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Reproductive status, antioxidant defences and lipid peroxidation in *Octopus tehuelchus* (Cephalopoda: Octopodidae) females

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ABSTRACT

As in other semelparous cephalopods, *Octopus tehuelchus* females enter a senescent state after spawning. In several taxa, damage to macromolecules that result from an imbalance between antioxidant defences and the production of reactive oxygen species (i.e. oxidative stress) has been postulated as one of the physiological costs of reproduction and one factor that contributes to ageing. In this work, we evaluate whether enzymatic and non-enzymatic antioxidant defences are conditioned by reproductive status in *O. tehuelchus* obtained from a wild population. The main antioxidant defences, catalase (CAT), superoxide dismutase (SOD), reduced gluthation (GSH) and lipid peroxidation (TBARS), were assessed in somatic and reproductive tissues from octopus females, at immature, mature and spent stages. Oviducal glands showed an increase in TBARS that coincides with a decrease in GSH when females reach a spent stage. No significant changes were observed in the assayed parameters on the ovary wall, digestive gland, mantle and gills along maturity stages. CAT activity was undetectable in most analysed tissues, SOD activity and GSH were similar to or lower than in other cephalopods, while average TBARS levels in somatic tissues were at least one order of magnitude higher than levels found in other molluscs. Our results show that during sexual maturation and after natural oxidative stress conditions (spawning), only oviducal glands show changes that can be linked with the physiological processes of different reproductive status. The low antioxidant defences and the flat protective reactions after spawning could be associated with the semelparous life history of this species.

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Introduction

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defences. If the production of ROS is beyond an organism's capacity to quench them, oxidative damage to macromolecules occurs. This

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damage can compromise the functions of the macromolecules and can even lead to cell death (Finkel and Holbrook 2000; Turrens 2003; Abele and Puntarulo 2004; Balaban et al. 2005; Halliwell 2007; Kregel and Zhang 2007). Enzymatic and non-ezymatic antioxidant defences play a key role in the prevention of oxidative stress by averting the effects of ROS. The main enzymatic antioxidant defences are superoxide dismutase (SOD) and catalase (CAT). SOD catalyses the conversion of superoxide into O_2 and hydrogen peroxide, while CAT accelerates the inactivation of hydrogen peroxide by promoting its conversion into H_2O and O_2. In addition, the most important non-enzymatic antioxidant is the endogenous reduced glutathione (GSH) that contains a thiol group which acts as a reducing agent (Turrens 2003; Halliwell 2007; Kregel and Zhang 2007). Membrane lipids are one of the most sensitive targets, with ROS attack resulting in an increase in lipid peroxidation products. Once peroxidation is initiated it continues until it reaches the final product, malondialdehyde (Abele and Puntarulo 2004; Martin and Grotewiel 2006; Kregel and Zhang 2007), that is commonly used to assess the degree of oxidative damage (Zielinski and Pörtner 2000; Di Salvatore et al. 2013; Le Pabic et al. 2015).

Many semelparous species undergo an extremely rapid senescence on completion of the reproductive process (Kirkwood 2005). Most cephalopods are short-lived and semelparous organisms (Boyle and Rodhouse 2005). Except for *Nautilus* spp., there is no cyclical regression and re-growth of gonads after spawning or an extended spawning that takes place in more than a single breeding season (Boyle and Daly 2000). Octopus females grow fast, mature and, after mating, they spawn, brood the eggs and enter a senescent state. Therefore, octopus lifespan is linked to the reproductive event (Anderson et al. 2002).

It has been proposed that oxidative stress status plays a role in senescence (Sohal et al. 2002; Turrens 2003; Balaban et al. 2005; Kregel and Zhang 2007), particularly triggered by an increase in oxidative damage, and in some cases accompanied by a decrease in antioxidant defences (Harshman and Zera 2007; Kregel and Zhang 2007). On the other hand, a rapid decline in organisms' health has been observed in invertebrates and mammals after reproduction, which has been related to a sudden process of aging (Harshman and Zera 2007; Monaghan et al. 2009).

Oxidative stress parameters have been assessed in few studies in cephalopods. In post-hatching cuttlefish *Sepia officinalis*, cultured under Zn-exposed conditions, Le Pabic et al. (2015) detected perturbations in oxidative stress parameters. In addition, Semedo et al. (2012) showed that antioxidant enzyme activity is induced by metal accumulation in wild populations of *Octopus vulgaris*. Zielinzky and Pörtner (2000) assessed antioxidant defences in *S. officinalis* and *Lolliguncula brevis* reared in aquaria and showed that the activity of antioxidant enzymes from gills, mantle and brain were not uniform with age. Garrido et al. (2017) demonstrated the influence of diet on some *Octopus vulgaris* paralarvae antioxidant defences. However, there are no studies in cephalopods that evaluate antioxidant defences or the profile of oxidative damage related to sexual maturation and to the post-spawning state.

Octopus tehuelchus (D'Orbigny 1834) is a small benthic octopus that inhabits the coast from the south of Brazil to central Atlantic Patagonia (Ré and Ortiz 2008). Reproductive biology and population structure have been studied in north Patagonia (Ré 1998; Storero et al. 2010). The life cycle of this species has been estimated to be between 24 and 30 months, and since it is a terminal spawner, the spawning moment is an inflection point in the animal's life (Pujals 1986; Storero et al. 2012). Knowledge of

these population characteristics allows the prediction of the reproductive stage of animals in a certain season, including the post-spawning stage during which females perform parental care on the egg clutches for more than 4 months (Alves and Haimovici 2011; Ré 1998; Storero et al. 2010) up to the end of their lives. These characteristics make *O. tehuelchus* a suitable model for this study.

