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Reproduction and oxidative metabolism in the brooding sea star *Anasterias antarctica* (Lütken, 1957)

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ABSTRACT

The oral-brooding sea star *Anasterias antarctica* is distributed on the coasts of South Patagonia and north of Antarctic Peninsula. This species is a potential predator of shellfish *Mytilus chilensis*, an important commercial resource for Beagle Channel region. The aims of this work were to study the variation in the gonad index (GI), to establish the brooding season, to determine the reproductive effort of both sexes, and to assess the concentration of liposoluble antioxidants and production of reactive oxygen species (ROS) in male and female gonads and embryos of *A. antarctica* during the year. Four samplings were performed: three during the brooding season and one during the non-brooding season. Individuals sampled during the brooding season were assigned to one of the three groups: brooding females, non-brooding females, and males. Individuals sampled during the non-brooding season were assigned to one of the three groups: sexually mature females, sexually non-mature females and males.

Histological observations allowed determining that only females incubate the embryos on the oral surface for seven months. Males showed a strategy characteristic of broadcast spawners, whereas females spawned only a small number of eggs, as it is characteristic of brooders and as observed in other species of asteroids. Males show a significant increase in GI before spawning (from 1% to 15%) and then a marked decrease. During gametogenesis, testes showed a low level of β-carotene and high production of ROS. Between May and October; brooding and non-brooding females showed similar values of ROS production and concentration of antioxidants. Mature females had a significantly higher GI and lipid soluble antioxidant concentration than non-mature females. ROS production was higher in non-mature females. In *A. antarctica* embryos, ROS production increased and liposoluble antioxidant defenses decreased along development. The reproductive effort of males was about 25% lower than that of females, probably because of brooding costs. Gonadal maturation occurred in summer in both sexes, in concordance with an increase in the concentration of liposoluble antioxidants and the minimum values of ROS production. This suggests a strategy of oxidative damage prevention and gamete protection through allocation of antioxidants to mature gonads. The β-carotene and α-tocopherol accumulation in ovaries would protect not only maturing oocytes but also embryos, given that these antioxidants would be transferred to eggs. Along the reproductive cycle, *A. antarctica* gonads are protected from oxidative processes, mainly by β-carotene. Further, during gonad maturation, protection increases with the increase in α-tocopherol concentration and the decrease in ROS, whose highest and lowest values coincide with maximum gonadal maturation.

1. Introduction

The evolution of traits related to fitness establishes that a beneficial change in the expression of one trait often involves a negative change in the expression of another. These compensations are very common in nature, and their existence is commonly explained in terms of limited resources and energy balance. The cost of reproduction is the most prominent life history trade off, because costs are paid in terms of survival and future reproduction and can also limit the amount of energy available to immune and antioxidant functions (Stearns, 1992). Several studies, mainly in birds, point to the role of reactive oxygen species (ROS) in reproduction. Particularly, an increase in the reproductive effort leads to a higher susceptibility to oxidative stress (Alonso-Alvarez et al., 2004). Nevertheless, very little is known about ROS production

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and antioxidant defenses during reproduction in invertebrates (Pérez et al., 2011; Petes et al., 2008).

The generation of ROS, such as superoxide anion, hydrogen peroxide and hydroxyl radical, takes place continuously in living cells, mainly as a byproduct of respiration (Halliwell and Gutteridge, 1989). Once produced, ROS may damage cellular components and tissues, particularly proteins, lipids and nucleic acids, which often leads to cumulative organ injury (Lushchak and Bagnyukova, 2006). This process can be understood as a situation derived either from an enhanced rate of ROS generation or from a diminished level of antioxidant defenses. Endogenous antioxidants are synthesized by an organism whereas exogenous antioxidants such as lipid soluble α-tocopherol and carotenoids are obtained from food (Tummeleht et al., 2006). β-carotene is recognized as a lipid antioxidant, i.e. a free radical trap and quencher of singlet oxygen. Fluctuations in ROS production in aquatic organisms have been attributed to: (a) exogenous factors such as hypoxia, hyperoxia, pollution, poisoning, UV radiation, and availability and quality of food (Abele-Oeschger et al., 1994; Geracitano et al., 2004; Keller et al., 2004; Malanga et al., 2007; Power and Sheehan, 1996; Wilhelm-Filho et al., 2001) and (b) endogenous factors such as feeding rate, growth, locomotor activity, age, sex, metabolic rate, oxygen consumption (Abele et al., 1998; Livingstone et al., 1990; Winston and Di Giulio, 1991) and reproduction (Pérez et al., 2011; Petes et al., 2008). Petes et al. (2008) reported that mussels accumulate high concentrations of carotenoid pigments in their gonadal tissues to potentially protect gametes from the damaging oxidative stress experienced during aerial exposure. Moreover, it has been previously demonstrated that oxidative damage of the gonad of the sea urchin *Loxechinus albus* increases during gametogenesis, where the concentration of lipid-soluble antioxidants decreases and lipid oxidation increases (Pérez et al., 2011).

