



# Feeding rates and carbon and nitrogen partitioning in crown-of-thorns sea star larvae (*Acanthaster cf. solaris*) during development

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## Abstract

Crown-of-thorns sea star (CoTS; *Acanthaster cf. solaris*) outbreaks are a significant cause of coral decline. Enhanced food supply for the larvae via eutrophication is implicated as a cause of outbreaks, yet larval feeding ecology is poorly understood. In this study, feeding experiments were carried out at two algal food concentrations of 1000 cells mL<sup>-1</sup> (~1.52 µg chl *a* L<sup>-1</sup>) and 3000 cells mL<sup>-1</sup> (~4.56 µg chl *a* L<sup>-1</sup>) across six successive larval stages to investigate the effect of food concentration on filtration rate and ingestion rate for these stages. Filtration rate increased with larval stage and more than tripled from 127 ± 32 µL larva<sup>-1</sup> h<sup>-1</sup> (mean ± SE) of the youngest (2–3 dpf) larvae to 497 ± 109 µL larva<sup>-1</sup> h<sup>-1</sup> at late brachiolaria stage (9–10 dpf). Ingestion rate increased with food concentration and larval age, with advanced brachiolaria larvae consuming 313.5 ± 39.1 cells larva<sup>-1</sup> h<sup>-1</sup> in the higher algal food treatment. Organic carbon (C) and nitrogen (N) measured in larvae and their food indicated that the youngest feeding larvae ingested 13% their body carbon content daily, with that number almost doubling to 24% by advanced bipinnaria stage. The C/N ratio decreased sharply for brachiolaria larvae, reflecting developmental changes and greater dependence on exogenous nutrition. These results add to our understanding of the role food concentration plays in the growth and survivorship of CoTS larvae in the field.

**Keywords** Larval feeding ecology · Filtration rate · Planktotrophic larvae · Phytoplankton · Carbon · Nitrogen

## Introduction

The corallivorous crown-of-thorns sea star (CoTS; *Acanthaster* spp.) still remains a threat to coral reefs in the Indo-Pacific region which is further exacerbated by the cumulative pressures of climate change (Mellin et al. 2019; Pratchett et al. 2021). Periodic outbreaks of CoTS have been recorded since the early 1960s (Pearson and Garrett 1976), with the Great Barrier Reef (GBR), Australia, currently experiencing its fourth outbreak. Each outbreak (~1962, 1979, 1993 and 2009) is believed to begin in the

far Northern GBR in an area between Cairns and Lizard Island, termed the initiation zone (Brodie 1992; Pratchett et al. 2014). There has been debate over the causes of these primary outbreaks and whether they are linked to human activities. One of the hypotheses to explain CoTS population outbreaks is the ‘nutrient limitation hypothesis’ also referred to as ‘terrestrial runoff hypothesis’ (Birkeland 1982; Brodie 1992; Brodie et al. 2005; Fabricius et al. 2010). Recent work by Kroon et al. (2023), however, has proposed oceanic processes, such as upwelling and resuspension, as another potential source of nutrients in the ‘initiation zone’. Regardless of the source of nutrients, the ‘nutrient limitation’ hypothesis posits that survival of planktotrophic CoTS larvae is limited by food and under increased nutrient concentrations more algal food is available. As a consequence, CoTS larvae are released from food limitation, leading to a shorter development cycle and increased chance of metamorphosis and settlement (Birkeland 1982; Pratchett et al. 2014). This hypothesis is supported by several studies that have established that greater survival and development of CoTS larvae are linked to increased phytoplankton concentrations with

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a general agreement that chlorophyll *a* (chl *a*) levels below  $0.5 \mu\text{g L}^{-1}$  delay larval development (Okaji 1996; Okaji et al. 1997; Uthicke et al. 2015, 2018; Wolfe et al. 2015b). In terms of cell numbers, severe limitations in development have been observed mostly with food concentrations  $< 1000$  cells  $\text{mL}^{-1}$  (Wolfe et al. 2015b; Uthicke et al. 2018). High phytoplankton concentrations ( $> 10,000$  cells  $\text{mL}^{-1}$ ) can also have detrimental effects on larvae (Wolfe et al. 2015b; Pratchett et al. 2017a). Conversely, CoTS larvae experiments conducted *in situ* under oligotrophic conditions found evidence that larvae did not experience food limitation under natural conditions (Olson 1985, 1987). However, results of the latter experiments were questioned by Okaji (1996) who used the same setup and found that phytoplankton in the incubation chambers was enriched compared to surrounding waters. There is evidence that CoTS larvae can undergo phenotypic plasticity, extending ciliated feeding appendages in response to low food conditions (Wolfe et al. 2015a). Since phytoplankton abundance and distribution vary both spatially and temporally, CoTS larval development may be limited by the availability and quality of the food source (Mellin et al. 2017; Wolfe et al. 2017). Moreover, food limitation often leads to longer larval development, and greater time CoTS larvae spends during the pelagic phase could lead to increased mortality through predation (Cowan et al. 2017).

Like many other marine benthic invertebrates, CoTS are broadcast spawners with a feeding larval phase that spend varying times from 10 days to several weeks in the water column depending on several factors. These factors include food availability (Wolfe et al. 2015b; Pratchett et al. 2017a) and temperature (Uthicke et al. 2015). Their reproductive strategy depends on the success of the planktotrophic larval stage for dispersal, and the offspring that survive to metamorphosis has direct implications for adult population size (Birkeland 1982; Brodie 1992; Deaker and Byrne 2022). After spawning and fertilization, embryos hatch to gastrula stage at 6–9 h and undergo transition through two distinct pelagic phases: (1) bipinnaria stage is characterized by the development of functional gut and ciliated bands for swimming and feeding (Lucas 1982; Keesing et al. 1997) on particulate food sources (phytoplankton 2–20  $\mu\text{m}$ ) (Ayukai 1994; Okaji et al. 1997); however, larvae can develop to advanced bipinnaria in the absence of particulate food by utilizing endogenous sources ('maternal provisioning') and/or alternative nutrition sources, i.e. dissolved organic matter (DOM), detritus (Hoegh-Guldberg 1994; Nakajima et al. 2016) and bacterial associations (Carrier et al. 2018) and (2) brachiolaria stage requires exogenous food sources to complete development to metamorphosis and is characterized by brachiolaria arms and adhesive disc for attachment (Caballes and Pratchett 2014).

CoTS are one of the most studied echinoderm species due to their destructive nature during population outbreaks

(Pratchett et al. 2014, 2017b). However, there has been limited investigation into the link between larval feeding ecology and these outbreaks. Previous work that investigated CoTS larval feeding ecology concentrated on food size selectivity (Ayukai 1994; Okaji et al. 1997), food type selectivity (Mellin et al. 2017) or effects of food concentration on feeding (Lucas 1982).

Two important physiological parameters for planktonic filter feeders are (1) filtration rate (*FR*) (volume of water cleared of particles per larva per unit of time) which is considered a key parameter for planktonic filter feeders and is a measure of feeding behaviour (Coughlan 1969; Riisgard and Larsen 2001) and (2) ingestion rate (*IR*) (the number of phytoplankton cells ingested per larva per unit of time). Previous studies have shown that several species of asteroid larvae increased filtration and ingestion rate with increasing ciliated band size and hence larval size. A study observing direct capture of polystyrene microspheres by several species of asteroid larvae found that filtration rate increased with larval size (Hart 1996). Similar results were observed in asteroid larvae feeding on the phytoplankton *Amphidinium*, and ingestion rate increased in proportion to food concentration to a maximum then declined with increasing food concentrations (Strathmann 1971). In preparation for metamorphosis, larvae undergo changes in morphology such as rudiment formation and exhibit substrate-searching behaviours; at this stage, echinoderm larvae have been shown to reduce feeding (Lucas 1982) or reach a plateau (Fenaux et al. 1985). Filtration rates in CoTS larvae also vary with food concentration and food type (Mellin et al. 2017) and food size (Ayukai 1994). Lucas (1982) reported that CoTS larvae decreased *FR* with increasing food concentrations, while the oldest larvae at brachiolaria stage were able to increase *FR* at concentrations below approximately 700 cells  $\text{mL}^{-1}$  (Lucas 1982). Filtration rate for marine invertebrate larvae generally reaches a plateau and then decreases with increasing food concentration as larvae cannot capture or process increasing food concentrations (Coughlan 1969; Marin et al. 1986).

Increased knowledge and understanding on the feeding response of CoTS larvae to nutrition availability is of importance to the understanding of major factors impacting this critical early life history phase potentially amendable to management strategies. The objectives of the present study were to investigate the filtration rate (*FR*) and ingestion rate (*IR*) in response to two different satiating food concentrations (Uthicke et al. 2018) (1000 cells  $\text{mL}^{-1}$  and 3000 cells  $\text{mL}^{-1}$ ) during complete CoTS larval development. In addition, organic carbon (C), nitrogen (N) and C/N molar ratio of algal food and CoTS larvae were measured during the pelagic development cycle from eggs to advanced brachiolaria larvae to increase the understanding of biochemical changes during development. These values were also used to calculate C and N biomass-specific ingestion rate ( $\text{d}^{-1}$ )

for the 3000 cell mL<sup>-1</sup> treatment. Patterns in growth and C/N described for the Antarctic sea urchin *Sterechinus neumayeri* found a decrease in C/N during development, which suggested a decrease in lipid reserves and increase in N-rich, structural protein (Marsh et al. 1999).