The aim of this work is to evaluate in *O. tehuelchus* females from a wild population whether the main antioxidant defences and lipid peroxidation in somatic and reproductive tissues are conditioned by their reproductive status.

Materials and methods

Sampling sites, condition of females and assignment of maturity stages

Octopus tehuelchus females were collected using pot-longlines at 10 m depth from a low-anthropic-impact environment (Di Salvatore et al. 2013) in Playa Fracasso in the San Jose Gulf (Argentina) (42.716°S, 65.000°′W), on the north Atlantic Patagonian coast. Animals were obtained from May to August in 2009 and 2010. Water temperature was measured at the time of sampling with a digital thermometer (Table 1). During these months it is possible to find at least two cohorts: immature animals that belong to a younger cohort, and spent females which belong to an older cohort (Ré 1998). After capture, octopuses were transferred to the laboratory in seawater. In accordance with ethical considerations and animal welfare during experimental manipulations (Mather and Anderson 2007; Moltschaniwskyj et al. 2007; Andrews et al. 2013), they were anesthetised and killed by gradually adding ethanol (up to 5% for 1 hour) to the seawater.

Females were weighed and examined macroscopically to determine their maturation stage, and to look for prey remains in their crops and stomachs or the presence of spherical white cysts with coccidia (1–2 mm diameter) in the crops, intestines or cecum. Octopuses with apparent white cysts were considered infected (Sardella and Re 1990; Sardella et al. 2000; Storero and Narvarte 2013). Animals that either had prey remains or were infected were not used for biochemical analyses

Table 1. Maturity stages and macroscopic maturation characteristics of *Octopus tehuelchus* females, used in this work, along with number of samples used, month and temperature of capture.

Macroscopic maturity stages	Characteristics (*)	Number of animals sampled	Sampling months and temperature (°C)
Immature (IM)	Ovary of medium size (1.8–3 cm) with no vitellogenic or early vitellogenic oocytes. Yellowish oviducal glands of 3–4.5 mm width. Distal oviducts with sperm and slightly widened.	11	March (15) May (13) September (9)
Mature (MA)	Ovary of large size (> 3 cm) with advanced and mature oocytes. Dark oviducal glands of 4.5–6 mm. Distal oviducts with sperm and very widened.	14	April (14.5) May (13) June (13)
Spent (SP)	Ovary is flaccid, reduced in size (1.5–2 cm), without mature oocytes inside. Thinned distal oviducts. Oviducal glands reduced. Samples used at this stage, correspond to brooding females captured along with egg clutches containing eggs in embryonic development stages between XI and XIII, according to Naef (1928).	13	June (13) August (11) September (9)

* from Pujals (1986).

because the digestive process and the presence of parasitism may interfere with physiological parameters (Castellano-Martinez and Gestal 2013). A total of 38 *O. tehuelchus* females were selected and classified into three clearly different stages regarding the macroscopic maturation characteristics of the species defined by Pujals (1986), as immature (IM), mature (MA) or spent (SP). For the latter stage the embryonic developmental stage of the egg captured along with the brooding females was also considered (Table 1).

Samples

Biochemical assays were undertaken on the main tissues related to reproduction, and on somatic tissues expected to show peaks of activity in different stages of the octopuses' life. Tissue samples were taken immediately after capture and stored at −20°C until processing. Samples of reproductive tissue were taken from the ovary wall and the oviducal glands (which act as spemathecae and as secretory organs at different maturity stages; Pujals 1986). Somatic tissue was sampled from the mantle, digestive gland and gills. Samples were weighed and homogenised (1:5 w/v) in 0.154 M KCl with 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 0.2 mM benzamidine, as protease inhibitors. Homogenates were centrifuged at 4°C for 20 min at 10,000 × *g* (Sabatini et al. 2015). The homogenate supernatants were immediately used or otherwise stored at −20°C and used within 1 month. All measurements were made in duplicate. All expressed weights correspond to fresh weight.

Lipid peroxidation

Lipid peroxidation was determined by measuring thiobarbituric acid-reactive substances (TBARS) through a modified protocol of Ohkawa et al. (1979), adapted to cephalopods (Fassiano et al. 2012). Briefly, a 50 µL aliquot of the supernatant was mixed with the reagent containing 0.3% (w/v) thiobarbituric acid (TBA), 4% (w/v) trichloroacetic acid (TCA) and 0.01% (w/v) butylated hydroxytoluene (BHT), following incubation at 100°C for 20 min. After cooling on ice, the reaction mixture was centrifuged for 10 min at 8000 × *g* and supernatant absorbance was measured at 535 nm. TBARS concentration was estimated using the extinction coefficient of the TBARS–thiobarbituric acid complex (156 mM^{-1} cm^{-1}). Results were expressed as TBARS µmol g^{-1}.

Reduced glutathione (GSH) content

GSH levels were measured following the Anderson (1985) procedure. A sample aliquot of 200 µL was acidified with 100 µL of 10% sulfosalicylic acid. After centrifugation at 8000 × *g* for 10 min, supernatant (acid-soluble GSH) aliquots of 100 µL were mixed with 6 mM 5,5-dithiobis-(2-nitrobenzoic) acid (DTNB) in 0.143 M buffer sodium sulfate (pH 7.5), containing 6.3 mM disodium ethylenediaminetetraacetate (Na_2EDTA). After 30 min incubation at room temperature, absorbance at 412 nm was measured. GSH content was determined using a calibration curve prepared with a GSH standard solution. Results were expressed as GSH nmol g^{-1}.

