THE ENVIRONMENTAL STRESS EFFECTS ON THE REPRODUCTION OF A POLYCHAETE ANNELID PERINEREIS CULTRIFERA IN EAST COAST OF ALGERIA

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Abstract - Seasonal changes in (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 amounts of protein, carbohydrate, and lipid, were determined inside these ones. This study shows that the period of reproduction unroll in spring (April, May) ; the oocyte diameter and the monthly amounts of the different components are affected in polluted sites; however, the site of Skikda presents the lowest values.

Keywords - Polychète●reproduction ●oocytes ●stress ●metabolites

I. INTRODUCTION

The organisms living undergo many types of stressors: natural (climate change), 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; they have been conventionally used as bio-indicators in the biomonitoring sediment toxicity (Lotufo et al., 2001). These communities are formed bya large majority of sedentary species, which are all integrators agents temporal effects of various environmental stresses, whether natural or anthropogenic origin.There productive biology of Nereidae was recently studied for their commercial value (Zribi, 2007 ; Rettob, 2012), this family has a gonochoric mode of reproduction which present not well differentiated genitals ,however, they have masses of gametic cells (Martin et al., 1995) free in the coelom and will bere moved by snapping the integument corresponding to the épitoquy (Golding and Yuwono, 1994) ;

In this context, we evaluated the impact of environmental pollution on the reproduction of female of Perinereis cultrifera considering morphometric and biochemical study of three populations collected monthly at three sites along the east coast of Algeria.

II. 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.

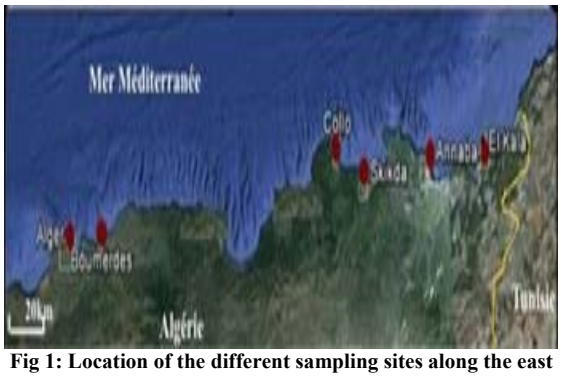


Fig 1: Location of the different sampling sites along the east Algerian coast; El-Kala, Annaba, Skikda.

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 (Rouabah A, Scaps P., 2003b).

2.3. 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. 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.

2.4. Biochemical analysis

Proteins, carbohydrates and lipids were extracted from the same gonad sample (oocytes) following the procedure of(Shibko et al,. 1966). In brief, after incision, each sample of oocytes was individually homogenized in 1 ml of trichloroacetic acid (20%) and then centrifuged (5000 g for 10 min at 4°C).

The supernatant was used for the determination of carbohydrates as described by Duchateau & Florkin (1959) using anthrone as reagent and glucose as standard, while the pellet added with a mixture of ether and chloroform (1V/1V) was subjected to a second centrifugation (5,000 g for 10 min at 4°C).

The resulted supernatant was used to quantify the lipids based on the vanillin method of Goldsworthy et al., (1972), finally, protein concentration was determined in resulting pellet using the Bradford (1976) assay with blue brilliant of coomassie (G 250, Merck) as reagent and bovine serum albumin (Sigma) as standard. Data were expressed as µg of protein, lipids or carbohydrates per mg of oocytes.