## Material and methods

### CoTS spawning and larval culturing

Adult CoTS were collected on SCUBA from John Brewer Reef (18° 38' S, 147° 02' E) between October and November in 2019 and 2020 and transported to the Australian Institute of Marine Science. They were maintained at 26.5 °C in 1000 L flow through tanks. CoTS were spawned by making a small incision and removing 4–5 gonadal lobes (Uthicke et al. 2015). Sex was determined macroscopically, and ovary lobes were placed in 200 mL of filtered sea water (FSW) with 10<sup>-5</sup> M 1-methyladenine added to give a final concentration of 50<sup>-9</sup> M 1-methyladenine to induce maturation (Kanatani 1969). Testes lobes were placed in dry 6-well plates with a cover. Matured eggs were washed over a 500-µm mesh and combined to make a stock solution of approximately 400 eggs mL<sup>-1</sup> diluted with FSW. Two microlitres of dry sperm from each male was combined and diluted using ~ 15 mL of FSW, and 1 mL of this diluted sperm solution was added to the egg stock solution, resulting in a final concentration of ~ 10<sup>6</sup> sperm mL<sup>-1</sup>. Fertilisation percent was immediately assessed and found to be consistently > 99%. The fertilised eggs were then stocked at 10–15 eggs mL<sup>-1</sup> in 70 L holding tanks at 28 °C with gentle aeration. After 24 h, a 100% water exchange was carried out and at 48 h when most embryos had developed to early bipinnaria stage, they were transferred to 16 L (14 L working volume) flow through Perspex culture chambers with a stocking density of at 1–2 larvae mL<sup>-1</sup> and fed via an automated feeding system (Uthicke et al. 2018). For the automated feeding system, the algae stock solution was calculated for a 24-h period and dosed into an algae feeder tank. A solenoid valve controlled by fluorometers dosed the algae from the feeder tank at a set chl *a* target value into a 500-L header tank which flowed into the 14-L culture chambers. This gave a continuous food supply to larvae. Each batch of larvae was sourced from 4 to 6 male and female broodstock with 1:1 sex ratio used for each batch.

The automated flow through the feeding system supplied a continuous mixture of *Dunaliella* sp. (Chlorophyte) and *Tisochrysis lutea* (Haptophyte) fed at a 1:1 ratio with respect to chl *a* content, equating to approximately 2000 cells mL<sup>-1</sup> in total with 70% *Tisochrysis lutea* and 30% *Dunaliella* sp. (0.8 µg chl *a* L<sup>-1</sup>, flow rate ~ 150 mL min<sup>-1</sup>). This concentration was chosen to ensure that the larvae were fed at

satiating conditions (Uthicke et al. 2018). The starter culture of *Dunaliella* sp. (CS-353) and *Tisochrysis lutea* (CS-177) was sourced from the Australian Algal Culture Collection (Hobart, Australia). Algae were grown under 12:12 light dark cycle at 24 °C and using F/2 nutrient medium.

The larvae were maintained in the flow through system until they were harvested for subsequent feeding experiments. Prior to each experiment, healthy swimming larvae were harvested by removing aeration and turning on lights above the culture chambers to attract them towards the light near the top of the culture chamber. The larvae were then siphoned, concentrated and counted before being dosed into 200 mL incubation chambers for the feeding experiments. For the feeding experiments, a total of six larval development stages were categorized and used for the experiments with five larval stages based on the criteria by Lucas (1982) and a sixth stage, mid bipinnaria, was defined as an intermediate stage between early and advanced bipinnaria. The corresponding larval ages in days post fertilization (dpf) are outlined in Table 5.

### Filtration and ingestion rate experiments

Two target algal food concentrations of 1000 cells mL<sup>-1</sup> (~ 1.52 µg chl *a* L<sup>-1</sup>) and 3000 cells mL<sup>-1</sup> (~ 4.56 µg chl *a* L<sup>-1</sup>) were used for each feeding experiment, which is consistent with the chl *a* concentration range (often used as a proxy for phytoplankton concentrations) reported on the GBR during flood plumes (Devlin et al. 2001, 2012). These cell numbers and chl *a* concentrations are both considered satiating with respect to growth and larval development speed (Wolfe et al. 2015b; Uthicke et al. 2018). For each experiment, 24 glass incubation chambers each containing 200 mL FSW were set up on shaker plates set at 40 rpm in a temperature-controlled room set at 28 °C, under dim light. Twelve chambers were assigned to the 1000 cells mL<sup>-1</sup> food treatment and twelve were assigned to the 3000 cells mL<sup>-1</sup> food treatment. The green algae *Dunaliella* sp. was chosen as the sole algae for the experiments because this algae species was commonly used for previous CoTS larvae feeding experiments (Lucas 1982; Ayukai 1994; Okaji et al. 1997; Uthicke et al. 2018); as well, it is easily discriminated on the flow cytometer for counting.

For each food concentration treatment level, six chambers contained larvae and the other six only contained algae (controls) to correct for algae-settling rate. The larvae were stocked at between 0.25 and 1.0 larvae mL<sup>-1</sup> and placed on shaker plates to acclimate and starve for approximately 24 h prior to the addition of *Dunaliella* sp. At the start of each experiment, a 1/500 dilution of *Dunaliella* sp. stock concentration was analysed on an Accuri C6 plus flow cytometer (Beckinson Dickinson Biosciences) with CSampler. Based on the concentration obtained, the required volume of the

*Dunaliella* sp. stock was dosed into the replicate chambers. Immediately after the addition of stock algae, two 1 mL samples were taken from each glass chamber using an in-house built plunger with a 100- $\mu\text{m}$  mesh to exclude larvae to confirm the algae concentration. Final samples were taken in the same manner at the end of each approximately 24-h experiment. At the end of each experiment, larvae from each chamber were concentrated, before the number and larval development stage were determined, under a dissection microscope. Flow cytometer settings for algae counts were as follows: 100–200  $\mu\text{L}$  sample volume, fast fluidics speed and detectors: FL1 (533/30 filter) and FL3 (670 LP filter).

Ingestion rate  $IR$  (cells larva  $\text{h}^{-1}$ ) and filtration rate  $FR$  ( $\mu\text{L}$  larva  $\text{h}^{-1}$ ) were calculated using the formula of Frost (1972) (Eqs. 3 and 5).  $C1^*$  and  $C2^*$  are the initial and final algae concentrations respectively, which were measured in the control chambers;  $k$  is a growth constant and  $t$  is the duration of the experiment. In Eq. (2),  $C1$  and  $C2$  are the initial and final algae concentrations measured in the treatment chambers with larvae, respectively, and a grazing coefficient  $g$  was determined for each of the treatment chambers. The  $FR$  was then calculated using Eq. (3) where  $V$  is the volume of water in the chamber and  $N_{larva}$  is the number of larvae in each chamber.  $IR$  was calculated based on Eq. (5) as the product of the  $C_{mean}$  concentration (cells  $\mu\text{L}^{-1}$ ) and  $FR$  obtained.

$$k = \frac{1}{t} \ln \frac{C1^*}{C2^*}, \quad (1)$$

$$g = \frac{1}{t} \ln \frac{C1}{C2} + k, \quad (2)$$

$$FR = \frac{V}{N_{larva}} g, \quad (3)$$

$$C_{mean} = \frac{C1(e^{(k-g)t} - 1)}{(k - g)t}, \quad (4)$$

$$IR = FR * C_{mean}. \quad (5)$$

### Larvae and phytoplankton C, N and chlorophyll *a* analysis

*Dunaliella* sp. was analysed for chl *a*, organic carbon (C) and nitrogen (N) content. Briefly, chl *a* was determined fluorometrically (Turner Designs 10 AU fluorometer), where duplicate 100  $\mu\text{L}$  stock algae samples were filtered onto 25 mm Whatman GFC filters, ground to a slurry with 90% acetone and incubated in the dark at 4  $^{\circ}\text{C}$  for 2 h (Parsons 1984). Organic carbon and nitrogen were analysed by taking

duplicate 100  $\mu\text{L}$  stock algae samples filtered onto pre-ashed (12 h @ 450  $^{\circ}\text{C}$ ) 25 mm GFC filters and stored at  $-20^{\circ}\text{C}$  until analysis. The samples were analysed using a Shimadzu TOC-V carbon analyzer, equipped with a solid sample module (SSM-5000A) after removing inorganic carbon using 2 M hydrochloric acid. Prior to each sampling, algae cell counts per volume were determined using a flow cytometer (Accuri C6 plus sampler). This enabled cell-specific values to be calculated for each chemical parameter.

Simultaneous to the feeding experiment, eggs and larvae from the same batch were analysed for C and N using a FlashSmart Elemental Analyzer (ThermoFisher scientific, USA), with aspartic acid (Sigma Aldrich) as standard and both in-house and certified reference materials used as standard checks. Larvae were harvested from the automated flow through feeding system and starved for 24 h to ensure that their stomachs were empty of algae prior to sampling. The feeding system provided a continuous supply of approximately 2000 cell  $\text{mL}^{-1}$  70% *Tisochrysis lutea* and 30% *Dunaliella* sp. mixture. Eggs were harvested after addition of 1-MA. The larvae were concentrated, and replicate ( $n=6$ ) subsamples were taken to calculate the average number of larvae per volume. The required volume (equating to sample size: 4000 eggs, embryos or larvae up to bipinnaria stage and 1000 larvae for later stages, per replicate) was then filtered on to pre-ashed 25 mm GFC Whatman filters ( $n=6$  replicates,  $n=3$  FSW blanks per collection) and stored at  $-20^{\circ}\text{C}$  until analysis. The samples were freeze dried prior to analysis. The molar C/N ratio was then calculated from the concentration of C and N.

