The effect of the reproductive cycle on the bioaccumulation of heavy metals and the induction of metallothionein in the polychaete *Perinereis cultrifera* of the coastline of El Jadida (Atlantic coast, Morocco)

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Introduction

The Annelid Polychaetes are the important representatives of the benthic community recognized as biological tools of choice to reveal the perturbations which affect coastal ecosystems.[1] The bioaccumulation of trace metals and their harmfulness was studied in different marine species. The detoxification of metals involves their liaison with different ligands, in order to limit their bioavailability and therefore their deleterious effects.[2] The induction of metallothionein-like proteins (MTLP) by the metals in these living organisms, plays a crucial role in their regulation and a in their mechanism of detoxification.[3]

Perinereis cultrifera (Grube, 1840) is a Polychaete present along the Atlantic coast of El Jadida. Their repartition, reproduction and population dynamics have been studied by our team.[4,5] The present work has therefore the objective to investigate to what extent the induction of MTLP by the bioaccumulation of trace elements is influenced by the reproductive cycle of the Polychaete *P. cultrifera*. This species was sampled in the epitokous (abrupt changes in the body structure of many worms of the class Polychaeta. The epitokous occurs in the period of sexual reproduction, when the worms become adapted to swimming) and atokous forms, which represents another reason for our choice of this study.

Materials and Methods

Samples of *P. cultrifera* were collected seasonally during 2004 in the intertidal zone at low tide, from two sites (Figure 1). The SM site (Mouilha Site) is located in the immediate vicinity of an urban sewage, and the SR site is located 10 km from the first. On our arrival to the laboratory, the animals were placed in the seawater after being filtered by flirted-paper and oxygenated for a purge period of 24 h.

Analysis of cadmium (Cd), zinc (Zn) and copper (Cu) is carried out on the samples of the worms dried in the oven. Digestion (with nitric acid) was done during 1 h with room temperature, and to 140°C in a sand bath for 3 h. The acid solutions thus obtained are filtered (flirted-paper) and analyzed by atomic absorption spectrophotometry (AAS). On the other hand, worms were homogenized in 20 mM TRIS, 150 mM NaCl solution adjusted to pH=8.6. The homogenate obtained was subject to the protocol of compartmentation:[6] the soluble and insoluble fractions were separated by centrifugation (25,000 X g for 55 min). The soluble heat-stable thiolic compounds (MTLP) were isolated by centrifugation of the soluble fraction (15,000 X g for 10 min) after heat-treatment (75°C for 15 min). The quantification of (MTLP) is done in the cytosolic fraction by differential pulse polarographic analysis (PAR Model 174 analyzer, PAR/EG&G Model 303 static mercury drop electrode).

The stage of sexual maturity of *P. cultrifera* is evaluated by the measurement of the average diameter of the oocyte, using a nearest micrometer (30 oocytes/female). For each oocyte, the diameter is estimated by the sum of the maximum and minimum length. In order to estimate the number and age distribution of the cohorts at a given season, seasonal measurements of fresh weight were also carried out for the entire population of SM.[4] The analysis of variance (ANOVA) and the test of Newman-Keuls have been used for expressing and comparing the results.

Results and discussion

In specimen tissues of *P. cultrifera* from the station SR, the total levels of Cd was highly variable. The minimum and maximum content is 0.087 and 0.719 g.g^{-1} respectively in autumn and summer (Figure 2). The quantity in Zn is 35.317 µg.g^{-1} in autumn while that of Cu shows no significant difference according to the seasons. For the animals of the station SM, the highest concentrations of Cd, Zn and Cu were 0.155; 65.5 and 2.807 g.g^{-1} in winter.

The quantification of MTLP shows that their concentration is highly significant in the spring in worms of SM, which is 940 µg.g^{-1}. The lowest value is 428 µg.g^{-1} in summer (Figure 3). The highest rate for worms of SR is 859 µg.g^{-1} in winter.