In most sea star species, both male and female gametes are released in large quantities into the sea; only a small number of sea star species are brooders, with females holding large eggs close to or inside their bodies and males broadcasting sperm into the water column (McClary and Mladenov, 1988). The oral-brooding sea star *Anasterias antarctica* is distributed on the coasts of South Patagonia and north of Antarctic Peninsula, and occurs from the intertidal zone to 150 m (Mah and Danis, 2009). It is a common predator of intertidal and shallow sublittoral communities in Tierra del Fuego, feeds mainly on *Mytilus chilensis*, *Pareuthria plumbea* and *Trophon geversianus* (Curelovich, Personal communication). The mussel *M. edulis* may be the main source of non-enzymatic antioxidants from the phytoplankton (Maoka, 2011; Schleder et al., 2008). The sea star *Anasterias minuta* (a junior synonym of *A. antarctica,* Romanelli Michel, 2014), presumably does not feed during brooding, whereas others such as *Anasterias rupicola* from Marion Island (Southern Ocean) does feed (Blankley and Branch, 1984). The development of *A. antarctica,* includes a non-feeding, lecithotrophic, modified brachiolaria (Gil et al., 2011).

The different reproductive strategies in both sexes in *A. antarctica* suggest that males and females differ in energy allocation to gonads and therefore in prevention of oxidative damage during reproduction. Although males seem to invest more in gamete production, only females breed. As food uptake is probably nil during the prolonged brooding season, brooding may impose a substantial energetic cost (Gil et al., 2011).

The aims of this work were to study the variation in the gonad index (GI), to establish the brooding season, to determine the reproductive effort of both sexes and to assess the concentration of liposoluble antioxidants and production of ROS in male and female gonads and embryos of *A. antarctica* during the year.

It is hypothesized that if oxidative stress acts as a constraint on reproduction, measurements of oxidative balance (pro-oxidants/antioxidants) before reproduction should be negatively related to the reproductive output and that if reproduction induces oxidative stress, the reproductive output should be positively related to the oxidative balance after reproduction.

2. Materials and methods

2.1. Sea star collection and processing

The individuals of *A. antarctica* were randomly collected in the intertidal zone of Ensenada Bay, National Park Tierra del Fuego, Beagle Channel (54°51′00, 14″S, 68°29′38, 79″W; Fig. 1). Four samplings were performed: three during the brooding season: (1) on 28 May 2009, at the beginning of brooding; (2) on 21 August 2009, in the middle of the brooding season; and (3) on 19 October 2009, at the end of brooding. The fourth sampling was carried out during the non-brooding season on 28 February 2010, and only adult females and males were collected. Seasonal samples of 30 individuals were collected. The specimens collected were transported to the Laboratorio de Ecología, Fisiología y Evolución de Organismos Acuáticos from the Centro Austral de Investigaciones Científicas-CONICET (Ushuaia, Argentina) for subsequent processing. Prior to dissection, each individual was superficially dried with tissue paper and weighed (total weight, ±0.01 g), and the distance from the tip of the longest arm to the opposite interradius (length) was measured using an electronic caliper (±0.1 mm). Then, animals were dissected and gonads and pyloric cecum (digestive glands) were weighed separately (±0.01 g). The indexes of different body components (GI: gonad index and PCI: pyloric cecum index) were calculated as organ wet weight (g) × 100/total wet weight (g). Samples of gonads and embryos mass to be used for biochemical analyses were immediately frozen and stored at −80 °C for 30 days, until analysis.

2.2. Determination of sex and brooding season

One gonad of each specimen was fixed in Bouin's solution for 12 h, and then water washed and transferred to 70% alcohol. A cross-section block was dehydrated in an alcohol series, cleared in benzene, embedded in Paraplast, sectioned at 5 μm and stained with Carazzi's hematoxylin and eosin (Pérez et al., 2008). Sections were examined microscopically and each individual was sexed. Individuals sampled during the brooding season were assigned to one of the three groups: brooding females, no brooding females, and males. Individuals sampled during the non-brooding season were assigned to one of the three groups: sexually mature females, non sexually mature females and males.

2.3. Biochemical measurements

2.3.1. ROS production

Gonads and embryo mass were homogenized separately (1:5 *w*/*v*) in a 100 mM Tris–HCl, pH 7.75 buffer, with 2 mM EDTA and 5 mM $MgCl_2$ (Gallagher et al., 1992). Measurements were conducted according to Viarengo et al. (1999) with modifications. Briefly, the homogenates were centrifuged at 4 °C for 20 min at 10,000 *g* and only the supernatants were conserved. To quantify the ROS production, the fluorescent probe 2′,7′ dichlorofluorescein diacetate was added to the buffer (30 mM HEPES buffer at pH 7.2, with 200 mM KCl and 1 mM $MgCl_2$), in a final concentration of 40 μM. Then, after addition of 10 to 5 μl of the supernatant, the reaction mixture was incubated at 35 °C for 10 min. The fluorescent compound F-DA, generated by radical dependent oxidation of the probe, was detected by fluorescence Spectrophotometer Hitachi F 3010 at $\lambda_{ex} = 488$ nm and $\lambda_{em} = 525$ nm.

2.3.2. Concentration of lipid soluble antioxidants

The concentration of β-carotene and α-tocopherol in homogenates from gonads and embryo mass was quantified separately by reverse-phase high performance liquid chromatography (HPLC) with electrochemical detection using a Bioanalytical Systems LC-4C amperometric detector with a glassy carbon working electrode at an applied oxidation potential of 0.6 V (Desai, 1984). Samples were extracted with 1 ml of ethanol and 4 ml of hexane. After centrifugation at 600 *g* for 10 min,