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Effects of exposure duration on the response of *Pocillopora damicornis* larvae to elevated temperature and high pCO_2

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ABSTRACT

Efforts to evaluate the response of coral larvae to global climate change (GCC) and ocean acidification (OA) typically employ short experiments of fixed length, yet it is unknown how the response is affected by exposure duration. In this study, we exposed larvae from the brooding coral *Pocillopora damicornis* to contrasts of temperature (24.00 °C [ambient] versus 30.49 °C) and pCO_2 (49.4 Pa versus 86.2 Pa) for varying periods (1–5 days) to test the hypothesis that exposure duration had no effect on larval response as assessed by protein content, respiration, *Symbiodinium* density, and survivorship; exposure times were ecologically relevant compared to representative pelagic larval durations (PLD) for corals. Larvae differed among days for all response variables, and the effects of the treatment were relatively consistent regardless of exposure duration for three of the four response variables. Protein content and *Symbiodinium* density were unaffected by temperature and pCO_2, but respiration increased with temperature (but not pCO_2) with the effect intensifying as incubations lengthened. Survival, however, differed significantly among treatments at the end of the study, and by the 5th day, 78% of the larvae were alive and swimming under ambient temperature and ambient pCO_2, but only 55–59% were alive in the other treatments. These results demonstrate that the physiological effects of temperature and pCO_2 on coral larvae can reliably be detected within days, but effects on survival require ≥5 days to detect. The detection of time-dependent effects on larval survivorship suggests that the influence of GCC and OA will be stronger for corals having long PLDs.

1. Introduction

Pelagic larvae of marine invertebrates have been studied for nearly two centuries (Young, 1990), but the modern era of quantitative analyses arguably began with Thorson (1950). Larval biology has since played a central role in ecological investigations of the community structure of marine ecosystems (Grosberg and Levitan, 1992; Morgan, 2001), and spurred the creation of a unique field on investigation, supply-side ecology (Lewin, 1987; Underwood and Keough, 2001). Paralleling the rise in importance of studying the ecology of larvae has been interest in their functional biology, an emphasis that has included investigations ranging from analyses of larval structure (Young et al., 2002), development (Strathmann, 1985), and biomechanics (Chia et al., 1984), to studies of their metabolism (Hoegh-Guldberg and Emlet, 1997) and the genetic control of development (Raff, 2008).

While the discoveries from invertebrate larvae provide incentive to continue with this work, the impact of anthropogenic disturbances in the marine realm (Hoegh-Guldberg and Bruno, 2010) has heightened interest in this topic (Byrne, 2011). It is often inferred that larvae are more vulnerable to environmental conditions than adults (Byrne, 2011), and therefore are more strongly affected by degrading conditions. However, data to this effect are equivocal (Pechenik, 1999) and, indeed, adult provisioning of larval stages with defensive mechanisms can enhance resistance to environmental assaults (Hamdoun and Epel, 2006). Nonetheless, changes in marine ecosystems due to global climate change (GCC) and ocean acidification (OA) (Hoegh-Guldberg and Bruno, 2010) have brought new interest in the biology of larvae and the roles they play in the trajectories of change in population size (Albright, 2011; Byrne, 2011; Ross et al., 2011). Investigations of larvae in the context of GCC and OA are still in their infancy, but momentum in this direction has built quickly for tropical reef corals (Albright, 2011), driven by the desire to understand more about the consequences of the losses of coral that have taken place in the last 30 years (Gardner et al., 2003; Wilkinson, 2008), and the implications of warmer and less basic oceans (Hoegh-Guldberg et al., 2007; Veron et al., 2009). There are now about 20 studies of coral larvae that evaluate their response to physical and chemical conditions that are pertinent to understanding the impacts of GCC and OA (Table 1; Edmunds et al., 2011; Albright, 2011). One common theme of these investigations is the use of fixed exposure times varying in length from a few hours to several weeks, after which (and, sometimes, during) the response of the larvae to the treatments is

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Table 1
Summary of studies investigating the effects of temperature and CO_2 on coral larvae to show species studied, duration of incubations, treatment condition, dependent variables (DV) measured, and source. Duration: hours (h) or days (d); treatments: T, temperature (°C), pCO_2 (Pa, * = ambient pCO_2), Light [intensity: μmol quanta m^{-2} s^{-1}, † = natural light regime). nd = no data.

