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Crown-of-Thorns Starfish Larvae Can Feed on Organic Matter Released from Corals

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Abstract: Previous studies have suggested that Crown-of-Thorns starfish (COTS) larvae may be able to survive in the absence of abundant phytoplankton resources suggesting that they may be able to utilize alternative food sources. Here, we tested the hypothesis that COTS larvae are able to feed on coral-derived organic matter using labeled stable isotope tracers (^{13}C and ^{15}N). Our results show that coral-derived organic matter (coral mucus and associated microorganisms) can be assimilated by COTS larvae and may be an important alternative or additional food resource for COTS larvae through periods of low phytoplankton biomass. This additional food resource could potentially facilitate COTS outbreaks by reducing resource limitation.

Keywords: *Acanthaster*; COTS; coral reefs; coral mucus; food limitation; isotope analysis; Japan

1. Introduction

Outbreaks of Crown-of-Thorns starfish (COTS), *Acanthaster* spp., can have devastating effects on coral reefs throughout the Pacific and Indian Oceans [1,2]. Determining the causes and spatial variability of COTS outbreaks has proven to be a major challenge for coral reef managers [2,3]. Efforts to determine the causes of COTS outbreaks on the Great Barrier Reef have identified anthropogenic eutrophication as an important correlate [3–6]. The increased phytoplankton concentrations that result from high inorganic nutrient concentrations in the water are thought to promote abnormally high survival rates of COTS larvae by acting as an important food resource [4–6]. However, most coral reefs are often considered oligotrophic systems with low phytoplankton biomass, which may help keep COTS populations stable in unperturbed conditions [4]. Maximal survival of COTS larvae has been directly linked to specific concentrations of food resources [6–9]. In the studies using natural phytoplankton assemblages as food sources, COTS larvae have been found to benefit from increasing food availability from 0.25 to 0.8 μg chl-*a* L^{-1}, with the highest growth rates observed at concentrations of >0.8 μg chl-*a* L^{-1} and resource limited mortality occurring below 0.25 μg chl-*a* L^{-1} [6,8]. Furthermore, Wolfe et al. [7] recently reported using a single microalgae species that phytoplankton levels of 1 μg chl-*a* L^{-1} were optimal for COTS larval development success.

On many coral reefs, typical concentrations of chl-*a* are approximately 0.2–0.6 μg chl-*a* L^{-1} (Table 1), which may be limiting to the survival of COTS larvae [6,8]. In Okinawa, chl-*a* concentrations often fall below 0.25 μg chl-*a* L^{-1}, the critical concentration for COTS larval survival [10–14]. However, local

abundance of COTS suggests their larvae may be able to survive under low chl-*a* conditions, possibly by utilizing additional food sources [15,16]. Recent studies have reported that naturally-occurring COTS larvae were in the advanced developmental stages in the vicinity of coral communities [17–19], suggesting the utilization of organic matter derived from coral reefs by COTS larvae.

Table 1. Chlorophyll-*a* (Chl-*a*) concentrations in various coral reef waters. Some values were visually interpreted from figures. The values in parenthesis are the mean of several data in the study. GBR, Great Barrier Reef.

Site	Chl-*a* (μg L^{-1})	Reference
Miyako Island (Okinawa, Japan)	0.10–0.15	[10]
Miyako Island (Okinawa, Japan)	0.1–0.4	[11]
Sesoko Island (Okinawa, Japan)	0.11–0.77 (0.45)	[12]
West coast of Okinawa Island (Japan)	<0.05–1.79 (0.17)	[13]
Ishigaki Island (Okinawa, Japan)	0.09–0.55	[14]
Princess Charlotte Bay (GBR, Australia)	0.06–0.28 (0.16)	[20]
Princess Charlotte Bay (GBR, Australia)	0.40	[21]
Cairns-Innisfail sector (GBR, Australia)	0.03–0.64 (0.25)	[20]
Wet Tropics (GBR, Australia)	0.70	[21]
Central GBR (Australia)	0.19–0.72 (0.38)	[22]
Whitsunday Islands (GBR, Australia)	0.31–1.21 (0.79)	[23]
Uvea Atoll (New Caledonia)	0.23	[24]
The Southwest lagoon (New Caledonia)	0.25–2.14 (0.60)	[25]
Maître Island (New Caledonia)	0.26–0.42 (0.30)	[26]
Tikehau Atoll (Tuamotu, French Polynesia)	0.17	[27]
Takapoto Atoll (Tuamotu, French Polynesia)	0.23	[27]
Takapoto Atoll (Tuamotu, French Polynesia)	0.21–0.23 (0.22)	[28]
Fakarava/Rangiroa Atolls (French Polynesia)	0.008–0.25	[29]
Ahe Atoll (French Polynesia)	0.08–0.85 (0.34)	[30]
Tioman Island (Malaysia)	0.20–0.24 (0.22)	[31]
Bidong Island (Malaysia)	0.28–0.30 (0.29)	[32]

