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# Effects of hypoxia and elevated ammonia concentration on the viability of red snapper embryos and early larvae

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ABSTRACT

The effects of hypoxic conditions and elevated ammonia concentrations on the viability of embryos and newly hatched larvae of the red snapper (*Lutjanus campechanus*) were investigated. In all experiments, tested levels of hypoxia or ammonia concentrations were applied to embryos and unfed newly hatched larvae from three different spawns. Exposures began at 1 h post fertilization (pf) and lasted until all individuals in a group had expired. Survival rates were monitored daily in duplicates for each spawn in each treatment. Fertilized eggs exposed to 2 mg $L^{-1}$ dissolved oxygen (29% saturation) showed complete mortality before hatch while 81% of embryos in control groups (>85% saturation) hatched and subsequently maintained high survival until 5 days pf. Exposure to a moderate hypoxia (target 3 mg $L^{-1}$, 43% saturation) reduced significantly the hatch rate and subsequent survival rates; the magnitude of the difference in survival rate between control and exposed groups increased from 10% at hatch to 45% at 5 days pf. When oxygen concentration was maintained high (83% saturation) until 36 h pf and then progressively reduced to reach 3 mg $L^{-1}$ at 2 days pf, the survival of exposed embryos and larvae did not differ significantly from those recorded in control groups, although potential delayed or cumulative effects of the treatment after 4 days pf could not be evaluated in this experiment.

Embryos exposed to 10 mg $L^{-1}$ total ammonia (TA-N), which corresponded to unionized ammonia (UIA-N) concentrations ranging between 0.307 and 0.468 mg $L^{-1}$ in the conditions of the experiment, exhibited significantly reduced hatch rates and complete mortality between 3 and 4 days pf; the latter period corresponds to the onset of exogenous feeding of red snapper. In contrast, control groups (TA-N < 0.26 mg $L^{-1}$, UIA-N < 0.006 mg $L^{-1}$) maintained high survival rates beyond 5 days pf indicating potential to successfully initiate exogenous feeding. Exposure to 1 mg $L^{-1}$ TA-N (0.020 mg $L^{-1}$ < UIA-N < 0.054 mg $L^{-1}$) did not alter significantly survival with respect to control groups. Significant interactions between the spawn and the tolerance to hypoxia or elevated ammonia were detected in both experiments, indicating that variations among spawns need to be accounted for when determining safe levels for hatchery production.

*Statement of relevance*

Achieving a reliable supply of high quality eggs and larvae is one of the main challenges of the developing marine aquaculture industry.

Most studies to date have focused on maternal determinants of egg quality but the viability of embryos and newly hatched larvae can be impacted after fertilization if environmental conditions become unfavorable due to intensive hatchery conditions; this topic is poorly documented in marine fishes to date.

This study provides data on the effects of two major stressors acting under high density culture (hypoxia and elevated ammonia concentration) on embryos and newly hatched larvae of the red snapper; the results highlight the importance to consider variations among spawns/parents when determining safe levels for hatchery production and also the high sensitivity of red snapper to these stresses, suggesting that this topic should be investigated in other marine offshore species.

Relevance of the research to commercial aquaculture.

The research contributes to control egg quality.



## 1. Introduction

Achieving a reliable supply of viable fry for hatchery production is one of the main current challenges faced by the developing marine

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aquaculture industry (Mylonas et al., 2010). The viability of embryos and pre-feeding larvae is particularly difficult to control because it is determined by several factors (Brooks et al., 1997). To date, most studies have focused on the maternal determinants of egg characteristics, the survival potential of embryos, and the modulation of these maternal effects by parameters such as the age, the size, or the nutritional and stress status of the female parent prior to spawning (Bobe and Labbé, 2010). However, environmental factors can induce stress and affect the viability of eggs and larvae post fertilization (Brooks et al., 1997). In the wild, marine species generally release their gametes into an optimal buffered environment that ensures maximal survival and effective development of their offspring during the early larval period (Rijnsdorp et al., 2009). On another hand, intensive hatchery rearing conditions usually differ from the natural environment (Ashley, 2007), due to the incubation of embryos and newly hatched larvae at high density which can lead to rapid deterioration of the water quality. Depletion of dissolved oxygen (DO) and increase of ammonia concentration result from the metabolic activity (respiration and excretion) of embryos and hatched larvae. Aerobic processes involved in the degradation of dead and unfertilized eggs also contribute to oxygen demand and ammonia production. These changes in water quality have been linked to a reduction in hatch and early larval survival rates, along with other deleterious effects on offspring (Holt and Arnold, 1983; Shang and Wu, 2004). In such a situation, egg quality could be high at fertilization, but if embryos and larvae are cultured under deteriorated conditions, the viability and fitness of fry may be reduced. To date, information on the effects of such stressors on egg and larval quality is still limited in marine species (Yúfera and Darias, 2007). This topic is of central importance for hatchery production considering the limited respiratory and avoidance capacity at these early stages.

