The biology of larvae from the reef coral *Porites astreoides*, and their response to temperature disturbances

**Abstract** Pelagic larvae play a fundamental role in the life history of virtually all scleractinian corals, yet much of their biology remains unexplored. One aspect of coral larvae – their response to temperature perturbations – has potentially important consequences for understanding the effects of global warming and El Niño–Southern Oscillation events on coral recruitment. The present study tests the effects of temperature perturbations on coral larvae using *Porites astreoides* larvae as a model system. In June 1999, larvae were collected from 18 m depth on Conch Reef, Florida, and incubated at ambient (28°C), depressed (26°C), and elevated (33°C) temperatures in outdoor tanks shaded from direct sunlight. Treatments were repeated with new larvae every 24 h, and treatment effects quantified as larval motility, mortality, metamorphosis and metabolism. Elevated temperature significantly increased mortality and metamorphosis, and a similar trend was observed at the reduced temperature, although this was not significant. Neither elevated nor reduced temperatures affected larval motility. Gross photosynthesis (P) was significantly depressed by elevated and reduced temperatures, and respiration (R) varied proportionately with temperature (Q10≈2), although this effect was not statistically significant. At the highest temperature the P/R ratio declined to < 1, indicating that thermal stress reduces the potential for autotrophy. Together, these results suggest that elevated temperatures affect coral larvae by depressing photosynthesis and creating an energy shortage, which ultimately could reduce recruitment (by increasing mortality), shorten larval longevity and favor premature metamorphosis. An unexpected finding was that larvae differed physiologically among release dates. Although preliminary, this suggests that larval fitness in *Porites* spp. may vary depending on the day of release, a phenomenon that could have significant ramifications with respect to the population structure of adults.

**Introduction**

Most benthic marine organisms have a bentho-pelagic life cycle that includes a free-living larval stage (Pechenik 1999). Pelagic larvae play a critical role in dispersal and habitat selection (Thorson 1950; Pechenik 1999), but must settle and metamorphose in order to have the potential to contribute to adult demographics (Caley et al. 1996). Elucidating the relationship between larval supply and adult population structure has become a central goal of marine ecology (Underwood and Fairweather 1989; Caley et al. 1996; Hughes et al. 2000), yet this endeavor has proved complex and intractable. A long-standing explanation for this complexity has been the open nature of most marine systems (Thorson 1950; Caley et al. 1996), where larvae can originate from many sources and settle in a variety of destinations (Roberts 1997). The “openness” of marine systems has recently been challenged by evidence of larval retention near source populations (Cowan et al. 2000), a finding that is forcing a reappraisal of the factors contributing to an unpredictable relationship between larvae and adults. Although this relationship is thought to be strongly affected by pre-settlement events in the plankton (e.g. larval mortality and substratum selection) (Underwood and Fairweather 1989), relatively little is known about the processes governing larval success (Grosberg and Levitan 1992; Morgan 1995). Thus, it remains a formi-
dable task to predict how, or to what extent, environmental perturbations might affect larval survival and generate demographic effects.

Coral reefs provide a good example of an ecosystem where a better understanding of the larval biology of the dominant taxon, the Scleractinia, would be beneficial. Such information would, for instance, provide new insight into the processes creating diverse tropical communities (Connell 1978), as well as the potential for recovery from the recent world-wide declines in coral cover (Wilkinson 1999). Planula larvae are the product of sexual reproduction in scleractinians (but see Stoddart 1983) and, although asexual proliferation is important (e.g. fragmentation; Highsmith 1982), only planulæ have the potential to recruit to denuded areas and maintain genetic diversity. Coral planulæ have been studied for more than a century (de Lacaze-Duthiers 1873; Duerdon 1902), and their basic biology is relatively well known (reviewed in Harrison and Wallace 1990). However, given that coral larvae can be adversely affected by changes in salinity, temperature, light availability and pollution (Edmondson 1946; Coles 1985; Gleason and Wellington 1995; Richmond 1997), it is surprising how few quantitative analyses have addressed effects of environmental perturbations. Tropical corals live close to their upper thermal limit (e.g. Glynn 1993), and it is likely that elevated temperatures have negative effects on the physiology of their larvae (Coles 1985; Richmond 1997), as suggested by the depression of coral settlement by high temperatures (Jokiel and Guinther 1978). If temperature affects pre-settlement events such as larval mortality, longevity and substrate selection, then the predicted increases in temperature over the coming decades (Hoegh-Guldberg 1999) may have insidious and disproportionately large impacts on coral populations.

