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NOTE

Oxygen consumption of a single embryo/planula in the reef-building coral *Acropora intermedia*

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ABSTRACT: O_2 consumption of a single embryo/planula at each developmental stage was monitored in the reef-building coral *Acropora intermedia* using an optical O_2-sensing system with our original micro-chamber system (6.28 µl). The lowest rate of O_2 consumption was observed in unfertilized eggs. After fertilization, O_2 consumption increased and remained constant until the prawn chip blastula stage. However, O_2 consumption began to increase again during the bowl-shaped blastula stage, which involves the formation of 2 germ layers and corresponds to the beginning of gastrulation. The rate of O_2 consumption peaked during the teardrop-shaped planula stage. During this stage planulae are able to swim actively, especially in the vertical plane, so an increase in energy consumption during this stage is to be expected. O_2 consumption began to decrease gradually 5 d after spawning. At this stage, the larvae frequently touched the substrate with their concave aboral end, which features numerous spirocysts required for substrate attachment. When the planulae began to settle, 7 d after spawning, the rate of O_2 consumption dropped to that of unfertilized eggs, suggesting that the planulae slowly use stored energy for crawling/settlement behavior and/or post-settlement growth and survivorship.

KEY WORDS: Development · Dispersal · Energy · Larva · Lecithotrophic · Metabolism · Recruitment · Settlement-competency period

INTRODUCTION

In marine invertebrates, larval dispersal and recruitment affect the distribution and abundance of adult populations (Underwood & Keough 2001). The dispersal potential of planktonic larvae is physically dependent upon ocean currents and biologically dependent upon buoyancy in the water column (Morgan 1995), larval behavior (Harii & Kayanne 2003), survivorship (Isomura & Nishihira 2001), and the length of the settlement-competency period (Nozawa & Harrison 2005). In environments in which food is scarce, these biological factors are affected by the egg's energy content, in the form of lipids, proteins, and carbohydrates (Holland 1978, Chia et al. 1984), and by the metabolic rate at which these reserves are consumed during development (Richmond 1987, Bryan 2004). In coral, temporal changes in the lipid content have been reported in larvae of spawning species (Harii et al. 2007); however, little information is available regarding energy consumption (Richmond 1987, Ben-David-Zaslow & Benayahu 2000). The metabolic rate at each

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developmental stage is an important index for understanding larval dispersal and recruitment; such data indicate when the larvae use energy and how they allocate the maternally derived energy for dispersal potential, larval fitness, and post-settlement growth and survival (Richmond 1987, Hoegh-Guldberg & Manahan 1995).

A number of studies have examined the energetics of the development of planktotrophic and lecithotrophic species of sea urchin and abalone (Crisp et al. 1985, Marsh et al. 1999, Bryan 2004). However, the most common method of measuring O_2 consumption (i.e. an electrode) is unsuitable for small chamber volumes because the sensor itself consumes dissolved O_2 to measure the density of O_2 (Clark 1956). In addition, because numerous embryos or planulae compete with each other to consume excess energy in the chamber, the O_2 consumption rate per embryo or planula derived from this method is not reliable for analyzing the transitions between developmental stages. Here, we present data for O_2 consumption of a single coral embryo/planula at each developmental stage using an optical O_2-sensing system. This system has several advantages: no O_2 consumption, no membrane or electrolytes required (i.e. easy maintenance), high sensitivity and stability, and an adequate chamber volume (Nakaya et al. 2003, 2005). No previous data are available for O_2 consumption in coral embryos or planulae using the technique described here.

MATERIALS AND METHODS

We collected 9 fragments of *Acropora intermedia* (Wallace & Dai 1997) from 3 large colonies at a depth of 2 to 4 m from the back-reef in Bise, Okinawa (26° 3′ 52″ N, 127° 5′ 30″ E). All specimens were cultured together in a large tank (1.5 m high and 20 m^2 in area). To prevent stress from the orientation of attachment, each fragment was secured vertically in a PVC pipe attached to a plastic plate (Okubo et al. 2005). All branches spawned on 16 and 17 June 2007; the gametes that were spawned on 17 June were used in the experiment. To collect sperm and egg bundles from a single colony, when the bundles appeared, the fragments were removed from the large tank and placed in separate tanks. After spawning, bundles from a single colony were placed in Petri dishes and broken by pipetting to collect unfertilized eggs. For fertilization, sperm and egg bundles collected from all colonies were mixed together in 20 l holding containers.

