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The effect of predator exposure and reproduction on oxidative stress parameters in the Catarina scallop *Argopecten ventricosus*

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ABSTRACT

Predation is known to impact growth and reproduction, and the physiological state of the prey, including its susceptibility to oxidative stress. In this study, we investigated how prolonged exposure to predators modulates tissue specific antioxidant defense and oxidative damage in the short-lived epibenthic scallop *Argopecten ventricosus* (2 years maximum lifespan). Scallops that were experimentally exposed to predators had not only lower antioxidant capacities (superoxide dismutase and catalase), but also lower oxidative damage (protein carbonyls and TBARS = thiobarbituric acid reactive substances including lipid peroxides) in gills and mantle compared to individuals not exposed to predators. In contrast, oxidative damage in the swimming muscle was higher in predator-exposed scallops. When predator-exposed scallops were on the verge of spawning, levels of oxidative damage increased in gills and mantle in spite of a parallel increase in antioxidant defense in both tissues. Levels of oxidative damage increased also in the swimming muscle whereas muscle antioxidant capacities decreased. Interestingly, post-spawned scallops restored antioxidant capacities and oxidative damage to immature levels, suggesting they can recover from spawning-related oxidative stress. Our results show that predator exposure and gametogenesis modulate oxidative damage in a tissue specific manner and that high antioxidant capacities do not necessarily coincide with low oxidative damage.

1. Introduction

Sublethal effects of predation risk can markedly shape the morphology and life history of the prey at both individual and population levels (Luttbeg and Kerby, 2005). In scallops, mussels and snails, predator exposure has been shown to increase shell thickness, adductor muscle size (Delgado et al., 2002; Lafrance et al., 2003), and affect size and age at first reproduction (Reimer, 1999; Hoverman et al., 2005; Guerra et al., 2011). Chronic exposure of animals to the presence of predators in their natural habitat can further result in higher basal swimming activity and higher respiration rates. Some studies have reported a relation between respiration rates, rates of mitochondrial oxygen turnover and the production of reactive oxygen species (ROS) in fish and mammals (Ji, 1993; Asami et al., 1998; Ji, 1999; Aniagu et al., 2006; Powers and Jackson, 2008 for review). ROS are reactive intermediates that are produced primarily within mitochondria as unavoidable products of aerobic metabolism. If not counterbalanced by cellular maintenance mechanisms such as the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), ROS can damage enzymes, protein receptors, lipid membranes and DNA (Beckman and Ames, 1998; Barja, 2004) leading to cell dysfunction and oxidative stress.

In a study related to this topic, Philipp et al. (2008) investigated the effect of short-term swimming on oxidative stress levels in muscle tissues of young *Aequipecten opercularis* scallops after exposure to a potential predator (sea star). The authors showed that ROS generation increased during predator-induced burst swimming, but there was no evidence of oxidative damage, perhaps due to a parallel increase in the scallop's ROS scavenging capacities. In contrast, a longer-term study of the damselfly *Enallagma cyathigerum*, showed a decrease in the insect's antioxidant defense system (CAT capacities) after 5 days exposure to a predator (Slos and Stoks, 2008). Although the authors suggested that the decrease in CAT capacities together with an increase in respiration rate might inevitably result in oxidative stress, they did not directly determine the levels of oxidative damage. Predicting the effect of predator exposure on oxidative stress parameters in prey is challenging because the effects can vary between species but also with duration of exposure. Up to now, a mechanistic understanding of how long-term exposure to predators induces oxidative stress in marine ectotherms is lacking.

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Oxidative stress is also recognized as being associated with reproductive activities (Kim et al., 2009; Costantini, 2010; Bergeron et al., 2011; Guerra et al., 2012). Accumulation of oxidative damage and decreases in antioxidant protection during reproduction have been shown in fruit flies, birds, and also in bivalves (fruit flies: Wang et al., 2001; birds: Alonso-Alvarez et al., 2004, 2006; Bize et al., 2008; bivalves: Soldatov et al., 2008). Particularly scallops are known to invest so heavily into gametogenesis and spawning, that reproduction represents a major stress (Barber and Blake, 1991). In a precedent study conducted in the field, we showed that in *Argopecten ventricosus*, maturation is fueled by energy diverted from adductor muscle to the gonad and that high investments into reproduction are also connected to a peak increase in protein carbonyls and TBARS in scallops' muscle, mantle and gill tissues (Guerra et al., 2012). As gametogenesis in bivalves is strongly governed by environmental conditions such as temperature and food (Viarengo et al., 1991; Wilhelm Filho et al., 2001; Malanga et al., 2004; Guerra et al., 2012) it is difficult to disentangle the effects of reproduction and the effects of environmental factors on oxidative stress levels under natural conditions in the field. Within this context, in the present study we used a single cohort of *A. ventricosus* scallops that was reared under controlled temperature and food conditions in the laboratory.