Antioxidant enzyme activities

SOD activity was measured by the Beauchamp and Fridovich (1971) method. This method is based on the sample capacity to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The reaction mix contained, in a final volume of 3 mL: 0.1 mM Na_2EDTA, 13.3 mM DL-methionine, 75 µM NBT and 120 µM riboflavin, in 50 mM potassium phosphate (pH 7.8). A SOD unit was defined as the enzyme amount necessary to inhibit by half the rate of NBT reduction. Results were expressed as SOD units g^{-1}.

CAT activity was determined by the Aebi (1974) method. The method is based on the monitoring of H_2O_2 decay during 20 seconds at 240 nm. The extinction coefficient, on the assay conditions, is 40 M^{-1} cm^{-1}. The reaction mixture contained 30 mM hydrogen peroxide in 50 mM (pH 7.0) phosphate buffer. A CAT unit was defined as the enzyme amount necessary to decompose 1 mmol of H_2O_2 per min. Results are expressed as CAT units g^{-1}.

Statistical analyses

Data from different groups were compared by one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test. The assumptions of normality and homogeneity of variances were tested with Kolmogorov–Smirnov's and Bartlett's tests, respectively. Pearson correlations were used to analyse the relationship between the parameters of oxidative stress and animal weight (Sokal and Rohlf 1995). All data were expressed per gram of tissue as mean ± standard deviation to compare to data obtained by other authors (Zielinski and Pörtner 2000; Sukhotin et al. 2002; Gostyukhina 2013).

Results

Enzymatic activities were generally low. In order to discard the effects of technical error, samples were measured repeatedly. In some cases, the amount of homogenate was small and this led to different numbers of samples per tissue.

Pearson correlations were not significant ($p > 0.05$) between animal weight and the measured parameters, either for the whole data set or for each maturation group.

Ovary wall

SOD activity and GSH levels showed no difference among the different stages ($p > 0.05$; Figure 1(a,b)), although GSH showed a decreasing trend in SP, to almost half the level in IM ($p > 0.05$). Average values were 70.5 ± 48.0 SOD units g^{-1} and 130.4 ± 52.5 GSH nmol g^{-1}. TBARS levels were constant through maturation process ($p > 0.05$; Figure 1(b)); mean value was 11.8 ± 1.9 µmol TBARS g^{-1}. CAT activity was not detectable.

Oviducal glands

Values of SOD activity remained stable throughout maturity stages and showed no significant differences for the maturity stages analysed ($p > 0.05$; Figure 2(a)). The SOD mean was 499.5 ± 323.4 SOD units g^{-1}. Although GSH levels tended to decrease in SP compared to IM or MA, this variation was not significant ($p > 0.05$; Figure 2(b)). The

