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Irreversible behavioural impairment of fish starts early: Embryonic exposure to ocean acidification

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ABSTRACT

Long-term species responses to ocean acidification depend on their sensitivity during different life stages. We tested for sensitivity of juvenile fish behaviour to ocean acidification by exposing eggs to control and elevated CO_2 levels, and translocating offspring between treatments in a reciprocal design. After 12 weeks of exposure, activity, inactivity and anxiety levels of juveniles from control eggs were similar, whether juveniles had experienced elevated CO_2 conditions or not, and this pattern was consistent over time. However, juveniles raised as eggs under elevated CO_2 showed increased anxiety levels compared to those from control eggs. This response was not reversed when CO_2-exposed juveniles were translocated to control conditions. Our findings highlight the value of evaluating fish sensitivities to global change pollutants across different life stages, and indicate that sensitivity during the often-overlooked egg stage can be critical with long-lasting impairment of behaviours that are coupled to individual fitness and population persistence.

1. Introduction

Increasing atmospheric CO_2 levels due to human greenhouse gas emissions are projected to reach ~ 936 ppm by the year 2100 (Hoegh-Guldberg et al., 2014) and warm and acidify the world's oceans (Caldeira and Wickett, 2003; IPCC, 2013). Marine life is expected to be affected by these changing physico-chemical conditions in their environment (Lefort et al., 2014; Nagelkerken and Connell, 2015). Understanding how organisms respond across their alternate life stages is fundamental (Russell et al., 2012) as physiological, phenological, and behavioural alterations are often life-stage specific (Rijnsdorp et al., 2009; Hollowed et al., 2013; Bozinovic and Portner, 2015) and leave a legacy on older stages. Furthermore, differential sensitivity to environmental stressors across life stages can create bottlenecks for population growth and persistence (Munday et al., 2009b; Lucey et al., 2015; Marshall et al., 2016). As such, the capacity of each life stage to acclimate or adapt represents a critical component of how populations might respond to future climates (Munday et al., 2009a; Munday et al., 2012).

Whilst environmental change can alter the performance of marine organisms at distinct life stages, it is the early life stages that tend to be more sensitive to stressors than adults (Pineda et al., 2012; Marshall et al., 2016). The larvae and adults of a species not only differ in morphology and function, but also in the habitat they occupy and their habitat-specific environmental conditions (Marshall et al., 2016). The large surface to volume ratio of small larvae not only increase their exposure to environmental stressors (Baumann et al., 2012; Marshall et al., 2016), but also their less developed anatomy hampers their capacity to buffer these stressors (Marshall et al., 2016). Marine invertebrates are often tolerant to ocean warming during their gamete phase and during fertilization, while their embryos tend to exhibit high rates of mortality (Byrne, 2011). Likewise, for some fish species their eggs and larvae have narrower thermal windows than adults (Pörtner and Farrell, 2008; Rijnsdorp et al., 2009).

Early stages of marine organisms are disproportionately sensitive to enriched CO_2 because their acid-base mechanisms have not yet developed fully (Ishimatsu et al., 2005, Murray et al., 2014, Przeslawski et al., 2015, Munday et al., 2016). Most studies on early life stages, however, have focussed on calcifying organisms due to the perceived fragility of their skeleton during early development (Byrne, 2011; Kroeker et al., 2013). By contrast, fish have been considered to be more tolerant to ocean acidification because of their physiological capacity for acid-base regulation (Munday et al., 2016). Yet recent work suggests that fish are vulnerable during their embryonic and larval stages (Wittmann and Pörtner, 2013) and that there is potential for their harmful effects to carry over onto older life stages, many of which mediate population persistence. In fish, only a few studies have evaluated their potential to acclimate over longer-term periods and they are

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mainly based on tropical species (Welch et al., 2014).

In this study, we evaluated how ocean acidification can affect the behaviour of a temperate fish when exposed at two different life stages – embryonic and juvenile – and whether they show any degree of acclimation with increasing length of exposure (4, 8 and 12 weeks). Fertilized eggs of a mouth-brooding fish, *Vincentia badia*, were exposed to near-future levels of elevated CO_2. Because their larvae undergo direct development (personal observation from the field and laboratory), juvenile hatchlings may be more resistant to stressful conditions as their physiological machinery is more developed relative to those broadcast as spawned eggs and pelagic larvae (Lucey et al., 2015). Insight into the potential influence of ocean acidification on early developmental stages, particularly the impairment of essential behavioural traits (e.g. such as activity and anxiety levels) provides clues about future recruitment and population persistence.

