

Marine pollution effects on the reproduction process of *Perinereis cultrifera* (Annelida, Polychaeta) in Algeria

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The Mediterranean Sea is one of the busiest areas worldwide in terms of maritime activity and faces considerable anthropogenic disturbances, such as pollution by hydrocarbons and heavy metals. This study evaluated the environmental status of three sites on the Algerian coast using the annelid polychaete *Perinereis cultrifera* as a biomonitoring sentinel species. It included different reproductive parameters such as oocyte diameter, frequency distributions, sexual maturity index, biochemical quantitative parameters represented by the vitellogenins and vitellins, and a histological study of the coelome during the reproductive period. The results showed that the females collected from El-Kala (healthy site) have the highest diameter $304.1 \pm 24.3 \mu\text{m}$ during April but the values were increased in Annaba $290.0 \pm 21.0 \mu\text{m}$ and Skikda $245.3 \pm 26.7 \mu\text{m}$. Significant differences were observed between worms from the three study sites during the three months of the study. Moreover, the size-frequency indicated that a relatively high proportion of females containing mature oocytes was found in April in females collected at El-Kala 52.4%, 46.4% in females collected at Annaba and 36.7% in females collected at Skikda. In addition, regarding the sexual maturity index, the highest values were recorded in April (3.8, 2.9, and 1.9) at the El-Kala, Annaba, and Skikda study sites. However, the biochemical analysis demonstrated that the highest mean concentration of vitellogenin was in females of El-Kala ($5.3 \pm 0.3 \mu\text{g}/\text{mg}$ of coelomic fluid) in April, and the lowest mean concentration was measured in females of Skikda ($1.5 \pm 0.2 \mu\text{g}/\text{mg}$ of coelomic fluid). Therefore, the highest mean concentration of vitellin was measured in females of El-Kala ($4.4 \pm 0.2 \mu\text{g}/\text{mg}$ of oocytes) in April, while the lowest mean concentration of vitellin was measured in females of Skikda ($1.02 \pm 0.2 \mu\text{g}/\text{mg}$ of oocytes) in May. Significant differences were observed between worms from the three sites during the three months of the study. Furthermore, histological observations revealed that oogenesis was asynchronous, with oocytes at different stages of vitellogenesis simultaneously present. The structure of the oocytes of females collected at El-Kala showed intense vitellogenic activity due to the presence of very dense yolk cells. In contrast, the oocytes of the females collected from the two polluted sites (Annaba and Skikda) were small, vitellogenesis presented low intensity, and yolk cells were less frequent at the periphery and less uniform at the cytoplasmic mass; the nucleus was smaller, indicating a slower vitellogenic activity. These observations confirm the previously obtained results.

Keywords: *Perinereis cultrifera*; biometric; sexual maturity; vitellogenesis; Algeria.

Introduction

Urban water sources play an important role in industry, agriculture, and aquatic ecosystem conservation. However, human and industrial activities are responsible for the introduction of hazardous chemicals, such as pharmaceuticals and personal care products (PPCPs), to the environment through industrial effluents, domestic wastewaters, and diffused sources linked to agriculture (Vidal-Dorsch et al., 2012; Bahlmann et al., 2014; Evgenidou et al., 2015; Wang & Wang, 2016; Chen et al., 2020; Choi et al., 2021; Park et al., 2021).

The major environmental problems along the coastline of the Mediterranean Sea are urbanization, sewage and urban run-off, waste management, industrial effluents, marine transport, sand erosion and eutrophication (Scoullou & Ferragina, 2010; Belabed et al., 2017; Karadirek et al., 2019; Belfetmi et al., 2021; Boukari et al., 2021). Around one-third of the Med-Sea population lives in coastal regions whereas 65% of the population (around 120 million inhabitants) resides in coastal hydrological basins of the southern region of the Med-Sea, where environmental pressure is an increasing trend (EEA, 2015). Over the years, many research activities related to environmental problems in the Mediterranean region have been conducted by many scientists (Griffol et al., 2011; Aouini et al., 2017; Soud et al., 2017; Amamra et al., 2019; Drif et al., 2019). Since the beginning of the industrial revolution and the subsequent increase in industrial development, very large amounts of toxic pollutants have been discharged into coastal environments and estuaries, contaminating marine sediments with metals (Perkins et al., 1973; Farmer, 1991; Liu et al., 2003; Lavradas

et al., 2016; Inal et al., 2018). Local contamination is thus usual and can be due to several factors including pipeline construction, wastewater treatment and disposal, runoff, mining, industrial activities, ports, urban development (Taylor & McLennan, 1995; Balls et al., 1997; Morton & Blackmore, 2001). The Algerian coastal zone has experienced severe deterioration as a result of growing population and industrialization. Great industrial settlements in some areas (Skikda, Arzew, Algiers and Annaba) have been discharging their solid and liquid wastes directly into the sea or after a limited treatment. The maritime traffic and untreated domestic discharges from 16 million inhabitants along the Algerian coast are other factors influencing sea contamination. This situation exerts great pressure on the coastal marine ecosystem and worsening conditions can be observed on large sections of the coast, particularly in the gulfs close to the biggest agglomerations, such as Algiers, Oran and Annaba (Grimes, 2003) and near the industrial-harbour complexes, like Arzew, Bejaia, Ghazaouet, Skikda and Annaba (Belhadj et al., 2017; Amira et al., 2018; Amamra et al., 2019; Drif et al., 2019; Ziouch et al., 2020).

Benthic communities are directly exposed to contaminants adsorbed on the particle phase, and also to those dissolved in water at the sediment-water interface and, as a consequence, they have been conventionally used as bio-indicators in the biomonitoring of sediment toxicity (Lotufo et al., 2001; Guemouda et al., 2014; Meghlaoui et al., 2015; Snani et al., 2015; Tlili & Mouneyarc, 2019; Boumaza et al., 2021). These communities are formed by a large majority of sedentary species which are integrator agents of temporal effects of various environmental stresses either of natural or anthropogenic origin (Snani et al., 2015). Polychaete annelids are

well represented in marine environments and constitute a significant percentage of the total biodiversity and abundance of benthic macrofauna. Polychaetes are the dominant macrofauna within fine sediments (Fauchal, 1997). Polychaeta worms of the Nereididae family are one of the most common and representative species of the estuarine macrobenthos (Scaps, 2002), and they are classified as key species due to their important role as a food source and in biogeochemistry processes (Banta & Andersen, 2003; Amiard-Triquet, 2009).

