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Meriem Snani
Laboratoire de Biologie Animale Appliquée, Département de Biologie, Faculté des Sciences, Université Badji Mokhtar, Annaba, Algeria.

Zoubeida Meghlaoui
Laboratoire de Biologie Animale Appliquée, Département de Biologie, Faculté des Sciences, Université Badji Mokhtar, Annaba, Algeria.

Ouided Maamcha
Laboratoire de Biologie Animale Appliquée, Département de Biologie, Faculté des Sciences, Université Badji Mokhtar, Annaba, Algeria.

Tarek Daas
Laboratoire de Biologie Animale Appliquée, Département de Biologie, Faculté des Sciences, Université Badji Mokhtar, Annaba, Algeria.

Patrick Scaps
Laboratoire de Biologie Animale, Université des Sciences et Technologies de Lille, Villeneuve d'Ascq Cédex, France.

Correspondence:
Ouided Maamcha
Laboratoire de Biologie Animale Appliquée, Département de Biologie, Faculté des Sciences, Université Badji Mokhtar, Annaba, Algeria.

Laying period and biomarkers of the polychaete *Perinereis cultrifera* from the eastern coast of Algeria subjected to marine pollution

Meriem Snani, Zoubeida Meghlaoui, Ouided Maamcha, Tarek Daas, Patrick Scaps

Abstract
Seasonal changes in oocyte diameter and pollution biomarkers of the Polychaete *Perinereis cultrifera* (Nereididae, Polychaeta) were studied during twelve months from January to December 2012 in the east coast of Algeria. The samples were collected monthly at three sites: El-kala, a site far from any source of pollution, Annaba and Skikda, sites located near human and industrial wastes. The oocytes took away females are measured and the biomarkers selected during this study were the activities of acetylcholinesterase as neurotoxicity marker and glutathione S-transferase as phase II enzyme. The results show differences between sites compared with the reference samples the period of reproduction unroll in spring (April, May). This approach confirms that individuals from Skikda and Annaba have been submitted to highly polluted environment. Individuals collected from Skikda showed the highest reduction of diameter oocyte as well as the highest inhibition or induction of enzyme activities indicating a highly contamination status.

Keywords: *Perinereis cultrifera*, reproduction, oocytes, Pollution, Biomarkers

1. Introduction
Large amounts and variety of xenobiotics have been and are introduced into the aquatic environment. They are accumulated in sediments, which then constitute true reservoirs of contaminants [1]. Algeria experienced an urban, agricultural, industrial and tourism significant development, which threatens the quality of the marine environment. Indeed, the coast receives anthropogenic releases associated with various human activities [2]. Organisms living in this environment undergo numerous factors of stress of different types: natural (climate changes), anthropogenic (pollution) causing alterations in the marine ecosystem. Benthic communities are directly exposed to contaminants adsorbed on the particle phase, but 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 [3]. 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. Polychaete annelids are well represented in marine environments and constitute a significant percentage of total biodiversity and abundance of benthic macrofauna. Polychaetes are the dominant macrofauna within fine sediments [4]. The polychaete *P. cultrifera* (Nereididae) was described for the first time by [5] from the Adriatic Sea. It occurs along the north-western coasts of Europe and the Mediterranean. This species has also been described in the Indian and Pacific Oceans [6]. According to the geographical location of the populations, mode of reproduction differs largely [7]. Reproduction in the English Channel and the Atlantic is of an epitokous type [8] as in the Mediterranean Sea at Salammbô near Tunis [9], as at Annaba on the Algerian Mediterranean coast near the Tunisian border [10], and in the Venice Lagoon in Italy [11]. However, on the west coast of Algeria in the Bay of Algiers, the reproduction has been described as atokous [12], as on the Moroccan Atlantic coast [13] and the Gulf of Marseille [14]. *P. cultrifera* is an intertidal poor disperser polychaete. It is reported to be a free-spawner. According to [15], *P. cultrifera* has a bentho-pelagic life cycle with a brief semi-pelagic phase.
The pollution of marine environments by the vast number of xenobiotics has increased during the last decade as a direct consequence of a wide variety of anthropic activities [16]. Such contamination represents a serious threat to the overall health of aquatic ecosystems [17].