2. Materials and methods

2.1. Study site and fish collection

The benthic scarlet cardinalfish, *Vincentia badia*, inhabit shallow subtidal seagrasses and nearshore reefs of Western and Southern Australia (Baker et al., 2010). We used a seine net to collect fish from November 2016 to January 2017 at Port Vincent (34°46′30.7″ S, 137°51′36.7″ E). Six adult scarlet cardinalfish with fertilized eggs in their mouth were placed at ambient or elevated CO_2 levels in 40 l nally bins with two pieces of PVC pipe per fish that acted as shelter. Adult fish were kept in two tanks under ocean acidification (OA) conditions: tank 1 housed two parents, and tank 2 one parent. The three parents with eggs inside the two tanks were exposed to elevated CO_2 conditions for 13 and 26 days, respectively. Exposure time of parents with eggs was determined by the time from capture until the egg hatched. For the control treatment one tank housed two parents that were kept under ambient conditions for 7–13 days. Additionally, there was a second control group were one parent spat out the eggs/hatchlings when it was captured, but the juveniles could still be used for the experiment. Upon hatching, juvenile fish from the ambient and elevated CO_2 treatments were transplanted reciprocally to an ambient (Control) or elevated CO_2 (OA) treatment using 20 l nally bins. This configuration resulted in four treatments that incorporate an embryonic phase followed by the juvenile phase: Control → Control (n = 5, 5, and 4, for week 4, 8, and 12, respectively), Control → OA (n = 4, 4, 2), OA → Control (n = 5, 5, 2), and OA → OA (n = 5, 5, 3). Cardinalfish offspring were fed with Artemia (*Artemia salina*) twice a day ad libitum during the 12 week period of the experiment.

2.2. Water chemistry

The 20 l tanks that housed the fish were placed inside temperature-controlled water baths of 300 l. Water temperature was kept at an average of 18.2 °C (approximate seawater temperature at the time of fish collection) using submersible titanium heaters with an automated temperature controller (Weipro 500 W). Each tank was provided with two air stones, one supplying ambient air and the other supplying either ambient air or a mix of air and CO_2 (average pH: 7.9; pCO_2: 1068 μ atm) using a Pegas 4000 MF gas mixer. Temperature and pH were measured every day using a 913 Metrohm pH meter and salinity was measured using a StarterPen conductivity meter (IC-ST10C-C). Total alkalinity values were estimated by Gran titration from 40 ml water samples at before the beginning of behavioural experiments, and after one month samples were taken three more times weekly. Samples were processed on the same day of collection. Mean pCO_2 water values were calculated using CO2SYS (Pierrot et al., 2006) for Excel with constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987) (see Table 1 for a summary of water parameters).

2.3. Behavioural experiments

The effects of ocean acidification on activity levels were assessed by quantifying the behaviour of juveniles after four, eight and twelve weeks. Each fish was removed from its tank and placed individually at the end of a rectangular 20 l bin, with the same water chemistry conditions as their treatment. Due to the small number of juvenile fish the same individuals were used at weeks 4, 8 and 12. A weighted mesh was positioned in front of the fish to prevent the fish from swimming to a different position of the bin, maintaining the same start position for each fish (with an area of 30 cm long × 10 cm wide). After an acclimation time of 3 min. (Huijbers et al., 2012; Jutfelt et al., 2013), a PVC pipe (4 cm diameter × 9 cm long) was provided as shelter and the mesh removed. To avoid observer's bias and effects of observer presence on fish behaviour, juvenile fish behaviour was remotely recorded for 3 min. From the top of the bin, using either a Canon Legria HF-R406 or a Canon Legria HFM52 camera attached to a metal frame. Three behaviours were considered for this study. 1) swimming: defined as the forward movement of the juvenile fish through the water column as realised by caudal fin action (Vollset et al., 2011). 2) floating: defined as the lack of movement by the fish or movements no greater than the fish body length. 3) hiding: fish entering the PVC pipe or positioning itself within the shadow of the pipe. Recordings were recorded using VLC media player 2.1.3. Swimming, floating and hiding behaviours were quantified in each video as the proportion of time they spent performing each activity. Experiments were performed under The University of Adelaide Animal Ethics Committee approval # S-2016-165.

2.4. Statistical analyses

Generalized linear mixed models were used to compare the proportion of time the juveniles spent swimming, hiding, and floating among embryonic treatment, juvenile treatment, and time (fixed effects). One model was performed for each behaviour. Embryonic acclimation time in their respective treatment (control or elevated CO_2) was included in the models as a random effect. Assumptions were tested with fitted residual and normality plots. The response variables were treated with a beta distribution, and the models were fitted with a log-it link function. Likelihood ratio tests were used to evaluate differences among treatments.

3. Results

Juveniles raised under ambient CO_2 as eggs and transferred to enriched CO_2 at hatching did not differ in their swimming activity, inactivity (floating) or hiding behaviour compared to juveniles that were raised both as eggs and hatchlings under control conditions (Table 2, Fig. 1a, b, c). Similarly, behaviours of juveniles exposed as embryos to enriched CO_2 did not differ when they were raised after hatching in control vs. elevated CO_2 conditions (Table 2). Activity and inactivity levels of juveniles which experienced embryonic CO_2 enrichment were similar to those that experienced control embryonic conditions (Table 2, Fig. 1b,c). However, the percentage of time that fish spent hiding was higher for all juveniles that had experienced elevated CO_2 embryonic exposure compared to ambient CO_2 embryonic exposure (Table 2, Fig. 1a).

Returning juveniles that had experienced CO_2 enrichment during the embryonic stage to control conditions did not reverse the opposing effects of elevated CO_2 on anxiety levels (Table 2, Fig. 1a). The observed responses for all four embryonic/juvenile treatments were maintained during the 12 week exposure (Fig. 1a, b, c), and showed no significant effect of time (Table 2).

Different embryonic acclimation times to treatments had no effect on the variability of fish responses, as random effect variation was close to 0 for all the models (Sup. Table 1).