Scleractinian corals release a large amount of organic matter in the form of mucus [33] as a result of basic metabolic activities and as a protective mechanism against various stresses [34]. Coral mucus particles contain carbohydrates, proteins, lipids, and numerous microorganisms [33,35]. When coral mucus particles become suspended in the water column it aggregates various organic particles (such as microbes and phytoplankton) and becomes more enriched in organic matter over time [33,36,37]. These coral mucus aggregates are considered to be one of the major contributors to the origin of particulate organic matter (POM) in reef waters [36,38]. These mucus aggregates have also been reported as an important food source for various reef animals, such as fish, zooplankton, and several benthic taxa, such as coral crabs and brittle stars [39]. Experimental evidence has shown that copepods and mysids, two common zooplankton taxa, directly utilize coral mucus aggregates as a food resource [40,41]. The release of mucus by corals increases with increasing ambient light and water temperature [42] as it originates from by-products of the photosynthesis of zooxanthellae [43]. Thus, mucus production is likely to be maximized in summer (July–August in Okinawa) which corresponds with the peak spawning of COTS (July in Okinawa). Coral mucus could, therefore, be an important food source for COTS larvae that develop on or near coral reefs during periods of low phytoplankton biomass.

Here, we examined whether COTS larvae are able to feed on coral-derived organic matter. Since understanding the key food sources of COTS larvae is critical for determining future recruitment of adults [7], it is important to investigate all possible food sources in the natural environment. If COTS larvae feed on organic matter released by corals, this would represent an additional food source not previously considered. This additional food resource may enhance the survival of COTS larvae and subsequent recruitment of adults in areas that are naturally low in phytoplankton abundance and/or during times of reduced phytoplankton biomass.

2. Materials and Methods

2.1. Collection of COTS Larvae

Adult COTS (ca. 25 cm diameter) were collected on shallow reefs around Onna Village (N26.508496; E127.854283), Okinawa, Japan, in June 2015. Individuals were immediately transferred to the flow-through indoor aquaria at the Sesoko Station, Tropical Biosphere Research Center (University of the Ryukyus). We refer to the individuals from the Pacific Ocean clade COTS used in this study as *Acanthaster* cf. *solaris* [44,45].

COTS larvae were obtained using the method of Birkeland and Lucas [1]. Several mL of 1-methyladenine solution (1×10^{-3} M) were added to a crystallization dish with mature ovary lobes from the collected adults to release the oocytes. The oocytes released were pipetted into a separate glass dish filled with filtered seawater. Two drops of dense spermatozoa suspension were then added and gently stirred to fertilize oocytes. Fertilized oocytes were repeatedly rinsed with filtered seawater and introduced into a 9 L cylindrical plastic container with filtered seawater. After approximately 24 h, actively swimming gastrula larvae were transferred to another container where they were reared in filtered seawater at 28 °C with enriched phytoplankton using *Dunaliella tertiolecta* at a cell density of 500 cells mL^{-1} (= 0.9–1.0 μg chl-*a* L^{-1}), until they reached the advanced bipinnaria stage. The algae *D. tertiolecta* (NRIA-0109) was provided by GeneBank of the Japan Fisheries Research and Education Agency (Yokohama, Japan). Daily seawater changes were carried out by gentle reverse filtration using a 60 μm mesh screen.

2.2. Collection of Coral Mucus

Colonies of the branching corals, *Acropora muricata* and *A. intermedia* were collected from the reefs of Sesoko Island, and raised in the outdoor aquaria facility at the Sesoko Station for more than a year. Two weeks before the experiment, the coral colonies were transferred to a 30 L flow-through outdoor aquarium tank. Fresh seawater was directly pumped from the reef to the aquaria at the flow rate of 2.5 L min^{-1}.