The polychaete worms, as benthic fauna are biological parameters that indicate the overall aquatic fertility of the sediments, and the study of the polychaetes may be used as baseline information to evaluate the demersal fish stocks, as they form a major food item in the nutrition of the bottom feeders. In addition, and more importantly they could be used as good biological indicators of marine pollution. As a common and abundant species, *Perinereis cultrifera* could be a good choice for any prospective study. With a wide distribution and numerical dominance in some benthic communities, it occurs along the north-west of Europe, the Mediterranean Sea, the Atlantic Ocean, the Indian Ocean, the Pacific Ocean and the Red Sea (Fauvel, 1923; Herpin, 1925; Durchon, 1957; Cazaux, 1965; Cabioch et al., 1968; Wu et al., 1985).

The mode of reproduction, the age of sexual maturity and the characteristics of populations differ among populations. According to the literature, *P. cultrifera* has a lifespan of three years (Fauvel, 1916; Herpin, 1925; Durchon, 1951) and it reproduces with epitokous type in the Atlantic Ocean and the English Channel (Cazaux, 1965; Scaps et al., 1992), whereas, on the Algerian coast, Marcel (1962) pointed out that the lifespan of this species did not exceed two years and it reproduces without epitoke. The species is gonochoric and reproduces from epitokous or atokous forms. Based on that, Marcel (1962) thought of splitting the species into two physiological races. *P. cultrifera* is characterized by a monotelic (semelparous) pattern of reproduction in which a single, climatic reproducti-

ve event is followed by death. Concerning oogenesis, four phases could be recognized: oogonia (active mitosis), premeiotic, previtellogenic and vitellogenic (Olive & Clark, 1978), when primary oocytes appear, they accumulate nutritive resources (lipids, glycoprotein and finally lipovitellin) and will grow to a maximum oocyte diameter. Growth and gametogenesis are controlled by a neuro hormone produced by the cerebral neuroendocrine system. Neuroendocrine activity is high during the early stages of life, later reduces resulting in the accumulation of maturing gametes (Golding & Yuwono, 1994).

The aim of this study is to evaluate the effects of marine pollution on the progress of reproduction and on vitellogenesis by a biometric and histological study of three populations monthly sampled at three sites of the Algerian east coast during the reproductive period as part of the monitoring of this region of Mediterranean.

Material and methods

Study area. In this study, three sites were chosen: two sites (Annaba and Skikda), according to their position on potential sources of pollution, and a third site (El-Kala), which is a relatively clean area, far from any urban or industrial influence (Fig. 1). El-Kala (36°54'00.14" N, 08°28'12.48" E) is located in the extreme eastern part of the Algerian coast. This site is part of a national park, away from all the sources of pollution. Annaba (36°55'0.88" N, 07°46'5.96" E) is located approximately 78 km away from El-Kala. The site is exposed to various pollutants from domestic, agricultural, and industrial sources. Skikda (36°53'49.51" N, 6°52'50.39" E) is located approximately 182 km away from El-Kala. This site is in contact with the petrochemical industry, where raw petroleum and refined hydrocarbon products contaminate the surrounding areas via atmospheric pollution as well as effluents, which are dumped into marine ecosystems.

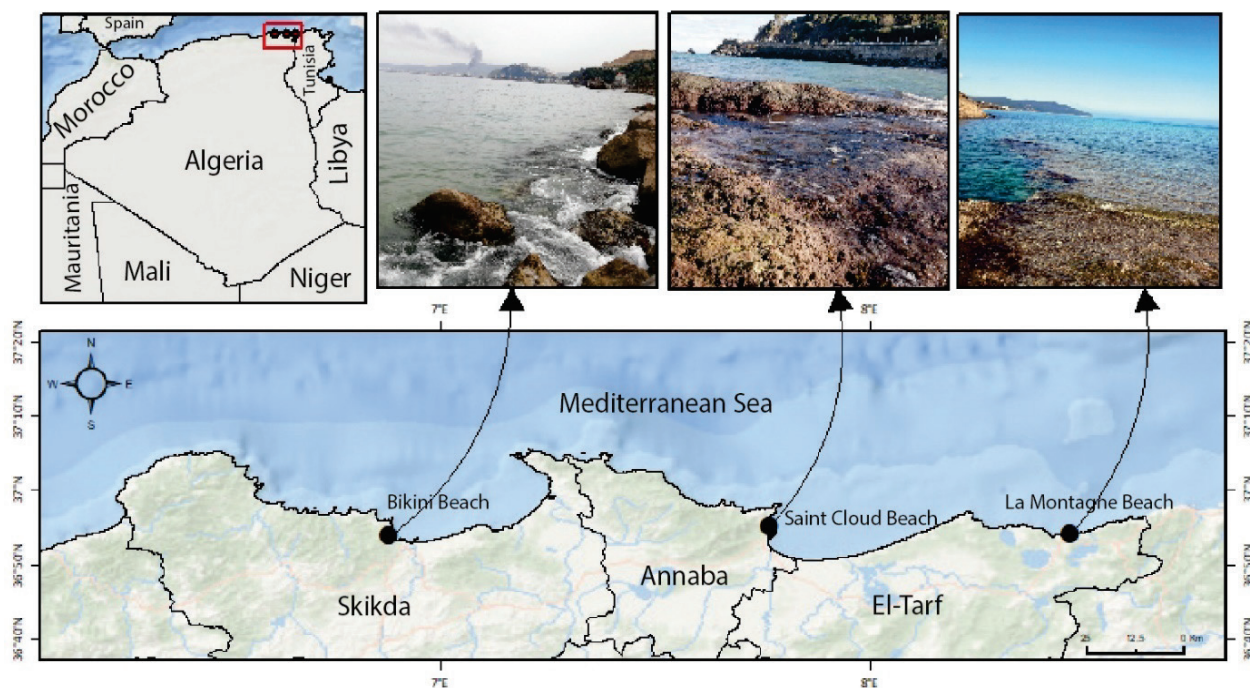


Fig. 1. Map of the study area showing the location of sampling sites

Sampling procedure. The specimens were collected monthly from March to May 2019. They were found within Rhodophyceae on algal-covered hard bottoms. They live in the low intertidal zone and extend down into the sublittoral zone. Consequently, the intertidal and shallow sublittoral hard bottoms were sampled methodically by scraping algae and looking for individuals (Rouabah & Scaps, 2003).