To reliably measure the metabolic rate at each developmental stage, each embryo or planula was removed from the 20 l holding container and washed in Millipore-filtered seawater (MFSW, 0.2-µm pore diameter) in a Petri dish. The embryo was then placed in our original acrylic micro-chamber (cylindrical hole: 1 mm radius × 2 mm high) filled with MFSW (6.28 µl). This system was optimized to measure the O_2 consumption rate of a single embryo or planula of *Acropora intermedia*. The chamber was placed in a water bath (Personal-11, Taitec) maintained at 27 ± 0.08°C (mean ± SD). The metabolic rate of a single embryo or planula was measured as the rate of O_2 consumption using the optical O_2-sensing system (Fibox3, PreSens). The sensor was calibrated against a zero solution (0.01 M sodium sulfate) and air-saturated MFSW (Nakaya et al. 2003) before the analysis of the various developmental stages. Data were collected using a personal computer and converted into absolute values. The rate of change (V_{O2}) in the dissolved O_2 concentration was calculated using simple linear regression ($r^2 > 0.9$). We measured V_{O2} over the course of 6.5 to 7 min and created the linear regression using data from the third to sixth min (3 min total), during which oxygen consumption was stable. The chamber was wrapped in aluminum foil during measurement. Control values were measured for 10 min using a chamber filled with MFSW alone. Each value for O_2 consumption is expressed as the mean ± SD × 10^{-5} ml O_2 egg/embryo/planula^{-1} h^{-1} at 27°C. After measurement, the embryo was returned to the 20 l holding container.

Differences in O_2 consumption across developmental stages were analyzed using the Kruskal-Wallis test with a correction for ties. Where significant differences were detected, Dunn's multiple comparison was used to determine which group(s) differed.

RESULTS AND DISCUSSION

As a general observation and a useful baseline, we noted that embryogenesis in *Acropora intermedia* was similar to that in other *Acropora* spp. (Fig. 1) (Hayashibara et al. 1997, Okubo & Motokawa 2007). The lowest rate of O_2 consumption was measured in unfertilized eggs at 0.97 ± 0.26 × 10^{-5} ml O_2 egg^{-1} h^{-1} at 27°C (Figs. 1A & 2). After fertilization, O_2 consumption in the fertilized eggs increased to 1.48 ± 0.17 × 10^{-5} ml O_2 $embryo^{-1}$ h^{-1} at 27°C. Moreno & Hoegh-Guldberg (1999) also reported that respiration in 3 congeneric seastars (*Patiriella,* Verrill) increases soon after fertilization. After the first cleavage and the formation of a heart-shaped zygote at 2 h (Figs. 1B & 2), O_2 consumption remained at the same level as in fertilized eggs until the prawn chip blastula stage (Dunn's multiple comparison, $p > 0.05$, Figs. 1B–I & 2). However, O_2 consumption began to increase in the bowl-shaped blastula (Figs. 1J,K & 2), which requires the formation of 2

