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Acute hypoxic exposure affects gamete quality and subsequent fertilization success and embryonic development in a serpulid polychaete

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ABSTRACT

Hypoxia likely compromises the reproductive success of those marine organisms carrying out external fertilization because their gametes and embryos are inevitably exposed to the external environment. *Hydroides elegans*, a dominant serpulid polychaete in Hong Kong waters, can spawn throughout the year but the number of recruits drops during summer when hypoxia commonly occurs. This study attempted to explain such observation by investigating the gamete quality, including sperm motility, egg size, complexity and viability, after 1-h hypoxic exposure (1 mg O_2 l^{-1}). In addition, how gamete quality affects fertilization success and embryonic development was examined. After 1-h hypoxic exposure, sperm motility was significantly reduced, compromising fertilization success. Although the eggs remained viable, more malformed embryos and retarded embryonic development were observed. We interpreted that the harmful effect of hypoxia on embryonic development was attributed to the teratogenicity and induced oxidative stress, ultimately causing the reduction in recruitment during summer.

1. Introduction

Hypoxia, a condition in which the dissolved oxygen (DO) level in the water body is below 30% of O_2 saturation (Levin et al., 2009), can be a natural event in light of temperature and salinity stratification in the water column (Stanley and Nixon, 1992). However, reported increases in the occurrence of hypoxia worldwide are usually associated with the anthropogenic input of nutrients and organic matters into the coastal waters with poor water circulation because microorganisms can rapidly consume the oxygen in the water during decomposition (Diaz and Rosenberg, 1995, 2008). This globally pressing issue is not uncommon in Hong Kong, especially in sheltered harbors, fish farms or embayments in the summer when wind and tidal mixing are slight. For example, a routine monthly monitoring programme conducted by the Environmental Protection Department, Hong Kong (http://epic.epd.gov.hk/ca/uid/marinehistorical/p/1), revealed that the bottom water in Tolo Harbour in the east of Hong Kong suffered from hypoxia in July and August from 2008 to 2011. It is surmised that the occurrence of hypoxia would be further aggravated due to increase in human activities and global warming (Nixon, 1990; Wu, 2002).

It has been well-documented that hypoxia can pose harmful effects on the population dynamics of marine ecosystems, including massive mortality of marine organisms, changes in species composition and reduction in biodiversity (Pihl et al., 1991; Diaz and Rosenberg, 1995; Gray et al., 2002). Under severe hypoxia (<1 mg O_2 l^{-1}), the community structure would be dominated by few tolerant, opportunistic species since most marine species are eliminated within few days (Llansó, 1992; Diaz and Rosenberg, 1995). The effect of hypoxia is even more devastating when hypoxia coincides with the spawning period of marine organisms, resulting in temporary exclusion of the sensitive species in particular (Rosenberg and Loo, 1988).

Fertilization and embryonic development are important processes in determining the abundance of local populations. Unfortunately, for those marine organisms carrying out external fertilization, their gametes are inevitably exposed to external environment. As gametes are very sensitive to hypoxia (Lutz et al., 1994), fertilization success would be compromised due to impairment of gamete quality. For example, Wu et al. (2003)

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reasoned that hypoxia can worsen sperm quality, in terms of swimming velocity, leading to reduction in fertilization success. Eggs are also susceptible to hypoxia. For instance, the eggs of bay anchovy suffered approximately 50% mortality during 12-h exposure to 2.8 mg $O_2\,l^{-1}$ (Chesney and Houde, 1989). Even worse, planktonic eggs tend to sink into the hypoxic bottom layer (Breitburg, 2002), which greatly reduces the chance of successful fertilization.

A serpulid polychaete, *Hydroides elegans*, was chosen as the study species. This widespread, gregarious species is characterized by rapid growth, proliferation and short life cycle (Carpizo-Ituarte and Hadfield, 1998). In this regard, it constantly creates nuisance to many man-made structures through fouling, thereby incurring huge economic cost of cleaning and application of anti-fouling agents (Townsin, 2003). As the eggs of *H. elegans* are negatively buoyant (Kupriyanova et al., 2001) and fertilization occurs shortly after spawning, the fertilized eggs will sink to the sea bottom and experience hypoxia which normally develops at the sea bottom and extends upward. In Hong Kong, the mortality of *H. elegans* was very high and there were nearly no recruits in summer in areas when hypoxia prevails (J.Y.S. Leung, pers. obs.). Our previous study revealed that the fertilization success was reduced by more than 30% while only about 30% of the embryos managed to develop into blastula under hypoxic condition (2 mg $O_2\,l^{-1}$) (Leung et al., 2013). Nonetheless, we could not explicitly explain how hypoxia leads to the reduction in fertilization success and delayed embryonic development. The present study aimed to ascertain the effect of acute hypoxic exposure on the gamete quality and its relationship with the subsequent fertilization success and embryonic development. We hypothesized that the gametes would be impaired under hypoxia, resulting in reduction in fertilization success and rate of embryonic development.