Examination of coelomic punctures and oocyte examination. Before oocyte sampling, polychaetes were anesthetized with 8% formalin for a few minutes. A sample of the coelomic fluid (~1 mL) was collected using a 1 mL syringe. The needle was inserted laterally into the polychaete body

between chaetigers 25 and 30, and the coelomic fluid was extracted and examined under a “Leica DM500” microscope, at $\times 4$ magnifications. For each individual, the number of oocytes was counted, and the diameter was measured. Oocyte diameter was used as an indicator of the maturation stage.

Frequency distribution of oocyte diameters. For the analysis of the frequency distribution of oocyte diameters, the oocyte diameter of 10 mature females was randomly measured under a “Leica DM500” microscope. The oocyte percentage for each month is equal to: number of oocytes for each range / total number of oocytes) $\times 100$. The oocyte diameter fre-

quencies for investigated worms can be grouped by mean oocyte diameter into 8 classes: 0–50, 50–100, 100–150, 150–200, 200–250, 250–300, 300–350 and 350–400.

Sexual maturity index (SMI). Since the sex ratio (about 80%) is greatly in favour of females, the investigation was carried out only on female worms. To determine the sex of the worm and assess the sexual maturity of females, an incision was made using a scalpel approximately 30 segments behind the head. The segment content was released on a glass slide and the coelomic fluid was examined under a microscope. The dimensions measured were the maximum diameter of each oocyte to the nearest micrometre. The reproductive status of the specimens was characterized by a stage of maturity ranging from 1 to 4 (Table 1). The sexual maturity index (SMI) was calculated as:

$$SMI = [(1 * n_1) + (2 * n_2) + (3 * n_3) + (4 * n_4)] / N,$$

where n_x is the number of individuals in stages x and N is the sum of n_x (Durou & Mouneyrac, 2007).

Table 1

Description of the development stage of sexual maturity of female worms *Perinereis cultrifera*

Stage coefficient	Stage description	The aspect of sexual products	Oocyte diameter range, μm
1	Indifferent	absence of germ cells in the coelomic cavity	–
2	Proliferation	small oocytes and oogonial cluster in the coelomic cavity	15–60
3	Growth (previtellogenesis)	growing oocytes freely suspended in the coelomic fluid	65–190
4	Vitellogenesis	vitellogenesis (undergoing)	190–250
5	Mature	oocyte large and spherical (mature oocytes)	250–450

Histological processing. Tissue samples were chemically fixed in formalin solution (9%) for 48 h, dehydrated in an alcohol series of progressive concentrations (70%, 80%, 90%, and 95%), cleared with xylene, embedded in paraffin wax (72 °C), and sectioned at a thickness of 3 μm using a microtome (Thermo Scientific). The sections were stained with Meyer's hematoxylin solution and eosin, mounted on glass slides, and viewed under a "Leica DM500" microscope.

Biochemical analyses (quantification of vitellogenin and vitellin). The coelomic content obtained by puncturing the animals was centrifuged at 1000 g for 3 min. The supernatant (coelomic fluid) was removed and the sediment of cellular elements (oocytes-eleocytes) was mixed with 2 mL of 0.05 M phosphate buffer pH 7.4, containing 0.55 M NaCl. Oocytes were then separated from eleocytes using sterile 35 μm nylon nets (Baert & Słomianny, 1987). Vitellogenin and vitellin content was measured following the procedure described by Fabre et al. (1990). Briefly, oocyte samples were placed in 500 μL of Tris-HCl-NaCl and homogenized using ultrasound. After centrifugation (5000 rpm for 10 min), three distinct layers were separated, and the intermediate layer containing vitellogenin and vitellin was collected in a clean Eppendorf tube and stored at –20 °C until analysis. The quantification was made according to Bradford (1976) using Coomassie G-250 (BBC) brilliant blue as a reagent and bovine serum albumin as a standard. The absorbance was read at 595 nm, and the results were expressed in $\mu\text{g}/\text{mg}$ of coelomic fluid and $\mu\text{g}/\text{mg}$ of oocytes for vitellogenin and vitellin, respectively.

Statistical analysis. All data are presented as mean (\bar{x}) \pm standard deviation (SD). Normality was checked using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was conducted to analyze the differences between sites ($P < 0.05$). Statistical analysis of data was performed with R (version 4.2.1; R Core Team, 2022) and RStudio (RStudio Team, 2022). Pearson's correlation tests were performed to understand the relationships between the different parameters.

Results

Biometric study of oocyte growth. As shown in Figure 2, the oocytes of the females collected from El-Kala, have an average diameter of $254.9 \pm 28.1 \mu\text{m}$ during March, from which it begins to increase to reach

the value of $304.1 \pm 24.3 \mu\text{m}$ during April and then it decreases during May, when $271.5 \pm 30.8 \mu\text{m}$ are recorded. The same growth is noted in Annaba and Skikda, the oocytes evolve in maturity from a value of $234.3 \pm 25.9 \mu\text{m}$ in Annaba and $219.6 \pm 34.8 \mu\text{m}$ in Skikda during March to reach the value of $290.0 \pm 21.0 \mu\text{m}$ in Annaba and $245.3 \pm 26.7 \mu\text{m}$ in Skikda during April, followed by a decrease in diameter during May when the value of $256.5 \pm 27.3 \mu\text{m}$ in Annaba and $227.8 \pm 25.7 \mu\text{m}$ in Skikda was recorded. Significant differences were observed between worms from the three study sites during the three months of the study ($P < 0.05$).

