NOTE

Juvenile age and available coral species modulate transition probability from herbivory to corallivory in *Acanthaster* cf. *solaris* (Crown-of-Thorns Seastar)

Rachel C. Neil[1,2,3] · Maria Gomez Cabrera[2] · Sven Uthicke[2]

Abstract Prior to transitioning to a coral diet, juvenile *Acanthaster* cf. *solaris*, the Crown-of-Thorns Seastar (COTS), feed on crustose coralline algae. Although a detailed understanding of juvenile ecology is crucial to predict and prevent outbreaks, the exact timing of the transition is unresolved. Two experiments were conducted to measure time and size of COTS at the transition, and investigate potential modulating effects of different coral species. COTS began early transitions at similar sizes (7.5–8.5 mm), and these first transitions were observed around 136–145 d. Between 175 and 191 d, a 50% transition in the presence of *Acropora tenuis* was measured. After 175d, the percentage of COTS cohorts that had transitioned was significantly lower in *A. millepora* (38%) and *Stylophora pistillata* (7%) compared to *A. tenuis* (51%). These data fill important knowledge gaps in juvenile ecology, and the influence of coral species on transition suggests an undescribed feedback mechanism between prey and predator.

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Rachel Neil and Maria Gomez Cabrera are contributed equally to this work.

✉ Rachel C. Neil
  rachel.neil@my.jcu.edu.au; r.neil@aims.gov.au

[1] College of Science and Engineering, James Cook University, 1 Angus Smith Drive, Douglas, QLD 4814, Australia

[2] Australian Institute of Marine Science, Cape Cleveland, QLD 4811, Australia

[3] AIMS@JCU, James Cook University, DB17-148, Townsville, QLD 4811, Australia

Introduction

Corallivorous Crown-of-Thorns Seastars (COTS), *Acanthaster* sp., are significant contributors to coral reef decline in the Indo-Pacific region (De'ath et al. 2012; Kayal et al. 2012). Predicting and managing COTS outbreaks requires a thorough understanding of their life cycle, particularly as the causes of outbreaks remain unresolved (Pratchett et al. 2014; Westcott et al. 2020). After a planktonic larval period, juvenile COTS settle and metamorphose among crustose coralline algae (CCA) covered substrate, where they remain feeding on biofilms and CCA until transitioning to corallivory at 4–12 months post-settlement (Yamaguchi 1974; Zann et al. 1987; Deaker et al. 2020a; Wilmes et al. 2020b).

The transition to coral is an important bottleneck in the life cycle of the COTS. This is the point at which COTS become a threat to corals, as well as driving an increase in COTS growth-rate and a transition from juvenile to adult morphology (Yamaguchi 1974; Wilmes et al. 2020a). During this time COTS may be damaged or killed by corals as the seastars start to actively seek them (Deaker et al. 2021), as well as changing the COTSs' habitat and exposure to other forms of predation (Wilmes et al. 2019). Aquarium studies found that this transition occurs as early as 4-months, once COTS reached $\sim$ 8 mm in diameter (Yamaguchi 1974), while studies of in situ juveniles estimated the first point of transition occurred 13 – 15 months post-settlement (Zann et al. 1987).

While past studies have identified the approximate age and size that COTS begin their transition, field studies are

hampered by the difficulty of ageing juveniles (Wilmes et al. 2017; Deaker et al. 2020b), and aquarium-based studies typically begin their investigations into the behaviour of transitioning COTS well after they have surpassed the reported minimum size and age (Yamaguchi 1974; Johansson et al. 2016; Deaker et al. 2021). Clarifying this early transition point and behaviour of cohorts during this period is important for predicting outbreaks of COTS, as most aspects of juvenile ecology critical for understanding and modelling outbreaks are still 'largely unresolved' (Pratchett et al. 2017, 2021), and population models require knowledge of the transitions between life history stages and the factors influencing these.