This study focuses on recently released planula larvae (hereafter referred to as larvae), and their response to fluctuations around the mean ambient seawater temperature for June (in the upper Florida Keys). The common Caribbean coral *Porites astreoides* was selected for this study because it broods its larvae and releases them on a predictable schedule making them relatively easy to collect. Our experiments were designed to characterize *P. astreoides* larvae and to test the effects of temperature on: (1) mortality, motility (i.e. swimming speed) and metamorphosis, and (2) selected physiological traits. Ultimately we wanted to gain insights into the proximal effects of temperature on coral larvae and the likely consequences for settlement. Our results identify a strong effect of temperature on the larvae of *P. astreoides*, and reveal unexpected differences among larvae released on different days.

**Materials and methods**

**Larval collection and experimental design**

Larvae were collected in June 1999 using demersal plankton nets that were placed over colonies of *Porites astreoides* at 18 m depth on Conch Reef, Florida (24°56.819′N; 080°27.292′W). Traps were made from a plastic collar (∼20 cm diam. Vexar), a nylon mesh tube, and a cod-end made from two 50 ml plastic centrifuge tubes (for details see Brazeau et al. 1998). Small (≤15 cm diam., n = 52) colonies of the brown morph of *P. astreoides* were chosen haphazardly and enclosed with a trap between 10 and 21 June. This period spanned the new moon (13 June) when *P. astreoides* releases planulae (McGuire 1998). Nests were checked daily (at ∼1000 hours) for planulae, which, when found, were collected and returned to the laboratory on Key Largo. Larvae were transported in plastic tubes fitted with 250 μm mesh windows, and were kept in darkened plastic barrels containing 120 l seawater during transit. On shore the larvae were pooled among colonies and allocated to experimental treatments (described below). Larvae collected the morning following release are described as “recently released”. As *P. astreoides* generally releases larvae after dark (Gleason, unpublished data), recently released larvae had been in the water column for ∼15 h when collected at ∼1000 hours, assuming it was dark by 1900 hours the night before. The true age of the larvae could not be determined, as the date of fertilization was unknown.

Temperature experiments were completed using 451 tanks (n = 6), each with independent water circulation, that served as statistical replicates in all analyses. The tanks were located outdoors and exposed to sunlight screened to levels similar to those found at 18 m depth (Lesser 2000); between noon and 1400 hours the mean irradiance was 545 ± 42 μmol photons m⁻² s⁻¹ (± SE, n = 16). Each tank was fitted with a water pump, heater and filter, and four were equipped with chillers (1.6 HP, West Coast Aquatics). The heaters and chillers were used to maintain two tanks at ambient seawater temperature (∼28°C), two at a reduced temperature (∼26°C) and two at an elevated temperature (∼33°C, using heaters alone). The ambient and reduced temperatures are ecologically relevant for the Florida Keys (McGuire 1998), but the elevated temperature is about 2°C above the highest temperature recorded in this location (McGuire 1998). The reduced temperature was depressed relative to the ambient seawater temperature when the experiments were completed, but it is much higher than the cool end for this region (25°C, McGuire 1998). The tanks were filled with seawater collected 10 km offshore, and 50% water changes were completed daily using freshly collected seawater that was equilibrated to treatment temperatures. Salinity was assessed periodically using specific gravity, and temperature was recorded every 10 min with data loggers (Onset Optic Stowaways).

The effects of temperature on the larvae were investigated using 24-h incubations; larvae were returned to the laboratory at ∼1400 hours and placed into treatment tanks between 1500 and 1600 hours. Incubations were carried out in 50-ml plastic tubes fitted with 250 μm mesh windows. Ten or fifteen larvae (depending on availability) were placed into each tube, and one tube was added to each of the six tanks. After 24 h, larvae within the tubes were inspected to quantify mortality, motility and metamorphosis. Five motile larvae were selected haphazardly for the determination of respiration and photosynthesis (i.e. metabolism) and, afterwards, were frozen to quantify protein and zooxanthellae content. To characterize recently released larvae, additional samples were processed for metabolism and tissue composition immediately after returning to the laboratory.

**Protein and zooxanthellae content**

Protein and zooxanthellae content were determined using larvae frozen in batches of five as described above. Each sample was thawed and sonicated (Ultrasonic Processor GEX600) to provide a slurry of ruptured animal cells and intact zooxanthellae. Microscopic inspection revealed that zooxanthellae were not damaged by this procedure. The volume of the slurry was measured, 50 μl was removed for protein analysis, and the remainder was used to count zooxanthellae. The protein content was determined with the Coomassie Brilliant Blue Assay (Bradford 1976) after solubilizing the protein under alkaline conditions (0.01 M NaOH with heating at 50°C for 5 h) and neutralizing (pH 7.0). Assays were completed in...