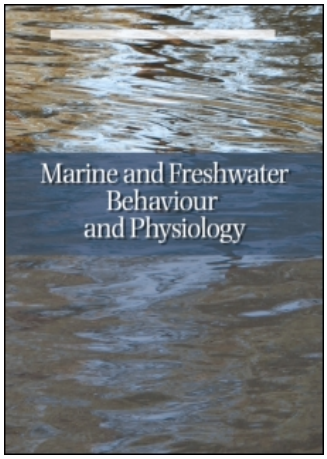

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POSTMETAMORPHIC GROWTH AND METABOLISM OF LONG-SPINED BLACK SEA URCHIN (*DIADEMA ANTILLARUM*) REARED IN THE LABORATORY

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Postmetamorphic growth and metabolism measurements were obtained on two cohorts of laboratory-reared *Diadema antillarum*. The cohorts grew linearly from less than 1 mm to over 43 mm. Daily growth averaged 0.097 and 0.11 mm d^{-1}, respectively, for the two cohorts, and was found to differ significantly. Urchin metabolism was examined by a series of simultaneous measurements of oxygen consumption and ammonium excretion over 16 days on starved juveniles ranging 16.5 to 18.3 mm. Metabolic activity under conditions of starvation was used as a test of the viability of urchins reared in the laboratory with cultured food resources. Catabolic activity differed from the first week of starvation compared to the second. Metabolic response included: (1) a 2.2-fold increase in oxygen consumption rate; (2) 50% decline in ammonium excretion rate; and (3) a 5.1-fold increase in oxygen to nitrogen ratio. These measurements are consistent with a shift from almost pure protein catabolism during the first seven days of starvation to a lipid : protein catabolic ratio of 1 : 1 after the first week. Growth and metabolism experiments of this type are seen as a first step towards optimizing laboratory culture techniques of this species.

Keywords: Sea urchin; Postmetamorphic growth; Metabolism; Starvation test; Aquaculture; Threatened species; *Diadema antillarum*

INTRODUCTION

Historically, the long-spined black sea urchin, *Diadema antillarum* (Philippi: Echinoidea) ranged throughout the tropical and subtropical coastal waters of the Atlantic Ocean (Ogden and Carpenter, 1987). Until the early 1980s, it was a conspicuous component of coral reef, sea grass and hard bottom habitats within the Caribbean Sea and adjacent waters (Lessios *et al.*, 1984). However, between 1983 and 1984, the

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species experienced catastrophic mortality that apparently spread from Punta Galeta, Panama, to eventually affect the entire tropical western Atlantic basin (Lessios *et al.*, 1984). By February 1984, the species had experienced from 94 to 99% mortality within the western Atlantic region (Lessios *et al.*, 1984). While the cause of the mass mortality is suspected to be a water bourn pathogen, this has yet to be confirmed (Bauer and Agerter, 1987).

Diadema antillarum is a grazer of macroalgae and occasionally live coral polyps, and as such, it has been described as having a positive, mediating role in coral reef ecosystems (Carpenter, 1988; Lessios, 1988; Edmunds and Carpenter, 2001). However, high *D. antillarum* densities have been observed to clear large areas of sea grass and to bioerode major portions of coral reefs as well as reduce coral recruitment as newly-settled spat are fed upon by these sea urchins (Bauer and Agerter, 1987; Lessios, 1988). Conversely, low *D. antillarum* densities can lead to unregulated macroalgal growth which subsequently out-competes coral polyps for space on the reef and thus reduces coral growth and recruitment (Lessios *et al.*, 1984).

