Effects of sessile Protozoa on intracapsular oxygen tension and embryonic shell calcification in the muricid Chorus giganteus

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ABSTRACT: Clusters of egg capsules deposited by some common marine Mollusca may suffer problems of a low diffusive oxygen supply to the embryos they contain, especially if the capsules are exposed to hypoxic seawater or attachment and growth of marine biofouling organisms. The present study was undertaken to determine the effects of severe biofouling by sessile Protozoa on intracapsular oxygen tension (IPO₂) and the development of embryos contained in the egg capsules of the muricid snail Chorus giganteus. We also investigated the effects of ambient oxygen tension (EPO₂) on IPO₂. The presence of sessile Protozoa attached to the outer wall of the egg capsules significantly reduced the IPO₂ compared to capsules not fouled by Protozoa. Clean capsules containing embryos showed an IPO₂ of about 105 mm Hg, compared with about 92 mm Hg for protozoan-fouled capsules when both were immersed in air-saturated seawater at 12°C. The embryos in capsules without Protozoa grew normally, hatching in about 70 d as velicong larvae, whereas the development of larvae in protozoan-fouled capsules showed impairment of shell formation and delay in hatching for up to 5 mo. Pre-hatch embryos at 60 d measured about 922 μm and had an ash content near 18 μg embryo⁻¹; embryos in capsules covered by micro-organisms measured only about 783 μm, with an ash content of about 3 μg embryo⁻¹ over the same time period. Our study suggested that the lack of larval calcification observed in the presence of sessile Protozoa on the outer wall of the egg capsules was probably related to reduced IPO₂. Similarly, any factor reducing oxygen supply to encapsulated embryos (i.e. exposure to water masses with low oxygen content, biofouling, reduced water movement) could impair embryonic development, a significant phenomenon thus far not reported in C. giganteus.

KEY WORDS: Biofouling · Egg capsules · Mollusc · Embryonic development · Hypoxia

INTRODUCTION

Several recent papers have revealed striking changes in dissolved oxygen concentration within specific marine microhabitats, such as in tide pools and close to rocky substrata (Rosemb erg & Loo 1988, Shashar et al. 1993, Diaz & Rosemb erg 1995). As a consequence of such fluctuations, some organisms occurring close to the substratum may be exposed to extensive periods of hypoxia or even anoxia (Pihl et al. 1991). Many marine molluscs not only attach their eggs to the substratum but also lay them in clusters and in large numbers enclosed in either egg masses or egg capsules (Gallardo 1981, Pechenik et al. 1984). The encapsulated development, common in some gastropods, suggests a priori that the contained embryos may be subjected to problems of oxygen supply, which may constrain both the shape of the capsules and the number of embryos they contain. Accordingly, Strathmann & Strathmann (1995) have shown that the diffusive supply of oxygen is a limiting factor for embryos clustered in gelatinous egg masses. Development rate in 3 species of opisthobranch gastropods was retarded when exposed for 10 to 24 h to oxygen concentration below 10% air saturation. Some opisthobranch embryos exposed to low oxygen con-
centration demonstrated a reduction in shell size at hatching. Moreover, supply of oxygen, rather than elimination of wastes, limited the rate of development within masses of aggregated embryos (Strathmann & Strathmann 1995). The demand for oxygen could also explain why Conus species the number of embryos increased in proportion to surface area of the capsule rather than to its volume (Perron & Corpuz 1982). Similarly, in 3 species of gastropods the number of embryos decreased as the thickness of the egg mass increased (Lee & Strathmann 1998). In an artificial gel matrix that simulated egg masses, development rate of central embryos became increasingly retarded in relation to peripheral embryos with increasing embryonic numbers and with increasing thickness of the gelatinous egg masses (Strathmann & Strathmann 1989). Roller & Stickle (1989) suggested that the relatively large number of veliger larvae inside egg capsules of Thais haemastoma caniculata (Gray) might generate anaerobic stress that could influence the respiration rates of hatched larvae.