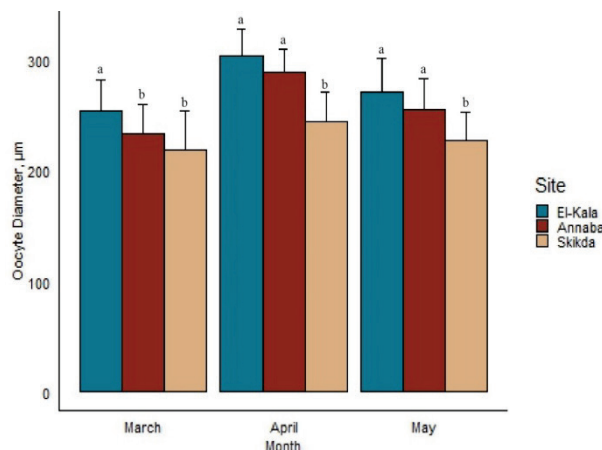


Fig. 2. Oocyte diameter of females collected from El-Kala, Annaba, and Skikda during the 2019 reproductive period: different letters indicate differences between sites within one month of observations (Tukey's test, $\bar{x} \pm \text{SD}$, $P < 0.05$)

Size-frequency. It has been found that a relatively high proportion of females containing mature oocytes was found in April in females collected at El-Kala 52.4% (Fig. 3a), 46.4% in females collected at Annaba (Fig. 3b) and 36.7% in females collected at Skikda (Fig. 3c). Females with small oocytes (diameter less than 50 μm) were present throughout the study period, with 0.02% in El-Kala, 4.1% in Annaba, and 4.7% in Skikda in March.

Sexual maturity index. The results showed that the highest values of the sexual maturity index were recorded in April (3.8, 2.9, and 1.9) at the El-Kala, Annaba, and Skikda study sites (Fig. 4). However, the lowest values were recorded in March (3.1, 2.3 and 1.5) respectively at the three study sites of El-Kala, Annaba and Skikda.

Histological analysis. Histological observations revealed that oogenesis was asynchronous, where oocytes at different stages of vitellogenesis were simultaneously present (Fig. 5a, 5c, and 5e). These oocytes are surrounded by nurse cells (eleocytes), are at an advanced stage of vitellogenesis, and are covered by a thin vitellin membrane (Fig. 5b, 5d, and 5f).

The structure of oocytes from females collected at El-Kala showed intense vitellogenic activity due to the presence of very dense yolk cells at the periphery of the vitellin membrane, which were evenly distributed over the surface of the cytoplasm surrounding the nucleus. The nucleus located at the center is large and round, indicating intense nuclear activity. These oocytes were ready to rupture the integument to be released (epitoky) for swarming and fertilization (Fig. 5a and 5b). In contrast, oocytes from the females collected from the two polluted sites in Annaba (Fig. 5c and 5d), and Skikda (Fig. 5e and 5f) were small in diameter, vitellogenesis was of low intensity, and yolk cells were less frequent at the periphery and less uniform at the cytoplasmic mass; the nucleus was smaller, indicating a slower vitellogenic activity.

These observations confirm the results of oocyte diameter, sexual maturity index, and oocyte size frequencies, which were lower in oocytes from sites affected by pollution. Moreover, the sum of the histological results allowed us to observe oocyte growth where vitellogenesis was significantly disrupted.

The concentrations of vitellogenin (Vg) and vitellin (Vt) in *P. cultrifera* females from each sampling site are shown in Figure 6a and 6b. The highest mean ($\pm\text{SD}$) concentration of vitellogenin was the El-Kala

site at, $5.3 \pm 0.3 \mu\text{g}/\text{mg}$ of coelomic fluid in April, with values ranging from 4.9 to $5.5 \mu\text{g}/\text{mg}$ of coelomic fluid while the lowest mean ($\pm\text{SD}$) concentration of it was measured at the Skikda site at $1.5 \pm 0.2 \mu\text{g}/\text{mg}$ of coelomic fluid in May, with values ranging from 1.3 to $1.8 \mu\text{g}/\text{mg}$ of coelomic fluid. Significant differences were observed between worms from the three sites during the three months of the study ($P < 0.05$).

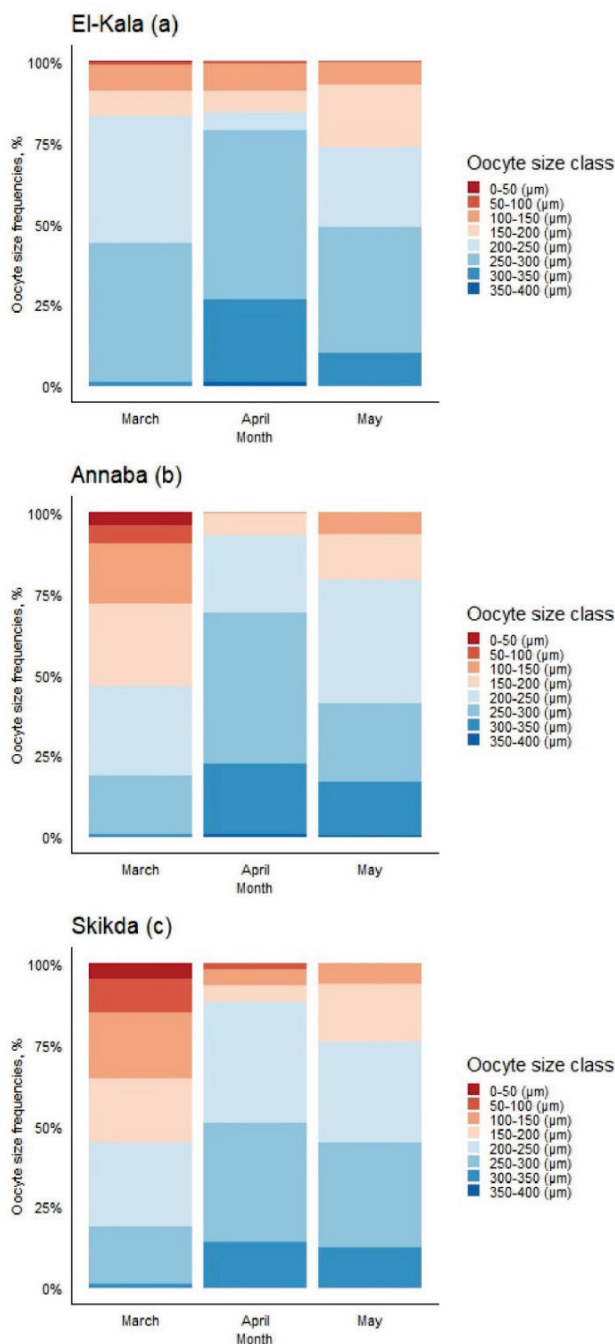


Fig. 3. Size-frequency distribution of *Perinereis cultrifera* oocytes from El-Kala (a), Annaba (b), and Skikda (c) during the study period

