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Impact of ocean acidification on the early development and escape behavior of marine medaka (*Oryzias melastigma*)

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ABSTRACT

Ocean acidification is predicted to affect a wide diversity of marine organisms. However, no studies have reported the effects of ocean acidification on Indian Ocean fish. We have used the Indian Ocean medaka (*Oryzias melastigma*) as a model species for a marine fish that lives in coastal waters. We investigated the impact of ocean acidification on the embryonic development and the stereotyped escape behavior (mediated by the Mauthner cell) in newly hatched larvae. Newly fertilized eggs of medaka were reared in seawater at three different partial pressures of carbon dioxide (pCO_2): control at 450 μatm, moderate at 1160 μatm, and high at 1783 μatm. Hatch rates, embryonic duration, and larval malformation rates were compared and were not significantly different between the treatments and the control. In the high pCO_2 group, however, the yolks of larvae were significantly smaller than in the control group, and the newly hatched larvae were significantly longer than the larvae in the control. In the moderate pCO_2 group, the eye distance decreased significantly. No significantly negative growth effects were observed in the larvae when exposed to pCO_2 levels that are predicted as a result of ocean acidification in the next 100–200 years. Larvae reared under control conditions readily produced C-start escape behavior to mechano-sensory stimuli; however, in the moderate and high pCO_2 experimental groups, the probabilities of C-start were significantly lower than those of the control group. Therefore, the sensory integration needed for the C-start escape behavior appears to be vulnerable to ocean acidification. Altered behavior in marine larval fish, particularly behaviors involved in escape from predation, could have potentially negative implications to fish populations, and, further, to the marine ecosystems at the levels of CO_2 projected for the future.

1. Introduction

The mean atmospheric carbon dioxide (CO_2) levels have recently surpassed 400 ppm (www.esrl.noaa.gov/gmd/ccgg/trends/), and levels are expected to reach 1000 and 1900 ppm by the years 2100 and 2300, respectively (Caldeira and Wickett, 2003, 2005; IPCC, 2007). One third of CO_2 in the atmosphere is absorbed by the ocean, which is causing a decrease in pH, a process known as ocean acidification (Feely et al., 2004; Sabine et al., 2004; Orr et al., 2005). The increase in the dissolved CO_2 into the ocean results in a change in carbonate chemistry in the water, such as increased concentrations of H_2CO_3 (carbonic acid), HCO_3^- (bicarbonate ions), and H^+ (hydrogen ions), and decreased concentrations of CO_3^{2-} (carbonate ions) (Fabry et al., 2008). Meta-analysis of more than 400 studies revealed that, ocean acidification can cause a reduction in calcification, alter developmental processes, and affect growth in ways that could result in decreased abundance across a broad range of marine organisms (Kroeker et al., 2013). To date, the effects of ocean acidification on calcification have been investigated the most.

In general, fishes appear to be more tolerant to the effects of ocean acidification compared to marine invertebrate groups (Pörtner et al., 2004; Melzner et al., 2009). Adult fish actively adjust in vivo acid-base equilibrium through bicarbonate accumulation and ion exchange across the gills (Claiborne et al., 2002; Evans et al., 2005; Melzner et al., 2009). However, early life stages of fishes may

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be more vulnerable to ocean acidification than later stages because of their higher surface-to-volume ratio and lack of specialized mechanisms for pH regulation (Kikkawa et al., 2003; Ishimatsu et al., 2008). Negative effects of ocean acidification on embryonic development, larval growth, metabolism and survival have been reported in some species (Franke and Clemmesen, 2011; Baumann et al., 2012; Frommel et al., 2012; Bignami et al., 2013; Pimentel et al., 2014; Bromhead et al., 2015). However some studies have not detected any effect upon embryogenesis, hatching, growth and development, or swimming ability (Munday et al., 2011; Hurst et al., 2012, 2013; Harvey et al., 2013; Maneja et al., 2013, 2014).

Other recent studies have shown that projected CO_2 levels for the near future have a negative impact on sensory systems and alter behaviors of marine fishes (Clements and Hunt, 2015; Nagelkerken and Munday, 2016). Behavioral changes include increased activity and boldness (Munday et al., 2013; Jutfelt et al., 2013), loss of behavioral lateralization (Domenici et al., 2012; Jutfelt et al., 2013), impaired olfactory capacity (Munday et al., 2009a; Dixson et al., 2010; Ferrari et al., 2011), altered auditory responses (Simpson et al., 2011) and slowed retinal function (Chung et al., 2014). These behavioral alterations may have a serious impact on predator-prey interactions, population recruitment, and the overall balance of an ecosystem.