Efforts to culture *D. antillarum* for reintroduction in the wild, the aquarium trade, coral–*Diadema* interaction research, or for isolation of the disease-causing agent(s) have been hampered by their limited availability and status as a threatened species. For the most part, echinoid gametes have been obtained invasively via dissection, chemical injection, rapid temperature change, and/or low voltage electrical shock (Strathmann, 1987). While each of the above methods can be successful, all have related levels of parental mortality and questionable larval viability. Recently, adult *D. antillarum* have been induced to spawn spontaneously in the laboratory (i.e., noninvasively) and the resulting offspring have been reared through metamorphosis to maturity (Capo *et al.*, 2001).

The purpose of this study was to evaluate the urchin rearing methodology in terms of growth and nutritional health of cohorts reared in the laboratory for subsequent release into South Florida coral reef ecosystems. Metabolic response to starvation is an indication of the nutritional status of the sea urchins when reared in the laboratory as well as to provide insight into their initial reaction upon reintroduction into the wild. Here, we describe preliminary growth trials and associated metabolic experiments on postmetamorphic *D. antillarum* reared under laboratory conditions. Specifically, we examined (1) the growth trajectories of two cohorts from shortly after metamorphosis until age 594 days; and (2) simultaneous oxygen consumption and ammonium excretion rates for 306-day-old individuals starved for a period of 16 days. From the latter measurements, oxygen to nitrogen (O : N) ratios were calculated to provide an index of nutritional condition and health (Mayzaud and Conover, 1988; Brockington and Peck, 2001). Previous studies have measured the effects of long term (Brockington and Clarke, 2001) and medium term (Kaneko *et al.*, 1981) starvation on adult sea urchins to determine differential losses of various tissues within the organism. In this study, we observed shifts in metabolic substrates over the short term in juvenile sea urchins to determine the effects of starvation on urchin energy reserves and differential substrate catabolism as starvation progressed (Mayzaud and Conover, 1988). Our overall objective was to evaluate the health and nutritional status of our laboratory-reared urchins and thereby gain insight into their suitability as experimental animals, as stock enhancement candidates or as items for the saltwater aquarium trade.

MATERIALS AND METHODS

Urchin Culture

Adult *D. antillarum* (> 50 mm test diameter, TD) were collected from the Florida Keys (24.6185 N, 81.3998 W) and held in a conditioning tank system previously described by Wisner *et al.* (1996). After a period of 28 to 63 days in the conditioning tanks, the urchins spawned spontaneously, providing eggs and larvae continuously for a full year (Capo *et al.*, 2001). Eggs were collected in a 20 μ-mesh bag filter, and then placed in lightly aerated 550 L conical incubation tanks overnight. Echinopleuteus larvae were collected from the incubation tanks, rinsed and transferred into 200 or 550 L fiberglass culture tanks. Upon metamorphosis, urchins were placed in a flow-through seawater system (Capo *et al.*, 2002) for further grow out. The sole food source provided to postmetamorphic urchins was a strain of the macroalga *Gracilaria ferox* (Capo *et al.*, 1999).

Growth

Postmetamorphic growth rates were determined for two laboratory-spawned cohorts: the first was spawned on February 2, and the second on April 20, 2001. Urchins ranging from 0.5 to 13.0 mm test diameter (TD) were measured to the nearest 0.01 mm with the aid of a dissecting microscope fitted with an ocular micrometer; urchins > 13 mm TD were measured with vernier calipers. The test diameters of 20 to 30 individuals were measured within two weeks of metamorphosis and then again on six occasions up to age 539 days. Plots of urchin size (i.e., test diameter) against age indicated growth was linear for both cohorts, thus analysis of covariance was used to test for growth rate differences between them. Water temperature was monitored daily and salinity weekly during growth trials and their possible effect on growth rates were examined.