The highest mean ($\pm\text{SD}$) concentration of vitellin was measured at site of El-Kala at, $4.4 \pm 0.2 \mu\text{g}/\text{mg}$ of oocytes in April, with values ranging from 4.1 to $4.6 \mu\text{g}/\text{mg}$ of oocytes while the lowest mean ($\pm\text{SD}$) concentration of vitellin was measured at Skikda at $1.02 \pm 0.20 \mu\text{g}/\text{mg}$ of oocytes in May, with values ranging from 0.7 to $1.2 \mu\text{g}/\text{mg}$ of oocytes. Significant differences were observed between worms from the three sites during the three months of the study ($P < 0.05$).

Correlations between different studied parameters. The Pearson test allowed us to confirm that there is a positive and highly significant correlation between the four studied parameters at El-Kala (Fig. 6a), and very significant at Annaba (Fig. 6b) and Skikda (Fig. 6c), with lower values.

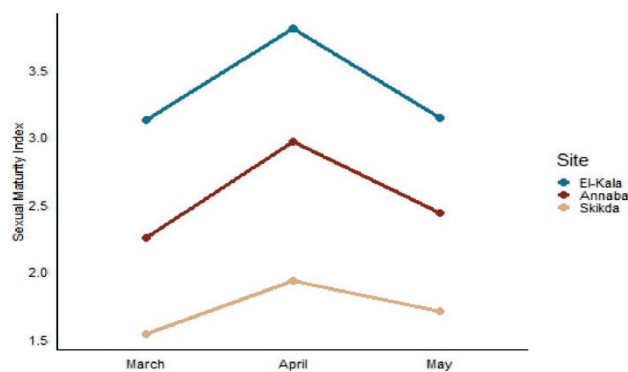


Fig. 4. Sexual maturity index variations in *Perinereis cultrifera* females at different study sites during the study period

Discussion

The aquatic ecosystem is an important environment that supports the livelihoods of millions of people, and its contamination is a major threat and concern (Ahmed & Thompson, 2019; Yu et al., 2020). The constructions along these ecosystems discharge several types of pollutants, such as metals, pesticides, microorganisms and microplastics (Abelouah et al., 2021; Chahouri et al., 2021). The complex mixtures of those pollutants cannot be assessed (Hamza-Chaffai, 2014) due to their potential synergistic/antagonistic effects. Their occurrence has become a major threat to the health of this ecosystem (Cappello et al., 2015). The Algerian coast, like several Mediterranean wetlands, is subject to increasing pressure in anthropogenic activities (urbanization, industry, pollution, aquaculture, tourism and overfishing). The consequences can be detected on the general states of ecosystems, mainly in macrofauna that is more sensitive and more exposed (Ben Mustapha et al., 1999; Ayari & Afli, 2003; Guemouda et al., 2014; Boucetta et al., 2016).

Many species have been used as bioindicators of pollution especially the bivalves (Sifi et al., 2007; Bensouda & Soltani-Mazouni, 2014; Hamdani et al., 2020) and the polychaetes (Rouhi et al., 2013; Snani et al., 2015; Diaz-Jamillo et al., 2017; Ramdani et al., 2020). The littoral is highly vulnerable to a wide assortment of contaminants and micropollutants directly released into the seas and oceans, to which are added those released into the air and drained by soils and rivers (Bensouda-Talbi, 2015). Polychaetes, located in unpredictable environments, have been deeply studied to understand the characteristics of their life cycle. Fauchald (1977) has divided polychaetes into three general reproductive lifestyles. Later, Wilson (1991) described a two-factor classification system for types of reproductive modes within the Polychaeta based on the type of larval development and the fate of the female gametes (free spawned or brooded in a variety of ways). Mettam (1980), Olive (1985), and Prevedelli & Cassai (2001) related this diversity of reproductive traits to the importance of variation in life-history traits related to the characteristics of brackish environments, presence or absence of epitoky, and reduction or disappearance of the dispersal phase.

In polychaetes, a specialized type of coelomic cells (eleocytes) assumes a central role in the process of oogenesis. Eleocytes synthesize yolk protein, vitellogenin (Fischer, 1979) which is secreted into the coelomic fluid and transferred to the oocytes, which internalize it by a specific uptake system (Fischer et al., 1991).

The observation of the oocytes of *P. cultrifera* during the three months of study (March, April and May 2019) showed an oogenesis presenting an asynchronous evolution where one meets in the same individual oocytes of different sizes and belonging to different stages of vitellogenesis.

The oocyte diameters at El-Kala increase in March to reach a peak in April then they gradually decrease during May. The impact of anthropogenic and industrial effects in Annaba and Skikda causes the reduction of oocyte diameter and this results in the imbalance of the storage of reserves during the process of oogenesis in comparison with the El-Kala site considered as a reference site in our study due to its remoteness from any kind of pollution.

