cycles and ending each time with their emission. The second phase is that of sexual repose, with the accumulation of energy reserves that are necessary to restart a new gametogenesis **(Benomar *et al*., 2006; Bhaby *et al*., 2014)**.

The *Mytilus galloprovincialis* species has been the subject of several studies by our team, including the bioaccumulation of trace metals **(Merzouki *et al*., 2009; Merzouki & Sif, 2012)** and the impact of xenobiotics on the biology and physiology of this species **(Sif *et al*., 2016; Khalil *et al*., 2019)**. Local knowledge of the different reproductive stages of *M. Galloprovincialis* is essential.

The objective of the present work was to expand the previous data of our research team by focusing on the reproductive cycle of the mussel *Mytilus galloprovincialis* from the coast of El Jadida region. The study was based on the bimonthly measurement of the oocyte diameter, the gonad index and the condition index of *M. galloprovincialis* off the coast of El Jadida.

MATERIALS AND METHODS

Study sites and sampling

Two study sites were selected in the coastal area of El Jadida (Fig. 1):

- Site of Haouzia (H) which lies about 1km north of the city of El Jadida (33°14'40.87''N, 8°28'38.597''W).
- Site of Jorf-Lasfar (J) situated about 25km south of the city of El Jadida (33°04'24.3 ''N 8°40'03.0 ''W).

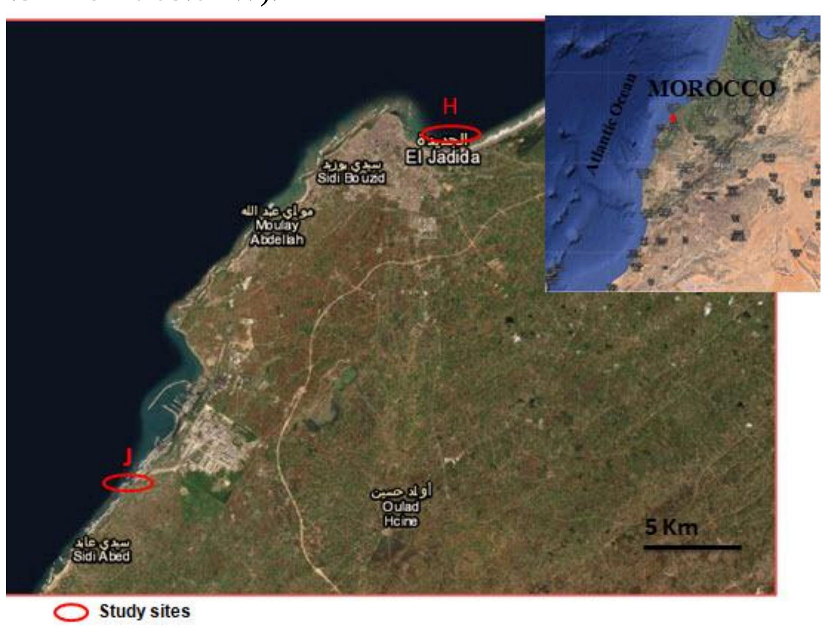

Fig.1. Geographical location of the study sites

Approximately, 20 mussels of *Mytilus galloprovincialis* per site were collected in bi-monthly increments from January through December 2019. Sampling took place at low tide in the intertidal zone. The size class is 4- 5cm. On arrival at the laboratory, the animals were selected, washed and then transferred to tanks containing oxygenated seawater for a period of acclimatization from 24 to 36 hours in an ambient temperature.

Abiotic parameters measurement

Water temperature and pH were measured at both research sites. The measurements were made on site using a multiparameter probe (Multi 340i).

Measuring biological parameters

Sex ratio (SR)

The sex ratio is defined as the proportion of male or female individuals compared to the total. The sex of the mussels was determined by macroscopic examination of the mantle color.

$$\mathbf{SR\ F = (F / (M + F)) \times 100}$$

$$\mathbf{SR\ M = (M / (M + F)) \times 100}$$

Where, **F = females** and **M = males**

Oocyte diameter

After opening the shells and identifying the sex, a fragment of the mantle of the female individuals was taken and deposited on a drop of sea water carried by a slide. The fragment was then covered by a slide. The observation and the measure of the oocyte diameter were realized using the optical microscope equipped with a micrometric eyepiece. A total of 30 diameter measures were performed for about six individuals.

The reproduction of bivalves includes a sequence of sequence of events from the development of the gonad to its regression; Table (1) presents the different stages of reproduction according to lubet 1959.