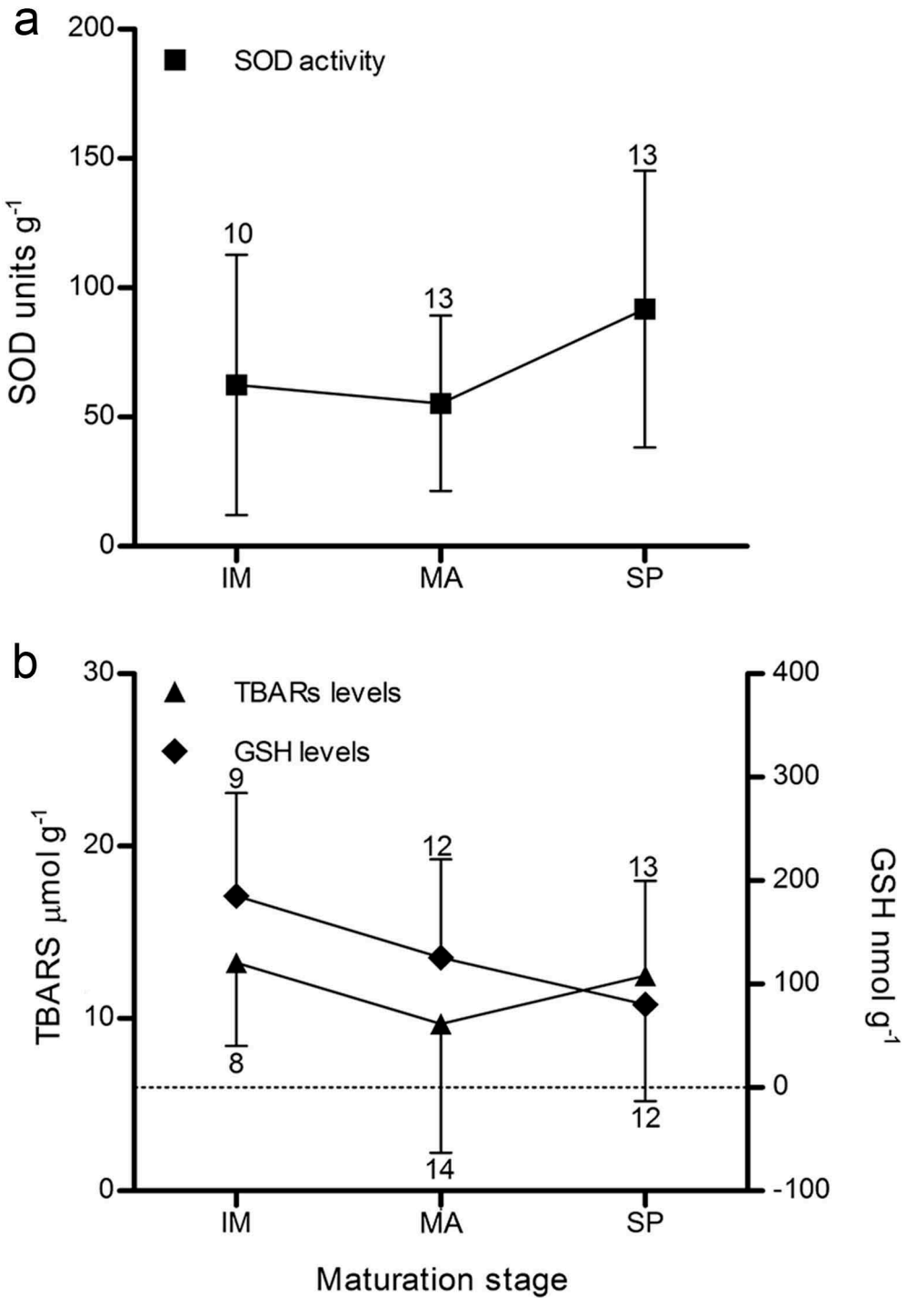

Figure 1. Antioxidant defences and lipid peroxidation parameters in the ovary wall of female *Octopus tehuelchus* in different maturity stages. SOD: superoxide dismutase; GSH: reduced glutathione; TBARS: thiobarbituric acid reactive substances; CAT: catalase; IM: immature; MA: mature; SP: spent stage; (a ●) SOD activity (SOD units g^{-1}), (b ▲) TBARS levels (TBARS µmol g^{-1}) and (♦) GSH levels (GSH nmol g^{-1}). Significant differences are indicated by asterisks: * $P < 0.01$ with respect to IM and ** $P < 0.01$ with respect to MA. Numbers on top of bars = *n*. S

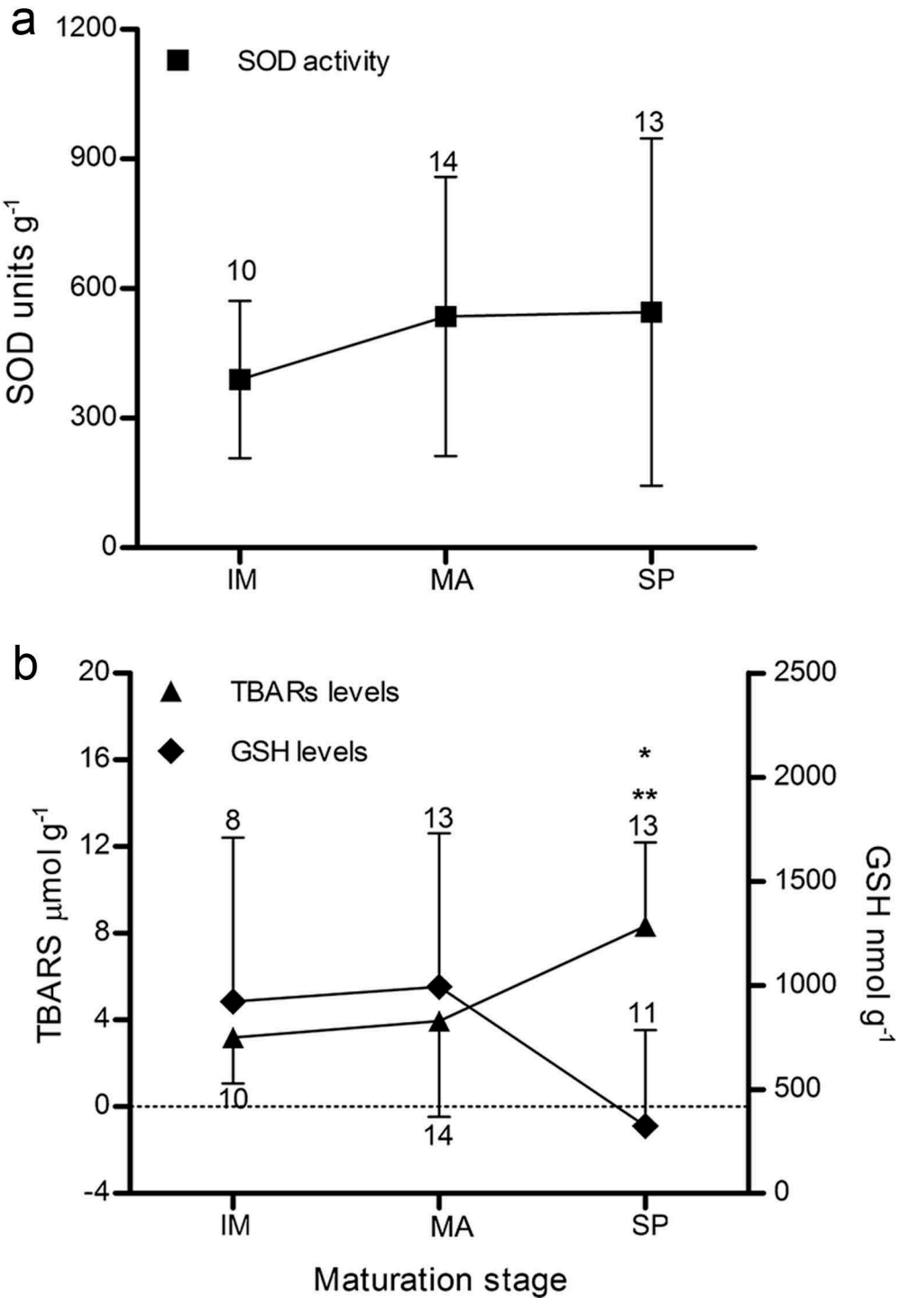

Figure 2. Antioxidant defences and lipid peroxidation parameters in oviducal glans of female *Octopus tehuelchus* in different maturity stages. SOD: superoxide dismutase; GSH: reduced glutathione; TBARS: thiobarbituric acid reactive substances; CAT: catalase; IM: immature; MA: mature; SP: spent stage; (a ■) SOD activity (SOD units g^{-1}), (b ▲) TBARS levels (TBARS µmol g^{-1}) and (♦) GSH levels (GSH nmol g^{-1}). Significant differences are indicated by asterisks: * $P < 0.01$ with respect to IM and ** $P < 0.01$ with respect to MA. Numbers on top of bars = *n*.

average value was 746.2 ± 368.2 GSH nmol g^{-1}. TBARS showed a significant variation through the maturation stages ($p < 0.01$), with a 2-fold increase of the TBARS levels in the SP stage as compared to the other two (Figure 2(b)). The mean value was 5.1 ± 2.7 µmol TBARS g^{-1}. CAT activity was under the detection limits (0.05 CAT units g^{-1}).

