



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



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### ARTICLE INFO

#### Article history:

Received 13 August 2014

Received in revised form 25 November 2014

Accepted 28 November 2014

Available online 18 December 2014

#### Keywords:

*Anasterias antarctica*

Antioxidants

Brooding

Oxidative metabolism

Reproduction

ROS production

### 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  $\beta$ -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  $\beta$ -carotene and  $\alpha$ -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  $\beta$ -carotene. Further, during gonad maturation, protection increases with the increase in  $\alpha$ -tocopherol concentration and the decrease in ROS, whose highest and lowest values coincide with maximum gonadal maturation.

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### 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  $\alpha$ -tocopherol and carotenoids are obtained from food (Tummeleht et al., 2006).  $\beta$ -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*, *Pareruthria 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,  $\pm 0.01$  g), and the distance from the tip of the longest arm to the opposite interradius (length) was measured using an electronic caliper ( $\pm 0.1$  mm). Then, animals were dissected and gonads and pyloric cecum (digestive glands) were weighed separately ( $\pm 0.01$  g). The indexes of different body components (GI: gonad index and PCI: pyloric cecum index) were calculated as organ wet weight (g)  $\times$  100/total wet weight (g). Samples of gonads and embryos mass to be used for biochemical analyses were immediately frozen and stored at  $-80^\circ\text{C}$  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  $\mu\text{m}$  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  $\text{MgCl}_2$  (Gallagher et al., 1992). Measurements were conducted according to Viarengo et al. (1999) with modifications. Briefly, the homogenates were centrifuged at  $4^\circ\text{C}$  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  $\text{MgCl}_2$ ), in a final concentration of 40  $\mu\text{M}$ . Then, after addition of 10 to 5  $\mu\text{l}$  of the supernatant, the reaction mixture was incubated at  $35^\circ\text{C}$  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  $\lambda_{\text{ex}} = 488$  nm and  $\lambda_{\text{em}} = 525$  nm.

#### 2.3.2. Concentration of lipid soluble antioxidants

The concentration of  $\beta$ -carotene and  $\alpha$ -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,