III. STATISTICAL ANALYSIS

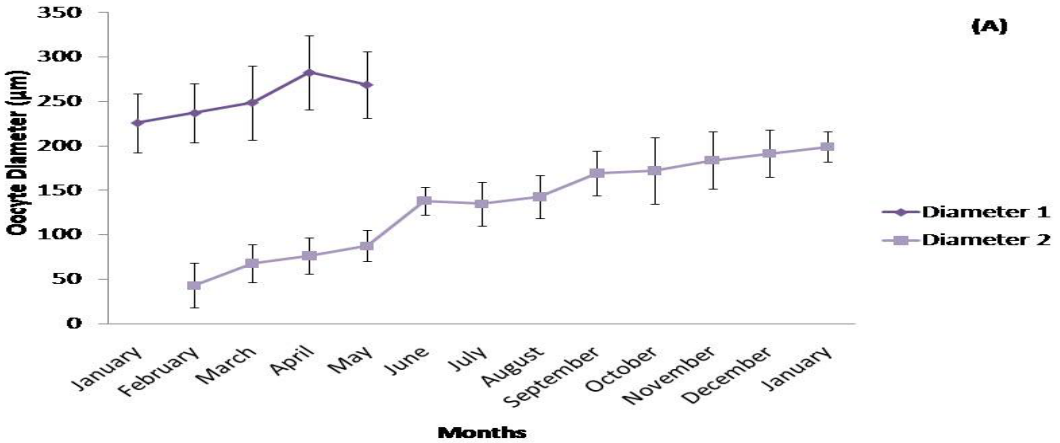
Data were expressed as Mean ± Standard Deviation (M ± SD). Comparison of mean values was estimated by Student's t-test. The effects of time and sampling sites were tested by a two-way analysis of variance (ANOVA). The relation between the oocyte diameter and biochemical components was also examined. All statistical analyses were performed using MINITAB Software (Version 14, PA State College, USA) and $p<0.05$ was considered to be statistically significant difference.