2. Materials and methods

2.1. Collection and rearing of adult Hydroides elegans

Adult *H. elegans* were collected from a fish farm in Yung Shue O, Hong Kong (22°19′N, 114°16′E). Other foulers, such as tunicates and oysters, were carefully detached from the clumps. The specimens were kept in the laboratory under the following conditions: temperature: 21.0 ± 0.5 °C, DO: 8.32 ± 0.12 mg $O_2\,l^{-1}$, salinity: 32.0 ± 0.5 psu and pH: 8.15 ± 0.05. The seawater was renewed fortnightly and *H. elegans* were fed daily with approximately 1×10^5 cells ml^{-1} *Isochrysis galbana* (Qiu and Qian, 1998).

2.2. Physical parameters in the experiment

The desired DO levels were achieved by pumping an appropriate amount of nitrogen and air into the experimental system (Leung et al., 2013). The flow rate of the gases was controlled by digital flow meters (Voegtlin GCR-B3SA-BA20). The DO level in the seawater was monitored by the Stable Optical Oxygen System (TauTheta Instruments, SOO-100-OEM, USA) to maintain the desired DO levels. Normoxia was maintained by pumping air only. The temperature for all experiments was maintained at 28 °C, the average summer temperature of bottom waters in Hong Kong (EPD, 2012), by a heating bath circulator. The salinity for all experiments was 32.0 ± 0.5 psu.

2.3. Experiment 1 – Effect of acute hypoxic exposure on gamete quality

Spawning was induced by carefully breaking the calcareous tube near the abdominal region of adult *H. elegans* with a pair of forceps under a dissecting microscope. The sperm (creamy white) from 3 males and eggs (orange) from 8 females were transferred into separate 15 ml centrifuge tubes, followed by diluting to 3 ml with filtered seawater (FSW). The DO level of the sperm and egg suspensions was then adjusted to 1 mg $O_2\,l^{-1}$ (hypoxia) or maintained at 6 mg $O_2\,l^{-1}$ (normoxia) for 1 h. The sperm which was exposed to hypoxia is denoted as 'hypoxic sperm' hereafter, and a similar term was also applied to eggs.

The swimming velocity of sperm was analyzed as the indicator of sperm quality. One drop of sperm suspension (ca. 0.1 ml) was added on a glass slide which was then placed under a compound microscope (Axioplan 2 imaging, ZEISS, Germany) equipped with a video camera. The swimming behavior of the sperm was videotaped immediately. Three different types of swimming velocity, namely curvilinear velocity (VCL), straight-line velocity (VSL) and average path velocity (VAP), were determined using the CRISMAS sperm motility analysis system (Image House A/S, Denmark). VCL is defined as the time-average velocity of a sperm along its actual trajectory and considered as the actual swimming velocity. VSL is the time average velocity of a sperm along a straight line between its first detected and last detected position and considered as the dispersal velocity. VAP is velocity with the distance calculated by adding straight-line values between every 2 frames. A total of 40 sperms were randomly selected from each slide for measurement. Three centrifuge tubes with sperm suspension were prepared for each DO level as replicates.

Egg size, complexity and viability were determined as the indicators of egg quality. A total of 50 eggs were randomly selected from each centrifuge tube with egg samples and individual diameter, which represents egg size, was measured under a compound microscope using the software AnalySIS LS Professional 5.0 (Olympus Soft Imaging Solutions GmbH, Germany). Egg complexity and viability were analyzed by flow cytometry. The eggs in the centrifuge tube were centrifuged at 1000 rpm for 5 min. The FSW was carefully removed and 2 ml phosphate buffered saline (0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4 at 25 °C) with 5 ppm propidium iodide (PI) were added into the centrifuge tube. The buffered saline was used to maintain constant pH and osmotic pressure. Dead cells and living cells can be differentiated by their nuclei after being stained by PI that the nucleus of a dead cell will fluoresce. The egg suspension was then injected into the flow cytometer (Facscalibur, Becton Dickinson, USA) for measurement. Egg complexity, which represents the extent of granules and other substances inside the eggs, was determined as the intensity of side scatter from a light beam of wavelength 488 nm on the eggs in suspension. For egg viability, PI was used as a DNA stain, which can penetrate cells with damaged membranes and bind to DNA. The fluorescence excitation of the eggs stained with PI was determined using a fluorescence detector with a detection range of 585 ± 21 nm. Three centrifuge tubes with egg suspension were prepared for each DO level as replicates.

2.4. Experiment 2 – Fertilization success and embryonic development

To study the relationship between gamete quality and fertilization success, a 2 × 2 factorial design (egg × sperm) was deployed. Sperm from 2 males and eggs from 3 females were transferred into separate 15 ml centrifuge tubes containing 1 ml FSW. The DO level of the gamete suspension was then adjusted to 1.0 mg $O_2\,l^{-1}$ (hypoxia) or 6.0 mg $O_2\,l^{-1}$ (normoxia) for one of the following durations: 15, 30 and 60 min. After the exposure, the eggs were transferred into a petri dish (BD Falcon, 35 mm in diameter, 10 mm in height), followed by adding 1 ml sperm suspension uniformly into the petri dish and a timer was switched on. The DO level of the FSW in the petri dish was maintained at 6 mg $O_2\,l^{-1}$ in the course of fertilization and embryogenesis. Micrographs were taken at 100× magnification at 30th, 60th, 90th,

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