This study aimed to address this knowledge gap by investigating the age and size juvenile COTS transition from CCA feeding to corallivory in two independent experiments. We aimed to identify the earliest and smallest size that transition would occur, and how long it takes for 50% and 100% of a cohort to transition. In addition, based on known feeding preferences (Johansson et al. 2016) and the potential of injury through coral defences (Deaker et al. 2021), we tested if offering *Acropora tenuis*, a species preferred by juveniles, *Acropora millepora*, a neutral species, or *Stylophora pistillata*, a species avoided by juveniles, would alter transitions.

Methods

We obtained COTS (*Acanthaster* cf. *solaris*) juveniles for both experiments by rearing larvae derived from spawning 5 males and 5 females as described in Uthicke et al. (2018). Larvae were settled and juveniles raised as per Balu et al. (2021) and Kwong et al. (2021).

Age and size of COTS transitioning to preferred food *A. tenuis*

In this experiment conducted in 2020, 24 5L flow-through aquaria were continuously supplied with 27.5 °C filtered seawater at 0.25L min^{-1}. Each aquarium was fitted with a 100 μm outlet filter to prevent COTS from escaping the tanks, and light was supplied by eight Aqua Illumination® Hydra® LEDs at an intensity of 100 μmol m^{-2} s^{-1}, in a 9:15 light:dark cycle.

Two fragments of *Acropora tenuis* ∼ 5 cm and two chips of CCA ∼ 2 cm in diameter were added to each aquarium. *A. tenuis* was chosen as juvenile COTS show a preference for feeding on this species, thus it seems logical that juveniles would preferentially transition to this food source (Johansson et al. 2016). Corals were collected from Backnumbers Reef (18° 31.043'S, 147° 8.475'E) on the Great Barrier Reef under permit G12/35236.1, and

introduced to the experimental systems with the CCA 10 days prior to the start of the experiment. This allowed time for corals and CCA to acclimate to the systems, and during this time corals that showed signs of stress were removed and replaced. Twenty-four juvenile COTS (116 d post settlement) were measured, and their arms counted via high-resolution photography using a Leica MS5 dissecting microscope with a calibrated ToupCam digital camera, then one COTS was added to each experimental tank. At this age, juvenile COTS averaged 3.24 mm ± 0.22 mm (mean ± s.e., n = 24), less than half the size reported in the literature that they transition to corals (∼ 8 mm), but large enough that they could be observed with the naked eye (Yamaguchi 1974; Lucas 1984; Wilmes et al. 2020a). Juveniles were then monitored weekly for evidence of transition from feeding on CCA to feeding on coral (Fig. 1B). After active feeding on coral fragments was observed, the age of the COTS was recorded, then the COTS photographed and measured (Fig. 1A). This experiment was run until all COTS had transitioned to determine the time period required for all individuals to transition.

Effect of available coral species on transitioning COTS cohorts

The second experiment was conducted in 2021, offering three different coral species: *Acropora tenuis*, *Acropora millepora* and *Stylophora pistillata*. This allowed us to test the effect of preferred species *A. tenuis* compared to less preferred *A. millepora* and typically avoided *S. pistillata* (Johansson et al. 2016) on the behaviour of the COTS as they began to feed on corals. Twelve 5 L flow-through aquaria were set in a similar manner as per experiment 1 in relation to light, light cycle and water flow. Ten juvenile COTS at 119 days post-settlement, averaging 3.41 mm ±
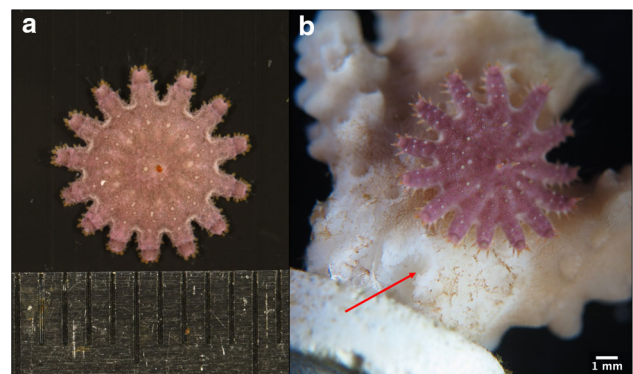