Table 1. The different stages of reproduction in the mussel

Stage of reproduction	description
Stage 0	Stage of sexual rest. Almost transparent gland. Development of the connective tissue. The follicles are still present but their diameter has decreased.
Stage I	Organization of follicles, multiplication of gonads. Macroscopically, visceral mass still transparent, but the pattern of the follicles forms a slight whitish network.
Stage II	The visceral mass has become opaque and whitish, we can find all stages of gametogenesis.
stage III A 1	The gland is very swollen, gametogenesis is very advanced.
stage III A 2	The excitations cause an emission.
stage III B	Oviposition or ejaculation have taken place. Macroscopically, the gland is flattened, and the color has become uniform.
Stage III C	The restoration stage is located between two successive issues.

Gonad index

In mussels, the gonad is mainly contained in the lobes of the mantle **(Mikhaïlov *et al.*, 1996)**. After the animals were shelled, the soft mass and mantle lobes were weighed using a precision balance (0.001g).

The gonad index (GI) is usually expressed as a percentage (%) by the ratio of gonad tissue weight to total soft mass weight.

GI = (Gonad weight / Total body weight) × 100

Condition index

The condition index (CI) is a biometric index commonly used in shellfish aquaculture to report the degree of shell filling the animals. The total weight and the weight of the soft mass were taken for about fifteen animals. According to AFNOR NF V 45056, (September 1985), the condition index was expressed in percentage (%) as follows

CI = (Soft tissue weight without mantle water / Total body weight) × 100

Statistical treatments

The different measurements of oocyte diameter, gonad index and condition index were expressed as means ± MSE. For inter-site and temporal comparisons, the statistical test used is the one parametric Newman-Keuls test, performed with STATISTICA 10 software as well as the analysis of variance (ANOVA).

RESULTS

Abiotic parameters

The temperature of the seawater at sites H and J remains comparable. In January, it fluctuates between 17°C and 18°C. A significant increase in temperature was noted from mid-May (25.9°C) and continued in June, July and September when the temperature reached 26.6°C (Fig. 2). From October onwards, the seawater temperature decreased to 17°C (Fig. 2).

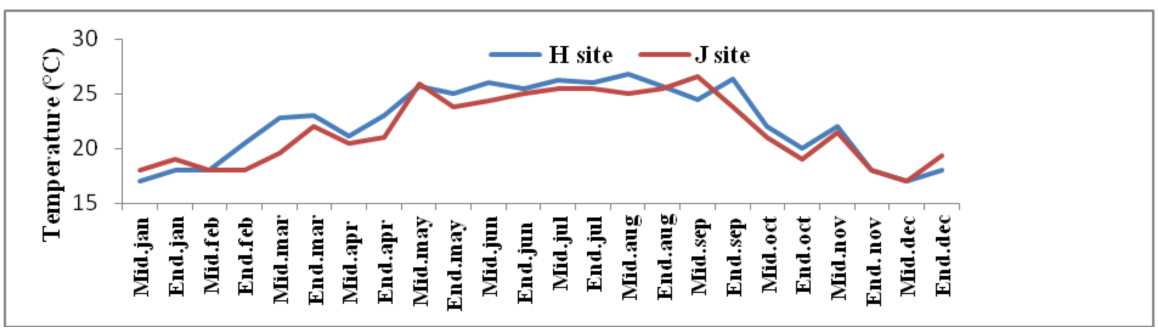

Fig.2. Spatio-temporal variation of seawater temperature at the study sites

The pH measurement of the seawater at the Haouzia site (H) was 8.41 as the minimum value, and the maximum was 9.5 at the end of March (Fig.3). In the site of Jorf-Lasfar (J), the pH oscillates between 7.26 in January.