In marine environments, hypoxia has been defined as the condition whereby dissolved oxygen levels in the water fall below 2.8 mg DO $L^{-1}$ (Wu, 2002). However, because the availability of oxygen strongly depends on other parameters, particularly water temperature and salinity, hypoxia is better characterized by the oxygen saturation level (Chabot and Claireaux, 2008). European sea bass *Dicentrarchus labrax* and Atlantic cod *Gadus moruha* juveniles avoid waters where oxygen saturation is less than 45–50% (Claireaux et al., 2000; Schurmann et al., 1998) which is considered to be a level of moderate hypoxia (Chabot and Claireaux, 2008). Severe hypoxia (between 15 and 20% saturation) results in rapid mortality of Atlantic cod juveniles (Claireaux et al., 2000). The effects of hypoxia also depend on the type of exposure (e.g., chronic or acute) and the status of the affected organisms (e.g., active swimming, digestion, stress) which determines their oxygen demand. Fish embryos depend exclusively on cutaneous respiration and their surface area for gas exchange is limited (Elshout et al., 2013). Their sensitivity to hypoxia is therefore expected to be higher than that of adults and juveniles, a hypothesis that is supported by empirical data across several freshwater species (Elshout et al., 2013). To date, information on the tolerance to hypoxia of embryos and larvae of marine fish species is still very limited. Incubation of eggs and larvae under intensive conditions is expected to result in increased levels of dissolved ammonia due to two main processes: (1) ammonia is the main nitrogenous metabolic waste product excreted by teleost fish, and thus is naturally produced in a healthy aquaculture tank, and (2) the degradation of unfertilized eggs and dead embryos also generates ammonia during proteolysis. Sub-lethal or lethal levels can occur as a result of accumulation over time, unless water is renewed at high rates or dissolved ammonia is actively removed by use of biological filters in recirculating aquaculture systems. Total ammonia nitrogen (TA-N) consists of the highly toxic un-ionized form of ammonia (UIA-N, or $NH_3$) in equilibrium with the relatively non-toxic form ($NH_4^+$, Aubrey et al., 2014). The concentration of the two forms of ammonia is regulated primarily by water pH and temperature. Maximum levels of UIA-N between 50 and 200 μg $L^{-1}$ were recommended for marine finfish species by Person-Le Ruyet et al. (1997) and Lemarié et al. (2004), but these recommendations were based on juvenile and adult fish while information on embryos and larvae is still very limited. Available data suggest that the development of embryos can proceed to hatch even in the presence of elevated concentrations of UIA-N in the water, but newly hatched larvae seem much less tolerant to ammonia than eggs (Chen et al., 2012) and can experience mortality with UIA-N levels as low as 0.31 mg $L^{-1}$ to 0.55 mg $L^{-1}$ (Holt and Arnold, 1983). To date, knowledge of the tolerance of embryos and larvae of marine fishes to elevated levels of ammonia remains very limited.

The red snapper *Lutjanus campechanus* is a candidate for marine aquaculture in the southeastern United States. The current hatchery protocol for this species involves stocking embryos for incubation at a density of 1 egg $mL^{-1}$ under gentle aeration and moderate water turnover (<20% $h^{-1}$). Larvae are typically transferred 24 h post hatch to larval tanks where rearing density is lowered to 0.1 larva $mL^{-1}$ or less. Incubation of red snapper eggs at a density of 2 eggs $mL^{-1}$ or higher was shown detrimental to larval survival estimated 36 h post hatch (Bourque and Phelps, 2007), but the actual changes of water quality parameters such as levels of dissolved oxygen and un-ionized ammonia induced by elevated density were not reported in this experiment. In addition, this study did not document the kinetics of mortality during development, which prevented evaluating the viability of larvae surviving the early phases of exposures and determining the cumulative effects of prolonged exposures.

The objective of this study was to evaluate the tolerance of the red snapper eggs and larvae to individual environmental stressors resulting from high stocking density in intensive aquaculture. This work focused on the two main water quality parameters discussed above (concentration of dissolved oxygen and total ammonia) and studied the kinetics of mortality during exposures to provide data for the management of water quality during incubation and early larval rearing.

## 2. Materials and methods

Each experiment was performed using embryos from three different wild-caught females serving as biological replicates. Females were induced for gamete maturation with human chorionic gonadotropin (hCG) following a protocol based on the method developed by Minton et al. (1983). Eggs were collected from each female by manual stripping at ovulation, and immediately fertilized in vitro with the sperm of one or two wild-caught males. Conditions at fertilization were: temperature 26 ± 0.5 °C, salinity 30 ± 1 psu, dissolved oxygen at 85% saturation or greater (>6 mg $L^{-1}$), and total ammonia (TA-N) 0 mg $L^{-1}$. At 1 h post-fertilization (pf), random subsamples of each spawn were transferred to experimental 1-L beakers for challenges. Artificial seawater (BIOSEA® Marinemix, Aqua Craft, Hayward, CA) was prepared using deionized water (Mako RO system, Aquatic Ecosystems) for all experiments.

### 2.1. Hypoxic challenges

Exposures of embryos to hypoxic conditions were performed in an oxygen chamber I-Glove incubator glovebox (Biospherix, Lacona, NY), which allowed uninterrupted control of dissolved oxygen (DO) levels in the water. A gas control module PROOX model 360 was used to lower dissolved oxygen concentration in the water to the desired level by injecting nitrogen gas into the oxygen chamber. Infusion of gas exactly matched chamber leakage to hold oxygen level constant. Accordingly, only one reduced (hypoxic) oxygen concentration could be tested per trial.

Three hypoxic challenges were performed, each exposing embryos and larvae to a different hypoxic treatment that was contrasted to control groups unexposed. For each challenge, the eggs from three females were transported in separate closed container at a density of 1 egg $mL^{-1}$ to the location of the oxygen chamber where they were immediately stocked in experimental beakers (approximately 100 eggs