The corals were labeled with enriched concentrations of stable carbon and nitrogen isotopes by incubating corals in isotopically-enriched seawater following the methods of Naumann et al. [46]. The water inflow was stopped at 1000 h, and the aquarium was treated with $NaH^{13}CO_3$ and $Na^{15}NO_3$ (Cambridge Isotope Laboratories, MA, USA, 98 atom%) to a final concentration of 20 mg L^{-1} and 1 mg L^{-1}, respectively. After a 5 h (at 1500 h) incubation, the flow-through seawater was resumed. The tank was aerated with a powerful air-pump for sufficient seawater agitation during each of the incubation periods. The labeling procedure was repeated for three days. On the final incubation day, labeled mucus was collected at 1600 h, one hour after the incubation period. To collect the mucus, corals were removed from the tank and washed with unlabeled filtered seawater [46]. The corals were then exposed to air and hung in direct sunlight to trigger mucus production [33]. The released mucus was collected in sterilized 50 mL Corning tubes. The collection of the labeled mucus was conducted twice, on July 18 and 25. The mucus was used immediately after each collection for the following feeding experiments. Triplicate subsamples of the labeled mucus from each collection period were placed in pre-weighed tin capsules and dried for 24 h at 60 °C, then stored in a desiccator until isotopic analysis was performed.

2.3. Feeding Experiments

Feeding experiments were conducted following each of the mucus collections using bipinnaria larvae. During the first feeding experiment, five-day-old (fertilized on July 13) and 14-day-old (fertilized on July 4) larvae were used (Experiment 1). For the second feeding experiment only five-day-old larvae (fertilized on July 20) were used (Experiment 2). Prior to the experiments, actively-swimming larvae were gently siphoned out, concentrated, and rinsed thoroughly with GF/F (Whatman, NJ, USA) filtered seawater. These larvae were kept in GF/F filtered seawater for 24 h to empty their guts. Feeding incubations were conducted using 12 polycarbonate bottles filled with 1 L

of GF/F filtered seawater. In Experiment 1, six bottles were used for five-day-old larvae and the other six for 14-day-old larvae. In each set of six bottles, three bottles treated with 2 mL of labeled mucus and the other three bottles were used as controls (without mucus). The feeding experiments contained approximately 300 starved larvae in each bottle. All bottles were incubated in the laboratory for 24 h keeping the water temperature at 28 °C. The bottles were rotated on a plankton wheel (Model II, Wheaton Instruments, NJ, USA) during the incubation to keep larvae and coral mucus particles in suspension. In Experiment 2, five-day-old larvae were introduced to all 12 bottles (300 individuals per bottle) and filled with 1 L of GF/F filtered seawater. Of these 12 bottles, six were used for the feeding treatment (with mucus) and the other six were used for the control treatment (without mucus). This second incubation was conducted for 48 hours, and three bottles were collected from each treatment at 24 and 48 h.

After the incubations, the COTS larvae were collected on a 60 μm mesh screen and washed with unlabeled Milli-Q water (Whatman, NJ, USA) 10 times to remove remaining labeled external mucus material. The larvae were then transferred into 15 mL Corning tube full of Milli-Q water. These tubes were refrigerated until the larvae settled to the bottom. The settled larvae were gently pipetted out (this procedure allowed for the collection of all larvae within a sample in a small volume of water), then placed into a tin capsule, dried (40 °C, 24 h), and stored in a desiccator until isotopic analysis was performed. Due to low biomass, COTS larvae collected from triplicate bottles for each feeding treatment were pooled to ensure a good signal. Therefore, only four samples for each feeding treatment were analyzed. Samples were not acidified with HCl to remove possible inorganic carbon because the acid could have damaged the larvae and altered their isotopic signature.