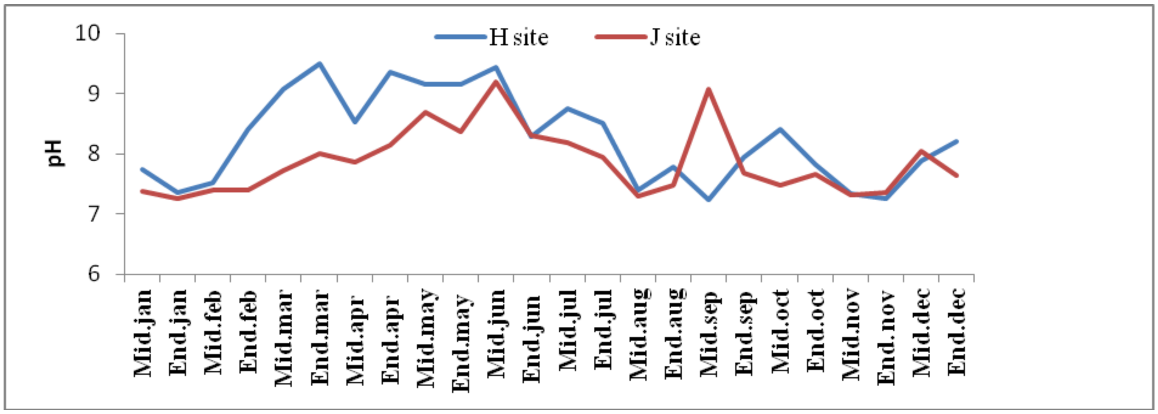

Fig.3. Spatial and temporal variation of seawater pH at the study sites

Biological parameters

Sex ratio (SR)

For the sex ratio for 340 mussels sampled in site H, the annual female rate was 61%, compared to 38% as a male rate (Fig.4). At the Jorf-Lasfar site, the percentage of females was 64 and that of males was 35.

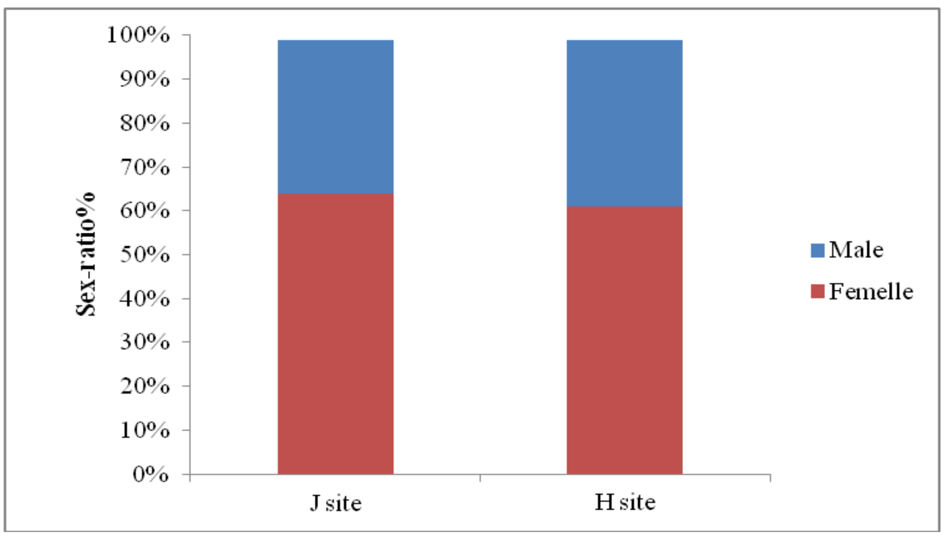

Fig.4. Annual rates of masculinity and femininity in *Mytilus galloprovaincialis* from the study sites

Oocyte diameter

The temporal evolution of the mean oocyte diameter of mussels at the two sites (H and J) was almost similar (Fig.5). The maximum value was recorded at the end of March; 63.25µm and 61.67µm, respectively, for the individuals of J and H. This indicates the stage of sexual maturity of individuals. The mean oocyte diameter shows a minimum value of 20.5µm recorded in mid-May in individuals from J and 24.25µm in those collected from site H. This is the gamete emission stage. This stage is significantly earlier for individuals of site J (Fig. 5).

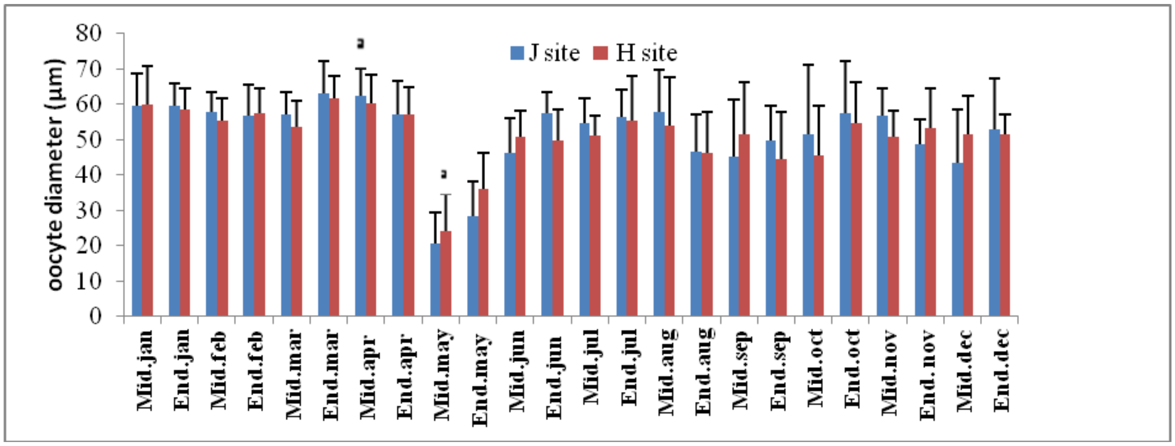

Fig.5. Temporal evolution of the average oocyte diameter of *Mytilus galloprovaincialis* from the surveyed sites