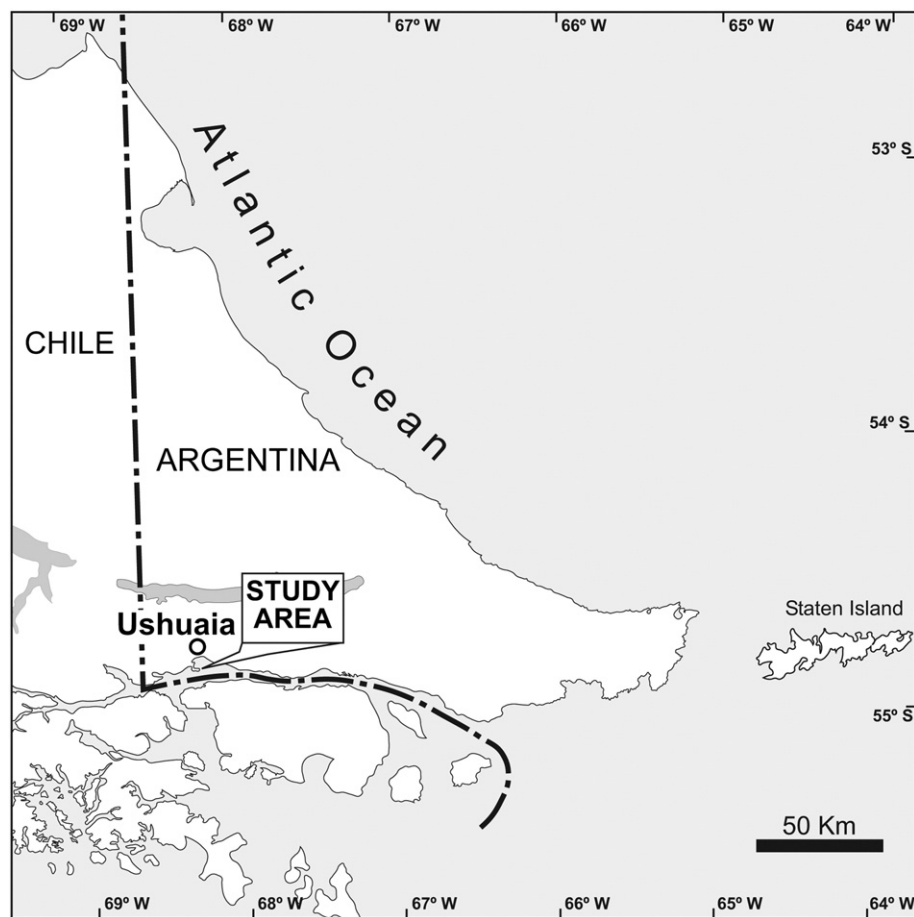


Fig. 1. Sampling area in Ensenada Bay, National Park Tierra del Fuego, Tierra del Fuego, Argentina.

the hexane phase was removed and evaporated to dryness under  $N_2$ . Extracts were dissolved in methanol/ethanol (1:1) and injected for HPLC analysis (Desai, 1984). D,L- $\alpha$ -tocopherol (Sigma) and  $\beta$ -carotene (Sigma) were used as standards.

#### 2.4. Reproductive effort

The reproductive effort was calculated according to Raymond et al. (2007). For males, it was calculated as the decrease in the mass of the gonads after spawning as a percentage of total body mass (1). In females, reproductive effort includes the maintenance costs during brooding as well as the mass decrease due to loss of gametes during spawning. Chia (1969) reported that, in *Leptasterias hexactis* females, the pyloric cecum is the main body component supplying energy for maintenance during brooding. Thus, the reproductive effort (RE) was calculated in females of *A. antarctica* as the decrease in the mass of the gonads and pyloric cecum during the reproductive period and brooding as a percentage of total body mass (2).

$$RE_{(M)} = \text{Average } GI_{(Mature)} - \text{Average } GI_{(Post-spawned)} \quad (1)$$

$$RE_{(F)} = [\text{Average } GI_{(Mature)} - \text{Average } GI_{(Post-spawned)} + \text{Average } PCI_{(Mature)} - \text{Average } PCI_{(Post-spawned)}] \quad (2)$$

#### 2.5. Statistical analysis

In males, seasonal variations in GI, ROS production and concentration of lipid soluble antioxidants ( $\beta$ -carotene and  $\alpha$ -tocopherol) were analyzed using one-way ANOVAs, followed by Tukey HSD comparisons.

The females sampled during brooding (May, August and October) were analyzed using a two-way ANOVA for GI, ROS production and concentration of lipid soluble antioxidants to test for significant differences between the season and reproductive condition (brooding and no brooding females). Pair-wise differences between seasons were analyzed by unplanned Tukey HSD multiple comparisons for unequal N with brooding females. In females sampled during the non-brooding season (February), differences in GI, ROS production and concentration of lipid soluble antioxidants between mature and non-mature females were tested using the unpaired *t* test. During embryogenesis, variations in ROS production and concentration of lipid soluble antioxidants were analyzed using a one-way ANOVA, followed by Tukey HSD comparisons for an unbalanced design. Assumptions of normality (Kolmogorov–Smirnov test) and homogeneity of variances (Bartlett test) were previously verified (Zar, 1984).

Relationships between ROS production and concentration of lipid soluble antioxidants were studied through Pearson correlations (Zar, 1984).

Statistical analyses were performed using STATISTICA 6.0 Package.

### 3. Results

Histological observations allowed us to determine that only females incubate the embryos on the oral surface for 7 months. Females showed two reproductive conditions simultaneously. During brooding season two population were present, brooding and non-brooding females while on non-brooding season there were only mature and non-mature females