C- and N-specific ingestion rates ( $\mu\text{g C ugC}^{-1} \text{larva d}^{-1}$  and  $\mu\text{g N ugN}^{-1} \text{larva d}^{-1}$ , respectively) were calculated by dividing the ingestion rate (in  $\mu\text{g C}$  or  $\mu\text{g N}$ ) for a 24-h period in the 3000 cells  $\text{mL}^{-1}$  food treatment by the average larvae C or N concentration for each larval stage.

### Statistical analysis

All statistical procedures were carried out in R v4.2.2 (R-Core-Team.). Two physiological parameters, filtration and ingestion rate, and C and N content per larva or egg were evaluated using a generalised linear mixed-effect model (GLMM) (Bolker et al. 2009) with the *glmmTMB* package (Brooks et al. 2017) modelled against a gamma error distribution. Conformity to statistical assumptions was tested using the *DHARMa* package (Hartig 2021). All models included the following nested random factors: year ( $n=2$ ), larva batch ( $n=5$ ) and experiment ( $n=14$ ). The models for filtration rate and ingestion rate included larval stage (categorical: five levels), algal concentration treatment (categorical: two levels) and larval density (continuous) as fixed effects. Mid brachiolaria were removed from the dataset for statistical purposes (only a single replicate was

available for the 1000 cells mL<sup>-1</sup> algae concentration treatment). We assessed the significance of fixed effects using Type II Wald's Chi-square tests. Contrasts were investigated with pairwise estimated marginal means with the *emmeans* package (Lenth 2020). Model strengths were assessed using the marginal  $R_m^2$  (variance explained by fixed effects) and conditional  $R_c^2$  (variance explained by both the fixed and random effects) values. Larval stage by food concentration interactions was tested and left in the final model if significant at 0.05 level.

Carbon and nitrogen content per larva was modelled using the same statistical procedures as described above for filtration and ingestion rate. These models included egg and larval stage (categorical: eight levels) as fixed effects.

## Results

### Filtration rates

The filtration rates (*FR*) of six larval development stages were measured at 3000 cells mL<sup>-1</sup> and 1000 cells mL<sup>-1</sup> algal food concentrations. The model predicting *FR* from the fixed effects of larval stage, food concentration, their interaction and larval density (in culture chambers) explained 90% (conditional  $R_c^2$ ) and 60% (marginal  $R_m^2$ ) of the variation in *FR* (Table 1). Filtration rates were not found to differ significantly between the 3000 cell mL<sup>-1</sup> and 1000 cell mL<sup>-1</sup> food treatments for all larval development stages except for the oldest larvae where the *FR* (mean ± SE) increased from 374 ± 81 μL larva<sup>-1</sup> h<sup>-1</sup> ( $n = 18$ ) in the 3000 cell mL<sup>-1</sup> treatment to 497 ± 109 μL larva<sup>-1</sup> h<sup>-1</sup> ( $n = 23$ ) in the 1000 cell mL<sup>-1</sup> food treatment ( $p < 0.01$ ). However, this is most likely

due to large variability in the data at this larval development stage. The *FR* averaged over both algal food treatments increased for each larval stage and had a significant increase between early bipinnaria larvae to advanced bipinnaria larvae ( $p < 0.05$ ), early brachiolaria larvae ( $p < 0.05$ ) and advanced brachiolaria ( $p < 0.05$ ) larvae with an approximate 150%, 167% and 230% increase in *FR*, respectively (Fig. 1). The *FR* (μL larva<sup>-1</sup> h<sup>-1</sup>) for each larval developmental stage was 127 ± 32 ( $n = 24$ ), 184 ± 65.9 ( $n = 10$ ) and 320 ± 52.2 ( $n = 53$ ), for early, mid and advanced bipinnaria, respectively, and 341 ± 70.2 ( $n = 36$ ) and 425 ± 90.1 ( $n = 41$ ) for early brachiolaria and advanced brachiolaria, respectively. The highest (± SE) *FR* was for advanced brachiolaria larvae of 497 ± 109 μL larva<sup>-1</sup> h<sup>-1</sup> ( $n = 23$ ) under the 1000 cells mL<sup>-1</sup> food concentration treatment, while the lowest *FR* was early bipinnaria of 126 ± 32.6 μL larva<sup>-1</sup> h<sup>-1</sup> ( $n = 12$ ) in the 1000 cells mL<sup>-1</sup> food concentration treatment. Mid brachiolaria was not included in the statistical analysis because only one replicate was available for the 1000 cells mL<sup>-1</sup> food treatment, yet the raw data suggest filtration and ingestion rates from the 3000 cells mL<sup>-1</sup> algae food treatment sits as an intermediate between that of the early and late brachiolaria. Filtration rate had a significant negative relationship with larval density with higher *FRs* under lower larval density.

### Ingestion rates

Like filtration rates, ingestion rates (*IR*) were measured for six successive larval development stages at two algal concentrations. All fixed effects and the interaction had a significant effect on *IR* and explained 95.77% (conditional  $R_c^2$ ) of the variation in *IR*. Ingestion rate from the 1000 to

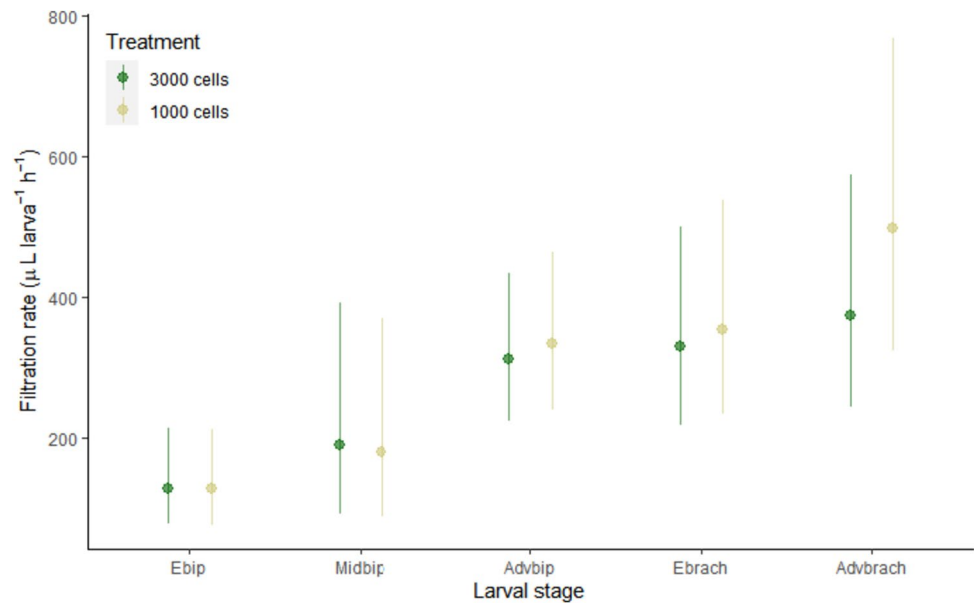
**Table 1** Summary of generalized linear mixed-effects models (GLMM) testing the effects of larval density, food concentration, larval stage and interaction on filtration rate and ingestion rates of *Acanthaster cf. solaris*

Predictors	Filtration rate			Ingestion rate		
	Estimates	CI	<i>p</i>	Estimates	CI	<i>p</i>
(Intercept)	189.4	109.84–326.60	< <b>0.001</b>	512.45	368.17–713.26	< <b>0.001</b>
Larval stage [Midbip]	1.48	0.61–3.56	0.385	1.16	0.71–1.90	0.556
Larval stage [Advbip]	2.43	1.33–4.44	<b>0.004</b>	1.04	0.74–1.47	0.809
Larval stage [Ebrach]	2.57	1.34–4.94	<b>0.005</b>	1.2	0.83–1.74	0.327
Larval stage [Advbrach]	2.93	1.51–5.68	<b>0.002</b>	1.46	1.00–2.14	<b>0.049</b>
Food treatment [1000 cells mL <sup>-1</sup> ]	0.99	0.85–1.15	0.877	0.3	0.26–0.34	< <b>0.001</b>
Larval density mL <sup>-1</sup>	0.5	0.36–0.69	< <b>0.001</b>	0.22	0.17–0.28	< <b>0.001</b>
Larval stage [Midbip] × Food [1000 cells mL <sup>-1</sup> ]	0.96	0.72–1.28	0.769	1.12	0.88–1.43	0.342
Larval stage [Advbip] × Food [1000 cells mL <sup>-1</sup> ]	1.08	0.89–1.32	0.423	1.2	1.01–1.41	<b>0.034</b>
Larval stage [Ebrach] × Food [1000 cells mL <sup>-1</sup> ]	1.09	0.89–1.34	0.409	1.2	1.01–1.43	<b>0.039</b>
Larval stage [Advbrach] × Food [1000 cells mL <sup>-1</sup> ]	1.35	1.09–1.66	<b>0.006</b>	1.59	1.33–1.90	< <b>0.001</b>