The abilities to detect and evade predators are critical to the survival of fish, especially to developing larval fish. During a predator-prey interaction, many fish employ a stereotyped escape response that consists of rapid-acceleration away from a threat, which is often described as a startle response or “C-start” (Domenici and Blake, 1997). C-start escape behavior in teleost fish is triggered by abrupt and unexpected stimuli. The short-latency escape behavior is under the command of a pair of Mauthner cells (also called the M cells) which are “decision-making” reticulospinal neurons (Eaton, 1991). Each of the paired M cells receive mechanosensory, visual, and other sensory input (Zottoli and Faber, 1979; Mirjany et al., 2011; Preuss et al., 2006). The large axon of M cell crosses the midline to synapse on motoneurons that innervate trunk muscle (Faber et al., 1989). Therefore, it allows one action potential in the M cell to initiate a fast escape response by generating a tail flip. In order to escape predatory aggression successfully, the nervous system of fish must detect and integrate multiple sensory cues to make appropriate and timely behavioral decisions. Ocean acidification significantly affected the predator-prey interactions of reef fish (Allan et al., 2013). For example, at the concentration of CO_2 projected to occur by 2100, the kinematics of the escape response of juvenile reef fish (*Amphiprion melanopus*) showed that the behaviors were adversely affected (Allan et al., 2014).

The marine medaka (*Oryzia melastigma*), also known as the Indian medaka, is becoming a promising model organism in laboratories for studies of ecotoxicology in the marine environment (Kong et al., 2008; Bo et al., 2011; Dong et al., 2014; Kim et al., 2016). Most ocean acidification studies have been conducted on tropical reef fish or on colder water species in the northern hemisphere (Frommel et al., 2013; Maneja et al., 2013; Hamilton et al., 2014) and in Antarctic region (Flynn et al., 2015), but not on coastal species like the medaka in the Indian Ocean region.

In this study, we first investigated the impact of predicted environmental CO_2 levels on the embryonic development of marine medaka, focusing on the development of the eyes and brains in newly hatched larvae. In view of previous work and the importance of escape behavior for larval fish, we also analyzed the C-start escape behavior parameters in the larvae under different pCO_2 levels (450 μatm, 1160 μatm and 1783 μatm). The moderate CO_2 levels (1160 μatm) and the high CO_2 levels (1783 μatm) represented open ocean projections for the years 2100 and 2300, respectively. However, many coastal habitats, like where marine medaka occur, experience substantial CO_2 fluctuation which can result in CO_2 levels greater than those projected to occur in open ocean environments by the end of the century (Dutta et al., 2013). This study provides a baseline understanding of how this species responds to acute ocean acidification exposure.

2. Material and methods

2.1. Study species and maintenance

In nature, the marine medaka (*O. melastigma*) is found in some areas of India and some riverine areas of Bangladesh (Dutta et al., 2013). There are a number of features that make the species a promising model marine fish for ecotoxicological research. These features include its small size, high fecundity, transparent embryos, distinct sexual dimorphism, and ease of culturing in the laboratory.

In 2011, we received ten pairs of adult marine medaka (*O. melastigma*) as a gift from State Key Laboratory of Marine Environmental Science (Xiamen University, Xiamen, China). Since then, a self-propagating and highly inbred population of marine medaka stock has been established in our lab. Fish in our laboratory were maintained in aerated 30‰ artificial seawater at 25 ± 2 °C in a 14 h light: 10 h dark cycle. The average CO_2 concentration was 450 ± 30 ppm as tested by a Thermo-Hygro-Ndir CO_2 Meter (TES-1370, TES Electrical Corp.). Artificial seawater was made with Red Sea salt (Red Sea Aquatics, HongKong). At a salinity of 31‰, the levels of pH and ALK (^{o}dKH) in the artificial seawater were 8.2–8.4 and 6.8–7.2, respectively. Fish were reared in holding tanks (60 cm × 45 cm × 40 cm) with circulating water and fed three times daily with brine shrimp.

Under optimal growth and breeding conditions, adult marine medaka can generate large numbers of genetically homogeneous offspring. The batch of adult marine medaka used in the present study was the tenth filial generation in our laboratory. Each female medaka spawned 20–40 eggs per day. Egg clusters remained attached to the belly of the females for about 2 h and then fell into the holding tanks. In this study, the fertilized eggs were collected from 100 breeding pairs cultured in three holding tanks. All the eggs collected from the three holding tanks were mixed together, rinsed with seawater, and the egg clusters were separated with a pair of scissors. After separation, the bad eggs were removed and the good ones were divided into each flask.

2.2. Experimental setup and water chemistry

Newly fertilized medaka eggs were reared in three 5-L flasks until hatching. Each flask contained seawater maintained with different levels of pCO_2: ambient (control) at 450 μatm, moderate at 1160 μatm, and high at 1783 μatm. A total of 350 eggs (collected within 6 h of being fertilized) were incubated in each flask, and three replicate flasks for each group were placed in a 100-L water bath at a temperature of 28 ± 1 °C. Because the eggs collected in one day were not enough for all the groups, the moderate pCO_2 and control group (control-1) experiments were conducted on April 22, and the high pCO_2 and control group (control-2) experiments were conducted on May 20, 2014.

Using the CO_2 enricher (CE-100-5, Ruihua Instrument and Equipment Co., Ltd., Wuhan, China), the seawater carbonate system in the water was maintained at a stable level by aerating with ambient air (450 ppm CO_2) and CO_2-enriched air (1200 or 1800 ppm CO_2) at a flow rate of 0.6 L/min through a fine-pore airstone. Rearing flasks were sealed across the top with a clear plastic film to limit CO_2 exchange with the atmosphere. Stable CO_2 levels in the sea water (variation < 5%) were achieved within 1 h,