Gills

SOD activity and GSH levels did not differ among maturation stages ($p > 0.05$; Figure 3(a,b)). Average values were 174.2 ± 61.5 SOD units g^{-1} and 161.7 ± 43.7 GSH nmol g^{-1}. TBARS levels decreased significantly in SP ($p < 0.05$) to nearly half the level in IM (Figure 3(b)). The average value was 11.7 ± 5.5 µmol TBARS g^{-1}. CAT activity was under the detection limits.

Mantle

CAT activity could not be detected in this tissue either, while the other antioxidant defences assayed, SOD and GSH, showed no significant variations among the maturation stages analysed ($p > 0.05$; Figure 4(a,b)). Average values were 96.4 ± 54.1 SOD units g^{-1} and 100.5 ± 22.36 GSH nmol g^{-1}. TBARs levels showed a significant decrease ($p < 0.01$) from IM to MA and a significant increase ($p < 0.01$) from MA to SP (Figure 4(b)). The average value was 3.1 ± 1.3 µmol TBARS g^{-1}.

Digestive gland

CAT activity, despite being low, was detectable, although differences were not significant ($p > 0.05$) (Figure 5(a)). The mean value was 4.7 ± 0.45 CAT units g^{-1}. Antioxidant defences and TBARS showed no significant differences through maturation ($p > 0.05$; Figure 5(a,b)). The average values were 587.9 ± 311.4 SOD units g^{-1}, 102.2 ± 26.4 GSH nmol g^{-1} and 74.9 ± 11.7 µmol TBARS g^{-1}.

Discussion

Empirical evidence often supports the prediction that long-lived species have high resistance to oxidative stress by producing less ROS or having lower oxidative damage levels and antioxidants (Constantini 2014). Since *Octopus tehuelchus* is a short-lived species (Storero et al. 2012), its antioxidant defences or oxidative damage would be expected to be higher than in other invertebrates. When compared even to other cephalopods, average TBARS levels in somatic tissues (gills and mantle) of *O. tehuelchus* were one order of magnitude higher than in the squid *Lolliguncula brevis* and the cuttlefish *Sepia officinalis* (Zielinski and Pörtner 2000). In addition, TBARS in the digestive gland of *O. tehuelchus* were 5-fold higher than in *Octopus vulgaris* (Semedo et al. 2012), and even 10–100 times higher than in samples taken from whole bivalves (Sukhotin et al. 2002). On the other hand, GSH levels in somatic tissues, gills and digestive gland of *O. tehuelchus* were similar to those of bivalves *Anadata inaequivalvis* and *Mytilus galloprovincialis* (Gostyukhina 2013). However, levels of general enzymatic antioxidant defences were lower than in other molluscs. Average SOD activity in somatic tissues was 3 times lower when compared to *S. officinalis*, and similar to *L. brevis*