Acetylcholinesterase (AChE) is an enzyme essential to the correct transmission of nerve impulses. An inhibition of this enzymatic activity has been used to detect and measure the biological effects of organophosphorus and carbamates in the marine environment [18]. Moreover, AChE may be also inhibited by heavy metals [19]. The glutathione-S-transferases (GSTs) are a multiple-enzyme family involved in phase II detoxification processes and are used as biomarkers of organochlorine pesticides and PCBs pollution in invertebrates [20].

The aim of this study was to test if the polychaete *P. cultrifera* could be used as a bioindicator in biomonitoring programs on the eastern coast of Algeria. In order to test this hypothesis we studied the effects of environmental pollution on indicators of reproduction (mean oocyte diameter) and enzymatic biomarkers (AChE, GST activities)

2. Materials and Methods

Experiments have been conducted on females *P. cultrifera* collected monthly during the year 2012.

2.1. Sampling sites

Sampling sites were chosen because of their geographical locations in eastern Algeria (Figure 1). Site selection was based on the level of pollution as well as ease of access to the study area and abundance of species. El-Kala (36°53'44" N; 08°26'35" E) is close to the Tunisian border (10 km). This site is part of a national park and is not urbanized, therefore, it was considered as the healthy reference site. Annaba (36°54'27" N; 7°45'26" E) is located about 80 km from the Tunisian border. This site is exposed to the pollution by pesticides and/or heavy metals released from the FERTIAL factory and the port activities [21]. Skikda (36°45'0" N06°49'60" E) is located about180 km from the Tunisian border. This site is near from Stora port that is characterized by an intense maritime traffic. This site is exposed to the pollution by PAH due to the presence of an important petrochemical complex.

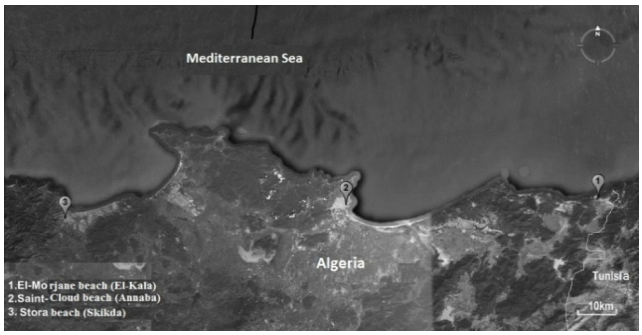


Fig 1: Location of the different sampling sites along the east Algerian coast; El-Kala, Annaba, Skikda.

2.2. Collection of individuals

Individuals were collected monthly from January to December 2012. They were found within Rhodophyceae, in algal-covered hard bottoms. They occur low in the intertidal zone and extend down into the sub littoral; in consequence, the intertidal and shallow sublittoral hard bottoms were sampled methodically by scraping algae and looking for individuals [10].

3.3. Examination of coelomic punctures

To study the reproductive cycle, the individuals were fixed in the laboratory with 8% neutral formaldehyde and examined for the presence of sexual products in the coelom. A short incision was made in the body wall at about the twentieth chaetigerous segment and a drop (~ 1 ml) of the coelomic fluid was removed with a Pasteur pipette and examined under a binocular microscope (Figure 2). When possible forty oocytes were measured using a calibrated eye piece graticule. The longest and the shortest diameter of oocytes were determined, and the average value was used as an estimate of oocyte size.