The maximum induction of MTLP observed in worms of SM coincided with the oocyte diameter (Figure 4). The two parameters show a significant positive correlation (r=0.73; $P<0.05$) and a very clear seasonal variation. In effect, the highest oocyte diameter is 316 µm noted in the spring, and 940 µg.g^{-1} for the MTLP of animals (Figure 3). This profile follows a gradual evolution from summer to spring. The evolution of the population of *P. cultrifera,* reprsented by frequency histograms of weight during the four seasons, shows that the structure of the population is normal and unimodal, except in the spring. The population is formed by three cohorts (C1, C2 and C3) where the first corresponds to least aged individuals, the second to those who are older and the third represents the youngest specimens of the new generation. The measurement of the fresh weight means of cohorts showed a progressive increase from summer to spring, respectively, 13.51±4.68 g.10^{-2} and 42.28±12.64 g.10^{-2}. This situation allows to say that *P.cultrifera* passes from a young population of medium size and moderately sip of genital products in the summer, to a population perfectly mature in the spring.

In the light of these results, the sexual maturity of *P. cultrifera* deriving from SM is highest in spring (old population and oocytes perfectly mature). In parallel, the concentration of MTLP follows almost the same time profile, this relationship seems to indicate that the reproductive cycle of these animals influences induction of MTLP. Therefore, two hypotheses are suggested: the first assumes that the MTLP induced would be due to the bioavailability of heavy metals, and the second, MTLP vary between the period of reproduction (oogenesis) and periods of sexual rest.[7] Among these metals which induce strongly the synthesis of MTLP, Cd and Zn.[8] Seasonal variation of their tissue levels has already been suggested by several authors, as well as for the bivalves and for the polychaete.[9] The second hypothesis

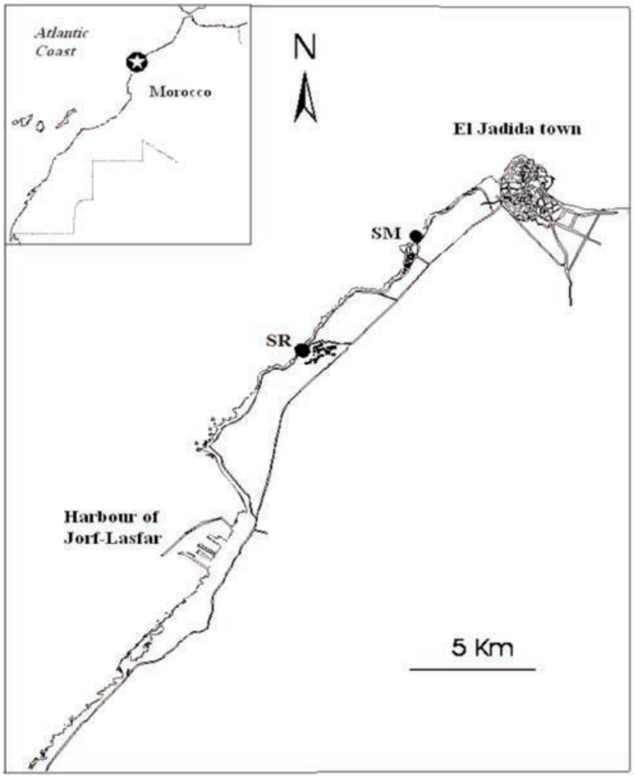

Figure 1. Location of the two sampling sites of *Perinereis cultrifera,* Morocco.