Species	Duration	Treatments	DV	Source
Porites astreoides	2 h	T = 26; pCO_2 = ~38.5, ~56.7, ~81.1; Light = 0	Respiration	Albright and Langdon, 2011
Acropora palmata	1–6 d	T = 28; pCO_2 = 49.8, 66.3, 88.8; Light = n/d	Settlement	Albright et al., 2010
Porites astreoides	7 d	T = 25.4–26.6; pCO_2 = 51.6, 58.5, 69.7; Light = 10	Settlement	Albright et al., 2008
Porites panamensis	10 d	T = 28.4–29.6; pCO_2 = 49.3–96.3; Light = 135	Settlement	Anlauf et al., 2011
Acropora digitifera	2 h–7 d	T = 26.2–26.6; pCO_2 = 33.5, 118.8, 295.0; Light = †	Settlement, survival, respiration	Nakamura et al., 2011
Acropora digitifera, *Acropora tenuis*	7 d	T = 26.8; pCO_2 = 40.5–363.3; Light = †	Survival	Suwa et al., 2010
Stylophora pistillata	12 h	T = 22.6, 25.4, 28.6; pCO_2 = *; Light = 139–165	Photophysiology, settlement	Putnam et al., 2008
P. damicornis	12 h	T = 24.0, 28; pCO_2 = *; Light = 581	Photophysiology	Putnam et al., 2010
Porites astreoides	1 d	T = 26, 28, 33; pCO_2 = *; Light < 545	Mortality, metamorphosis, respiration, photosynthesis	Edmunds et al., 2001
Pocillopora damicornis *Seriatopora hystrix* *Stylophora pistillata*	<2 h	T = 26.4–28.9; pCO_2 = *; Light = 0	Respiration	Edmunds et al., 2011
Acropora millepora	<10 h	T = 24, 28, 31; pCO_2 = *; Light = nd	Respiration, gene expression	Rodriguez-Lanetty et al., 2009
Pocillopora damicornis	17–19 d	T = 28.0; pCO_2 = *; Light = †	Respiration, photosynthesis structure	Gaither and Rowan, 2010

evaluated. Ideally, incubation times might be best matched to ecologically relevant pelagic larval durations (PLD) for the taxon under investigation, thereby realistically gauging the impacts of physical conditions over a period likely to culminate in settlement and metamorphosis.

For tropical scleractinians, PLD varies from a few minutes (Carlon and Olson, 1993), to hundreds of days (Graham et al., 2008) or even > 1 year (under laboratory conditions; Vermeij et al., 2009), and differ in minimum duration between brooding corals (which release larvae that can be immediately competent to settle) and broadcasting corals (which require several days in the plankton to develop to competency) (Harrison and Wallace, 1990). Nevertheless, modal PLDs for corals are likely to be ≤6 days (Gilmour et al., 2009; Harrison and Wallace, 1990; Miller and Mundy, 2003), and therefore it is relevant to ask whether similar durations of exposure to high temperature and elevated pCO_2 affect larval success. The goal of this study was to compare the effects of 1–5 day exposure on the response of brooded coral larvae to elevated temperature and high pCO_2. Ultimately we wished to improve the capacity to interpret research on the effects of GCC and OA on coral larvae – notably in studies (including our own) in which short (<24 h) exposures have been employed – and provide a temporal context for the interpretation of the impacts of physical conditions on coral larvae that might be used to improve future experiments.