### 3.1. Males

Male experienced variations in GI, ROS production and concentration of liposoluble antioxidants (Fig. 2) during the year. The mean male GI varied significantly between seasons (one-way ANOVA,  $P < 0.05$ ). The GI reached maximum values in January, when most individuals were sexually mature ( $P < 0.05$ ) (Fig. 2a). ROS production varied significantly between seasons (one-way ANOVA,  $P < 0.05$ ) (Fig. 2b). Animals sampled in January had significantly lower ROS production than individuals in the other three seasons ( $P < 0.05$ ). The mean  $\beta$ -carotene concentration varied significantly between seasons (one-way ANOVA,  $P < 0.05$ ), with the maximum value in January (Fig. 2c). The mean  $\alpha$ -tocopherol concentration of gonads did not vary significantly between seasons (one-way ANOVA,  $P > 0.05$ ) (Fig. 2d). The correlation between ROS production and concentration of lipid soluble antioxidants was not significant ( $\beta$ -carotene  $r$ : 0.5247 and  $\alpha$ -tocopherol  $r$ : 0.0077).

### 3.2. Females. Brooding season

The mean GI varied significantly between seasons ( $P < 0.05$ ), but it was not found significant differences between conditions (brooding non-brooding females,  $P > 0.05$ ). The GI reached its maximum in October (Tukey HSD multiple comparisons,  $P < 0.05$ ). The interaction between season and brooding and non-brooding females was not significant (two-way ANOVA,  $P > 0.05$ ) (Fig. 3a).

ROS production and concentration of lipid soluble antioxidants did not vary significantly with the season or condition of females (two-way ANOVA) (Figs. 3b, 3c and 3d). The ROS production was negatively correlated with  $\beta$ -carotene but not with  $\alpha$ -tocopherol ( $r$ : 0.7397 and  $r$ : 0.2213, respectively).

### 3.3. Females. Non-brooding season

Mature females had a significantly higher GI than non-mature females (Tukey HSD test  $P < 0.05$ ) (Fig. 4a). ROS production of gonads varied significantly between mature and non-mature females (Tukey HSD test,  $P < 0.05$ ), being higher in non-mature females (Fig. 4b). On the other hand, the concentration of  $\beta$ -carotene and  $\alpha$ -tocopherol was significantly different between mature and non-mature females during the non-brooding season (Tukey HSD test,  $P < 0.01$  and  $P < 0.0001$ , respectively), being higher in mature females (Fig. 4c, d). The negative correlation between ROS production and concentration of lipid soluble antioxidants was significant ( $\beta$ -carotene  $r$ : 0.5369 and  $\alpha$ -tocopherol  $r$ : 0.5936).