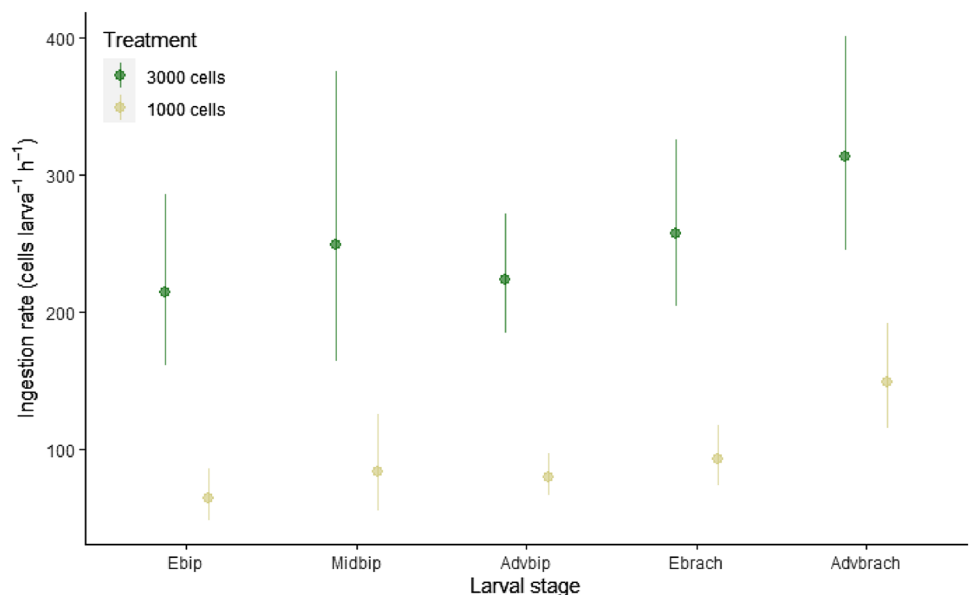
Bold indicates results which are statistically significant

*Ebip* early bipinnaria, *Midbip* mid bipinnaria, *Advbip* advanced bipinnaria, *Ebrach* early brachiolaria, *Advbrach* advanced brachiolaria

**Fig. 1** The effect of algal treatment (1000 and 3000 cells  $\text{mL}^{-1}$  *Dunaliella* sp.), larval density and CoTS (*Acanthaster* cf. *solaris*) larval development stage on filtration rate. Error bars represent 95% confidence intervals. *Ebip* early bipinnaria, *Midbip* mid bipinnaria, *Advbip* advanced bipinnaria, *Ebrach* early brachiolaria, *Advbrach* advanced brachiolaria



**Fig. 2** Results of GLMM testing the effect of CoTS (*Acanthaster* cf. *solaris*) larval development stage, algal treatment (1000 and 3000 cells  $\text{mL}^{-1}$  *Dunaliella* sp.) and larval density on ingestion rate. Error bars represent 95% confidence intervals. *Ebip* early bipinnaria, *Midbip* mid bipinnaria, *Advbip* advanced bipinnaria, *Ebrach* early brachiolaria, *Advbrach* advanced brachiolaria



3000 cells  $\text{mL}^{-1}$  food treatment was significantly higher for all larval stages with an approximate tripling for early, mid, advanced bipinnaria and early brachiolaria larvae and approximate doubling for the oldest larvae (Fig. 2, Table 1). During larval development, the *IR* (mean  $\pm$  SE) generally increased ( $n = 12, 214 \pm 31$  to  $n = 18, 314 \pm 39$  cells larva<sup>-1</sup> h<sup>-1</sup>) from the youngest to oldest larvae at the 3000 cells  $\text{mL}^{-1}$  food treatment, although the difference was not significant (Table 2). The *IR* in the 1000 cells  $\text{mL}^{-1}$  food treatment also increased ( $n = 12, 64 \pm 9.3$  to  $n = 23, 148.9 \pm 19$  cells larva<sup>-1</sup> h<sup>-1</sup>) during larval development and it was significantly greater for advanced brachiolaria larvae than that of early bipinnaria ( $p < 0.001$ ) and advanced bipinnaria larvae

**Table 2** Chemical analysis of *Dunaliella* sp. and *Tisochrysis lutea* on a per cell basis

Variable	<i>N</i>	<i>Dunaliella</i> sp.	<i>N</i>	<i>Tisochrysis lutea</i>
Chlorophyll <i>a</i> (pg cell <sup>-1</sup> )	2	1.52 (0.03)	2	0.35
Carbon (pg cell <sup>-1</sup> )	4	31.77 (4.89)	2	15.39
Nitrogen (pg cell <sup>-1</sup> )	4	5.72 (0.42)	2	2.53
C/N	4	6.47	2	7.09

Mean  $\pm$  SD

C/N represent molar ratio; *N* = number of samples analysed. *Tisochrysis lutea* C and N data from Mellin et al (2017)

( $p < 0.01$ ) by 132% and 86%, respectively (Fig. 2). The significant interaction is likely to be caused by *IR* increasing with larval stage in the 1000 cells  $\text{mL}^{-1}$  treatment, but not so in the 3000 cells  $\text{mL}^{-1}$  food treatment. However, the direction of the trend was similar in both cases. Ingestion rate had a significant negative relationship with larval density (Table 1).

### Chemical composition of larvae and phytoplankton

Organic carbon (C) and nitrogen (N) measured in larvae and eggs were modelled with larval development stage as the fixed effects and year/batch/experiment as nested random factors. Larval stage had a significant effect on C and N body content (Table 3) and showed a distinct pattern during larval development where C and N body content did not increase significantly from egg to advanced bipinnaria larvae ( $p > 0.5$ ), and then rapid gains were observed from advanced bipinnaria to early brachiolaria larvae, with 80% gain in C and 60% gain in N (C:  $p < 0.01$ , N:  $p < 0.01$ ). The C and N content then remained relatively constant during brachiolaria stage (Fig. 3).

The C content (mean  $\pm$  SE) between eggs and gastrula embryos ranged from  $0.64 \pm 0.09 \mu\text{g C egg}^{-1}$  ( $n = 5$ ) to  $0.97 \pm 0.011 \mu\text{g C larva}^{-1}$  ( $n = 17$ ) and to  $1.32 \pm 0.11 \mu\text{g C larva}^{-1}$  ( $n = 34$ ) for advanced brachiolaria stage larvae. The N content of eggs ranged from  $0.11 \pm 0.021 \mu\text{g N egg}^{-1}$  to  $0.17 \pm 0.023 \mu\text{g N larva}^{-1}$  for gastrula embryos to  $0.29 \pm 0.028 \mu\text{g N larva}^{-1}$  for advanced brachiolaria larvae (Fig. 3). During complete development from egg to metamorphosis, larvae biomass accumulated a C gain of 106% and an N gain of 164% (Table 4).

Molar C/N ratio of eggs and larvae (Fig. 4) calculated from C and N analysis indicated changes in the proportion of

C and N content during larval growth. The C/N ratio showed maximum values in eggs ( $\sim 6.83$ ) and minimum values in early brachiolaria stage ( $\sim 5.35$ ). This was due to an increase in N content from 14 to 18% of the total biomass (C + N combined) between eggs and the oldest larvae. The C/N ratio of larvae during development increasingly deviated from their algal food C/N ratio (*Dunaliella* sp., *Tisochrysis lutea*, C/N 6.47 and 7.09, respectively) (Fig. 4). When maximum ingestion rates of CoTS larvae feeding on *Dunaliella* sp. at a concentration of 3000 cells  $\text{mL}^{-1}$  were converted to biomass-specific rates (Table 3), it suggested that larvae ingested ca. 13% of their body C and N content per 24 h at early bipinnaria stage and this increased to ca. 24% and 21% for C and N, respectively, at advanced bipinnaria stage and then remained relatively constant in brachiolaria stage (Table 5).

## Discussion

### Ingestion and filtration rates

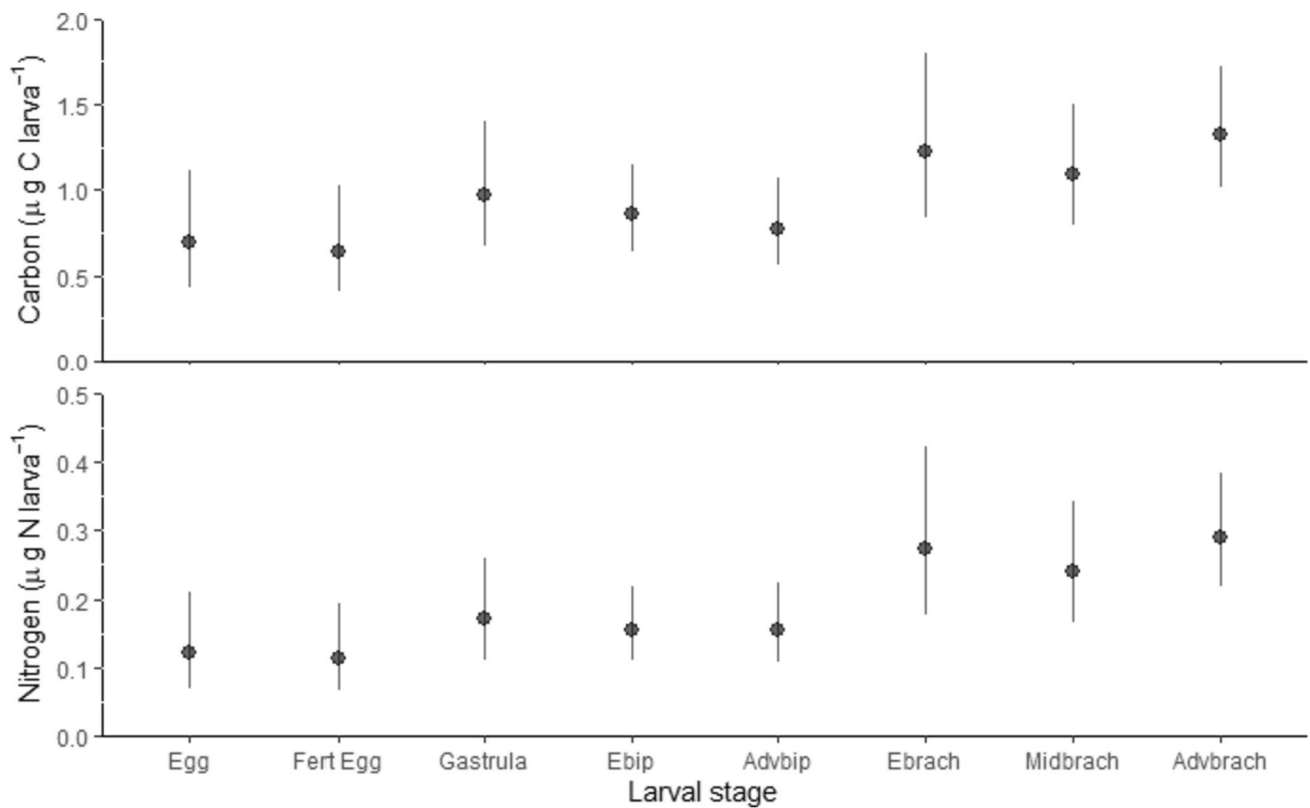
Increased understanding of the feeding response of *Acanthaster cf. solaris* (CoTS) larvae to environmental conditions is essential to elucidate the role food levels play in larval success and ultimately CoTS adult population size. In the present study conducted under two satiating algal food concentrations, we found that filtration rate (*FR*) (volume of water cleared of food particles) remained relatively consistent for both algal food concentrations (3000 cells  $\text{mL}^{-1}$  and 1000 cells  $\text{mL}^{-1}$ ) for each larval stage. The *FR* increased during larval development with an approximate tripling from early bipinnaria ( $127 \mu\text{L}^{-1} \text{h}^{-1}$ ) to advanced brachiolaria larvae ( $497 \mu\text{L}^{-1} \text{h}^{-1}$ ). Lucas (1982) measured rates of  $\sim 50$