Gonad index

Mussels from site J have a low gonad index, compared to animals from H; this difference is only significant in mid-May and late July (Fig. 6). The minimum value was 12.8% in mid-May, and the IG max. was 23.07% in late October. In individuals from site H, IG min. was 14.56% and 26.1% as maximum value.

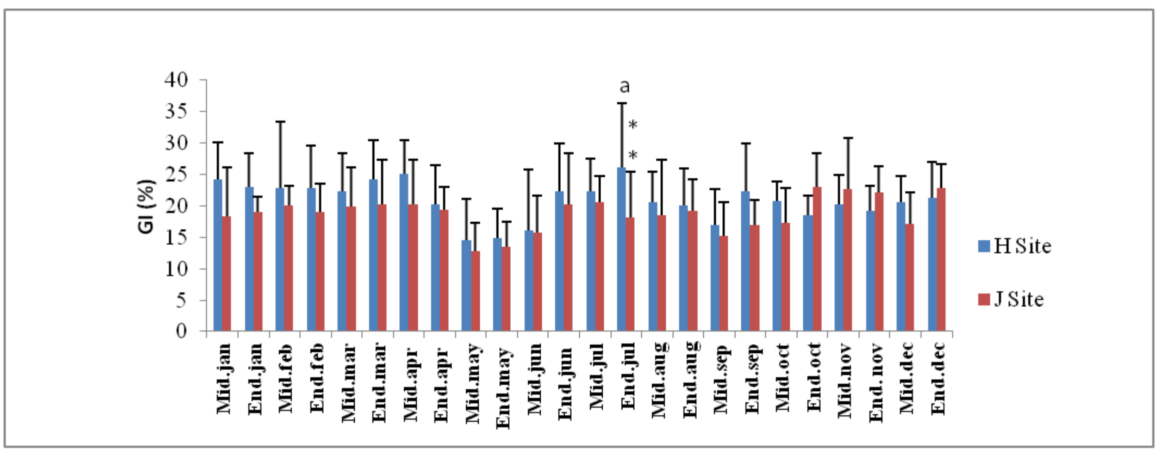

Fig. 6. Monthly variation in the mean gonad index of *Mytilus galloprovaincialis* from the surveyed sites

(*: Significant intersite difference at (**: P<0.0005; *: P<0.001; (a): Significant temporal difference at P < 0, 01).

Condition index

The shell filling degree of mussels from site H shows a minimum value of 23.11%, noted in mid-May; the maximum value of CI was 33.11% in the month of July in animals from sites J; the CI was 19.14% in mid-May, and the maximum value was 23.17% in mid-July (Fig.7).The results show a low reserve accumulation (weight) in individuals from both sites (J and H) in May and a maximum CI value in July (Fig.7).

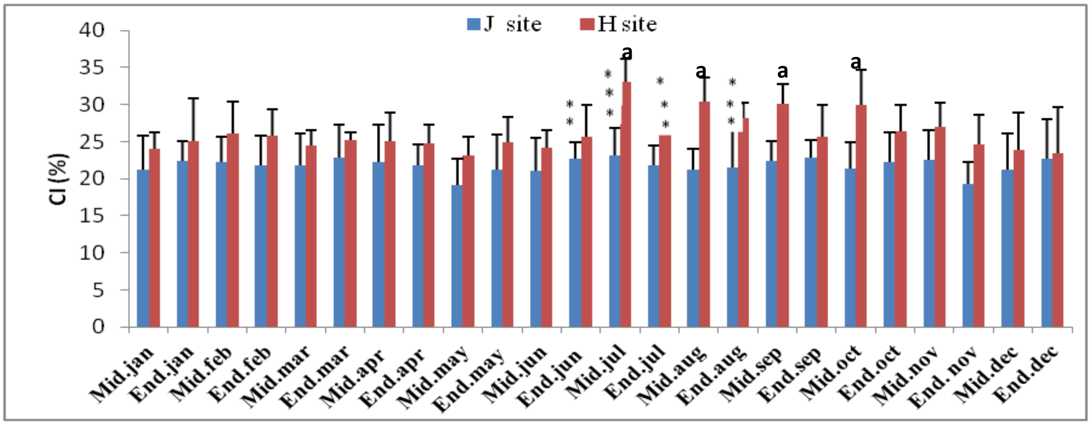

Fig.7. Monthly variation of condition index in Mytilus *galloprovaincialis* from study sites

(*: P<0.05; ** P< 0.001); ***: P< 0.00001. (a): Temporal significant difference at P< 0.00001).