2.4. Analysis

Carbon- and nitrogen-stable isotope ratios of the larvae were determined by elemental analysis/isotope ratio mass spectrometry (EA/IRMS) using a Flash EA1112-DELTA V PLUS ConFlo III System (Thermo Fisher Scientific, MA, USA) at SI Science Co., Ltd. (Saitama, Japan). The carbon and nitrogen isotopic ratios are expressed in δ notation (Vienna-PeeDee Belemnite limestone for carbon and atmospheric nitrogen for nitrogen) as the deviation from standards in parts per mill (‰) using the following equation: δX (‰) = [($R_{sample}/R_{standard}$) − 1] × 1000, where X is ^{13}C or ^{15}N and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The analytical error was less than ± 0.13‰ for carbon and ± 0.61‰ for nitrogen. Significant differences (at the $p < 0.05$ level) of $\delta^{13}C$ and $\delta^{15}N$ values between control and mucus were determined using Student's *t*-test.

3. Results and Discussion

The average value (± SE) of $\delta^{13}C$ and $\delta^{15}N$ of labeled coral mucus was 358.5 ± 92.7‰ and 1683.4 ± 112.1‰ for the first experiment and 707.4 ± 31.2‰ and 1983.8 ± 111.1‰ for the second experiment, respectively (Table 2). The $\delta^{13}C$ and $\delta^{15}N$ ratios of the labeled mucus showed higher values in the second experiment, likely because of label accumulation in coral tissue and/or the biological community in the mucous on the coral surface (e.g., bacteria and zooxanthellae) [46]. We measured the $\delta^{13}C$ and $\delta^{15}N$ values of raw coral mucus, which could contain bacteria and zooxanthellae. Therefore, we are not able to offer direct evidence of mucus labeling via coral tissue but rather the labeling of the aggregation of organic matter within the mucus particles. However, Naumann et al. [46] measured the $\delta^{15}N$ of coral mucus that was filtered through 0.2 μm filter to remove microorganisms, and showed coral mucus had been labeled successfully. Considering the $\delta^{15}N$ value in our labeled raw coral mucus (1.68‰–1.98‰) are similar to values reported in other studies ([46] ca. 2.00‰), we consider that the coral mucus in our experiment was successfully labeled.

After both the 24 and 48 h incubations, the COTS larvae in the mucus addition treatments exhibited highly-enriched $\delta^{13}C$ and $\delta^{15}N$ values compared to those in control treatments (Figure 1). The average value of $\delta^{13}C$ (40.7 ± 34.9‰) and $\delta^{15}N$ (133.4 ± 55.9‰) of larvae in the mucus treatments ($n = 4$) was significantly ($P = 0.017$ for $\delta^{13}C$ and 0.0040 for $\delta^{15}N$) higher than those in controls ($\delta^{13}C$, −16.8 ± 0.3‰; $\delta^{15}N$, 7.1 ± 0.2‰, $n = 4$) (Figure 1). Since $\delta^{13}C$ and $\delta^{15}N$ of the labeled mucus in the second experiment showed higher values, the following $\delta^{13}C$ and $\delta^{15}N$ values of COTS larvae also showed

higher values compared to those in the first experiment. These results indicate that $\delta^{13}C$- and $\delta^{15}N$-labeled mucus are transferred into COTS larvae, providing further evidence for the potential use of organic matter derived from corals as a food source.

Table 2. $\delta^{13}C$ and $\delta^{15}N$ signatures of labeled mucus released by *Acropora* corals. C/N ratios were determined as %C/%N of the samples. Bold values indicate mean (± SE) of triplicate measurements.

Collection Day (mm/dd/yy)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N
07/18/2015	288.9	1562.5	6.7
	463.7	1783.9	7.7
	322.9	1703.7	7.2
	358.5 ± 53.5	1683.4 ± 64.7	
07/24/2015	690.6	2091.3	7.8
	688.1	1869.4	7.2
	743.3	1990.6	7.3
	707.4 ± 18.0	1983.8 ± 64.1	