IV. RESULTS

4.1. Annual evolution of oocyte diameter

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



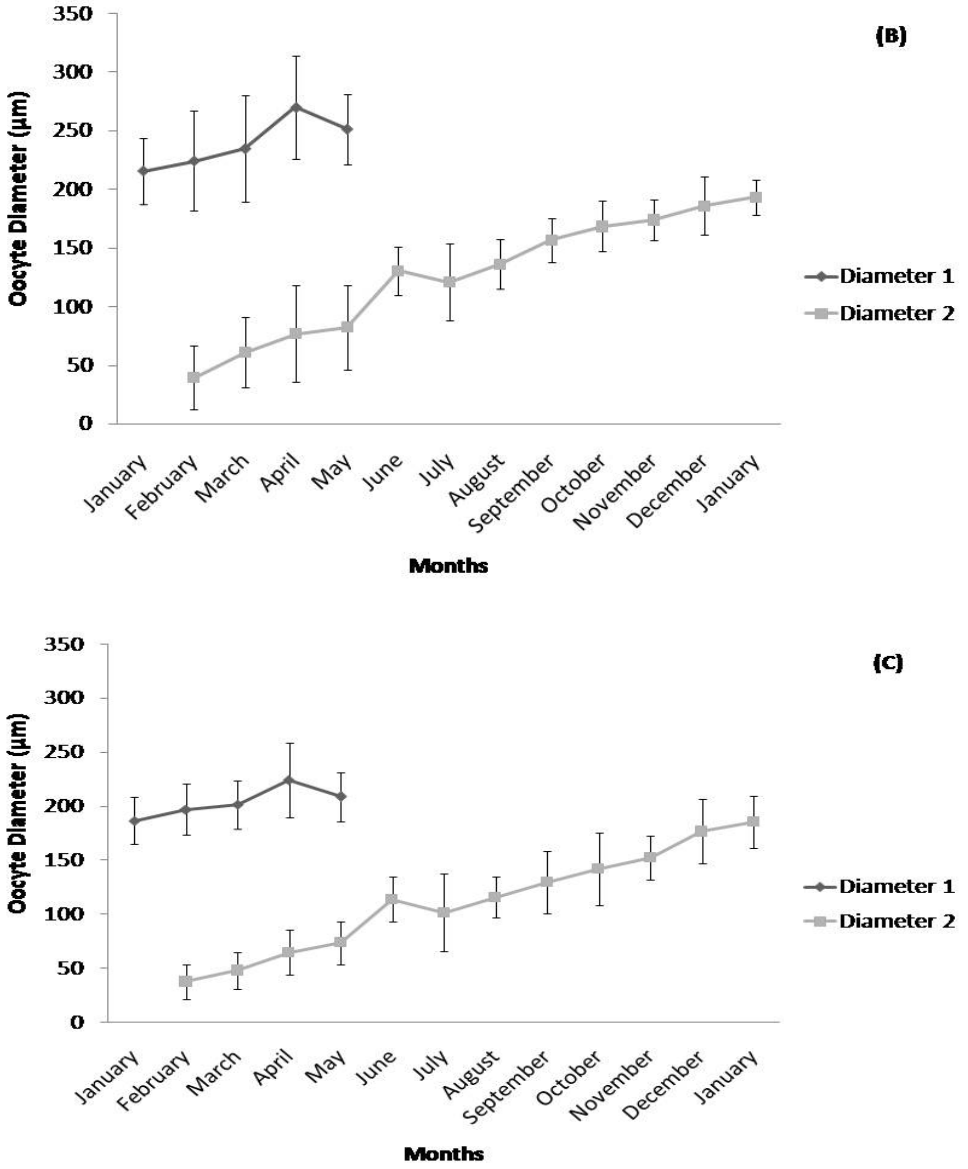


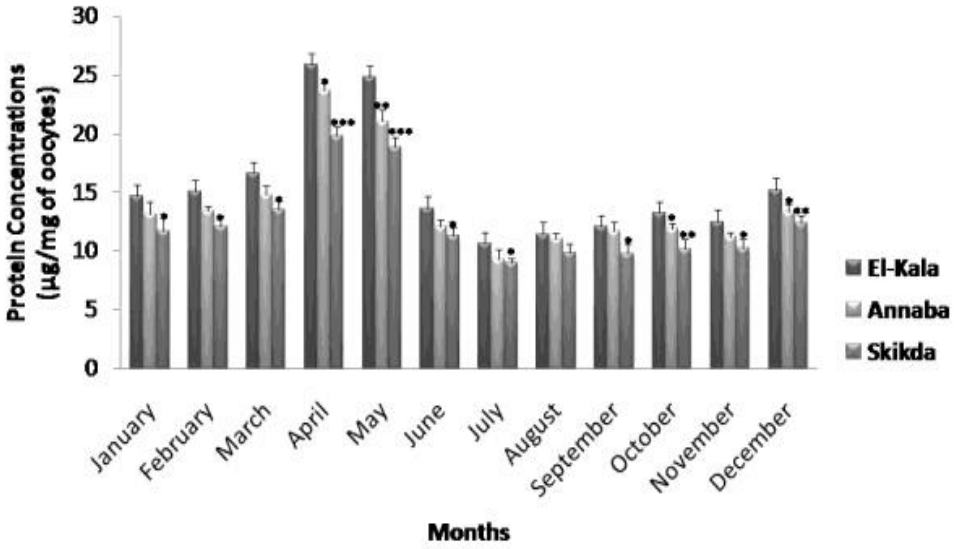
Fig 2: Oocyte growth from January to December of females collected from El-Kala (A), Annaba (B), and Skikda (C). Each data point represents mean ± standard deviation.

4.2. Annual analysis of the oocyte content metabolites

The quantification of the levels of energy reserves, namely proteins, carbohydrates, and lipids was determined monthly during the study period ranging a from January to December 2012 in the oocytes of female P.cultrifera, sampled at the three sites along the Algerian north east coast El-morjane beach (El-kala), St.Cloud beach (Annaba) and Stora beach (Skikda).