3.4. Biochemical analysis

Individuals were homogenized at 4 °C in a solution containing 38.08 mg EGTA, 0.1 ml Triton X 100, 5.845 g NaCl and 80 ml Tris/HCl buffer pH 7 for AChE and in 0.1 M phosphate buffer pH 6 for GST. Homogenates were then centrifuged at 9000 × *g* for 15 min at 4 °C for AChE activity and 13000 × *g* for 30 min at 4°C for GST activities. The supernatant of each sample was stored at -20°C. Total protein content in the homogenate was determined according to Bradford [22] at 595 nm using Bovine Serum Albumin as standard.

3.4.1. Acetylcholinesterase activity

AChE activity was determined according to Ellman *et al*. [23]. Reaction mixture contained 0.1 M sodium phosphate buffer (pH 7.5), 8 mM 2.4- dinitroochiocyanate benzene and the stock cytosolic solution containing acetylcholinesterase fractions. After pre-incubation, the reaction was started by addition of 8.25 mM acetylthiocholine (AtChl) as substrate. AChE activity was determined by kinetic measurement at 420 nm. Results were expressed as nanomoles (AtChl) hydrolyzed per minute per milligram protein.

3.4.2. Glutathione S-transferase activity

Glutathione S-transferase (GST) activity was determined according to Habig *et al.* [24] using 1-chloro-2,4-dinitrobenzene as substrate and glutathione (1 and 4 mM final concentration, respectively) in 100 mM sodium phosphate buffer, pH 7.5. All GST activity assays were realized in conditions of linearity with respect to incubation time. Results were expressed as micromoles produced per minute per milligram protein.

3.5. Statistical analysis

The results were expressed as means ± standard deviation (S.D). The normality of the distribution was tested using the Shapiro-Wilk test. To assess multiple comparisons, a parametric one-way analysis of variance (ANOVA) was performed on data with a Tukey's test. Statistical significance was defined at the $p \leq 0.05$ level. Statistical analysis was performed using Minitab (2000) Statistical Software version16.

4. Results

4.1. Annual evolution of oocyte diameter

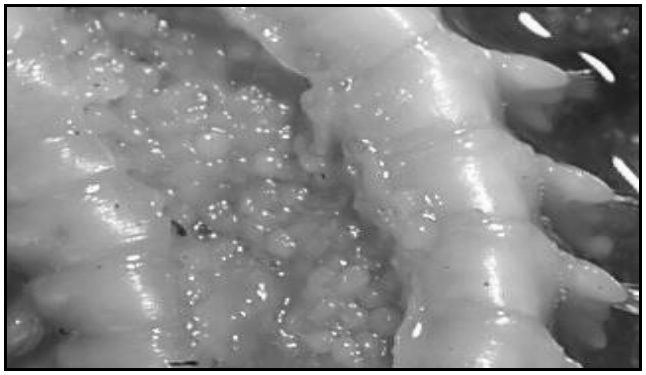


Fig 2: Appearance of a coelomic-puncture after incision (clusters of mature oocytes) (x 32)