**Table 3** Ingestion rate ( $\text{d}^{-1}$ ) converted to C and N units, biomass-specific ingestion rate ( $\text{d}^{-1}$ ) and C/N ratio, of CoTS (*Acanthaster cf. solaris*) eggs and larval development stages

Egg/larval stage	N	C/N	Ingestion rate			Biomass-specific ingestion rate	
			Cells $\text{larva}^{-1} \text{d}^{-1}$	$\mu\text{g C larva}^{-1} \text{d}^{-1}$	$\mu\text{g N larva}^{-1} \text{d}^{-1}$	$\mu\text{g C ug C}^{-1} \text{larva} \text{d}^{-1}$	$\mu\text{g N ug N}^{-1} \text{larva} \text{d}^{-1}$
Egg	5	6.83 (0.06)	–	–	–	–	–
Fert egg	6	6.84 (0.1)	–	–	–	–	–
Gastrula	17	6.56 (0.29)	–	–	–	–	–
Ebip	24	6.47 (0.17)	4672.43 (861.97)	0.15 (0.03)	0.03 (0.005)	0.13 (0.03)	0.13 (0.03)
Advbip	15	5.99 (0.58)	4801.63 (1109.15)	0.15 (0.04)	0.03 (0.006)	0.24 (0.03)	0.21 (0.03)
Ebrach	12	5.35 (0.08)	6951.59 (2307.25)	0.22 (0.07)	0.04 (0.013)	0.2 (0.07)	0.16 (0.06)
Midbrach	17	5.37 (0.09)	8747.95 (4045.88)	0.28 (0.13)	0.05 (0.023)	0.25 (0.13)	0.21 (0.11)
Advbrach	34	5.35 (0.39)	12,720.56 (5590.16)	0.40 (0.18)	0.07 (0.032)	0.26 (0.08)	0.21 (0.06)

Data presented as mean (SD),  $N=C$ , N sample size. Ingestion rates are based on mean ingestion rate at 3000 cells  $\text{mL}^{-1}$  ( $\sim 0.10 \mu\text{g C mL}^{-1}$ ) algal food treatment converted to C and N units (*Dunaliella* sp.  $C = 31.77 \text{ pg cell}^{-1}$ ,  $N = 5.72 \text{ pg cell}^{-1}$ , see Table 2)

Egg mature unfertilized egg, Fert egg fertilized egg (fertilization envelope present), Ebip early bipinnaria, Advbip advanced bipinnaria, Ebrach early brachiolaria, Midbrach mid brachiolaria, Advbrach advanced brachiolaria



**Fig. 3** GLMM results of the effect of CoTS (*Acanthaster cf. solaris*) egg and larval stage on carbon and nitrogen body content of larvae. Error bars represent 95% confidence intervals. *Egg* mature unfertilized egg, *Fert egg* fertilized egg (fertilization envelope present), *Ebip* early bipinnaria, *Midbip* mid bipinnaria, *Advbip* advanced bipinnaria, *Ebrach* early brachiolaria, *Advbrach* advanced brachiolaria

**Table 4** Summary of generalized linear mixed-effects models testing the effect of *Acanthaster cf. solaris* larval stage on carbon and nitrogen content on a per larva basis

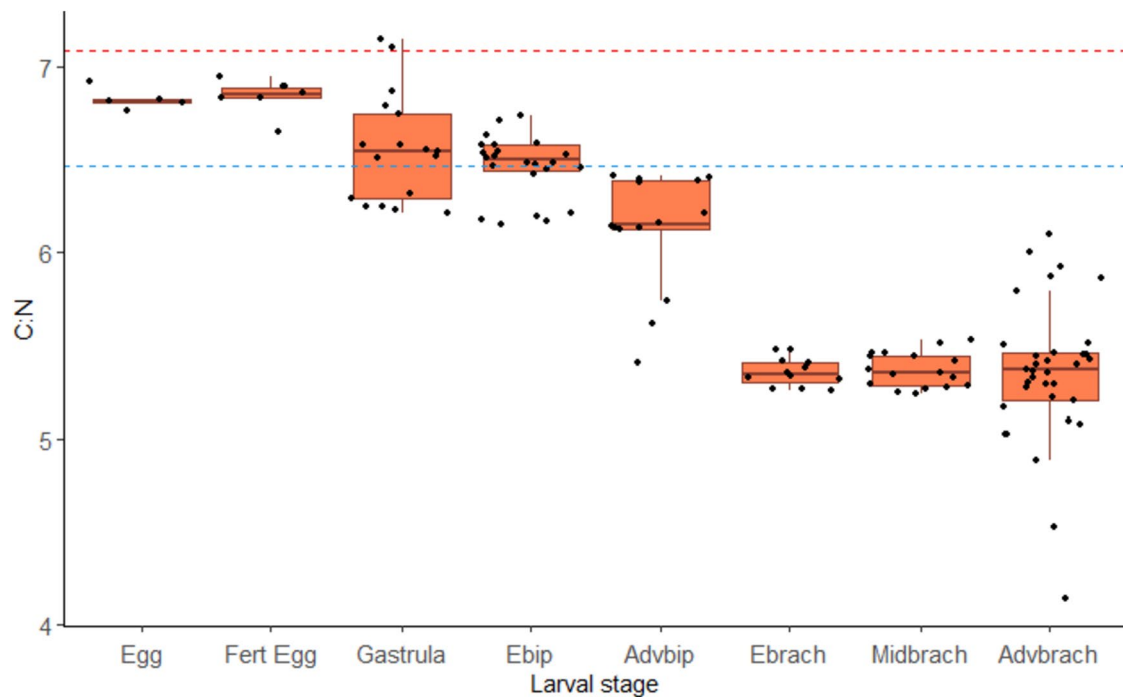
Predictors	Carbon content			Nitrogen content		
	Estimates	CI	<i>p</i>	Estimates	CI	<i>p</i>
(Intercept)	0.77	0.64–0.94	<b>0.009</b>	0.15	0.12–0.19	<b>&lt;0.001</b>
Egg	0.9	0.67–1.20	0.474	0.79	0.55–1.11	0.177
Fert egg	0.83	0.63–1.11	0.211	0.73	0.51–1.03	0.071
Gastrula	1.26	1.01–1.57	<b>0.042</b>	1.1	0.85–1.44	0.471
Ebip	1.11	0.92–1.33	0.275	1.01	0.81–1.26	0.951
Advbip	Reference			Reference		
Ebrach	1.59	1.26–2.01	<b>&lt;0.001</b>	1.78	1.34–2.35	<b>&lt;0.001</b>
Midbrach	1.41	1.15–1.73	<b>0.001</b>	1.55	1.22–1.98	<b>&lt;0.001</b>
Advbrach	1.71	1.43–2.05	<b>&lt;0.001</b>	1.88	1.51–2.33	<b>&lt;0.001</b>

Bold indicates results which are statistically significant  
 The models explained 88% and 90% (conditional  $R_c^2$ ) of the variance for carbon and nitrogen content, respectively  
*Egg* mature unfertilized egg, *Fert egg* fertilized egg (fertilization envelope present), *Ebip* early bipinnaria, *Advbip* advanced bipinnaria, *Ebrach* early brachiolaria, *Midbrach* mid brachiolaria, *Advbrach* advanced brachiolaria

$\mu\text{L}^{-1} \text{h}^{-1}$  up to  $\sim 250 \mu\text{L}^{-1} \text{h}^{-1}$  from the youngest to oldest larvae. We believe that the higher rates measured in the present study are the result of increased larval viability due to improvements in culturing techniques (e.g. flow through

system) combined with greater replication and precision in algae-counting technique (flow cytometry). Increasing *FR* with larval age and size has been reported in other planktotrophic echinoderm larvae, for example *Parastichopus*