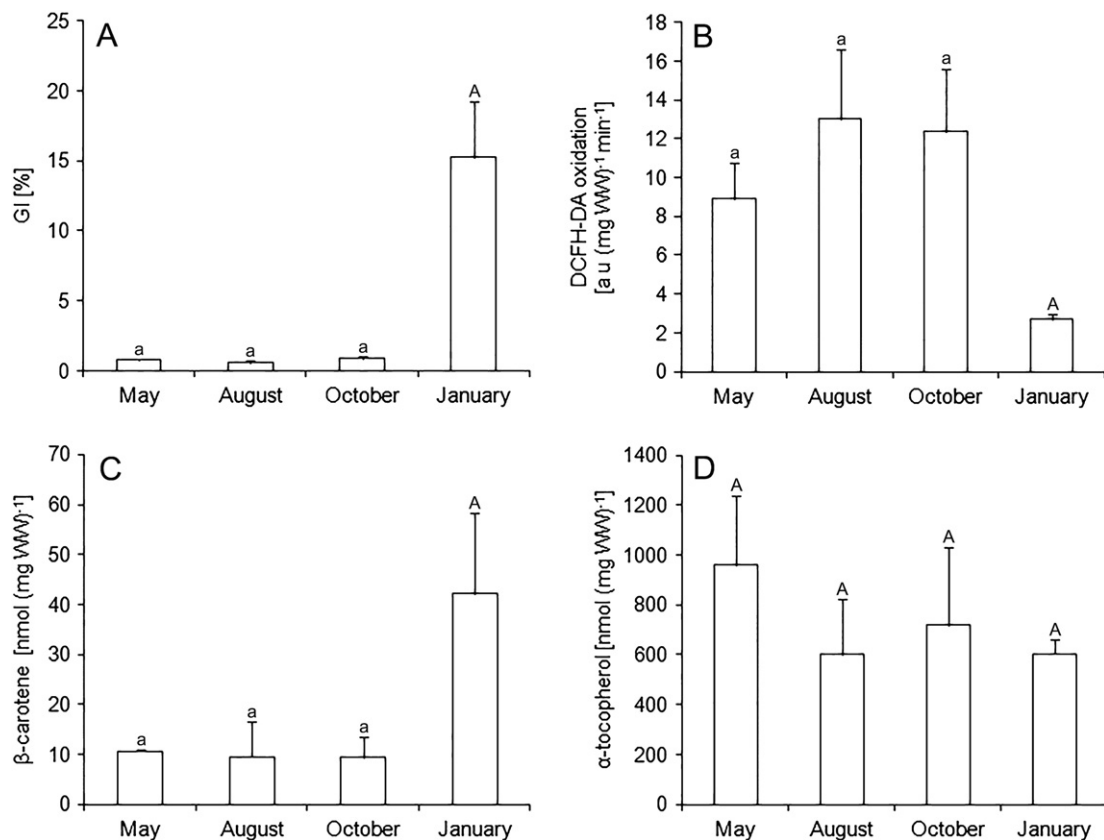
### 3.4. Embryogenesis

During the embryo development of *A. antarctica*, ROS production in the embryos showed a gradual, progressive and significant increase (one-way ANOVA,  $P < 0.01$ ) (Fig. 5a), whereas the concentration of lipid soluble antioxidants concentration decreased (one-way ANOVA;  $\beta$ -carotene  $P < 0.001$  and  $\alpha$ -tocopherol,  $P < 0.05$ ), being lowest at the end of the embryogenesis (Fig. 5b).

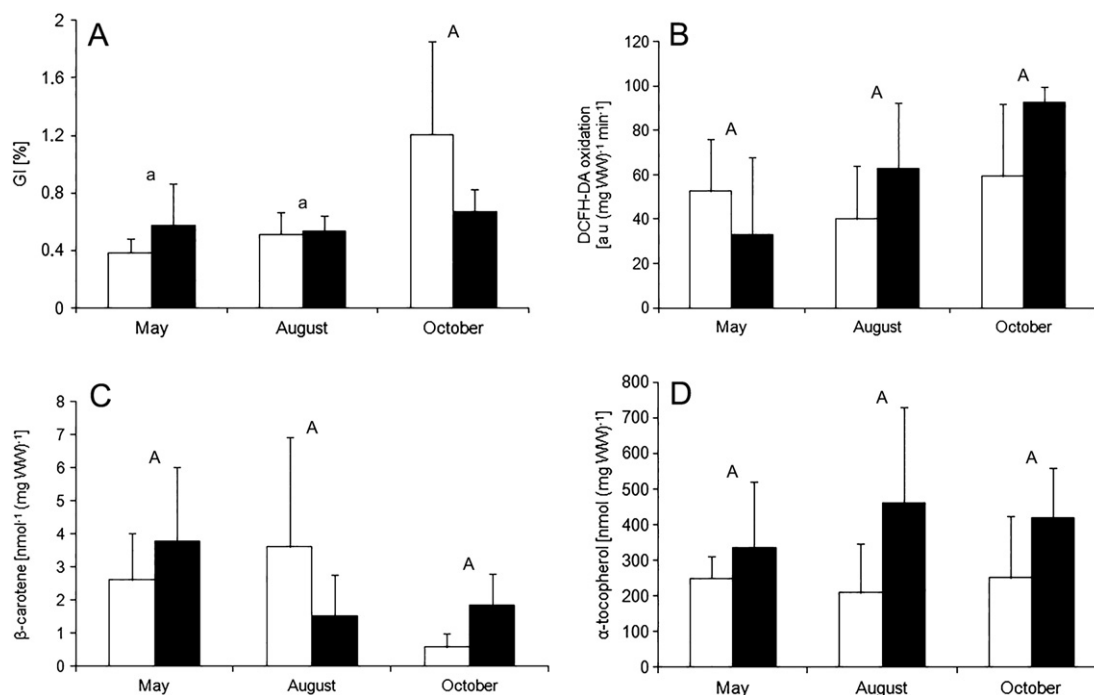
The statistical analysis showed a negative correlation between ROS production and  $\beta$ -carotene concentration ( $r$ : 0.9977); whereas the correlation between ROS production and  $\alpha$ -tocopherol concentration was not significant ( $r$ : 0.7207) (Fig. 5).