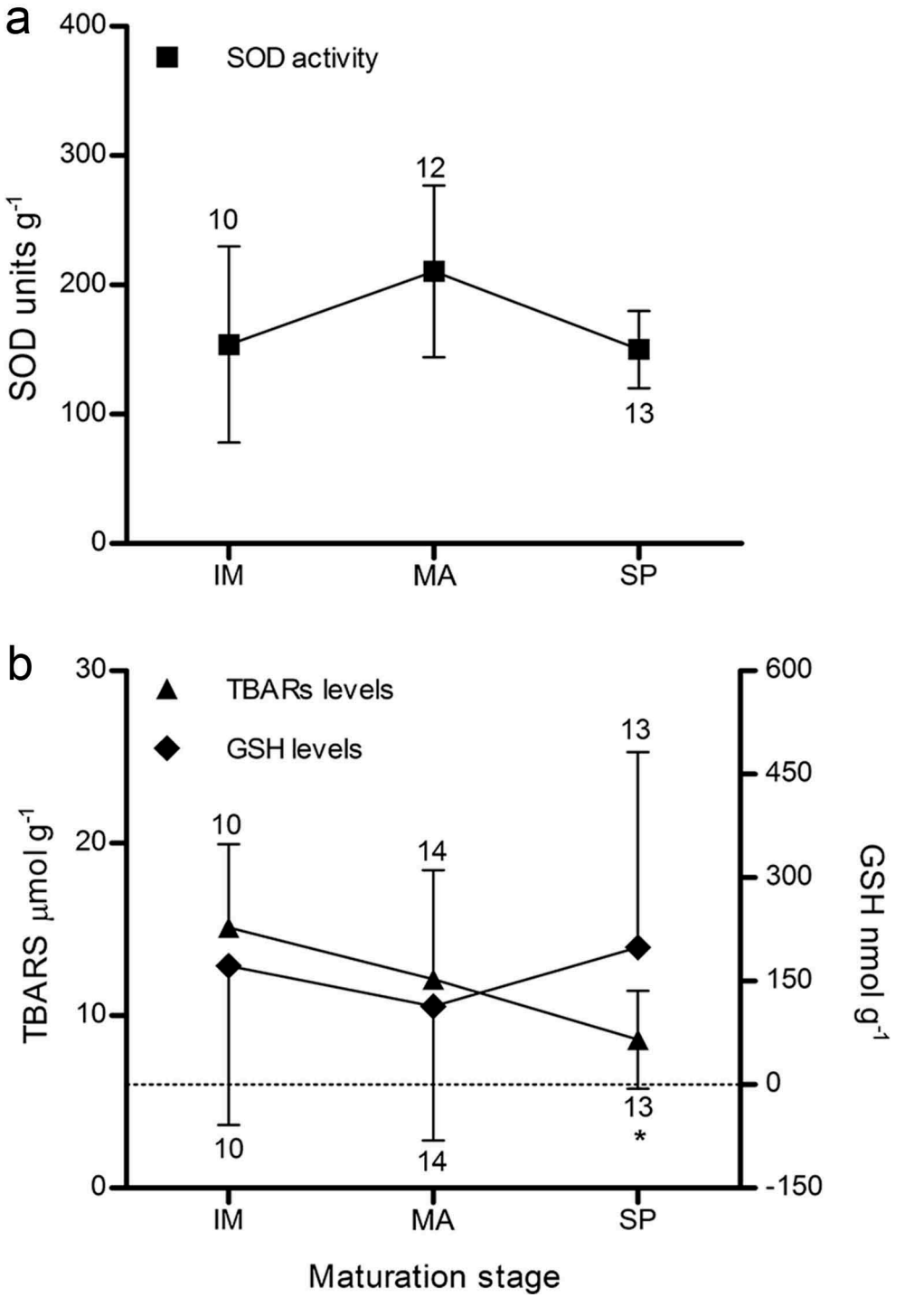

Figure 3. Antioxidant defences and lipid peroxidation parameters in gills of female *Octopus tehuelchus* in different maturity stages. SOD: superoxide dismutase; GSH: reduced glutathione; TBARS: thiobarbituric acid reactive substances; CAT: catalase; IM: immature; MA: mature; SP: spent stage; (a ●) CAT activity (CAT units g^{-1}), (■) SOD activity (SOD units g^{-1}), (b ▲) TBARS levels (TBARS µmol g^{-1}) and (♦) GSH levels (GSH nmol g^{-1}).Significant differences are indicated by asterisks: * $P < 0.01$ with respect to IM and ** $P < 0.01$ with respect to MA. Numbers on top of bars = *n*.