Metabolism

Urchin metabolism was estimated under conditions of starvation by measuring oxygen consumption and ammonium excretion of juveniles. Four experimental animals from the second cohort, 306 days of age and of similar size and weight (test diameter: 16.5–18.3 mm; live weight: 5.1–5.85 g) were isolated and tracked individually during 16-day starvation trials. Animals were considered "well-fed" at trial initiation. The first set of measurements were conducted on the animals directly after feeding and evacuation of their guts, these individuals were starved thereafter. Oxygen consumption was measured with a Strathkelvin Instruments^{TM} 928 system. The system consisted of an interface to communicate between oxygen electrodes and the computer for continuous recording of data. Oxygen concentration was measured with 1302 microcathode electrodes in closed 700 mL chambers. During each experimental run, six chambers were used simultaneously, four chambers housed the urchins individually and two were used (without animals) as blank controls. Urchins were kept within 1°C of 24°C.

On metabolic measurement dates, animals were gently transferred from their holding trays into the experimental chambers and acclimated for 1–2 H. Prior to oxygen and ammonium measurement, filtered seawater used in the experiments was saturated

with air for 5 to 10 min. Each experiment was run for 6 to 8 H, after which the animals were returned to their holding trays.

The water samples for the ammonium analysis were taken from all six chambers before and after each experiment, and frozen immediately at −18°C for later analysis. Ammonium concentration was determined by a colorimetric technique using the indophenol blue method (Ivancic and Degobbis, 1984) and analyzed with a Molecular Devices® Thermo max microplate reader. Ammonium excretion rates were determined as the difference between concentration in the control blank chambers and concentrations in chambers holding experimental animals, divided by the experiment duration:

$$E = \frac{[(C_{AF} - C_{BF}) - (C_{AI} - C_{BI})]}{\text{time}}$$

where E is ammonium excretion rate ($\mu M\,ind^{-1}\,h^{-1}$), time is in hours, and C_{AI} and C_{AF} (μM) are initial and final ammonium concentration in chambers with animals, respectively. Since two blank controls were used, the values were averaged and subtracted from the values of each chamber containing urchins. The average initial and final ammonium concentration (μM) are given as C_{BI} and C_{BF}, respectively. Atomic oxygen to nitrogen ratios (O : N) were calculated from simultaneous oxygen consumption and ammonium excretion measurements to determine the effects of starvation on urchin energy reserves and differential substrate catabolism as starvation progressed (Mayzaud and Conover, 1988).

RESULTS

Average (± 1 standard error) test diameters of the February-spawned cohort increased linearly from 0.58 ± 0.013 mm at age 79 d to 46 ± 1.11 mm at age 539 d. Similarly, mean test diameters of the April-spawned cohort increased linearly from 0.57 ± 0.01 mm at age 114 d to 39.2 ± 0.57 mm at age 480 d. Mean linear growth rates of the February- and April-spawned cohorts were 0.097 and 0.11 mm d^{-1}, respectively (Fig. 1). Analysis

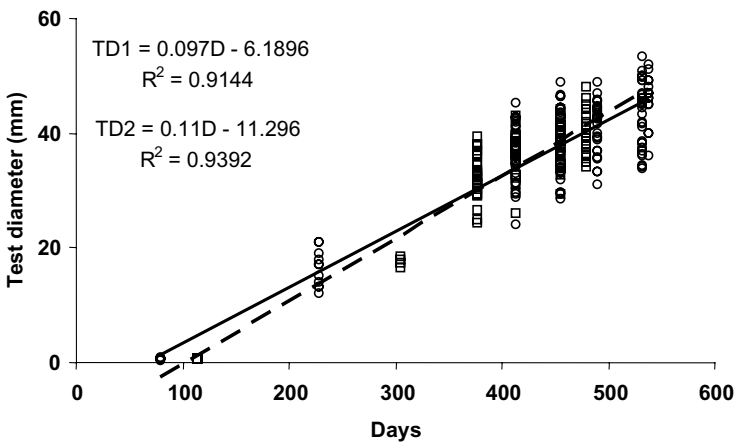

FIGURE 1 Growth trajectories as test diameter (TD, mm) over time (D, days) of two cohorts (*Diadema antillarum*) spawned in the laboratory (Cohort 1: ○, solid line, 2, February 2001, $N = 120$; Cohort 2: □, dashed line, 20, April 2001, $N = 90$). Sample sizes for each cohort–age combination ranged from 20 to 30 individuals.

of covariance indicated the above growth rates differed significantly ($P < 0.01$). Mean water temperatures during the first and second cohort's grow out periods were virtually identical 25.01 ± 0.04 and 24.81 ± 0.05°C, respectively. Salinities ranged from 33 to 36‰ during grow out, averaging 34‰ for both cohorts.