### 3.5. Reproductive effort

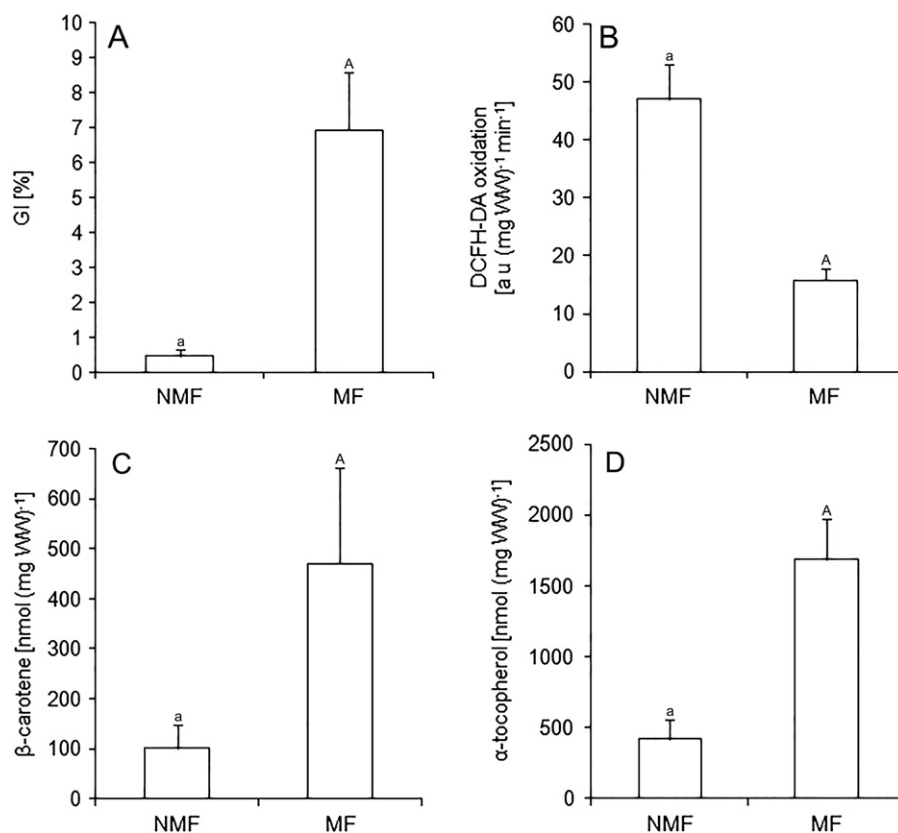
The reproductive effort of males was 9.31% of gonad mass, whereas that of females was considered as both the loss of ovary mass during



**Fig. 2.** Seasonal variation from males of *Anasterias antarcticacxx* A, Gonad index (GI); B, ROS production in gonads (DCFH-DA: 2',7'-dichlorofluorescein diacetate); C,  $\beta$ -carotene concentration in gonads; D,  $\alpha$ -tocopherol concentration in gonads. a u: arbitrary units. Mean and SD. Significant differences are indicated by the same capital and small letter.