**Fig. 4** Molar carbon and nitrogen ratio of CoTS (*Acanthaster cf. solaris*) egg and larval stages. *Egg* mature unfertilized egg, *Fert egg* fertilized egg (fertilization envelope present), *Ebip* early bipinnaria, *Midbip* mid bipinnaria, *Advbip* advanced bipinnaria, *Ebrach* early brachiolaria, *Advbrach* advanced brachiolaria. Boxplot line repre-

sents median, box indicates interquartile range (IQR) and whiskers denote  $1.5 \times$  IQR. Black dots represent measured data. Dashed lines represent C/N ratio of algal food used for rearing larvae in the automated feeding system (blue: *Dunaliella* sp. = 6.47.; red: *Tisochrysis lutea* = 7.09)

**Table 5** Larva age (dpf = days post fertilization) for each development stage for filtration and ingestion rate experiments and C/N analysis

Larva age dpf	Development stage
0.75	Gastrula
2–3	Early bipinnaria
4	Mid bipinnaria
4–6	Advanced bipinnaria
6–7	Early brachiolaria
7–8	Mid brachiolaria
9–10	Advanced brachiolaria

*californicus* (Holothuriodea), *Luidia foliolata* (Asteroidea) and *Ophiopholis aculeata* (Ophiuroidea) (Strathmann 1971). Also, a study using CoTS larvae with short (3–10 min) incubation periods also found that *FR* increased with larval age and peaked at late brachiolaria stage (Ayukai 1994). Increasing *FR* with larval age likely relate to increased length of feeding ciliated band in proportion to larval size (Strathmann 1971) as has also been reported for CoTS larvae (Wolfe et al. 2015a; Caballes 2017) and as such, it is most likely the reason for increased feeding rates with larval development observed in this study. To our knowledge, there are no

data available on whether the density of cilia also increases in asteroid larvae.

The oldest larvae showed the greatest variability in their *FR*, which is likely due to difference in competency for metamorphosis (larvae closer to settlement cease feeding) of the larvae as the larvae decrease the size of feeding appendages and internal changes occur with preparation for metamorphosis. Similar results have been observed previously with CoTS larvae (Lucas 1982) as well other planktonic larvae approaching metamorphosis, i.e. oyster (Rosa and Padilla 2020), gastropod (Hansen and Ockelmann 1991) and sea urchin (Pedrotti 1995).

Ingestion rate (*IR*) at each larval development stage was dependant on algal concentration and increased at higher concentrations as expected under constant *FR*. These results corroborate with previous results and are linked to greater number of algal cells filtered per unit of water when algae are provided at higher concentrations (Strathmann 1971; Lucas 1982; Ayukai 1994; Mellin et al. 2017). The youngest larvae, early bipinnaria, showed the largest relative increase (235%) in *IR* when exposed to the higher algal concentration as compared to the lower concentration, while the oldest larvae, advanced brachiolaria, increased by 110%, suggesting that the younger larvae may be able to utilize increased food resources more efficiently. Ingestion rate showed a general

trend of increase with larval development, but the difference was not statistically significant for the 3000 cell mL<sup>-1</sup> food concentration treatment, which is likely due to high variability in data at the oldest larval stage.

Larval density during incubations had a significant effect on both the *IR* and *FR*, with increasing larval densities having a decreasing effect on both *IR* and *FR*. This suggests either some competitive processes at higher larval density or larvae effecting other larvae in their feeding activity. This effect of slight variation in larval densities has been removed in our statistical models (by including 'larval density' as a covariate). In laboratory experiments, larval density has been found previously to affect larval growth, with increasing larval density reducing the probability of CoTS larvae reaching late brachiolaria stage (Uthicke et al. 2018), which was suggested to be due to density-dependant culturing artefact or heightened food competition among larvae at high larval densities. However, larval densities used in the laboratory are several orders of magnitude higher than those in nature (Uthicke et al. 2019).

### Carbon and nitrogen partitioning

CoTS larvae can commence feeding as soon as their alimentary canal is developed, which usually occurs by 2–3 days after hatching or early bipinnaria stage (Keesing et al. 1997). However, larvae can develop to advanced bipinnaria stage, with complete larval structures without requiring phytoplankton as a nutrition source (Lucas 1982; Brodie 1992). After this point, CoTS larvae require phytoplankton food sources to continue development to achieve competency. The larvae used for our C and N analysis were reared under constant, nutrition conditions and fed an algae mixture of *Dunaliella* sp./*Tisochrysis lutea* from the time they had developed an alimentary canal or early bipinnaria stage. Hence, at no time were larvae food limited except for a period of starvation (ca. 24 h) prior to sampling, which was designed to remove algae from their stomachs so that not confounding the C and N analyses. Therefore, changes in the C and N partitioning can be considered as the result of developmental changes and preparation for metamorphosis and settlement. Overall, the C and N content increased steadily during larval development with a sharp increase from brachiolaria stage larvae, thus not long after feeding commences. Parallel data of C and N and biochemical composition (lipids, protein and carbohydrate) of echinoderm larvae are scarce; however, studies on planktotrophic decapod larvae have shown C and N content to be highly correlated with lipid and protein content, respectively, and can be used as crude estimates thereof (Anger et al. 1989). As such, the current results are consistent with previous research findings from other echinoderms. For example, George et al. (1997) observed the most rapid increase in

lipid and protein contents of *Arbacia lixula* and *Paracentrotus lividus* (Echinoidea) in the late stage larvae prior to metamorphosis. The significant increase in C and N content coincided with an abrupt decrease in the molar C/N ratio in brachiolaria larvae. The decreased C/N ratio was due to a larger relative increase in the N pool relative to the C pool. This suggests that more proteins accumulate in later-stage larvae coupled with a depletion of maternal lipid reserves (Hart 1996; Anger 2001), consistent with previous research findings that larvae are then reliant on exogenous food sources. A depletion of maternal lipid reserves over the course of development has been observed in other asteroid (Byrne and Cerra 2000) and echinoid species (Sewell 2005) with planktotrophic development. The higher protein content is most likely building up the body structures, which is consistent with the results of adult echinoderm tissue analysis which consists predominately of protein (~70%) (Lawrence and Guille 1982; McClintock et al. 1990). Studies on decapod crustacean larvae have shown that C/N ratio trends vary with developmental mode, with lecithotrophic larvae showing increasing C/N ratios during development (Anger 1998).

Given that the C/N ratio of the food algae is around 6.62 as for most phytoplankton (Redfield ratio), the C/N ratio of the larvae increasingly deviates from the C/N ratio of the food with larval development (see Fig. 4), suggesting that CoTS larvae have to cope with excess dietary C. Previous work on the copepod, *Daphnia magna* showed that respiration and excretion of dissolved organic carbon was the primary mechanism by which excess dietary C was expelled (Darchambeau et al. 2003). The elemental composition of some zooplankton has been found to correlate with algal food composition, while other studies have found no link (Andersen and Hessen 1991; DeMott et al. 1998).

The increase in C and N content between eggs and the non-feeding gastrula stage may support the uptake of dissolved organic matter (DOM) as a source of nutrition. A previous study suggested that CoTS gastrula-stage larvae can take up DOM in sufficient amounts to provide for growth and development (Hoegh-Guldberg 1994). Similar evidence was found in another study which demonstrated that CoTS larvae assimilate coral-derived DOM (Nakajima et al. 2016).

We found (Table 3) that from advanced bipinnaria, larvae ingested approximately twofold increase of C and N content in a 24-h period relative to their own body content. This again confers with the ingestion rate results and is most likely due to increased energy demand when larvae are depleting maternal reserves and are building the rudiment and preparing for settlement and metamorphosis. Studies on the energy requirements (i.e. oxygen consumption rates) during development showed that weight-specific metabolic rates in planktotrophic *Heliocidaris tuberculata* (Echinoidea) larvae peaked in early development and then remained relatively stable during the last part of development (Hoegh-Guldberg

and Emllet 1997), which is a similar pattern to our results. Similarly, the weight-specific respiration rates of *Dendraster excentricus* and *Asterina miniata* larvae increased during development (Hoegh-Guldberg and Manahan 1995). The respiration rates of *Asterina miniata* larvae increased during development and increased food concentrations, and used less energy for protein deposition, compared to larvae raised under lower food conditions (Pace and Manahan 2007).

## Ecological significance

Key factors considered to control variations in CoTS larvae recruitment success are food availability (Birkeland 1982; Brodie 1992; Fabricius et al. 2010) and predation (Cowan et al. 2017). Under field conditions, CoTS larvae must contend with a wide range of particle types and be able to discriminate nutritious particles from inedible ones. As well for most studies on the Great Barrier Reef (GBR), chl *a* is used as a proxy for phytoplankton biomass. In this study, chl *a* was approximately  $1.52 \mu\text{g L}^{-1}$  and  $4.56 \mu\text{g L}^{-1}$  for the 1000 cells  $\text{mL}^{-1}$  and 3000 cells  $\text{mL}^{-1}$  food concentration treatments, respectively, which is consistent with the range of chlorophyll *a* concentrations reported on the GBR during flood plumes (Brodie et al. 2005; Devlin and Brodie 2005). As well, following flood events and oceanic processes such as upwelling, larger phytoplankton species  $> 10\mu\text{m}$  dominate phytoplankton biomass which can be effectively utilized by CoTS larvae (Revelante and Gilmartin 1982; Brodie et al. 2007). Our investigation was conducted under satiating (assumed to be  $> 0.5\text{--}0.8 \mu\text{g chl } a \text{ L}^{-1}$ ,  $> 1000 \text{ cells mL}^{-1}$ ) conditions (Fabricius et al. 2010; Wolfe et al. 2015b; Uthicke et al. 2018). Although limited, the data available on phytoplankton cell numbers on midshelf reefs suggest that diatoms are usually  $< 500 \text{ cells mL}^{-1}$  (Revelante and Gilmartin 1982), thus below the satiation levels.