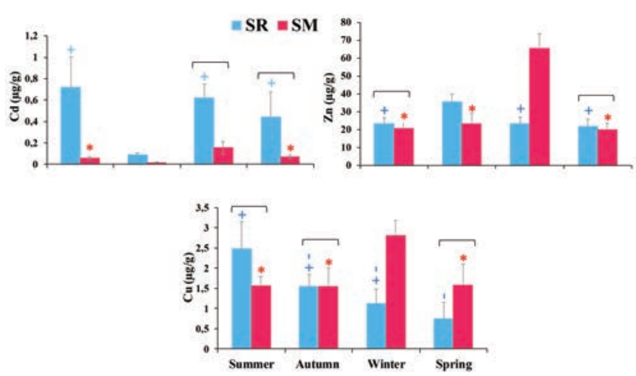

Figure 2. Seasonal variation of the concentration cadmium, zinc and copper in the *P. cultrifera.* (+/-) Meaning P <0.05 between seasons of SR; (*) Significance P<0.05 between seasons of SM; (bar) significance P<0.05 between SR and SM.

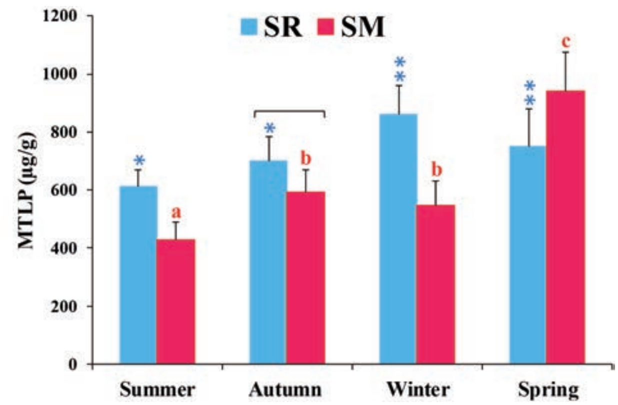

Figure 3. Seasonal evolution of metallothionein-like proteins (MTLP) in the polychaete *P. cultrifera.* (*/**) Meaning P<0.05 between seasons of SR; (same letter) significance P<0.05 between seasons of SM; (bar) significance P<0.05 between SR and SM.

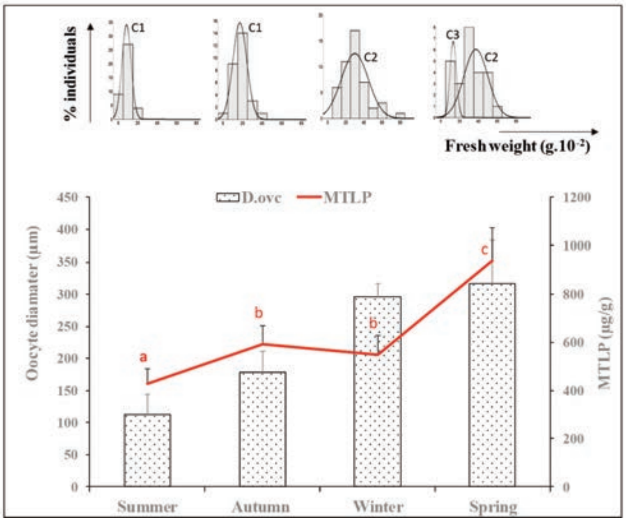

Figure 4. Seasonal evolution of frequency histograms of weight, oocyte diameter and MTLP concentration in the atokous form of *P. cultrifera* in SM station. (C_1: Cohort 1; C_2: Cohort 2 and C_3: Cohort 3).

suggests that during the course of the sexual maturation, the mechanisms of vitellogenesis require the intervention of a number of hormones (steroid hormones and others) and/or enzymes which will be activated by the metallic elements, consequently leading to the induction of MTLP. Contrary to what is observed in *Mya arenaria* (Molluscan shellfish)[10], the bivalve reproductive cycle (vitellogenesis and spawning) can modulate the expression of several biomarkers. In this situation, MT levels are lower to increase the availability of metals that are found in egg yolk proteins.

Conclusions

In conclusion, the Cd and consequently the MTLP are changed during seasons. The animals of the station SR are the most contaminated. In worms of the SM site, the highest rates are observed in winter and spring which coincide with the potential maturation of individuals.

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