We extended our use of brooded larvae from *Pocillopora damicornis* (Linnaeus, 1758) (Cumbo et al. 2012; Edmunds et al., 2011; Putnam et al., 2010) to conduct a 5 day experiment in which larvae were exposed to combinations of temperature and pCO_2 and sampled daily for protein biomass, respiration, *Symbiodinium* density, and survivorship. While larvae of *P. damicornis* can remain pelagic > 100 days, they can also settle within a few hours of release (Richmond, 1987) and, in general, probably conform to the aforementioned pattern for scleractinians of settling within days of release. Thus, we rationalized incubations lasting 5 days as ecologically relevant to evaluating the sensitivity of coral larvae to GCC and OA, and of use in interpreting existing studies of similar effects using incubations of varying (and usually short) duration (Table 1).

2. Methods

2.1. Experiment design, seawater chemistry, and larval collection

To test for the effects of exposure duration (hereafter "day"), temperature, and pCO_2 on *P. damicornis* larvae, an experiment was designed to test the null hypothesis that these factors have no singular or interactive effects on larval physiology and survivorship. The experiment crossed two temperatures with two pCO_2 regimes, and employed duplicate tanks nested within each treatment combination. Larvae were incubated in each tank, and sub-sampled daily, and the null hypothesis tested using ANOVA for the continuously distributed variables and the Kaplan–Meier method (Machin et al., 2006) for survivorship.

To obtain larvae, 8 colonies of *P. damicornis* ~20-cm diameter were collected from ~5 to 7 m depth on Hobihu Reef, Nanwan Bay (Taiwan, 25° 56.169′ N, 120° 44.824′ E) on 2nd March 2011, which was 3 days before the new moon and 13 days before the anticipated peak larval release for this spawning period (Fan et al., 2002). Colonies were transported to the National Museum of Marine Biology and Aquarium (NMMBA, Pingtun, Taiwan) and placed into individual aquaria supplied with flowing, sand-filtered seawater (50 μm) that carried larvae into cups fitted with plankton mesh bottoms. These aquaria were exposed to natural light augmented with 150 W metal halide lamps to create an irradiance of ~104 ± 2 μmol quanta m^{-2} s^{-1} ($n = 84$, measured as photosynthetically active radiation [PAR, 400–700 nm] with a Li Cor LI 1400 meter and LI-192 sensor). The cups were inspected daily at ~08:00 h for larvae, which are released before dawn in this species (Fan et al., 2006). We timed the experiment to utilize larvae released on or close to the peak of larval release, because our previous work demonstrated that the largest portion of each larval cohort is released at this time (Cumbo et al., 2012). Timing of peak larval release was predicted using records of spawning events (Fan et al., 2002) in conjunction with daily counts of larvae released during lunar March 2011. On the predicted day of peak release (14th March 2011), larvae from the 8 colonies were collected, pooled, stirred gently, and allocated haphazardly to the treatment tanks. As parental colonies release varying numbers of larvae, pooling among colonies ensured a haphazard selection of larvae relative to parents, but an unequal representation of maternal genotypes.

Treatments were created in eight 150 L tanks, each filled with 120 L of filtered (1 μm) seawater that was changed partially (20%) every day (at ~17:00 h). Tanks were individually heated (300 W heaters, Taikong Corporation) and chilled (Aquatech Ac11 or Shyeh Duwai Enterprise), with the temperature regulated using programmable, digital controllers (±0.1 °C, AquaControllers, Neptune Systems). Illumination was provided by metal halide (Phillips 150 W 10,000 k) and fluorescent (39 W, Phillips T5 460 nm) bulbs to create a mean light intensity of 268 ± 17 μmol quanta m^{-2} s^{-1} (±SE, $n = 64$). The light intensity was selected to approximate that found at the collection depth of the parent colonies in March, and the extent to which it is ecologically relevant depends on the amount of time *P. damicornis* larvae spend on the surface of the seawater at noon versus lower in the water column, or close to the benthos and engaged in substratum selection behavior. Unfortunately these details are not currently available.

Treatments were created by blending CO_2 with air, and continually assessing the mixture through an Infra Red gas analyzer (S151, Qubit Systems), which dynamically adjusted the flow of CO_2 to maintain desired levels. The gas mixture was supplied through an air stone