The results of this study suggest that CoTS larvae filter randomly and the filtration rate does not depend on algal concentration at the concentration range tested in this study. Furthermore, as the results also suggest that CoTS larvae under lower food conditions were not able to increase their filtration rate, these results support the idea that under oligotrophic conditions, CoTS larvae may not be able to adequately gain their nutrition requirements. Although the food concentrations tested in this study are considered higher than oligotrophic conditions, there are empirical studies which show that CoTS larvae growth is inhibited under lower food conditions (Wolfe et al. 2015b; Uthicke et al. 2018), which seems to also indicate that CoTS larvae are limited in their ability to adequately upscale their food intake by increasing filtration rate. Similarly, increased ingestion rates with higher food during all feeding stages suggest that CoTS larva energy intake can increase proportionally to the availability of food. However, we also found some support that at least

early larvae can also assimilate DOM. Future work investigating respiration rates of CoTS larvae will help provide a full depiction of larval energy requirements.

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**Author contributions** FP and SU designed the study. FP carried out the experiments. FP analysed the data and wrote the manuscript with significant contributions from all other authors.

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**Data availability** All data are available from the corresponding author upon request.

## Declarations

**Conflict of interest** FP declares that she has no conflict of interest. SU declares that he is an associate editor with Marine Biology.

**Ethical approval** Work with echinoderms does not require ethical approval.

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## References

- Andersen T, Hessen DO (1991) Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnol Oceanogr* 36:807–814. <https://doi.org/10.4319/lo.1991.36.4.0807>
- Anger K (1998) Patterns of growth and chemical composition in decapod crustacean larvae. *Invertebr Reprod Dev* 33:159–176. <https://doi.org/10.1080/07924259.1998.9652629>
- Anger K (2001) The biology of decapod crustacean larvae. AA Balkema Publishers
- Anger K, Harms J, Püschel C, Seeger B (1989) Physiological and biochemical changes during the larval development of a brachyuran crab reared under constant conditions in the laboratory. *Helgol Mar Res* 43:225–244. <https://doi.org/10.1007/BF02367901>
- Ayukai T (1994) Ingestion of ultraplankton by the planktonic larvae of the crown-of-thorns starfish, *Acanthaster planci*. *Biol Bull (lancaster)* 186:90–100. <https://doi.org/10.2307/1542039>
- Birkeland C (1982) Terrestrial runoff as a cause of outbreaks of *Acanthaster planci* (Echinodermata: Asteroidea). *Mar Biol* 69:175–185. <https://doi.org/10.1007/BF00396897>

- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* (amsterdam) 24:127–135. <https://doi.org/10.1016/j.tree.2008.10.008>
- Brodie JE (1992) Enhancement of larval and juvenile survival and recruitment in *Acanthaster planci* from the effects of terrestrial runoff: a review. *Mar Freshw Res* 43:539. <https://doi.org/10.1071/MF9920539>
- Brodie J, Fabricius K, De'ath G, Okaji K (2005) Are increased nutrient inputs responsible for more outbreaks of crown-of-thorns starfish? An appraisal of the evidence. *Mar Pollut Bull* 51:266–278. <https://doi.org/10.1016/j.marpolbul.2004.10.035>
- Brodie J, De'Ath G, Devlin M, Furnas M, Wright M (2007) Spatial and temporal patterns of near-surface chlorophyll a in the Great Barrier Reef lagoon. *Mar Freshw Res* 58:342–353. <https://doi.org/10.1071/MF06236>
- Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017) glmmTMB Balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* 9:378–400
- Byrne M, Cerra A (2000) Lipid dynamics in the embryos of Patiriella species (Asteroidea) with divergent modes of development. *Dev Growth Differ* 42:79–86. <https://doi.org/10.1046/j.1440-169x.2000.00486.x>
- Caballes CF, Pratchett MS (2014) Reproductive biology and early life history of the crown-of-thorns starfish. In: Whitmore E (ed) *Echinoderms : ecology, habitats, and reproductive biology*. Nova Science Publishers Inc, New York, pp 101–146
- Caballes CF (2017) Environmental influences on the reproductive biology and early life history of the crown-of-thorns starfish. Doctoral thesis, James Cook University
- Carrier TJ, Wolfe K, Lopez K, Gall M, Janies DA, Byrne M, Reitzel AM (2018) Diet-induced shifts in the crown-of-thorns (*Acanthaster* sp) larval microbiome. *Mar Biol* 165:157. <https://doi.org/10.1007/s00227-018-3416-x>
- Coughlan J (1969) The estimation of filtering rate from the clearance of suspensions. *Mar Biol* 2:356–358. <https://doi.org/10.1007/BF00355716>
- Cowan Z-L, Pratchett M, Messmer V, Ling S (2017) Known predators of Crown-of-Thorns starfish (*Acanthaster* spp) and their role in mitigating, if not preventing, population outbreaks. *Diversity* (basel) 9:7. <https://doi.org/10.3390/d9010007>
- Darchambeau F, Faerøvig PJ, Hessen DO (2003) How Daphnia copes with excess carbon in its food. *Oecologia* 136:336–346. <https://doi.org/10.1007/s00442-003-1283-7>
- Deaker DJ, Byrne M (2022) Crown of thorns starfish life-history traits contribute to outbreaks, a continuing concern for coral reefs. *Emerg Top Life Sci* 6:67–79. <https://doi.org/10.1042/ETLS20210239>
- DeMott WR, Gulati RD, Siewertsen K (1998) Effects of phosphorus-deficient diets on the carbon and phosphorus balance of daphnia magna. *Limnol Oceanogr* 43:1147–1161. <https://doi.org/10.4319/lo.1998.43.6.1147>
- Devlin MJ, Brodie J (2005) Terrestrial discharge into the Great Barrier Reef lagoon: nutrient behavior in coastal waters. *Mar Pollut Bull* 51:9–22. <https://doi.org/10.1016/j.marpolbul.2004.10.037>
- Devlin MJ, Debose J, Brodie J (2012) Review of phytoplankton in the great barrier reef and the potential links to crown of thorns. Centre for Tropical Water & Aquatic Ecosystem Research (Trop-WATER), James Cook University, Townsville
- Devlin MJ, Waterhouse J, Taylor J, Brodie J (2001) Flood plumes in the Great Barrier Reef: spatial and temporal patterns in composition and distribution. Research Publication No 68, Great Barrier Reef Marine Park Authority, Townsville
- Fabricius KE, Okaji K, De'ath G (2010) Three lines of evidence to link outbreaks of the crown-of-thorns seastar *Acanthaster planci* to the release of larval food limitation. *Coral Reefs* 29:593–605. <https://doi.org/10.1007/s00338-010-0628-z>
- Fenaux L, Cellario C, Etienne M (1985) Variations in the ingestion rate of algal cells with morphological development of larvae of *Paracentrotus lividus* (Echinodermata: Echinoidea). *Mar Ecol Progress Ser* (halstenbek) 24:161–165. <https://doi.org/10.3354/meps024161>
- Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol Oceanogr* 17:805–815
- George SB, Young CM, Fenaux L (1997) Proximate composition of eggs and larvae of the sand dollar *Encope michelini* (Agassiz): the advantage of higher investment in planktotrophic eggs. *Invertebr Reprod Dev* 32:11–19. <https://doi.org/10.1080/07924259.1997.9672599>
- Hansen B, Ockelmann KW (1991) Feeding behaviour in larvae of the opisthobranch *Philina aperta* - I. Growth and functional response at different developmental stages. *Mar Biol* 111:255–261. <https://doi.org/10.1007/BF01319707>
- Hart MW (1996) Variation in suspension feeding rates among larvae of some temperate, eastern Pacific Echinoderms. *Invertebr Biol* 115:30–45. <https://doi.org/10.2307/3226940>
- Hartig F (2021) DHARMA: residual diagnostics for hierarchical (Multi-Level/Mixed) regression models. R package version 0.4.4. <http://florianhartig.github.io/DHARMA/>
- Hoegh-Guldberg O (1994) Uptake of dissolved organic matter by larval stage of the crown-of-thorns starfish *Acanthaster planci*. *Mar Biol* 120:55–63. <https://doi.org/10.1007/BF00381942>
- Hoegh-Guldberg O, Emler RB (1997) Energy use during the development of a lecithotrophic and a planktotrophic echinoid. *Biol Bull* (lancaster) 192:27–40. <https://doi.org/10.2307/1542573>
- Hoegh-Guldberg O, Manahan D (1995) Coulometric measurement of oxygen consumption during development of marine invertebrate embryos and larvae. *J Exp Biol* 198:19–30
- Kanatani H (1969) Induction of spawning and oocyte maturation by -methyladenine in starfishes. *Exp Cell Res* 57:333–337. [https://doi.org/10.1016/0014-4827\(69\)90158-X](https://doi.org/10.1016/0014-4827(69)90158-X)
- Keesing JK, Halford AR, Hall KC, Cartwright CM (1997) Large-scale laboratory culture of the crown-of-thorns starfish *Acanthaster planci* (L.) (Echinodermata: Asteroidea). *Aquaculture* 157:215–226. [https://doi.org/10.1016/S0044-8486\(97\)00062-8](https://doi.org/10.1016/S0044-8486(97)00062-8)
- Kroon FJ, Crosswell J, Robson BJ (2023) The effect of catchment load reductions on water quality in the crown-of-thorn starfish outbreak initiation zone. *Mar Pollut Bull* 195:115255–115255. <https://doi.org/10.1016/j.marpolbul.2023.115255>
- Lawrence JM, Guille A (1982) Organic composition of tropical, polar and temperate-water echinoderms. *Comp Biochem Physiol B: Comp Biochem* 72:283–287. [https://doi.org/10.1016/0305-0491\(82\)90047-5](https://doi.org/10.1016/0305-0491(82)90047-5)
- Lenth R (2020) Estimated marginal means, aka least-squares means. <https://CRAN.R-project.org/package=emmeans>
- Lucas JS (1982) Quantitative studies of feeding and nutrition during larval development of the coral reef asteroid *Acanthaster planci* (L.). *J Exp Mar Biol Ecol* 65:173–193. [https://doi.org/10.1016/0022-0981\(82\)90043-0](https://doi.org/10.1016/0022-0981(82)90043-0)
- Marin V, Huntley ME, Frost B (1986) Measuring feeding rates of pelagic herbivores: analysis of experimental design and methods. *Mar Biol* 93:49–58. <https://doi.org/10.1007/BF00428654>
- Marsh AG, Leong PK, Manahan DT (1999) Energy metabolism during embryonic development and larval growth of an Antarctic sea urchin. *J Exp Biol* 202:2041–2050. <https://doi.org/10.1242/jeb.202.15.2041>
- McClintock JB, Hopkins T, Watts SA, Marion K (1990) The biochemical and energetic composition of somatic body components of