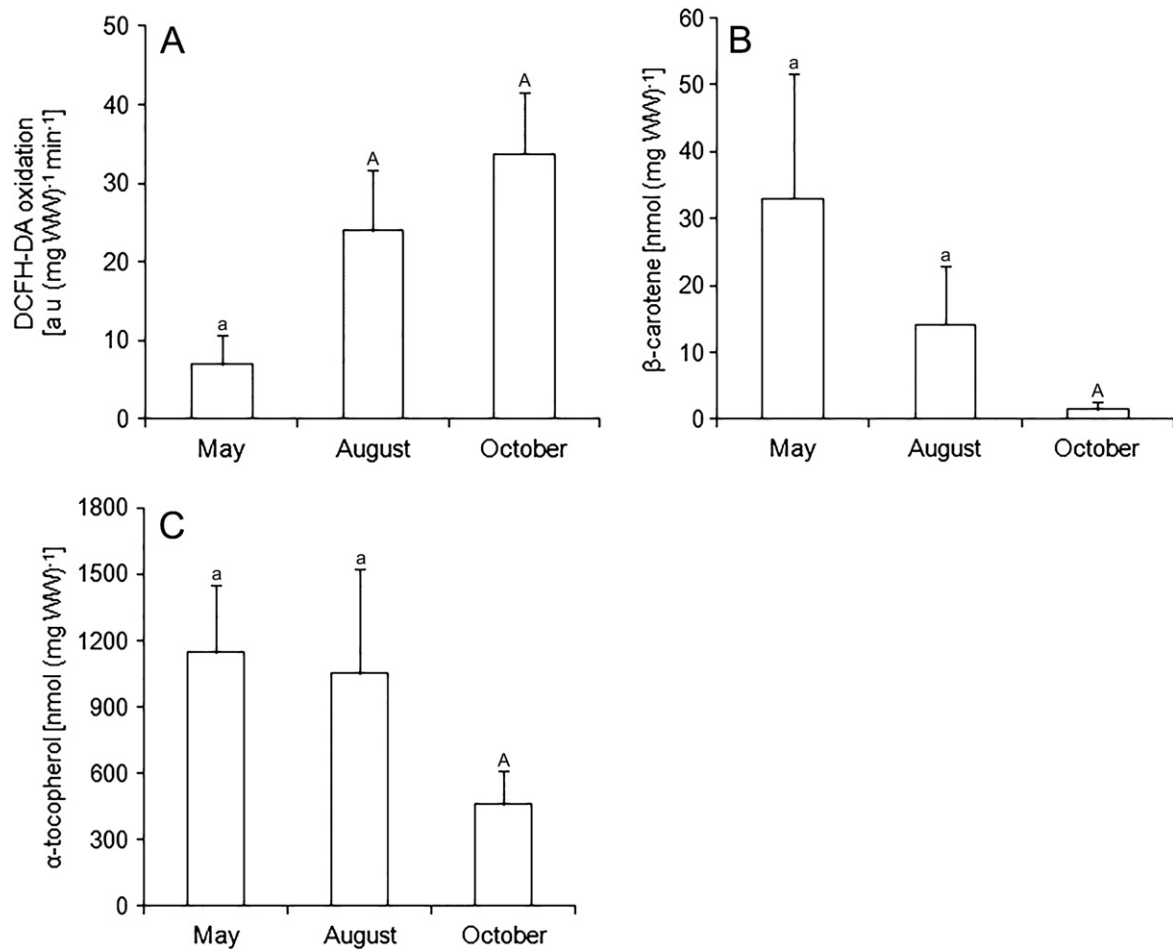


**Fig. 3.** Seasonal variation from females of *Anasterias antarctica* during the brooding season. A, Gonad index (GI); B, ROS production in gonads (DCFH-DA: 2',7' dichlorofluorescein diacetate); C,  $\beta$ -carotene concentration in gonads; D,  $\alpha$ -tocopherol concentration in gonads. a, u: arbitrary units. Mean and SD. Significant differences are indicated by the same capital and small letter. Non-brooding females □ and brooding females ■.



**Fig. 4.** Variation from females of *Anasterias antarctica* during the non-brooding season. A, Gonad index (GI); B, ROS production in gonads (DCFH-DA: 2',7' dichlorofluorescein diacetate); C,  $\beta$ -carotene concentration in gonads; D,  $\alpha$ -tocopherol concentration in gonads. a, u: arbitrary units. Mean and SD. Significant differences are indicated by the same capital and small letter. Non-mature females □ and mature females ■.