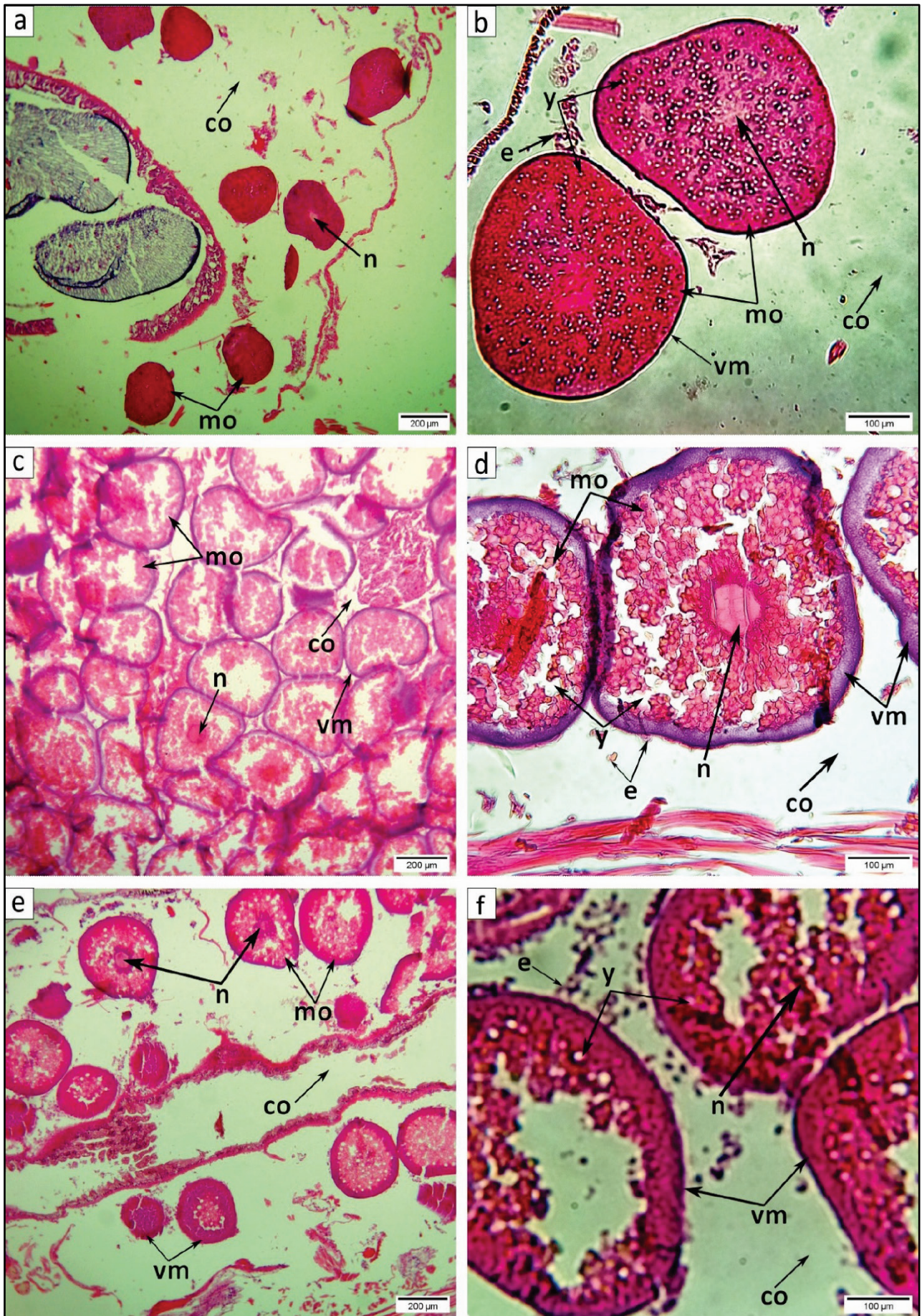


Fig. 5. Histological sections of female *Perinereis cultrifera* obtained from El-Kala (a, b), Annaba (c, d) and Skikda (e, f): a, c, e – maturing oocytes; b, d, f – mature oocytes; co – coelomic fluid; e – eleocytes; mo – mature oocytes; n – nucleus; y – yolk; vm – vitelline membrane

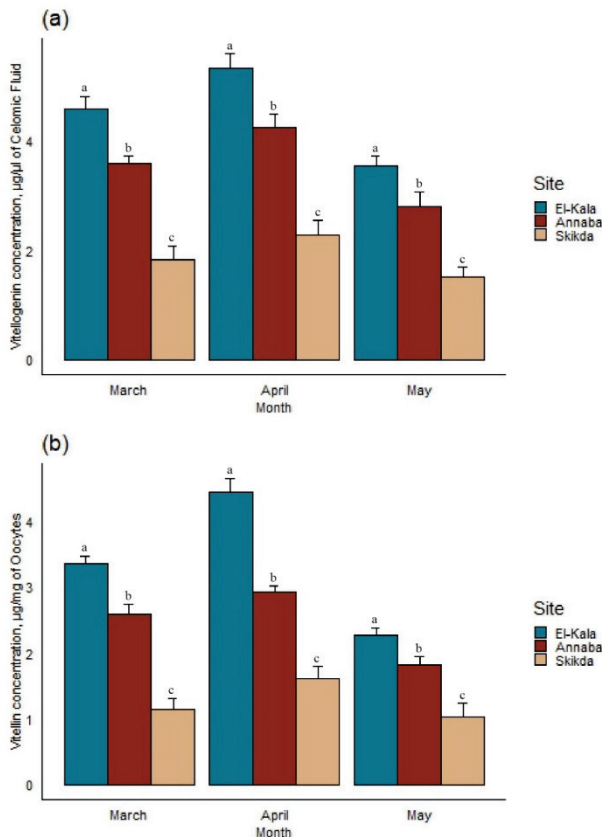


Fig. 6. Vitellogenin (a) and vitellin (b) concentrations in female *Perinereis cultrifera* sampled at the three study sites during the study period: different letters indicate the differences between the sites within one month of observations (Tukey test, $P < 0.05$); $\bar{x} \pm SD$, $n = 5$

The peaks recorded in April at the three sites can lead us to know the reproduction cycle of *P. cultrifera* and to confirm that this month is the period of emission of female gametes, which confirms the results of Daas et al. (2011).

The measurement of the diameter of the oocytes allowed us to determine the stage of sexual maturity. Nevertheless, it has been suggested that oocyte morphology could be a more sensitive indicator, particularly concerning hormonal activity (Schroeder et al., 1977).

