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Histology and histochemistry of sea anemones in environmental contamination studies

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Since contaminants such as metals, POPs (Persistent Organic Pollutants) and PAHs (Polycyclic aromatic compounds represent a risk to human health and to the environment, it is therefore extremely necessary to study their biological effects [1, 2, 3]. Man-made chemicals endocrine disruptors such as estrogens pose the potential to modulate endocrine function and thus adversely affect humans and other animal's reproductive development. In this work, sublethal toxicity tests were carried out with the sea anemones Actinia equina and *Anemonia sulcata* exposed to 17-β-Estradiol. A. equina and *A. sulcata* are species that present a wide geographic distribution and might possibly be effective pollution indicators. Histological and histochemical techniques were used to detect morphological changes in sea anemones in order to find histological parameters that could be useful as early biomarkers of environmental contamination [3]. The histological and histochemical procedures followed by standard methods to Hematoxylin and eosine (H & E), Periodic acid Shiff reagent (PAS) and Masson`s Tricrome (TMass) stained adaptated to Actiniidae conditions. Such as, the fixation (formalin and alcohol) time is high (96 hours), because this organisms are almost 98% of water body constitution. The slides obtained were observed by light microscopy means. The assemblage of methodologies described permitted the identification of several anomalies/pathologies in different parts of the sea anemones body, with special attention to reproductive structures. Results obtained for *A. sulcata* showed vitellogenic oocytes with anomalous dimensions, altered cytoplasm or without cellular membrane limits (Figure 1). It can also be observed lipid accumulations and cells membranes not always preserved. In certain areas oocytes presents small reactivity with atypical PAS low basophilic patterns. In the mesoglea the amoebocytes showed more eosinophilic cytoplasm or extracellular bodies suggesting necrosis or protein content. The effects at 10 µg/L concentrations show a considerable number of oocytes with germinal vesicles membranes and indistinct cytoplasm boundaries (Figure 1). Results obtained for *A. equina* showed some morphological changes in the spermatocytes of male gonads and in the germinal vesicles the female gonads. The effects observed at higher concentrations shows oocytes and ovarian tissues disintegration. The morphologic alterations observed suggested a delay in spermatogenesis [4] and although there have been no alterations in female vitellogenic granules, there are changes in their maturation. The whole effects lead to verify the role that the estradiol in the Anthozoan reproductive system.

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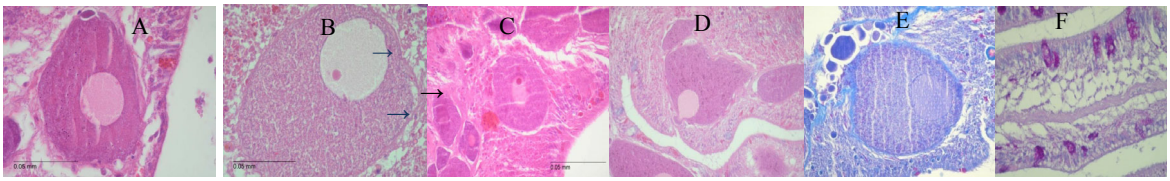

Figure 1. Sublethal 17-B-estradiol exposed to 96 hours on *Actinia equina* under control, 10, 100, 200 µg/ L. The appearance of some gonadal (H & E preparations) A- Control seawater; B- Control solvent (vitellogenic oocytes), C- Concentration 10µg/L: *A. sulcata* ovaries, nuclear oocytes membrane with indistinct boundaries (→), and primary oocytes (→); D-Concentration 100 µg/L (vitellogenic oocyte with irregular morphology), E-Vittelogenic oocytes with peripheral nuclei and visible nucleoli and oocyte in the first stage of vitellogenesis with cell membrane and irregular indistinct boundaries; F-Concentration 200 µg/L- Mesentery detail, with no effects at mucosal cells level and possible appearance of lipids accumulation areas (Bar: 0,05mm)

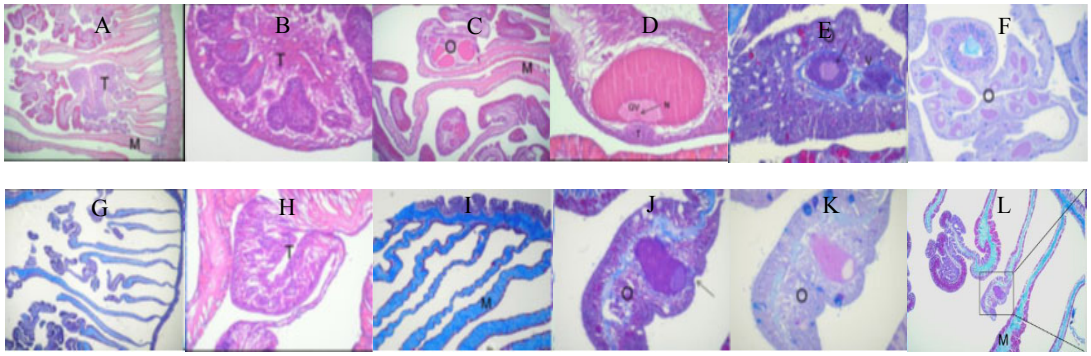

Figure 2. A, B, C and D with hematoxylin and eosine colorations controls: male gonads and mesenteries; B-Normal male gonad; C- Fertile female mesentery; D- Vitellogenic oocyte; E and F concentration 10µg/L: E-Ovary with vitellogenic oocyte showing irregularities in the nuclear membrane (Mass T) and F-Ovaries with mature oocyte (APAS); G and H concentration 100 µg/L: G- mesenteries, with ovaries in early stages of development (TMass) and H-Testis with anatomical changes (H & E) (L:Lise and T: Testis); I, J, K and L Concentration 200 µg/L: I- mesenteries infertile (TMass), J- oocyte with visible trophonema (TMass), K-Ovary with mucous cells as evidenced by the A / PAS and L- Ovaries (TMass) (Bar: 0,01mm).

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