**Fig. 5.** Seasonal variation from embryos of *Anasterias antarctica* during embryo development. A, ROS production (DCFH-DA: 2',7'-dichlorofluorescein diacetate); B, β-carotene concentration; C, α-tocopherol concentration. a.u.: arbitrary units. Mean and SD. Significant differences are indicated by the same capital and small letter.

spawning (7.17%) and the loss of the pyloric cecum due to maintenance costs during brooding (5.38%). Thus, the total reproductive effort (as % of body mass) for females was 12.55%, which is 25.82% higher than that for a standard male.

#### 4. Discussion

The females of *A. antarctica* brood the eggs in the oral area for seven months and do not feed during this period. Fasting during brooding has also been reported for other species, e.g. *Leptasterias polaris* (Himmelman et al., 1982) and *Leptasterias tenera* (Hendler and Franz, 1982). It is suggested here that spawning and winter brooding are related to a natural decrease in prey abundance and/or to the seasonal inactivity of predators in adult sea stars and their embryos during the cold season.

The males of *A. antarctica* show a strategy characteristic of broadcast spawners, whereas females spawn only a small number of eggs as it is characteristic of brooders, as in other species of asteroids (Mercier and Hamel, 2008; Raymond et al., 2004). Males showed a significant increase in GI prior to spawning from 1% to 15% and then a marked decrease, being the reproductive effort 9.31%. In contrast, mature females showed a much lower increase, from 0.5% to 7% in January, but the reproductive effort was 12.55%. The reproductive effort of males was about 25% lower compared reproductive effort of females (wet mass), probably due to brooding costs (Chia, 1969) given the reduction of the pyloric cecum during the prolonged fasting of brooding females. In contrast, the reproductive effort in *L. polaris* is 30% higher in males than in females (energy units, Raymond et al., 2004) and the

reproductive effort of both sexes is lower than in *A. antarctica* (7% and 8% for females and males respectively) (wet mass). These different reproductive effort values could be related to interspecific and/or habitat differences: *A. antarctica* inhabits the intertidal zone, whereas *L. polaris* inhabits the subtidal zone. In other systems it was observed that the reproductive effort was higher for females than for males; females could suffer more oxidative damage than males (Alonso-Alvarez et al., 2004). In *A. antarctica*, ROS production and concentration of antioxidants were one order of magnitude higher in females than in males. Then, the relation between ROS production and concentration of antioxidants remains relatively constant during the year for both sexes. Therefore, it was not found evidences of a trade-off between energy invested on reproduction (reproductive effort) and ROS/antioxidant protection.

Gonadal maturation occurs 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 accumulated in ovaries would protect not only maturing oocytes but also embryos, given that these antioxidants would be transferred to eggs. In sea urchins for example, carotenoids are transferred to the growing oocyte through nutritive phagocytes (Plank et al., 2002). Since the transference of antioxidants to embryos is related to the development and survival of embryos, the magnitude of the transference of antioxidants to embryos could be interpreted as a fitness component (Weiss et al., 2011).

During gametogenesis, testes showed a low level of β-carotene and high ROS production. On the other hand, between May and October,

brooding and non-brooding females showed no differences in ROS production or concentration of antioxidants. In addition, both populations of females showed a tendency to increase their ROS production and to decrease their concentration of  $\beta$ -carotene during the brooding season. These similarities between females at so different reproductive conditions (brooding/non-brooding) could be related to environmental factors. Then, ROS production would consequently increase the consumption of antioxidants and/or decrease their incorporation rate. It should be noted that exogenous antioxidants, such as lipid soluble  $\alpha$ -tocopherol and carotenoids ( $\beta$ -carotene, echinenone, astaxanthin), are obtained from food (Tummeleht et al., 2006). These results can also be explained by the annual cycle of phytoplankton in the Beagle Channel (Almandoz et al., 2011) and the lower feeding rate of *A. antarctica* in winter, as observed in *L. albus* (Pérez et al., 2010).