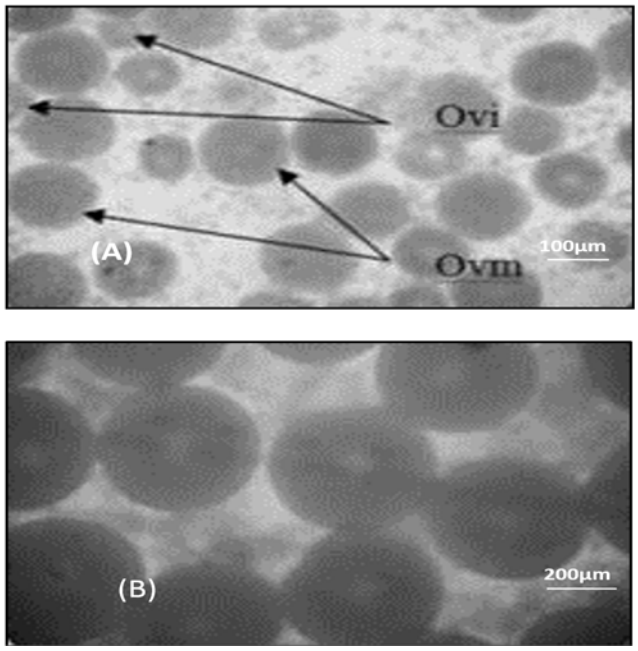
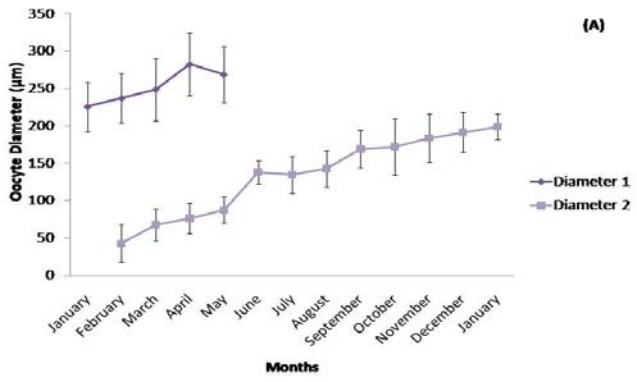


Fig 3: Microscopic appearance of a coelomic puncture of *P. cultrifera* showing oocytes at various stages of development. (A) Ovi: immature oocytes; Ovm: maturing oocytes (B) only mature oocytes

After incision of mature females during reproduction period, the coelomic-puncture presented many clusters of mature oocytes (Fig. 2); furthermore, microscopic appearance of this one showed different stages of oocyte development (Fig. 3)

Figure 4 A, B and C show the evolution of mean oocyte diameter for females collected in three different sites during the study period. The same trends were observed for the three populations. In January we only observed females bearing large oocytes (diameter > 175 µm). Then from February to May, two well-defined groups of females could be identified, one bearing small oocytes and the other bearing large oocytes (Figure 3A). So, in February, a new group of females with small oocytes (< 50 µm) started to emerge. In these females, the coelomic fluid also contained numerous coelomic corpuscles, but these gradually disappeared as the oocytes approaches maturity. From March to May, all the females bearing large oocytes (diameter > 175 µm) were epitokous (Figure 3B).The diameter of mature oocytes was about 250 µm (43.62–282.47µm and 39.76– 270.25µm) for females collected in El-Kala and Annaba and about 200 µm (37.76-224.37µm)for females collected in Skikda but the difference was not significant. In June, females bearing large oocytes disappeared due to the death of the mature epitokous females in May. The oocytes took about 16 months to develop fully (Fig. 4A, B and C).



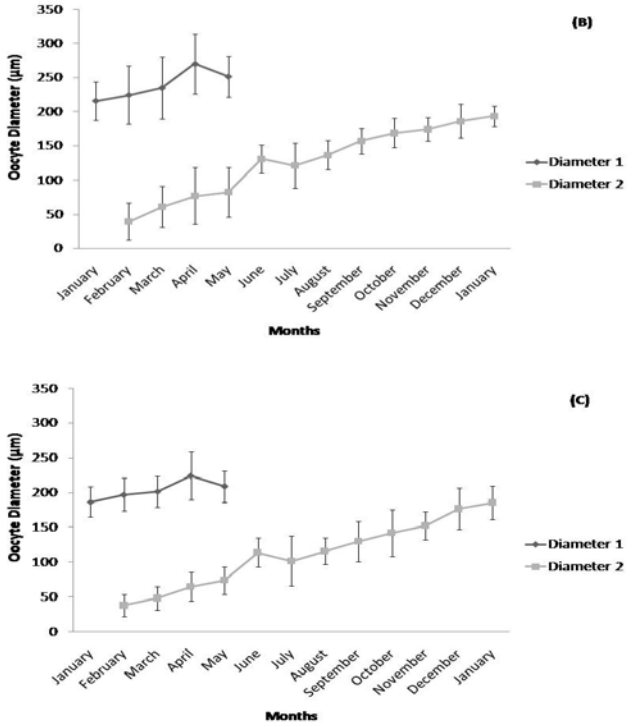


Fig 4: Oocyte growth from January to December of females collected from El-Kala (A), Annaba (B), and Skikda (C). Each data point represents mean ± standard deviation.