Evolution of protein concentrations

The results show the exchange rate oocytes proteins as function of time; however, a peak is clearly observed during the months of April and May at the three sites.the comparison of average monthly concentrations of protein a tthis three sites shows tha the largest amount of 25.84±0.75 mg/mg of oocytes was observed in April in females sampled from El-kala, while the lowest concentration in the range of 8.95±0.4 mg/mg of oocytes was recorded from females taken from Skikda in July. Furthermore, no significant difference was observed during the month of August ($P>0.05$) and for the three study sites; however, highly significant differences ($P <0.05$) were recorded respectively during the months of January, February, March, June, July, September and November at the site of Skikda and during the months of April to October and December at the Annaba site according to the reference site of El-kala; In addition, highly significant differences ($P<0.01$) were recorded in October and December at the site of Skikda and only in May at Annaba; and especially very highly significant differences ($P<0.001$) were respectively certified in April and May at the site of Skikda .The variance analysis with two classification criteria (site/ month) shows an effect site and month and interaction site/ month, all very highly significant($P <0, 001$).



. Fig 3: Monthly variations of protein concentrations expressed as µg/mg of oocytes for females collected at El-Kala, Annaba and Skikda from January to December 2012. Each data point represents mean ± standard error (n = 5).

Evolution of carbohydrates concentrations

From the beginning of growth, gradually evolution of oocyte carbohydrate contentis observed in females collected from El morjane beach (El-kala) reaching a maximum in the month of April. This was a ls observed in females collected from the other two study sites (Annaba and Skikda), followed by a decrease from the month of May until the end of the year. However, the maximum value (18.65 ±0.39 µg/ mg egg) was recorded in April at the El-Kala (healthy site), while the minimum value (10.18 ±0.41µg/mg oocytes) was reported in October in females collected at the site of Skikda (area affected by the pollution). The comparison of means between femalesof the three sites shows a very highly significant difference (P<0.001) during April at both sites of Annaba and Skikda, and a highly significant difference (P <0.05) duringthe months of January, May, June and November at the site of Skikda and June and September at theAnnaba site; where as no significant difference was observed during the remaining months of the year (Figure 6). Furthermore ,regarding the analysis of variance two classification criteria (location , month) ,it shows a site effect, month, and a very highly significant interaction web site/ month (P <0.001).

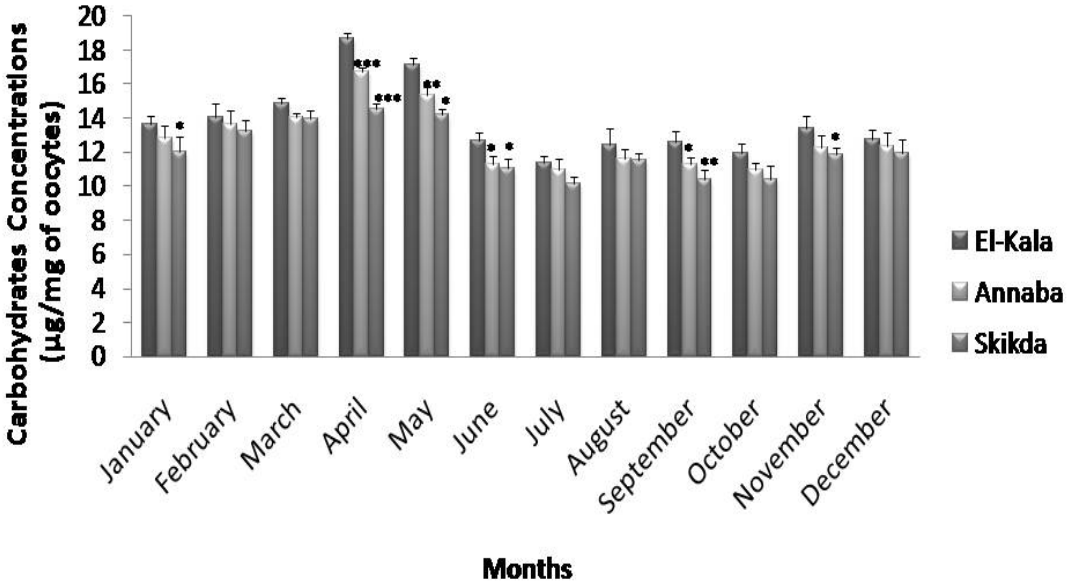


Fig 4: Monthly variations of carbohydrates concentrations expressed as µg/mg of oocytes for females collected at El-Kala, Annaba and Skikda from January to December 2012. Each data point represents mean ± standard error (n = 5).