The high-latitude seasonality in e.g. primary productivity, temperature and photoperiod (Clarke, 1987) influences biological functions such as reproduction, oxygen consumption rate and feeding (Pérez et al., 2010). In the Beagle Channel, minimum day length occurred in June and maximum in December. The increase in day length is followed by an increase in the water temperature (Pérez et al., 2008). The annual cycle of phytoplankton was characterized by a strong contrast between low density and biomass during the autumn–winter period and a significant increase during spring and summer (Almandoz et al., 2011). Since  $\alpha$ -tocopherol and  $\beta$ -carotene are only produced by photosynthetic organisms, and are required by higher trophic levels (Gao et al., 2014). During the winter, ROS production and to decrease the concentration of antioxidants in *A. Antarctica*, may be due to a low feeding rate and/or a to decrease in antioxidant content in preys (Almandoz et al., 2011).

Non-mature females do not show a favorable oxidative situation with high ROS production and low concentration of liposoluble antioxidants, which suggests low energetic investment during summer. The results suggest that these females brooded during the previous season, then allocate energy reserves to gonadal maturation during summer and to brooding the following season.

The coexistence of two populations of females during the year (brooding/non-brooding during the rest of the year and mature/non-mature during summer) could be due to a trade-off between the egg quality and the annual periodicity of reproduction in *A. antarctica* females. Given that reproduction/incubation are highly-demanding processes among life history strategies (Stearns, 1992), females would not reach the nutritional status necessary to attain high quality eggs every year and annual incubation periods, which also implies a long starvation period.

The development of *A. minuta* (a junior synonym of *A. antarctica*, Romanelli Michel, 2014) includes a non-feeding, lecithotrophic, modified brachiolaria (Gil et al., 2011). Since the *A. antarctica* embryos do not feed during development they utilize nutrients and antioxidants from mature oocytes (Plank et al., 2002). As the ROS production increases and the concentration of liposoluble antioxidant defenses decreases during development then embryos would be under oxidative stress, whose control is a component of fitness.

Given that  $\alpha$ -tocopherol increases (three-fold) only in mature ovaries and that its concentration in mature ovaries and early embryos is similar, it is suggested that *A. antarctica* females translocate  $\alpha$ -tocopherol from mature ovaries to embryos.  $\alpha$ -Tocopherol is considered one of the most critical factors to control lipid peroxidation in biological membranes (Niki, 2014), and its concentration has been used in many biological systems as an indicator of the membrane protection ability against harmful oxidants (Malanga et al., 2009). Moreover, there would be also a translocation of  $\beta$ -carotene to developing embryos. The functions of carotenoids are currently under study, since they are found among marine invertebrates and are frequently the reason for their coloration (Maoka, 2011). Carotenoid pigments quenching to singlet oxygen converting it into less-damaging  $H_2O_2$  (Krinsky, 1989) show strong influence on the growth and survival of organisms (George et al., 2001). In addition, the high concentration of these pigments in the gonads of many animal

species suggests the need for carotenoids in the reproductive cycle (Goodwin, 1980).

## 5. Conclusions

The females of *A. antarctica* brood the eggs in the oral area for seven months and do not feed during this period. The reproductive effort of males was about 25% lower compared the reproductive effort of females, probably due to brooding costs.

Gonadal maturation occurs 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. During gametogenesis (winter), both sexes showed a tendency to increase their ROS production and to decrease their concentration of antioxidants, which may be due to a low feeding rate and/or a decrease in antioxidant content in prey.

The embryos do not feed during development and utilize nutrients and antioxidants from mature oocytes. ROS production increases and the concentration of liposoluble antioxidant defenses decreases during development. Then, embryos would be under oxidative stress, whose control is a component of fitness.

## Acknowledgments

This research was supported by CONICET (PIP 0128) and Universidad Nacional de la Patagonia San Juan Bosco-Asociación de Parques Nacionales 997, Chubut, Argentina. Authors are grateful to Administración de Parques Nacionales Delegación Tierra del Fuego, to personal of Cátedra de Físicoquímica of Facultad de Farmacia y Bioquímica of Universidad de Buenos Aires, to personnel from Laboratorio de Ecología, Fisiología y Evolución de Organismos Acuáticos from CADIC-CONICET and to Dra. Juliana Giménez for facilitating the microscope. [SS]

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