We investigate whether long-term exposure to a predator influences the level of oxidative stress in different tissues (mantle, muscle and gill). As there is no always a strict relation between antioxidant capacities and oxidative damage formation (Costantini and Verhulst, 2009), oxidative stress was assessed by monitoring both, the levels of oxidative damage markers (TBARS and protein carbonyls) and the concurrent antioxidant capacities (SOD and CAT). To determine whether predator presence affected swimming intensity, we examined the effect of predator exposure on adductor muscle mass and the activity of two metabolic enzymes in the adductor muscle: octopine dehydrogenase (ODH) and citrate synthase (CS). ODH catalyses the anaerobic ATP production during burst swimming in scallops and CS is a Krebs cycle enzyme involved in aerobic metabolism during the recovery phase following burst swimming (De Zwaan et al., 1980; Livingstone et al., 1981; Bailey et al., 2003). As predator-exposed scallops survived the spawning event, we were able to measure antioxidant defense capacities and oxidative damage in mantle muscle and gills as well as muscle metabolic enzyme capacities (ODH and CS) in sexually immature, pre-spawning and post-spawned scallops.

2. Materials and methods

2.1. Experimental animals and culture conditions

A. ventricosus larvae were obtained and reared in the hatchery as described in detail in Guerra et al. (2011). Briefly, larvae were obtained from spawn of wild scallops. Juveniles were reared for 3 months in the hatchery until they reached 5–7 mm shell height. Subsequently, scallops were maintained in a flow-through system consisting of 8 parallel 70 L-aquaria under constant water flow of 210 L day^{-1} and a salinity of 33–36 ppt. Each aquarium contained an initial number of ~630 scallops. Temperature in the aquaria was adjusted each month following the natural conditions in the field to mimic seasonal temperature changes (Fig. 1). Field temperatures were recorded at the Rancho Bueno estuary (geographical position: 24°19′17.3″N, 111°25′37.3″W) located in Bahía Magdalena, Baja California Sur, Mexico, where optimal temperatures for *A. ventricosus* growth have been reported to occur (Sicard-Gonzàlez et al., 1999). The temperature in each aquarium was controlled using aquarium heaters (Hagen Aquaclear 22952) and recorded at 30 min intervals using temperature loggers (WTA32-5+37, Onset Computer Corp., Bourne, MA, USA). The animals were fed ad-libitum using a 1:1 mixture of *Chaetoceros calcitrans* and *Isochrysis galbana*.

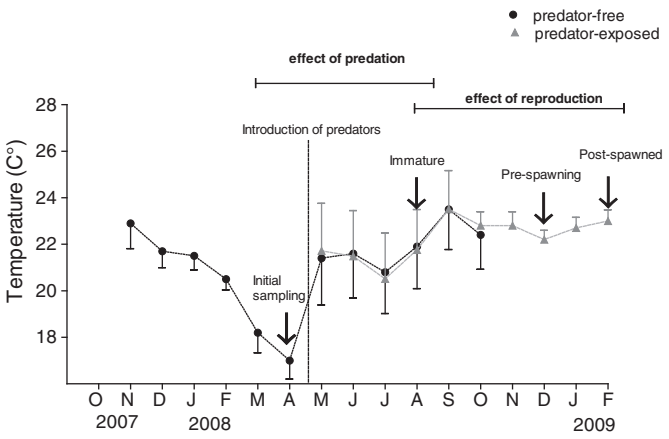

Fig. 1. Temperature pattern applied in the laboratory experiments. Arrows indicate time-points of sampling. The samplings in April and August of both groups were used to investigate the effect of predators on different parameters. The samplings in August, December and February of the predator-exposed group were used to investigate the effect of reproduction on different parameters. Dotted lines were added for a better overview of the temperature trend over time.

The first sampling was performed on April 8, 2008 at an experimental temperature of 17 °C when scallop mean shell height was 24 ± 5.3 mm. Six days after this initial sampling (14 April 2008), the blue crab *Callinectes sapidus*, a known predator of *A. ventricosus* in the natural environment (Ciocco and Orensanz, 2001), was introduced into each of four tanks used for the "predator-exposed treatment" (see Fig. 1). The crabs' pincers were tethered with rubber bands to prevent the crabs from eating the scallops. The crabs were fed squid every third night in a separate aquarium. Introducing *C. sapidus* into the aquaria immediately enhanced swimming activity in the scallops (personal observation). The effect prevailed throughout the entire 4 months of experimental period without observable signs of habituation in the scallops. The remaining 4 aquaria were maintained at the same temperature and feeding conditions as in the predator-exposed aquaria but without predators (predator-free treatment). After 4 months (16 August 2008), predator-exposed and predator-free scallops were sampled and the experimental temperature was 22 °C.

To investigate changes in oxidative stress during reproduction, immature, pre-spawning and post-spawned individuals of the predator-exposed groups were taken the 16 August (immature), the 14 December 2008 (pre-spawning) and the 20 February 2009 (post-spawned). Temperature was kept at 22–23 °C in order to eliminate temperature effects on oxidative stress parameters in the pre-spawning and post-spawned animals (Fig. 1). The animals in the predator-free treatment spawned earlier and invested more heavily into reproduction than the predator-exposed group (see Guerra et al., 2011). As spawning means an energetic drainage in scallops, reproduction resulted in higher mortalities in the predator-free group. Consequently, within this group, sampling was restricted to pre-spawning periods and the differences between immature, pre-spawning and post-spawned animals could not be examined.

During each sampling, two to three scallops were taken from each of the 4 replicate aquaria and dissected to obtain mantle, adductor muscle, gill and gonadal tissues. Tissues were weighed and mantle, adductor muscle and gills were frozen in liquid nitrogen for biochemical analysis. The shell height (distance from hinge to shell margin) was measured using calipers.

2.2. Gonad index

The gonad index (GI) was determined on each sampling date as (gonad weight/total shell weight) × 100 (see Lucas and Beninger,