Evolution of lipid concentrations

The results of oocyte lipid levels during the study years how fluctuations depending on the month; Moreover, the peak is clear lyidentifiedas for metabolites quantified previously, namely during the months of April and May fo rthe three study

sites.Indeed, the maximum value of the order of 16.52 ± 0.41 µg / mg egg was recordedin the month ofAprilat the site healthy (El-Kala), while the low est value corresponding tolipid 7.37±1.4mg / mg of oocytes was observed in July at the site of Skikda (polluted site). Statistical analysis of the rate shows no significant difference (P>0.05) during the study period at the Annaba site, except the month of Avril which it is very significant and May where it is highly significant (P<0.01); more over, at the site of Skikda, there is only the month of April a very highly significant difference (P<0.001) and a highly significant difference observed during the months of January, February, July, November and December (P <0.05); In addition, one highly significant difference was found in May atthe same site (Figure 7). The variance analysis with two classification criteria (location, month), reveals an effect and month very highly significant (P <0.001) against the effect by interaction site/month turns out not significant (P=0.877).

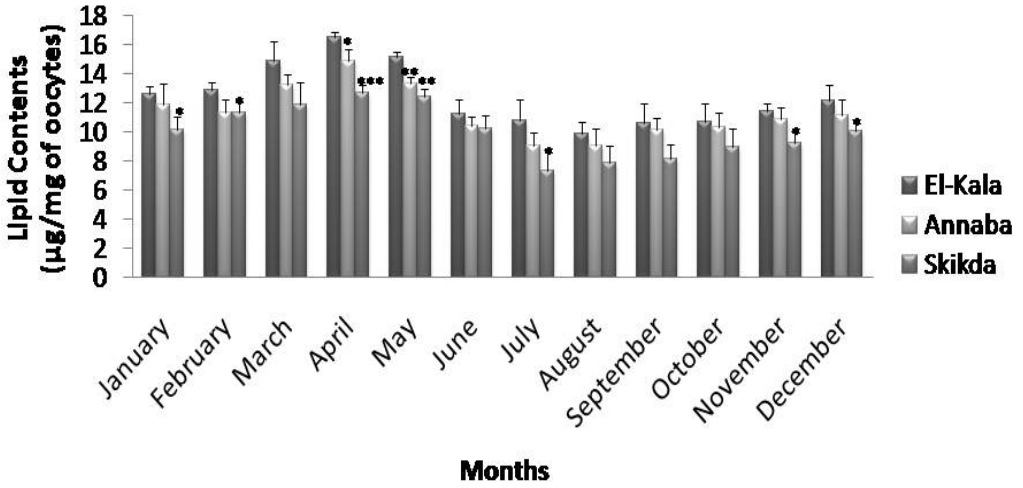


Fig 5: Monthly variations of lipid concentrations expressed as µg/mg of oocytes for females collected at El-Kala, Annaba and Skikda from January to December 2012. Each data point represents mean ± standard error (n = 5).

V. 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].

5.2. Changes in oocytes contents

Moreover, despite the differences in levels of protein, carbohydrates and fats, the monthly variationis similar for all three populations.In P.cultrifera, oogenesis has long been considered under th econtrol of a single hormone secreted by the brain,this hormone shows significant fluctuations depending on the stage of maturity; however, each state is a endocrine oocytes tate to allow the production of each cytoplasmic inclusion (proteins, carbohydrates, lipids), when the rateis high, the cytoplasm develops lipid and protein yolk; so when it is low or no carbohydrate reserves appear (Porchet and Dhainaut., 1977).

These observations are consistent with the literature, which indicates that in areas heavily polluted, many different species of plants and animals are able to cope with toxic chemicals that stress toleranceis supported by many different processes, such as the production of anti oxidant substrates (Durou et al, 2008), limiting the absorption bio transformation to less toxic forms, and increased excretion (Mason and Jenkins, 1995)

REFERENCES

[1] Bradford M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the

principle of protein-dye binding. Analy. Biochem., 72: 248-254.