Fig. 1 **A** Measurement photograph for a COTS; this individual is still in the CCA feeding phase. Scale bar increments are in millimetres. **B** COTS that has transitioned from feeding on CCA to feeding on *A. tenuis*. Note area of tissue loss on the coral indicated by the arrow, an old feeding scar

0.10 mm (mean $\pm$ s.e., n = 120) in diameter, were added to each tank with 2–3 CCA fragments $\sim$ 3 cm in diameter. Each tank was assigned a random coral species, then two coral fragments $\sim$ 5 cm in length were offered to the COTS per experimental tank (N = 4 tanks per coral treatment). Coral fragments were checked daily for COTS or any damage; if a coral was found damaged or if a COTS was found eating it, this would be replaced with a fresh coral fragment. COTS found eating coral were removed from the experimental tank, measured and age recorded, then placed in a separate tank. This experiment was terminated after 8 weeks, 175 d post settlement.

Statistical analyses

All statistical analyses were conducted in RStudio v1.3.1073 (R Core Team 2020). A Kaplan–Meier model was used to analyse the probability of COTS in experiment 1 transitioning at each time point (R-package "survival" (Therneau 2020)). The probability of juvenile COTS transitioning to coral at the end of the second experiment, and the survival of COTS under the different treatments, was tested with a generalised linear mixed effects model for binomial data (R-package "lme4" (Bates et al. 2015)). The model included a random effect representing the repeated (N = 4) tanks for each treatment. Differences between individual treatments were then tested using Tukey test for post hoc analysis (R-package "multcomp", (Hothorn et al. 2008)). A Welch two-sample t-test was also conducted to test for differences in COTS sizes at the start of each experiment.

Results and Discussion

In the first experiment, the earliest transition to coral feeding was recorded at 145 d for a juvenile 7.91 mm in diameter, while the smallest COTS to transition was 7.13 mm at 222 d (Fig. 2A). By 191 d, 50% (95% CI = 33.5%-74.6%) of the cohort had transitioned (pT_{50} = 191 d) to feeding on A. tenuis, and after 239 d all 24 specimens had transitioned (pT_{100} = 239 d) with no mortality occurring during the experiment (Fig. 2A). The mean size COTS transitioned to feeding on A. tenuis was 8.73 mm $\pm$ 0.23 mm (mean $\pm$ s.e., n = 24, ranging 7.13 mm to 11.16 mm) with 14.5 $\pm$ 0.217 arms (Fig. 2B).

During the second experiment, the timing of the first transition to coral (136 d) and size of juveniles (7.85 mm) was similar to the previous experiment (Fig. 3) (details in Supplementary Table 1). This experiment was concluded at 175 d ($\sim$ 6 months) post-settlement when > 50% of the A. tenuis cohort had transitioned to coral feeding. With one

exception (a < 4 mm specimen), individuals at the time of transition were 5.77—10.22 mm in diameter, with a mean diameter of 7.65 $\pm$ 0.20 mm, 14.4 $\pm$ 0.174 arms and first transition age of 162 $\pm$ 1.66 d (mean $\pm$ se).

Comparing the likelihood of COTS transitioning to feeding on different coral species, binomial generalised linear mixed effects models suggested significant differences between the coral treatments (χ^2 = 10.58, DF = 2, p = 0.0050). By 175 d the probability of transition was 0.38 when offered A. millepora, 0.51 with A. tenuis and only 0.07 (2 specimens) in the presence of S. pistillata. The probability of transition was not statistically different between the two Acropora species (Tukey's post hoc test, z = 1.141, p = 0.4803), whereas availability of both A. millepora (z = -2.506, p = 0.0313) and A. tenuis (z = -3.245, p = 0.003) resulted in significantly higher probabilities of transitioning to coral when compared to S. pistillata (Fig. 4). Thus, species available in the environment will have an important influence on the transition probability.