DISCUSSION

The results of the present study show that the mean oocyte diameter follows an evolution managed by the reproductive stages of *M. galloprovincialis* at both sites. On the other hand, it shows a variation of CI and GI as a function of the stage of the reproductive cycle.

The temperatures recorded in the two sites show a period of low temperature from January to March, followed by a period of high temperature from April to September, initiating the gamete emission stage. The pH values of the seawater show a decrease in pH at both sites during January and February, succeeded by an increase until July and a second decrease starting from August. The increase in pH of seawater is generally related to an increase in the biological activities and activities of plankton and other macrophytes and aquatic animals **(Mollo & Noury, 2013)**. Environmental parameters influence physiological processes of natural reproduction, population dynamics and gametogenesis in most marine species **(Äkesson & Costlow, 1991; Prevedelli & Simonini, 2003)** and in bivalves **(Fearman *et al*., 2010)** as well**.**

Sex ratio is a characteristic of the species whose variations are sometimes related to the environment **(Dermeche *et al*., 2009)**, a finding which is affirmed by our results showing the dominance of the female sex at site J and site H. Bimonthly measurement of oocyte diameter in *M. galloprovincialis* indicates the stage of genital maturity (stage III) in March and April, which is characterized by an increase in gonad index and condition index in mussels from both sites. The period of the emission of the principal gametes (stage IV) being situated in May, indicated by an emptying of the mantle. A second light emission; which remains however not significant are reported in August and September and in November/December. This shows that outside of the main spawning periods there is partial spawning. The variations in CI and GI of the mussel confirm the period of maximum spawning when the mantle becomes very thin without reserve. This correlation has already been identified in the study of **Bignell *et al*. (2008)** conducted on the English coast and in the mussels of southern Morocco **(Bhaby *et al*., 2014)**.

The reproduction cycle of this species from the coast of El Jadida is predominantly annual. Data from the literature have always shown a variability of the stages of the reproductive cycle in mussels, which is a function of the locality even for the same species. In the Mediterranean Sea and the Algerian coast **(Rouabhi *et al*., 2019)** *M. galloprovincialis* presents a bi-annual cycle, with a main emission stage in late autumn/early winter and a second emission in late spring/early summer. In the Moroccan Atlantic coast of the Agadir region, individuals present a single emission stage, mainly situated in spring **(Bhaby *et al*., 2014)** and at the level of the south coast of New Zealand, the gamete spawning stage is situated in spring and summer, and the gamete development occurred during autumn and early winter **(Smart *et al*., 2021)**. This observed difference may be related to environmental factors (food, presence of contaminants ...) and the physiological state of the mussels **(Dittami *et al*., 2010)**.

The pattern of variation of oocyte diameter and GI and CI shows a peak in the month of July in mussels from both sites; this sharp increase is followed by a decrease of the same indices in the month of September which is characterized by a high temperature indicates that there is a second partial spawning of gametes. These results are confirmed by a study showing that the rate of gametogenesis decreased with increasing the temperature **(Fearman *et al*., 2010)**, while oocyte diameter is decreased at warmer temperatures. The oocytes of 50 - 55µm diameter are more frequent at 7°C and 10°C, 40 - 45 µm are more numerous at 19°C, and those with diameters ranging from 35- 40µm are more responsive at 13°C and 16°C. These results suggest that the effect of temperature on the rate of gametogenesis is strongly influenced by the energy balance. At low temperatures, gametogenesis is faster, while at high temperatures, gametogenesis is slowed down **(Fearman *et al*., 2010)**.

CONCLUSION

The study of biological indices, notably the oocyte diameter, the condition index and the gonad index allowed studying the reproductive cycle of the mussel *Mytilus galloprovincialis* at the level of the coast of El Jadida. On the other hand, the measurement of oocyte diameter over time proved to be a good practical tool to study stages of maturity and gamete emission during the reproductive cycle in *M. galloprovincialis.* This method can be adapted to other marine mollusks.

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