All urchins used in the metabolic component of the study survived the starvation period. Rate of oxygen consumption was not significantly different among individuals, but did differ significantly over time (Table I and Fig. 2). Oxygen consumption

TABLE I Two-way ANOVA using individual urchins and time after starvation as the main treatment effects. No significant differences were found among individuals, therefore these probabilities are not shown

Measurement	*F-value*	*P*
Oxygen consumption	17.43	< 0.0001
Ammonium excretion	10.53	0.00017
O : N ratio	13.92	< 0.0001

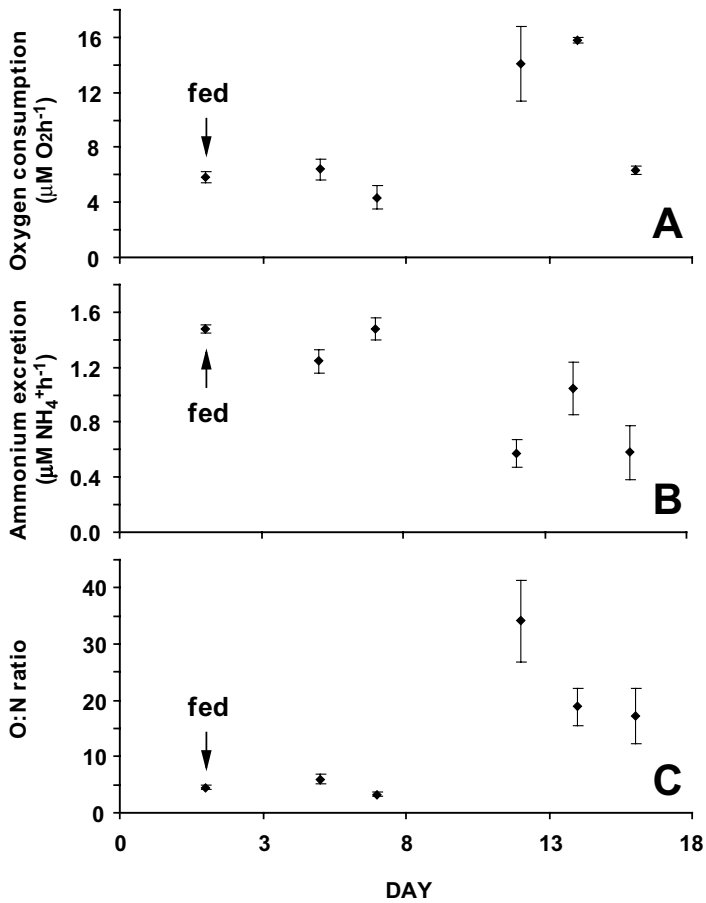

FIGURE 2 Metabolic response to starvation in 306-day old juvenile *Diadema antillarum*. A, Oxygen consumption. B, Ammonium excretion. C, O : N ratios. The first measurements represent fed individuals prior to starvation, subsequent measurements represent progressive starvation based on days after the first measurement time. Error bars represent ±1 standard errors.