[2] Daas T, Hamdi L, Daas-Maamcha O, Scaps P. Bmarqueurs de pollution chez l'annélide polychète ערPerinereis cultrifera de la côte algérienne. Bulletin de la société zoologique de France 2011; 136(1-4):117-130.

[3] Duchateau GH. and Florkin M., 1959. For trehalosemie of insects and its signification. Arch. Insect. Physiol. Biochem., 67: 306-314.

[4] Durou C., MouneyracC and Amiard-Triquet C., 2008.Environmental quality assessment in estuarine ecosystems: Use of biometric measurements and fecundity of the ragwormNereisdiversicolor (Polychaeta, Nereididae). Water Research.,42: 2157 – 2165.

[5] Fischer A, Dorresteijn A., 2004. The polychaete Platynereis dumerilii (Annelida): a laboratory animal with spiralian cleavage, life longsegment proliferation and a mixed benthic/pelagic life cycle, Bio Essays 2004, 26:31

[6] Golding D.W. and Yuwono E., 1994. Latent capacities for gametogenic cycling in the semelparous invertebrate Nereis. Proc. Nat. Acad. Sci. PhysiologyUSA.,91: 11777-11781.

[7] Goldsworthy GJ.,Mordue W. and Guthkelch J., 1972. Studies on insect adipokinetic hormones. Gen. Comp. Endocrinol., 18: 545-551.

[8] Lotufo G.R., Farrar J.D., Inouye L.S., Bridges T.S &Ringelberg D.B., 2001.Toxicity of sediment-associated nitroaromatic and cyclonitramine compounds to benthic

[9] Lotufo G.R., Farrar J.D., Inouye L.S., Bridges T.S &Ringelberg D.B., 2001.Toxicity of sediment-associated nitroaromatic and cyclonitramine compounds to benthic invertebrates. Environmental Toxicology and Chemistry., 20: 1762–1771.

[10] Mason A.Z.and Jenkins K.D., 1995. Metal detoxification in aquatic organisms. In: Tessier A., Turner D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. Wiley, Chichester, pp. 479–608.

[11] Mason A.Z.and Jenkins K.D., 1995. Metal detoxification in aquatic organisms. In: Tessier A., Turner D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. Wiley, Chichester, pp. 479–608.

[12] Porchet M and Dhainaut A., 1977. Effet de l'activité endocrine cérébrale sur la maturation de l'ovocyte des néréidiens (Annélides, Polychetes): Aspect cytologiques et biochimiques. Laboratoire d'Endocrinologie comparée des Invertébrés associé au C. N. R. S.,148: 31-49..

[13] Rettob M., 2012. Fecundity and the body length of Rag worm, P. cultrifera (Grube 1840) from wearlilir beach waters, small kei islands, southeast Maluku District. Journal of Coastal Development, Volume 16 : 84-88.

[14] Rouabah A, Scaps P. Life Cycle and Population Dynamics of the Polychaete Perinereis cultrifera from the Algerian Mediterranean Coast. P.S.Z.N.: Marine Ecology 2003b; 24(2):85-99.

[15] Rouabah L, Rouabah A, Ferroudj S, Scaps P. Comparison of the life cycles of two populations of the polychaete Perinereis cultrifera from the Bay of Algiers (Mediterranean Sea). Scientia Marina 2008; 72:769-778.

[16] Shibko S., Koivistoinen P., Tratyneck C., Newhall A. and Freidman L. 1966. A method for the sequential quantitative separation and glycogen from a single rat liver homogenate or from a sub cellular fraction. Analyt. Biochem.,19: 415-428.

[17] Zribi S., Zghal F andTekaya S., 2007.Ovogenèse de Perinereis macropus Claparède 1870 (Annélide, Polychète) dans le golfe de Gabès (Tunisie). C. R. Biologies., 330: 199-204.

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