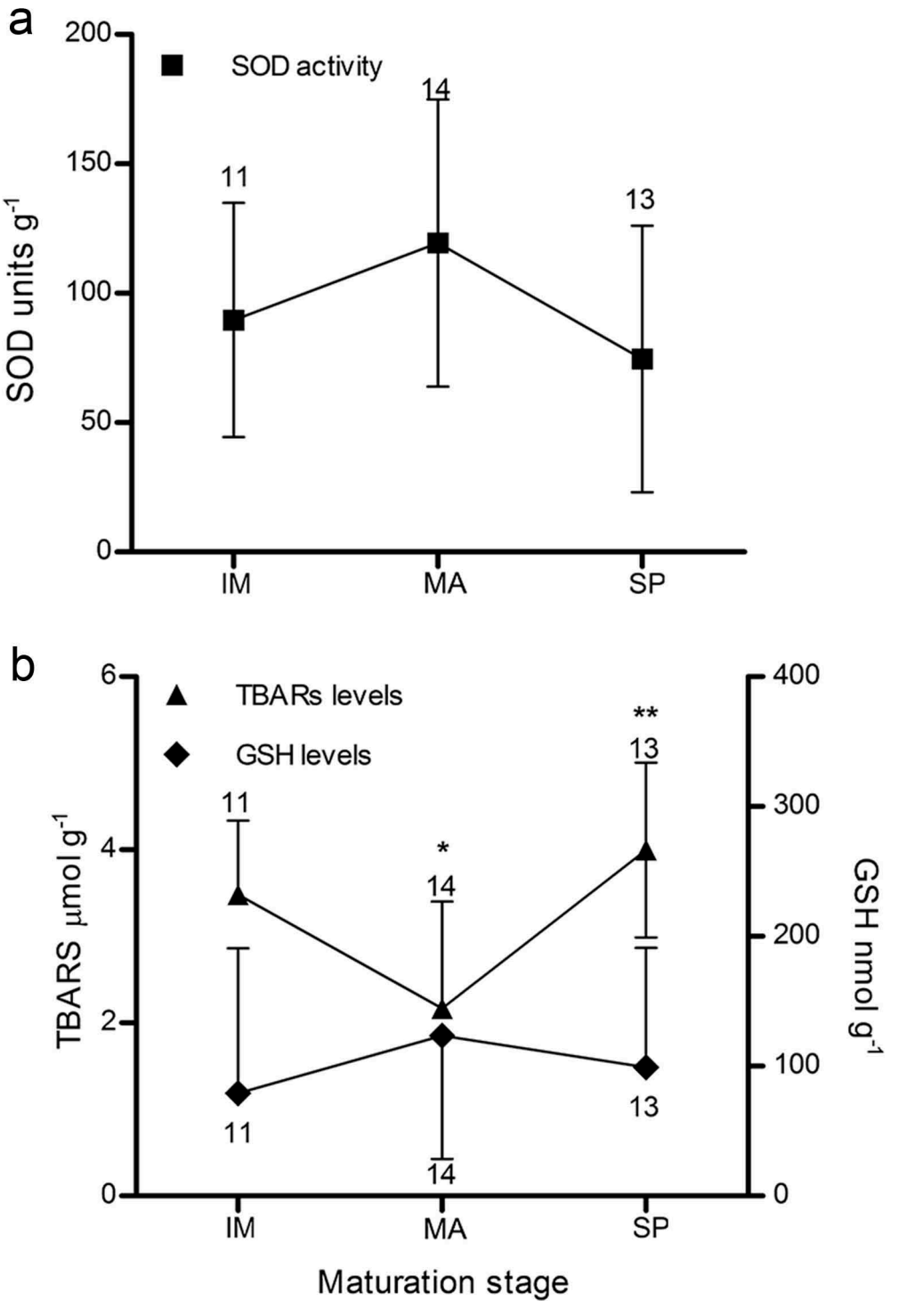

Figure 4. Antioxidant defences and lipid peroxidation parameters of female *Octopus tehuelchus* mantle in different maturity stages. SOD: superoxide dismutase; GSH: reduced glutathione; TBARS: thiobarbituric acid reactive substances; CAT: catalase; IM: immature; MA: mature; SP: spent stage; (a ■) SOD activity (SOD units g^{-1}), (b ▲) TBARS levels (TBARS µmol g^{-1}) and (♦) GSH levels (GSH nmol g^{-1}). Significant differences are indicated by asterisks: * $P < 0.01$ with respect to IM and ** $P < 0.01$ with respect to MA. Numbers on top of bars = *n*.

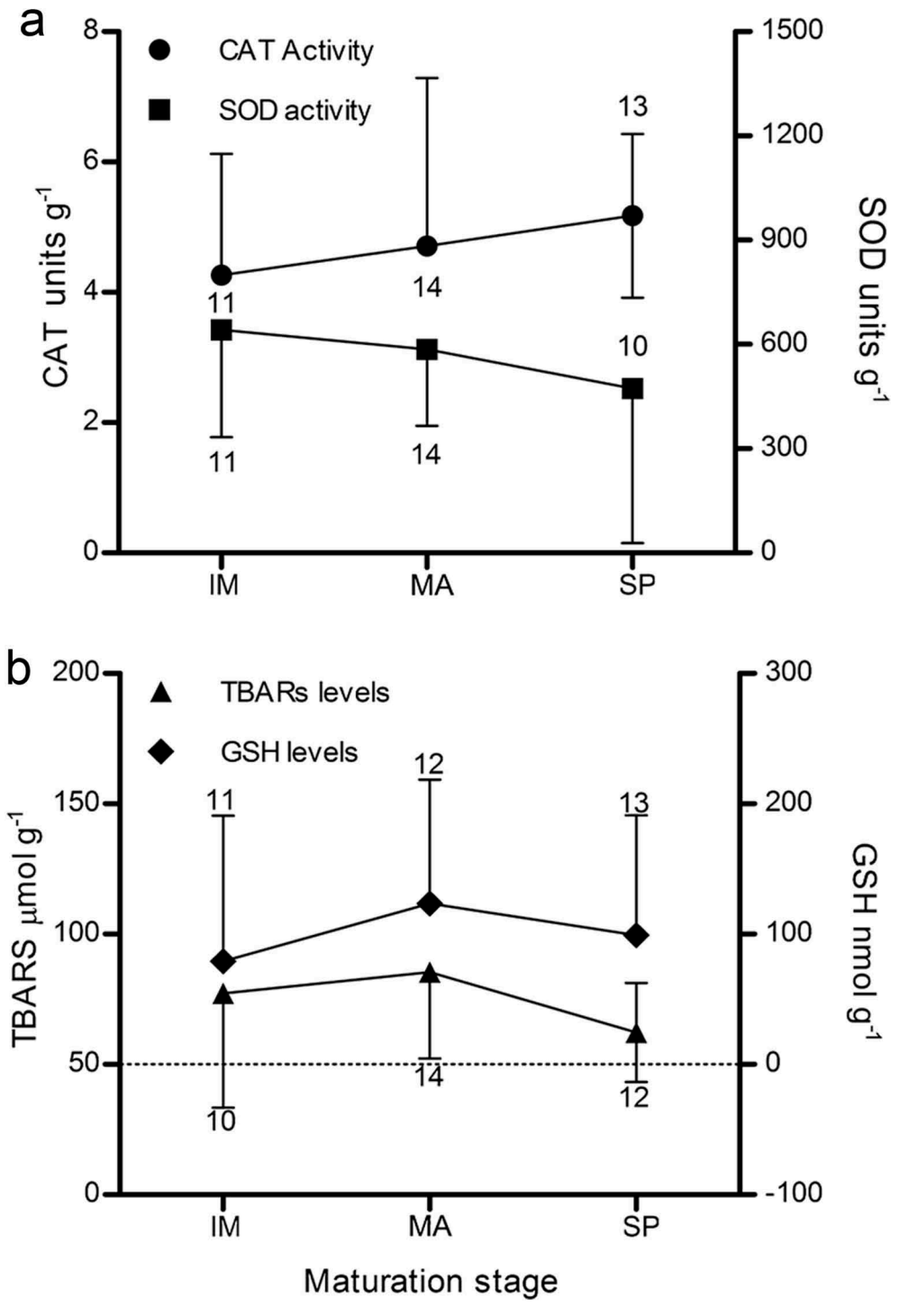

Figure 5. Antioxidant defences and lipid peroxidation parameters of female *Octopus tehuelchus* digestive gland at different maturity stages. SOD: superoxide dismutase; GSH: reduced glutathione; TBARS: thiobarbituric acid reactive substances; CAT: catalase; IM: immature; MA: mature; SP: spent stage; (a ■) SOD activity (SOD units g^{-1}), (b ▲) TBARS levels (TBARS µmol g^{-1}) and (♦) GSH levels (GSH nmol g^{-1}). Significant differences are indicated by asterisks: * $P < 0.01$ with respect to IM and ** $P < 0.01$ with respect to MA. Numbers on top of bars = *n*.

which has a shorter life expectancy than *S. officinalis* (Zielinski and Pörtner 2000). CAT activity remained unchanged with age in *S. officinalis* and *L. brevis*, although it was undetectable in *L. brevis* mantle (Zielinski and Pörtner 2000) and in most tissues in *O. tehuelchus*. In this sense, Hartman et al. (2003) showed an overall correlation between CAT levels and life span in recombinant strains of the nematode *Caenorhabditis elegans*, suggesting that life span, catalase levels, and resistance to oxidative stress are intimately associated. Thus, the short life cycle of *O. tehuelchus* and the low antioxidant defences, especially the lack of CAT activity, coupled with the high levels of polyunsaturated fatty acids found in octopus that are preferential targets for ROS (Navarro and Villanueva 2000; Abele and Puntarulo 2004), could be linked to the high baseline levels of TBARS found in this species.