The time for $\sim$ 50% to transition in the presence of A. tenuis (191 vs 175d) is comparable between the two experiments, albeit slightly earlier in the second experiment. There were no significant differences in the size of COTS at the start of each experiment (Welch two-sample t-test, $t_{34.339}$ = -0.70686, p = 0.4844), suggesting this difference was not because of variation in the sizes of COTS going into the experiments. The size of COTS that transitioned to A. tenuis was also similar across experiments, and similar to previously reported transitions around four months post-settlement at 8 mm in diameter (Yamaguchi 1974). The consistent results across different cohorts separated by several decades indicate that these values could be used to predict the behaviour of transitioning COTS, making this an important finding for COTS management and population modelling, as we have identified the age and size COTS enter the exponential growth period of their life and the point they become a threat to corals via predation.

Overall, mortality in the second experiment was 20.8% (25 total), with no significant differences between the different treatments (Pr(>|z|) > 0.05, details in Supplementary Table 2). In the first experiment, no mortality was observed among COTS. It is likely that the difference in mortality was due to the different experimental set-ups – less COTS in total (24 compared to 120) and only one COTS per tank compared to the 10 per tank in the second experiment, or potentially slight differences in batch quality. No escaped COTS were observed around the outsides of tanks or near the outlets, so it is likely that they died. Deaker et al. (2021) reported that within the first two-months of being offered coral, 37.8% of their 10-mo juveniles had been injured or killed by the Acropora sp.,

Fig. 2 A The probability of Crown-of-Thorns Seastar (COTS) transitioning to feeding on *A. tenuis* in the first experiment based on a modified Kaplan–Meier model. Green shading represents the 95% confidence interval. **B** Age and size of juvenile COTS when they first transitioned from feeding on crustose coralline algae to coral. Dashed line indicates the age COTS were introduced to the experiments, prior to which they had been feeding solely on CCA
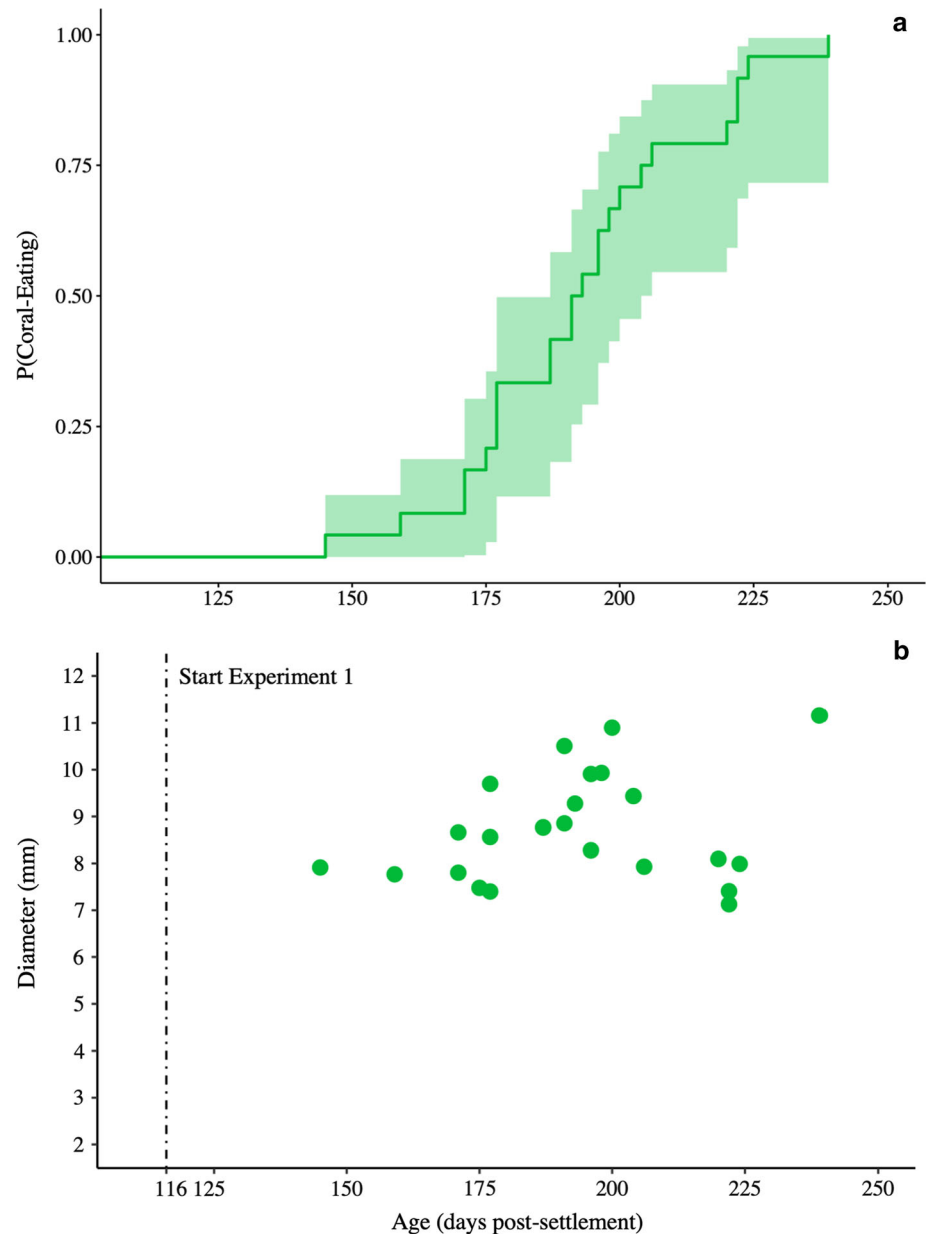
while Wilmes et al. (2019) found that in the wild, smaller COTS (3 mm) had higher incidence of predatory damage compared to larger (12 mm) juveniles from the same habitat. Mortality rates in wild juveniles have been reported as high as 2.6% d^{-1}, with most of it (73%) attributed to the effect of predators (Keesing et al. 2018). Since there were no predators in our experimental tanks, it is likely that the source of mortality in this study was injuries sustained from coral defences while transitioning to a coral diet (Deaker et al. 2021).