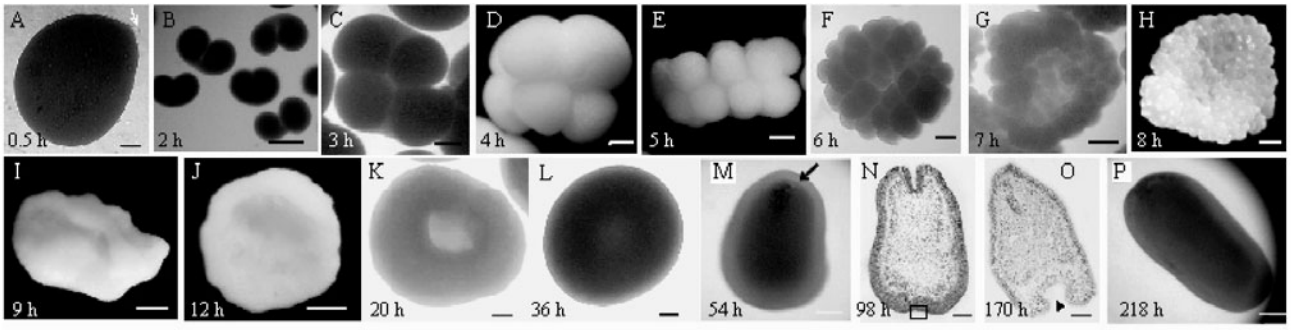

Fig. 1. *Acropora* spp. Embryogenesis (data from Okubo & Motokawa 2007). (A) External view of the egg and polar body (white arrow). Fertilization may not have occurred. (B) 2 cell stage. (C) 4 cell stage. (D) 8 cell stage. (E) Side view of the 16 cell stage. (F) 32 cell stage. (G) Morula stage. (H) Prawn chip blastula. (I) Smoothing of the cell surface. (J) Gastrulation begins as the blastula rolls inward. (K) Gradual thickening of the blastula. (L) Blastocoel is translucent and the blastopore is not externally visible. (M) Mouth forms at the animal pole during the planula stage. (N) Invagination of the outer germ layer and formation of the aboral concavity. Square indicates the aboral region with numerous spirocysts at this time. (O) Aboral concavity (arrowhead) becomes more conspicuous. (P) Elongated planula. Numbers indicate hours post-spawning. Scale bars = 100 µm (A, C–H, J–P), 50 µm (B, I)

germ layers and corresponds to the beginning of gastrulation (Okubo & Motokawa 2007). The blastocoel was formed (Fig. 1H,I, possible cavitation in coral) and was gradually filled with yolk and ingressing cells (Fig. 1J–L). In the lecithotrophic larvae of the sea urchin *Heliocidaris erythrogramma*, a transient spike in the metabolic rate is observed in late gastrulation (Hoegh-Guldberg & Emlet 1997). In mammals such as mice (Houghton et al. 1996, Ottosen et al. 2007), pigs (Sturmey & Leese 2003), cattle (Thompson et al. 1996), and humans (Butcher et al. 1998), O_2 consumption is relatively constant from the one-cell to morula stages, but sharply increases at the blastocyst stage, when the first cell differentiation event in mammalian embryogenesis segregates the inner cell mass lineage from the trophectoderm. These results suggest that the metabolic rate increases during major developmental changes such as gastrulation.

O_2 consumption peaked at the teardrop-shaped planula stage ($6.04 \pm 2.03 \times 10^{-5}$ ml O_2 planula^{-1} h^{-1} at 27°C, Figs. 1M & 2). This rate was statistically greater than those of all developmental stages before the round-shaped gastrula and after 6 d of development (Kruskal-Wallis test, $H = 94.7$, $p < 0.001$; Dunn's multiple comparison: $p < 0.05$ for each stage from unfertilized egg to bowl-shaped blastula versus teardrop-shaped planula stage; $p < 0.05$ for teardrop-shaped planula stage versus all stages from 7 to 20 d old planula). At this stage, the planulae swam actively, especially in the vertical plane, and the aboral region