- echinoderms from the northern Gulf of Mexico. *Comp Biochem Physiol a: Comp Physiol* 95:529–532. [https://doi.org/10.1016/0300-9629\(90\)90733-9](https://doi.org/10.1016/0300-9629(90)90733-9)
- Mellin C, Lugin C, Okaji K, Francis D, Uthicke S (2017) Selective feeding and microalgal consumption rates by crown-of-thorns seastar (*Acanthaster cf. solaris*) larvae. *Diversity*. <https://doi.org/10.3390/d9010008>
- Mellin C, Matthews S, Anthony KRN, Brown SC, Caley MJ, Johns KA, Osborne K, Puotinen M, Thompson A, Wolff NH, Fordham DA, MacNeil MA (2019) Spatial resilience of the Great Barrier Reef under cumulative disturbance impacts. *Glob Change Biol* 25:2431–2445. <https://doi.org/10.1111/gcb.14625>
- Nakajima R, Nakatomi N, Kurihara H, Fox M, Smith J, Okaji K (2016) Crown-of-thorns starfish larvae can feed on organic matter released from corals. *Diversity (basel)* 8:18. <https://doi.org/10.3390/d8040018>
- Okaji K, Ayukai T, Lucas JS (1997) Selective feeding by larvae of the crown-of-thorns starfish, *Acanthaster planci* (L.). *Coral Reefs* 16:47–50. <https://doi.org/10.1007/s003380050058>
- Okaji K (1996) Feeding ecology in the early life stages of the crown-of-thorns starfish, *Acanthaster planci* (L.). Doctoral thesis, James Cook University, Townsville, QLD <http://eprints.jcu.edu.au/27235>
- Olson RR (1985) In situ culturing of larvae of the crown-of-thorns starfish *Acanthaster planci*. *Mar Ecol Progress Ser (halstenbek)* 25:207–210. <https://doi.org/10.3354/meps025207>
- Olson RR (1987) In situ culturing as a test of the larval starvation hypothesis for the crown-of-thorns starfish, *Acanthaster planci*. *Limnol Oceanogr* 32:895–904
- Pace DA, Manahan DT (2007) Efficiencies and costs of larval growth in different food environments (Asteroidea: *Asterina miniata*). *J Exp Mar Biol Ecol* 353:89–106. <https://doi.org/10.1016/j.jembe.2007.09.005>
- Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, p 173
- Pearson RG, Garrett RN (1976) *Acanthaster planci* on the great barrier reef: general surveys 1972–1975. *Biol Cons* 9:157–164. [https://doi.org/10.1016/0006-3207\(76\)90006-9](https://doi.org/10.1016/0006-3207(76)90006-9)
- Pedrotti ML (1995) Food selection (size and flavor) during development of echinoderm larvae. *Invertebr Reprod Dev* 27:29–39. <https://doi.org/10.1080/07924259.1995.9672431>
- Pratchett MS, Caballes CF, RiveraPosada J, Sweatman H (2014) Limits to understanding and managing outbreaks of crown-of-thorns starfish (*Acanthaster* spp). *Oceanography and marine biology*. CRC Press, Amsterdam, pp 133–200
- Pratchett MS, Caballes CF, Dworjanyn S, Mos B, Thompson C, Blowes S (2017a) Larval survivorship and settlement of crown-of-thorns starfish (*Acanthaster cf. solaris*) at varying algal cell densities. *Diversity*. <https://doi.org/10.3390/d9010002>
- Pratchett MS, Caballes CF, Wilmes J, Matthews S, Mellin C, Sweatman H, Nadler L, Brodie J, Thompson C, Hoey J, Bos A, Byrne M, Messmer V, Fortunato S, Chen C, Buck A, Babcock R, Uthicke S (2017b) Thirty years of research on crown-of-thorns starfish (1986–2016): scientific advances and emerging opportunities. *Diversity*. <https://doi.org/10.3390/d9040041>
- Pratchett MS, Caballes CF, Cvitanovic C, Raymundo ML, Babcock RC, Bonin MC, Bozec Y-M, Burn D, Byrne M, Castro-Sanguino C, Chen CCM, Condie SA, Cowan Z-L, Deaker DJ, Desbiens A, Devantier LM, Doherty PJ, Doll PC, Doyle JR, Dworjanyn SA, Fabricius KE, Haywood MDE, Hock K, Hoggett AK, Høj L, Keesing JK, Kenchington RA, Lang BJ, Ling SD, Matthews SA, McCallum HI, Mellin C, Mos B, Motti CA, Mumby PJ, Stump RJW, Uthicke S, Vail L, Wolfe K, Wilson SK (2021) Knowledge gaps in the biology, ecology, and management of the Pacific crown-of-thorns sea star *Acanthaster* sp. on Australia's Great Barrier Reef. *Biol Bull* 241:330–346. <https://doi.org/10.1086/717026>
- R-Core-Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Revelante N, Gilmartin M (1982) Dynamics of phytoplankton in the Great Barrier Reef lagoon. *J Plankton Res* 4:47–76. <https://doi.org/10.1093/plankt/4.1.47>
- Riisgard HU, Larsen PS (2001) Minireview: ciliary filter feeding and bio-fluid mechanics- present understanding and unsolved problems. *Limnol Oceanogr* 46:882–891. <https://doi.org/10.4319/lo.2001.46.4.0882>
- Rosa M, Padilla DK (2020) Changes in food selection through ontogeny in *Crassostrea gigas* larvae. *Biol Bull (lancaster)* 238:54–63. <https://doi.org/10.1086/706821>
- Sewell MA (2005) Utilization of lipids during early development of the sea urchin *Evechinus chloroticus*. *Mar Ecol Prog Ser (halstenbek)* 304:133–142. <https://doi.org/10.3354/meps304133>
- Strathmann RR (1971) The feeding behavior of planktotrophic echinoderm larvae: mechanisms, regulation, and rates of suspension feeding. *J Exp Mar Biol Ecol* 6:109–160. [https://doi.org/10.1016/0022-0981\(71\)90054-2](https://doi.org/10.1016/0022-0981(71)90054-2)
- Uthicke S, Logan M, Liddy M, Francis D, Hardy N, Lamare M (2015) Climate change as an unexpected co-factor promoting coral eating seastar (*Acanthaster planci*) outbreaks. *Sci Rep* 5:8402. <https://doi.org/10.1038/srep08402>
- Uthicke S, Liddy M, Patel F, Logan M, Johansson C, Lamare M (2018) Effects of larvae density and food concentration on crown-of-thorns seastar (*Acanthaster cf. solaris*) development in an automated flow-through system. *Sci Rep* 8:642. <https://doi.org/10.1038/s41598-017-19132-w>
- Uthicke S, Fisher EE, Patel F, Diaz-Guijarro B, Doyle JR, Messmer V, Pratchett MS (2019) Spawning time of *Acanthaster cf. solaris* on the Great Barrier Reef inferred using qPCR quantification of embryos and larvae: do they know it's Christmas? *Mar Biol* 166:1–10. <https://doi.org/10.1007/s00227-019-3582-5>
- Wolfe K, Graba-Landry A, Dworjanyn SA, Byrne M (2015a) Larval phenotypic plasticity in the boom-and-bust crown-of-thorns seastar, *Acanthaster planci*. *Mar Ecol Prog Ser (halstenbek)* 539:179–189. <https://doi.org/10.3354/meps11495>
- Wolfe K, Graba-Landry A, Dworjanyn SA, Byrne M (2015b) Larval starvation to satiation: influence of nutrient regime on the success of *Acanthaster planci*. *PLoS ONE* 10:e0122010–e0122010. <https://doi.org/10.1371/journal.pone.0122010>
- Wolfe K, Graba-Landry A, Dworjanyn SA, Byrne M (2017) Superstars: Assessing nutrient thresholds for enhanced larval success of *Acanthaster planci*, a review of the evidence. *Mar Pollut Bull* 116:307–314. <https://doi.org/10.1016/j.marpolbul.2016.12.079>

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