In this work, the sexual maturity of females was defined by stages ranging from 1 to 4 after microscopic examination of an aliquot of coelomic fluid. According to stage 1 worms, germ cells are absent in the coelomic cavity. In stage 2 worms, germ cells are linked by cytoplasmic bridges and meiosis is not initiated. When meiosis is initiated, some primary oocytes appear in the coelomic fluid but the oogonial cluster is still present (stage 2). The minimum diameter of oocytes varies from 15 to 20 μm . Then, the oocytes initiate a phase of growth (stage 3), vitellogenesis, absorbing resources through the coelomic fluid. The oocytes are very heterogeneous in size (from 65 to 190 μm). Finally, the oocytes enter a maturation process and reach a maximum oocyte diameter equal to or greater than 250 μm leading to gametes that are mature and easily ready to be spawned (step 4). In general, Nereidae showed a wide range of oocyte diameter and according to Olive et al. (1998) from 170 to 200 μm for *Nereis virens* (M. Sars, 1835), while *N. diversicolor* (O. F. Müller, 1776) has a small range of oocyte diameter from 130 to 140 μm at three different latitudes in southwestern Portugal (Costa, 2003). The diameter of mature oocytes in *Perinereis nuntia brevicirris* (Grübe, 1867) ranges from 200 to 250 μm . Daas et al. (2011) indicated that oocytes of *Nereis falsa* took less than one year to develop fully, and the first mature oocyte diameter that occurred in the coelomic fluid of females was greater than 160 μm (approximately 180 μm). *Pseudonereis anomala* (Gravier, 1900) in Iskenderun Bay has a diameter of mature oocytes in specimens with epitokal modification that may reach 195 μm (Çinar & Ergen, 1995). Therefore, in the Barge canal, the maximum oocyte diameter of *P. anomala* was 207.9 μm ,

and the diameter of the mature oocytes ranged between 170.0 to 207.9 μm . However, on the Egyptian coast, *P. cultrifera* displayed lower fecundity and maturation percentage than *Platynereis dumerilii* (Audouin & Milne-Edwards, 1833), while the latter species recorded less biomass and smaller oocytes (Hamdy et al., 2020). Coelomic puncture in *P. cultrifera* females had a heterogeneous appearance and presented different oocyte diameters. Due to the asynchronous oogenesis of *P. cultrifera* females, we performed a biometric study of oocyte growth during the reproductive period.

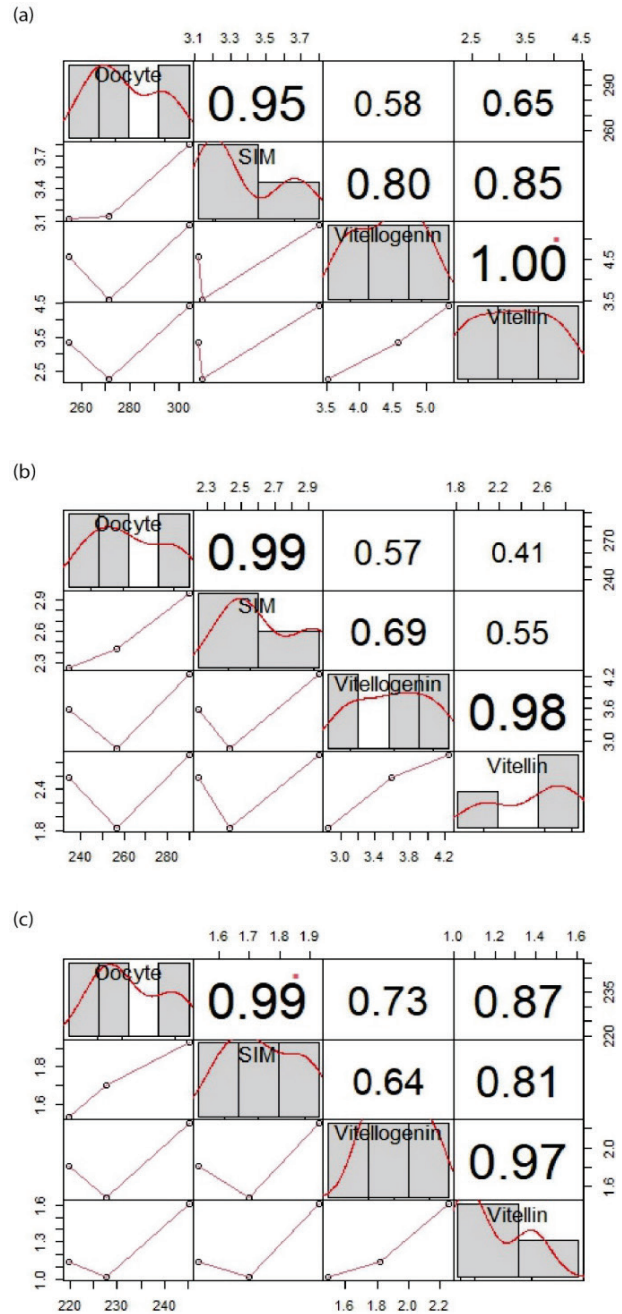


Fig. 7. Pearson correlation test between four parameters (oocyte diameter, sexual maturity index, vitellogenin, and vitellin) at El-Kala (a), Annaba (b), and Skikda (c)

The diameter of the oocytes present in the coelomic cavity was used as an indicator of the stages of maturation. In *P. cultrifera*, these germinal elements had a wide range of sizes, such that eight groups of oocytes were identified using a class gap size of 50 μm . Oocytes having completed vitellogenesis measured 280–300 μm . Females with small oocytes (diameter less than 50 μm) were present throughout the year. Thus, we can consider that the breeding period extends from March to May. Our results are in agreement with the work of El Barhouni et al. (2013) on *Marphysa*

sanguinea (Montagu, 1815). Oogenesis requires massive synthesis and storage of the female-specific lipoprotein, vitellogenin (VTG), a key protein for the growth and development of the oocytes. As in the other egg-laying animals, VTG is usually produced externally (extra-ovarian synthesis) and is taken up by the oocytes via receptor-mediated endocytosis and this is also true in annelids (Fischer et al., 1991; Hafer et al., 1992; Lee et al., 1997). Recent studies in *N. virens* (Schenk & Hoeger, 2011) have shown that VTG also carries the hemoglobin degradation product biliverdin. Biliverdin is formed in the eleocytes and is surprisingly converted to a novel conjugated form, glutathione biliverdin, which was termed nereioverdin. In female animals, vitellogenin bound nereioverdin is exported into the coelomic fluid and incorporated into the oocytes which become progressively green during growth.