4.2. AChE activity

We observed seasonal variations of AChE activity at each site. Globally, AChE activity was maximal in spring then it decreased in summer and remained more or less stable thereafter (Figure 5). The higher values of AChE activity were observed in April at the three study sites (37.59±1.89; 30.61±1.88 and 27.38±1.81nmol^{-1} min^{-1} mg protein for individuals collected in El-Kala, Annaba and Skikda respectively) while the lower values were observed in August (16.63±1.61; 14.89±1.54 and 11.54±2.04nmol^{-1} min^{-1}mg protein for individuals collected in El-Kala, Annaba and Skikda respectively).

AChE activity was significantly higher in El-Kala compared to Annaba and Skikda throughout the studied period except in January, March, June, August, and September where the difference was not significant between El-Kala and Annaba (Figure 5). AChE activity was significantly different in Annaba compared to Skikda throughout the studied period.

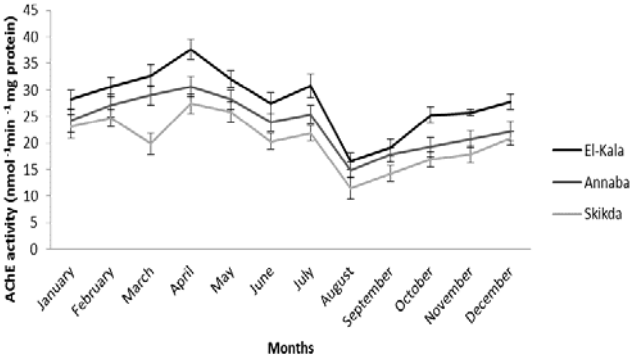


Fig 5: Monthly variations of AChE activity expressed as nmol^{-1}min^{-1} mg protein for individuals collected in El-Kala, Annaba and Skikda from January to December 2012

Each data point represents mean ± standard deviation (n=3)

4.3. GST activity

The results of seasonal variations of GST activity at each site showed that this one was maximal in spring and it decreased in summer. In the sites Skikda and Annaba, GST activity increased in late summer (September) and then remained more or less stable while GST activity remained low at El-Kala (Fig. 6). We observed also that the higher values of GST activity were recorded in April at the three study sites (8.78±0.69; 9.15±0.71 and 11.82±0.65µmol^{-1}min^{-1}mg protein for individuals collected in El-Kala, Annaba and Skikda respectively); in addition, the lower values were saved in August (2.81±0.55; 3.23±0.60 and 4.37±0.61µmol^{-1} min^{-1}mg protein for individuals collected in El Kala, Annaba and Skikda respectively).

Moreover, GST activity was significantly lower in El-Kala compared to Annaba and Skikda throughout the studied period (Figure 6). GST activity was significantly different in Skikda compared to Annaba (polluted sites) throughout the studied period except in the months February and July.

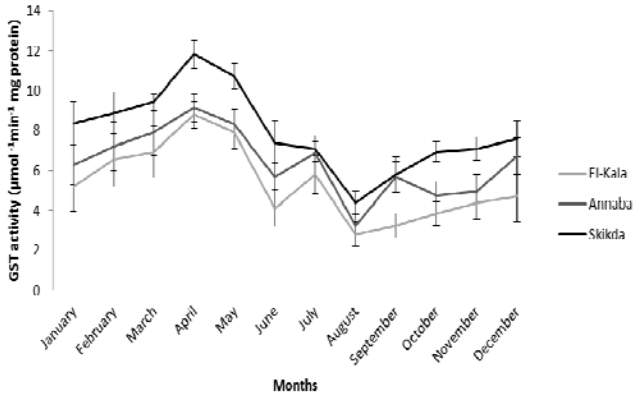


Fig 6: Monthly variations of GST activity expressed as µmol^{-1}min^{-1}mg protein for individuals collected in El-Kala, Annaba and Skikda from January to December 2012