TABLE II *T*-test for oxygen consumption (*R*) and ammonium excretion (*E*) measurements comparing the first and second weeks of starvation. Estimates are mean values ± standard errors

Measurement	*Days 1–7*	*Days 12–16*	*t-value*	*P*
R ($\mu M\, O_2\, ind^{-1}\, h^{-1}$)	5.54 ± 0.44	12.06 ± 1.49	4.2	0.001
E ($\mu M\, NH_4\, ind^{-1}\, h^{-1}$)	1.4 ± 0.05	0.73 ± 0.12	5.6	< 0.0001
O : N ratio	4.58 ± 0.45	23.43 ± 3.41	5.5	0.0002

remained relatively stable between 4 and 6 $\mu M\, O_2\, h^{-1}$ during the first week of starvation when compared to well fed animals (first measurement day: $5.4 \pm 0.4\, \mu M\, O_2\, h^{-1}$) (Fig. 2A). Oxygen consumption rate increased to $14.1 \pm 2.7\, \mu M\, O_2\, h^{-1}$ by day 12 and remained high until day 16 when it abruptly declined to $6.3 \pm 0.3\, \mu M\, O_2\, h^{-1}$ (Fig. 2A). There was a significant difference between the first period (days 1–7) and the second (days 12–16), where oxygen consumption rate increased 2.2-fold (Table II).

Ammonium excretion did not differ significantly among individuals, but differed significantly over time (Table I and Fig. 2B). The overall pattern observed for ammonium excretion rate over time was the reverse of that for oxygen consumption (Fig. 2). Ammonium excretion declined at a rate of $0.06\, \mu M\, NH_4\, d^{-1}$, with the greatest decline being between day 7 and day 12 (Fig. 2B). Ammonium excretion rate was 50% higher during the first week of starvation compared to the following period, this difference being significant (Table II).

Considering atomic O : N ratios, these increased significantly during days 12–16 of starvation by a factor of 5.1 in comparison to the first week of starvation (Fig. 2C and Table II). During the first week of starvation, the O : N ratio was 4.6 ± 0.45, which was similar to the first measurement day (fed animals: 4.5 ± 0.29). Afterwards, the O : N ratio increased to 23.4 ± 3.4, which was a significant increase after the first week of starvation and reflects the observed increase in oxygen consumption and decline in ammonium excretion after day 7 of the experimental period.

DISCUSSION

The postmetamorphic growth rates that we observed (i.e., 0.097 and 0.11 mm d^{-1}) for *D. antillarum* appear similar to others reported in the literature. Lewis (1966) collected two juvenile cohorts of *D. antillarum* off Barbados and held each for over a year. Feeding them a multispecies mix of macroalgae of the genus *Dictyota* at water temperatures ranging from 26 to 29°C, he observed growth rates from 0.06 to 0.11 mm d^{-1}. Bauer (1982) recorded monthly growth increment of two captive juveniles reared for 12 months in the laboratory. Their estimated overall daily growth rate was 0.10 mm d^{-1}, a value that tends to corroborate our results. Using length–frequency analysis, Lewis (1966) also estimated growth rates of a wild population of *D. antillarum*. His analysis yielded estimates ranging from 0.05 to 0.08 mm d^{-1}. The highest growth rates for juvenile *D. antillarum* were reported by Randall *et al.* (1964) from measurements conducted in field cage enclosures. Their growth rate values ranged from 0.12 mm d^{-1} for individuals grown from 23.2 to 33.7 mm (89 day period) to 0.22 mm d^{-1} for individuals grown from 8.8 to 27.1 mm (82 day period) (Randall *et al.*, 1964). In comparison, our lowest estimated growth rate was 0.045 mm d^{-1} for individuals grown from 36.1 to 39.2 mm (67 day period), and our highest recorded

growth rate was 0.21 mm d^{-1} for individuals grown from 17.5 to 32.3 mm (72 day period). All other factors being equal, higher growth rates in the laboratory and field enclosures *versus* in the wild might be expected for animals that do not need to expend energy on foraging for food and avoiding predators.