Vitellogenesis is the period of life during which the major yolk protein is being synthesized and deposited in developing oocytes. Among annelids, most nereids have for a long time been considered classic examples of polychaetes presenting autogenous vitellogenesis (Baert, 1985). This was based largely on the apparent absence or low levels of endocytotic activity as determined with electron microscopy (Dhainaut, 1967, 1976). In polychaete annelids, two types of vitellogenesis have been described: a heterosynthetic mechanism (in several different worms) and an autogenous process (other including *Nereis*) (Porchet et al., 1989). Recent biochemical results suggest that the heterosynthetic mechanism is more general than previously thought. In *Nereis*, the vitellogenin (the precursor) is synthesized in coelomocytes and after transfer through coelomic fluid incorporated into oocytes. In germinal cells, a conversion process, involving proteolytic cleavages of vitellogenin, produces mature vitellins which are accumulated in yolk granules. The neurohormones identified so far are not essential for vitellogenin synthesis. These neurohormones may regulate enzymatic activities in the oocytes. A recent study of *P. dumerilii* (Schenk et al., 2016) has shown that VTG synthesis is under control of the sesquiterpenoid hormone methyl-farnesoate (MF). MF suppresses VTG synthesis in eleocytes, thus acting as a juvenile hormone. In turn, declining titers of this hormone then lead to the initiation of sexual maturation. Vitellogenin (precursor of the major constituent of oocyte reserves) is secreted by eleocytes and absorbed by the oocytes during vitellogenesis (Bonnier & Baert, 1992). This uptake process is receptor-mediated endocytosis (Rees & Olive, 1999). In oocytes, a conversion process, involving particular protein cleavages, transforms the precursor (incorporated vitellogenin) into vitellin (Baert, 1985; Baert & Slomianny, 1987; Porchet et al., 1989; Rees & Olive, 1999; Andries, 2001). In the species *P. cultrifera*, vitellin occurs during the second stage of oogenesis "corticogenesis" which follows the stage of vitellogenesis. In addition, the results of in vitro metabolic studies show a progressive increase in the rate of secretion of vitellogenins (the precursor) newly synthesized by the coelomocytes (eleocytes) which produce this protein until the end of oocyte growth. Thus, contrary to the results of previous cytological and autoradiographic studies, current data clearly indicate that the vitellogenesis process is active not only during the vitellogenesis phase but also throughout corticogenesis (Baert & Slomianny, 2011).

Synthesized vitellogenin is incorporated into oocytes after transfer through coelomic fluid. In germ cells, a conversion process, involving proteolytic cleavages of vitellogenin, produces mature vitellins which are accumulated in the vitelline granules (Maurice et al., 2011).

The fact that the oocyte is capable of autonomously assuming its vitelline syntheses has often been considered as a primitive characteristic (Bilinski, 1976; Gremigni, 1979). Among the polychaete annelids, the nereidians have long seemed to meet this criterion. In marine worms, the results obtained by autoradiography have indeed shown that the oocyte is capable of synthesizing protein material intended for the vitelline globules (Dhainaut, 1967, 1970; Bertout & Dhainaut, 1971; Dhainaut & Porchet, 1977). The monthly monitoring of the rate of vitellogenin and vitellin shows a considerable increase in the quantity of vitellogenin and vitellin in females of *P. cultrifera* during April. A very highly significant difference was recorded concerning the rate of vitellogenin and a very highly significant difference for the vitellin between the reference site (El-Kala) and the polluted sites (Annaba and Skikda). Oogenesis is asynchronous where oocytes bathed in coelomic fluid are at different stages of vitellogenesis. The results show a slowing down of the passage of yolk constituents to the

oocytes of females from the Annaba site and that of Skikda. This could be explained either by a disturbance in the synthesis of the cerebral hormone (Durchon & Porchet, 1971) or by the impermeability of the vitelline membrane blocking the diffusion of vitellin materials, even dysfunction of eleocytes which have focused much more on immune responses compared to environmental stress. However, five major lipoglycoproteins (V1 to V5) identified as vitellins were detected in young oocytes of *P. cultrifera* (Baert, 1985). The histological study of the coelom of the females of the population of *P. cultrifera* during the month of April provides very interesting data on the ovogenetic cycle of this species.

This suggests that the period of reproduction is concentrated during the spring season and that oocyte growth is asynchronous since, in the same individual, we encountered oocytes presenting roughly similar diameters. This growth has also been observed in other species, in particular, *P. dumerilii* (Fisher & Dorresteijn, 2004); in addition, in a species similar to Nereidae such as *Perinereis macropus* (Claparède, 1870), oocyte growth is asynchronous where all stages of vitellogenesis are found in the same individual (Zribi et al., 2007). The results obtained show a very pronounced disturbance of oocyte growth by the slowed supply of essential elements of the yolk. However, previous studies have shown that oocyte metabolism takes place in two successive phases; during the first, the oocyte develops the first lipid and then protein reserve substances, followed by a second where it synthesizes mainly carbohydrate reserves (Porchet & Dhainaut, 1969). The cerebral ganglia secrete a hormone that controls oocyte development. This hormone exerts a double-action; on the one hand, it inhibits the growth of oocytes and on the other hand, it is essential for their metabolism (Porchet, 1972). Further cytological studies have shown that the forming yolk cells are located near the dictyosomes. There are numerous vesicles morphologically identical to those produced by the Golgi apparatus. These vesicles seem to materialize the process by which the transfer of protein material from the dictyosomes to the protein globules takes place, thus emphasizing the role of the Golgi apparatus in the construction of the yolk (Caro & Palade, 1964; Jamieson & Palade, 1967). The work of Baert (1985) indicates that the lipid globules represent 16.4% of the oocyte weight and which are essentially triglycerides and free sterols and that the most important amino acids are glutamine, leucine and alanine; these compounds are at the base of the vitelline as in insects (Hagedorn & Kunkel, 1979) and in *N. virens* (Fisher, 1979).

Conclusion

This study investigated the biometric, histological, and biochemical features of oocyte growth in female *Perinereis cultrifera* collected from three sites (El-Kala, Annaba, and Skikda) in Algeria. The study showed that oocyte diameter increased from March to April and decreased in May at all of the sites, with significant differences between the sites. The proportion of females with mature oocytes was the highest in April, and females with small oocytes were present throughout the study period. The sexual maturity index was the highest in April and lowest in March at all three sites. Histological observations revealed asynchronous oogenesis and disrupted vitellogenesis in the polluted sites. Biochemical analysis showed that the concentrations of vitellogenin and vitellin were the highest in April at the El-Kala site and lowest in May at the Skikda site, with significant differences between the sites during the study period. These results suggest that environmental pollution affects the reproductive health of *P. cultrifera*.

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