Recently, Cohen & Strathmann (1996) tested the hypothesis that oxygen supply to embryos in egg masses might be affected by the thin layer of fouling micro-organisms covering the egg mass and demonstrated that photosynthesis and respiration of such micro-organisms affected the supply of oxygen to embryos within the mass. They also suggested that under certain conditions the oxygen supply could become a limiting factor for development, although we have not found any publication dealing with the long-term effects of a low oxygen supply on embryonic development related to the presence of bio-films on egg capsules. In intensive artificial culture of egg capsules of the Chilean muricid Chorus giganteus (Lesson, 1829), sessile Protozoa of the genera Tubilopora and Vorticella have been found to colonise the outer surfaces of egg capsules. This provided ideal material to experimentally evaluate the effects that a reduction in oxygen supply, due to the presence of fouling microorganisms, might have on intracapsular oxygen tension (IPO₂) levels and their consequences for embryonic development.

Chorus giganteus is an endemic muricid on the Chilean coast, occurring subtidally on soft sediments and in areas with small rocks (Gallardo 1981). Embryonic development occurs within egg capsules, from which lecithotrophic veliger larvae emerge in 70 d at 13°C (Leiva et al. 1998). It has been found that embryos subjected to hypoxic environmental conditions (50% air saturation) developed more slowly, while shell formation and larval hatching from the capsule were prevented (J.M.C. et al. unpubl. data). The present study was undertaken to experimentally evaluate whether the presence of heavy protozoan fouling reduced oxy-


gen tension within the egg capsules of Chorus giganteus and whether such a reduction in oxygen tension was associated with impaired development of intracapsular larvae.

MATERIALS AND METHODS

Experimental material. Egg capsules were obtained from a laboratory population of Chorus giganteus maintained in culture conditions at the Universidad Austral, Valdivia (39°25'S, 73°10'W). About 100 capsules were harvested some 2 wk after oviposition and thoroughly washed with 5 μm filtered seawater. About half of the capsules were maintained in clean glass aquaria in a constant flow of 5 μm filtered seawater (60 l h⁻¹) at 12 ± 1°C and the remaining capsules were separately maintained in unfiltered seawater under similar conditions. Every 2 wk, 3 egg capsules were removed from each holding system and transported overnight to our laboratory in Concepción (36°45'S, 73°10'W) at 12°C. Measurements included capsule length and surface area. Embryos were measured for length, total dry weight, and total organic and inorganic matter content.

Embryos were collected on pre-ashed fiberglass filters, washed in ammonium formate solution isotonic with seawater and dried to constant weight at 80°C. Organic content was determined as the difference in dry mass before and after ashing for 4 h at 500°C. Embryo length was measured using an ocular micrometer with a light microscope. The surface area of individual capsules was determined by cutting the capsule into 2 pieces along its longest axis and drawing both pieces on an acetate slide. The drawing was cut out and weighed and converted to surface area by dividing by the weight of a known area of acetate slide.

Capsules were maintained until hatching occurred in order to obtain measurements throughout the entire capsular growth phase and to compare data obtained using clean (W/OP) egg capsules with those from protozoan-fouled (WP) egg capsules.

Oxygen tension. Oxygen measurements (Fig. 1) were carried out using a Strathkelvin Model 781 oxygen meter fitted with a 1302 SI Strathkelvin electrode to determine ambient oxygen tension (EPO₂) and a Clark-type model 768-20R needle microelectrode (Diamond General Development Co., diameter 890 μm) to determine intracapsular oxygen tension (IPO₂). Both electrodes were calibrated using nitrogen-purged, oxygen-free seawater to produce a zero value of oxygen tension (PO₂) and 100% air-saturated seawater (6.24 μl O₂ l⁻¹ at 12°C and 30% salinity) to give a PO₂ of 157 mm Hg. Measurements included the simultaneous evaluation of EPO₂ and IPO₂ first on W/OP egg