Each data point represents mean ± standard deviation (n=3)

5. Discussion

5.1. Oocyte growth

The biometric study of oocyte growth of the three populations of *P. cultrifera* showed that oocyte growth is asynchronous, and the reproduction is more intense from March to May, however the oocytes take 9 to 12 months to mature and their diameter at maturity is approximately 200µm; contrary to what is commonly admitted, since we met in the same individual oocytes with different diameters; moreover, in related species such as *Platynereis dumerilii* [25] and *Nereis virens* [26], oocyte growth is synchronous. Our results confirm previous observations on the mode of reproduction of *P. cultrifera* in the east coast of Algeria [27], who found that individuals assigned to *P. cultrifera* reproduce exclusively by epitoky. These results are in disagreement with those reported from the bay of Algiers [28] indicating that reproduction is of an atokous type. The mode of reproduction of *P. cultrifera* has been examined at other sites in the Mediterranean Sea by other authors. Reproduction is reported to be of an epitokous type at Salammbô near Tunis [9] and in the Venice lagoon [11], where as it is of an atokous type in the area of Marseille [14]. Mature individuals were found in May at Salammbô [9] and in March in the Venice lagoon [11].

In addition, observations on the benthic phase of the life cycle of *P. cultrifera* have been carried out in the English Channel and the Atlantic coast of France. Reproduction in the English Channel [8] and the Arcachon basin [29] is of an epitokous type and individuals assigned to *P. cultrifera* have 3-year life span. In the Arcachon basin, the reproductive season is short and spawning occurs from late April to early June [29]. *P. cultrifera* is widely distributed along the Mediterranean coast and frequently used in marine pollution studies [30, 31]. In the English Channel specific observations made by [8] indicated that reproduction takes place from May to June and sometimes July.

5.2. Changes in Biomarkers activities

We found an inhibition of AChE activity in individuals taken from altered sites. This inhibition may be the result of a neurotoxic effect by exposure to pollutants. Cholinesterase activities are known to be inhibited in the presence of some pesticides [18] and several studies have used AChE inhibition to evaluate the biological impact of organophosphate and carbamate pesticides [32]. Some studies have also demonstrated that AChE may be inhibited by heavy metals in various organisms [19]. PCBs induce cytochrome P450 (phase I) enzymes and the biotransformation products (including reactive electrophiles) could play a role in AChE enzyme inhibition [33]. According to Leiniö and Lehtonen [34] there is an increasing evidence of the potential use of AChE activity in ‘‘stress screening’’.

Inhibition of AChE activity from polluted sites have also been reported in two marine invertebrates *Nereis diversicolor* and *Patella vulgate* collected from Tangier’s Bay (Morocco) [35] and in the blue mussel *Mytilus edulis* and the female eelpout *Zoarces viviparous* from the southwestern Baltic Sea [36].

Moreover, we showed an increase of GST activity in worms sampled from altered sites. These findings are similar to those obtained for the worms *N. diversicolor* collected from polluted estuary of the Seine [37] and from the Oued Souss (Bay of Agadir, Morocco) before implantation of wastewater treatment [38]. GST is a phase II enzyme involved in the metabolism of lipophilic organic contaminants such as PAHs and PCBs detected at comparatively high levels in the Seine estuary [37]. In addition, this enzyme plays a role in cellular protection against oxidative stress which can be triggered by pollutants such as metals, PCBs and PAHs [39].

6. Conclusion

Our present study on the annual main oocyte diameter and biomarkers in *Perinereis cultrifera* revealed changes correlated to reproductive events in the east coast of Algeria. Moreover, there are differences between the three sites. This difference was related to their level of exposition to pollution.

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