Measured parameters in *O. tehuelchus* showed different values among the assayed tissues. Digestive gland showed elevated values among all tissues while mantle showed low values, consistent with other studies (Zielinski and Pörtner 2000; Soldatov et al. 2008; Gostyukhina 2013). Gill values were higher than mantle values for the measured parameters, as previously reported for other cephalopods (Zielinski and Pörtner 2000), and the ovary wall had the lowest values of SOD and GSH among all the tissues. In contrast, the oviducal glands exhibited the highest mean values for the measured antioxidant defences. As has been observed in bivalves (Soldatov et al. 2008), this would indicate that antioxidant defences of different tissues react differently as they are associated with different physiological roles and, therefore, have different needs in oxidative stress protection.

Antioxidant defences in tissues of *O. tehuelchus* do not show maturity-associated patterns in any of the analysed organs except one. In somatic tissues, GSH levels and SOD activity did not change, suggesting that they do not play a key role during MA and SP stages. The ovary wall shows a pattern similar to that of the somatic tissues assayed. Nonetheless, the oviducal glands do show specific changes associated with maturity stage transition. These changes involve a decrease in GSH and a simultaneous increase in TBARS when females reach the SP stage.

It has been reported in several taxa that in reproductive-related tissues there is an increase in lipid peroxidation associated with reduced antioxidant capacity after natural oxidative stress conditions (spawning) (Martin and Grotewiel 2006; Soldatov et al. 2008). Damage to membrane lipids is very relevant to the functional decline of reproductive organs (Hulbert et al. 2007). In *O. tehuelchus*, oviducal glands along with distal oviducts store sperm even from early stages of maturity and, once the female is ready to spawn, become darker and secrete mucoproteins and mucopolysaccharides that glue the eggs to the substrate (Table 1; Pujals 1986). After spawning, fertilisation has already occurred and the oviducal glands stop producing glue secretions. Considering that *O. tehuelchus* has a single reproductive event, the increased oxidative damage in this organ in SP stage could be associated with a loss of their secretory or sperm reservoir function.

On the other hand, in different biological models it was observed that lipid peroxidation in somatic tissues increases with longevity (Sukhotin et al. 2002; Hulbert et al. 2007; Abele et al. 2009). However, in *O. tehuelchus* TBARS levels decrease in gills and mantle at MA and SP stages, respectively, and remain constant in the digestive gland. In cuttlefishes (Zielinski and Pörtner 2000) and octopuses (Doubleday and Semmens 2011), peroxidised lipids are catabolised to lipofuscins. This physiological pathway could explain the decrease in TBARS levels observed in these organs, but this remains to be corroborated in *O. tehuelchus*.

As mentioned above, oxidative stress has been postulated as one of the physiological costs of reproduction and a factor that contributes to ageing (Harshman and Zera 2007; Kregel and Zhang 2007; Monaghan et al. 2009). In semelparous animals such as cephalopods, salmons and marsupials, a metabolic reorganisation and energy allocation occurs during the spawning period (Sawada et al. 1993; Semmens et al. 2004; Boyle and Rodhouse 2005; Naylor et al. 2008; Cook et al. 2014; Wilson et al. 2014; Taylor et al. 2015). However, there are few studies linking oxidative stress with semelparous life histories (Monaghan et al. 2009; Constantini 2014). Salmon populations of *Oncorhynchus nerka* that migrate different distances to spawn showed changes in the redox balance and antioxidant capacity that affect different tissues depending on the spawning site (Taylor et al. 2015). In *O. tschawytscha* (Chinook salmon) sampled from the field, ROS formation is increased in spawned salmon compared to non-spawned ones (Sawada et al. 1993). In addition, in *O. gorbuscha* (pink salmon), the antioxidant capacity and DNA damage are altered before and after the freshwater spawning migration on a tissue-specific basis, where some tissues, such as liver, show no changes while the brain is highly affected (Wilson et al. 2014). In wild *O. tehuelchus*, after spawning, oxidative stress parameters measured might be specifically linked with the physiological processes occurring in oviducal glands. Nevertheless, the low antioxidant defences and the flat protective reactions in somatic tissues and ovary wall might be associated with the semelparous life history of this species.

In natural environments, factors such as temperature or habitat complexity can affect many biological processes of cephalopods at different life-cycle phases (Boyle and Rodhouse 2005; Pierce et al. 2008; Semedo et al. 2012; Le Pabic et al. 2015). Since *O. tehuelchus*' maturity stages in the temperate waters of the Patagonian coast are coupled with seasonality (Ré 1998; Storero et al. 2010) (Table 1), results from ageing assays performed under laboratory conditions may differ from those obtained in wild animal populations (Abele et al. 2009). Studies under controlled culture conditions may be necessary to corroborate the low levels of antioxidant parameters observed under field conditions, and to clarify the role of oxidative stress in the life cycle of *O. tehuelchus*, particularly in post-spawning stages, when a rapid senescence is unleashed.

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Disclosure statement

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