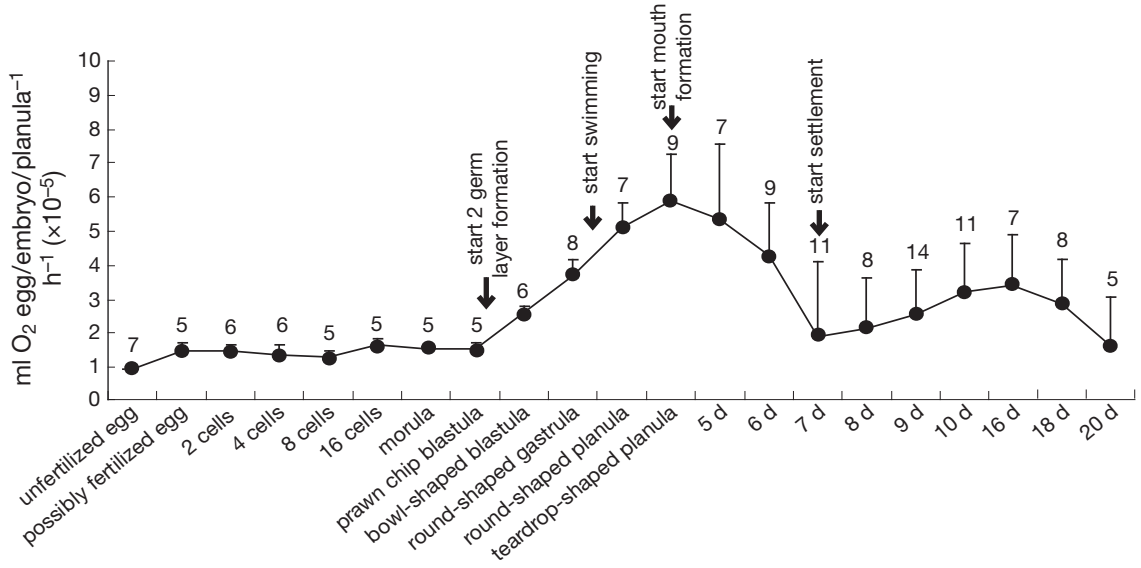

Fig. 2. *Acropora intermedia*. O_2 consumption of a single embryo/planula (mean ± SD). Numbers indicate replicates; vertical lines indicate +1 SD

became thicker and slightly concave in preparation for settlement (Fig. 1N) (Okubo & Motokawa 2007).

In echinoderms, specifically the sea star *Dendraster excentricus* and the sea urchins *Strongylocentrotus purpuratus* and *Strongylocentrotus franciscanus*, the rate of O_2 consumption peaks abruptly at hatching and then decreases sharply approximately 5 h after the beginning of hatching, returning to a level similar to that just prior to hatching (Hoegh-Guldberg & Manahan 1995). In *Acropora* planulae, however, such a dramatic change was not observed during the teardrop-shaped stage (Fig. 2) (Okubo & Motokawa 2007), but the total lipid content decreases dramatically 5 d after spawning (Harii et al. 2007). These results suggest that vertical swimming behavior requires increased energy consumption. We observed that O_2 consumption and energy use peaked during the stage characterized by vertical swimming (Fig. 1M,N). There are several explanations for this observation, including predator avoidance (Trager et al. 1994) and the need to locate a site with sufficient light intensity for growth after settlement (Mundy & Babcock 1998). In addition, planulae change their vertical position in the water column to disperse over significant distances while buoyant (Arai et al. 1993, Szmant & Meadows 2006). Although planulae are small, weak swimmers and cannot swim against horizontal currents, their ability to change the vertical position in the water column may have a significant influence on horizontal transport (Szmant & Meadows 2006), as has been demonstrated in fish larvae (Weinstein et al. 1980, Kingsford & Choat 1986, Dame & Allen 1996, Bradbury & Snelgrove 2001). Such behavior would increase larval survivorship and dispersal potential in the early life history of corals.

Beginning 5 d after spawning, O_2 consumption decreased gradually (Fig. 2) and the planulae began to touch the substrate with their concave aboral ends, where numerous spirocysts required for substrate attachment were developing (Will 1909, Mariscal et al. 1977, Yamashita et al. 2003) (Fig. 1O,P). Seven days after spawning, the planulae began to settle, and O_2 consumption decreased to levels observed in early developmental stages (Dunn's multiple comparison: $p > 0.05$ for each stage from unfertilized egg to round-shaped gastrula versus each stage from 7 to 20 d old planula). In *Acropora tenuis*, the lipid content remains at > 60% in 5 d old planulae (Harii et al. 2007). The maximum settlement period for spawning corals is reported to be more than 78 d after spawning (Wilson & Harrison 1998, Nishikawa et al. 2003, Nozawa & Harrison 2005). These studies suggest that planulae slowly consume stored energy for crawling/settlement behavior and/or post-settlement growth and survivorship. Further studies of O_2 consumption in settled polyps are required to clarify this issue.

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