We cannot attribute the growth discrepency between our two cohorts to differences in water quality because water temperatures and salinities during the cohorts' respective grow out periods were virtually identical. Rather, we suspect differences during the larval phase (e.g., larval density – in itself a serious challenge to measure) were ultimately responsible. For the purposes of stock enhancement or for research applications, the growth rate difference detected (statistically) may have little practical or biological significance given that mean sizes of the cohorts essentially converged at about age 350 days and 25 mm TD. Regardless of the inter-cohort growth difference, from a production standpoint, we are encouraged that our relatively high *D. antillarum* growth rates were achieved by feeding a single species of alga. Certainly, further investigation of the effects of water temperature, salinity and larval rearing conditions in combination with different diets is warranted on postmetamorphic growth, survival and maturation.

Our experiments on the metabolic activity of 306-day old juvenile *D. antillarum* suggest urchins shifted from near exclusive protein catabolism during the first week of starvation to approximately equal proportions of protein and lipid catabolism by the second week. This shift was reflected in increased oxygen consumption ($\times$ 2.2-fold increase) and depressed ammonium excretion ($\times$ 0.5-fold decrease), resulting in a 5.1-fold increase of the O : N ratio. The higher O : N ratios after 12 days of starvation suggest a substantial increase in the amount of lipid used for metabolic purposes – lipids possibly designated for gamete production upon sexual maturity. Similar starvation experiments have been conducted on the adults of polar and temperate sea urchins (Kaneko *et al.*, 1981; Lares and Pomory, 1998; Brockington and Clarke, 2001). Consistent among these studies is that starved urchins first use material in the digestive tract before turning to the gonads as an energy source. Working with the Antarctic sea urchin, *Serechinus neumayeri*, Brockington and Clarke (2001) found ammonium excretion to decline while O : N ratio increased over a 3-month starvation period. They compared starved individuals to those in the wild, which had low O : N ratios indicating significantly higher protein-based catabolic activity when feeding. Interestingly, Brockington and Peck (2001) reported a seasonal pattern in wild *S. neumayeri* of low O : N ratios during the austral summer *versus* high O : N ratios during the austral winter when little or no active feeding was apparent. In light of the results on metabolic activity of starved adult urchins from polar regions (Brockington and Clarke, 2001), our data suggest that the smaller, subtropical juveniles that we examined have higher metabolic rates, possibly smaller energy reserve capacities, and, therefore, react more rapidly to the stress of starvation in comparison to temperate and polar sea urchins.

Our findings on the growth and metabolism of *D. antillarum* juveniles have several implications, depending on the application. When considering the augmentation of wild stocks by releasing laboratory-reared juveniles into natural waters, knowledge of growth, metabolic activity and recovery from starvation is important for determining the optimal animal size, density, timing, and place of urchin release. From the metabolic component of this study, we tentatively conclude that juvenile urchins (16.5–18.3 mm TD) have the potential to survive in a new environment without food (or feeding) for a week before using their stored energy reserves. Presumably, larger

animals will have proportionally greater energy reserves, relatively lower weight-specific metabolic rates, and therefore, longer time periods to adjust to new conditions, but this needs to be examined through further experimentation. Certainly, once prerelease nutritional requirements and condition are established, postrelease experiments can ensure (e.g., growth experiments in cage enclosures), and perhaps, serve as measures of animal viability and thus the effectiveness of a given stock enhancement effort. Further measurement of, and experimentation with laboratory-reared urchins is warranted for researchers attempting to learn about the cause(s) of the disease that decimated wild populations and for those contemplating urchin rearing for the aquarium trade, as well as reef stock enhancement. Future comparisons of the physiology and starvation tolerances of laboratory-reared *versus* wild urchins are warranted, however, data from field-collected animals tend to be heterogeneous because the history of disease exposure, age, life stage, varied physiological adaptations to different environmental conditions are all unknown. In this regard, we view our growth and metabolism experiments as an essential first step towards optimizing laboratory culture techniques for year-round production of this species whether for commercial, experimental, or conservation purposes.

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