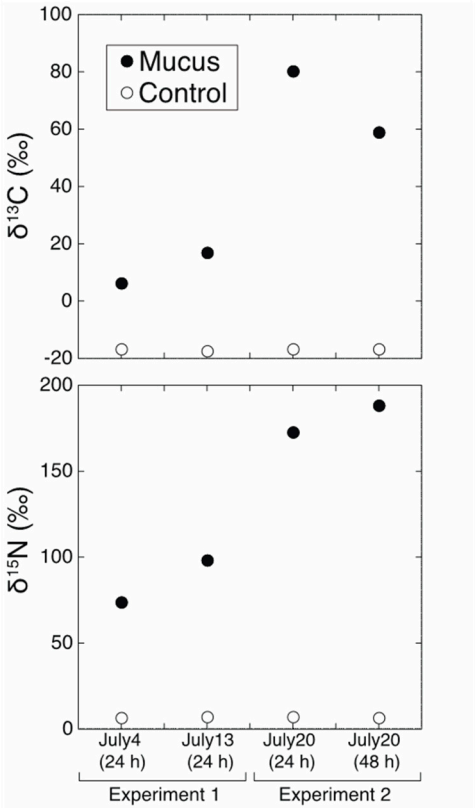

Figure 1. $\delta^{13}C$ and $\delta^{15}N$ signatures of COTS larvae after 24 h or 48 h of incubation with $\delta^{13}C$- and $\delta^{15}N$-labeled coral mucus and under control conditions. Closed and opened circles indicate fed larvae (with mucus) and unfed larvae (without mucus), respectively. Dates indicate the day of fertilization. Each plot comes from a single analysis.

This is the first study to show that COTS larvae can take up organic matter derived from corals. However, the raw coral mucus we provided to the COTS larvae may contain zooxanthellae and other microorganisms as mentioned above. Therefore, whether COTS larvae fed on whole coral mucus aggregates or selectively fed on the associated microorganisms remains unclear. Regardless, it is evident that the COTS larvae, in our feeding experiments, assimilated organic matter derived from corals, either directly (i.e., pure mucus), or indirectly via the associated microorganisms in/on the coral mucus aggregates (i.e., bacteria and zooxanthellae). It is also unclear whether COTS larvae fed on the particulate and/or dissolved fraction of the coral mucus, as we did not size-fractionate the coral mucus. Previous reports have shown that COTS larvae can utilize both POM and dissolved organic matter (DOM) [8,47,48]. Naumann et al. [46] also reported that coral associated epizoic acoelomorph

Wamioa worms utilized both the particulate and dissolved fraction of mucus released by corals. Thus, it is possible that COTS larvae are capable of feeding on both POM (mucus particles) and DOM released by corals.

Previous studies that have observed high survival rates of COTS larvae even when exposed to low concentrations of phytoplankton, suggesting that they may utilize other food sources (e.g., [8,9]). For COTS larvae in near-reef waters, organic matter derived from corals may provide an important and previously underappreciated source of nutrition. Peak spawning for COTS tends to occur in summer (July in Japan). During this period, elevated water temperature and light intensity may also cause higher organic matter release by corals [42]. In Okinawa, numerous advanced COTS brachiolaria larvae were found near coral communities [19] and it appears that some populations of COTS larvae are retained in near-shore waters due to prevailing along-shore currents in the archipelago in summer [17]. If COTS larvae are entrained near-shore they are more likely to encounter coral-derived organic matter. When the reef water residence time is short and the net transport of water is offshore, some fraction of coral mucus may be physically transported offshore before this material settles or is consumed within the reef environment [39,49]. Thus, coral-derived organic matter may also provide an important food source to COTS larvae in pelagic habitats adjacent to coral reefs. Consequently, coral mucus and its aggregates may provide one of the energy pathways for the conversion of coral primary production to COTS larvae.

Coral-derived organic matter may be a particularly important resource for COTS larval under oligotrophic conditions typical of many coral reefs where nutrients and phytoplankton biomass are low. While phytoplankton can be ephemerally high in areas with internal tidal influence or with episodic upwelling events, coral-derived organic matter may be a more consistent source of nutrition to COTS larvae on most reefs. Further, these larvae may be able to extend their planktonic durations during times of low phytoplankton biomass using endogenous nutrients, or by increasing their capacity for food capture by extending ciliary bands [50]. Although it is not likely that coral mucus, alone, is a direct causal factor of COTS outbreaks, it does seem likely that coral-derived organic matter could be an important food source, especially during periods of low phytoplankton levels. In sum, while more data are needed to understand the quantitative importance of coral-derived organic matter to COTS population dynamics, our results suggest that the feeding ecology of this species is more complex than previously thought. Given the nutritional quality of coral mucus and associated material, it is not surprising that COTS larvae, as well as many other species, take advantage of this resource in an ecosystem where nutrients are often limiting.

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