COTS that transitioned to feeding on *A. tenuis* (n = 18) and *A. millepora* (n = 12) were similar in size, 7.68 mm ± 0.31 mm and 7.48 mm ± 0.32 mm, respectively, while the two that transitioned to *S. pistillata* had a

larger mean size at 8.43 ± 0.10 mm (Fig. 3). These results emphasize that the availability of coral species plays a role in COTS diet transition. This follows past research suggesting some coral species (e.g., Echinopora sp.) can be lethal to COTS juveniles during transition (Johansson et al. 2016; Deaker et al. 2021), though even preferred *Acropora* corals can cause damage or mortality in COTS (Deaker et al. 2021). *Stylophora* coral, demonstrated here to delay a transition to coral diet, has previously been shown to be non-preferred food for juvenile COTS (Johansson et al. 2016). Some papers have suggested it is an important food source of adult COTS (Pratchett 2007; Pratchett et al. 2017), while other research suggests it is not preferred but also not actively avoided (Pratchett et al. 2014). The
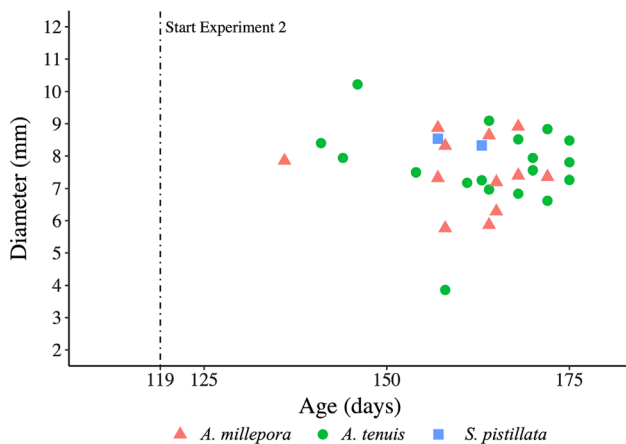
Fig. 3 Age and size of juvenile COTS when they first transitioned from feeding on crustose coralline algae to coral when offered *Acropora millepora*, *Acropora tenuis* and *Stylophora pistillata*. Dashed line indicates the age COTS were introduced to the experiment, prior to which they had been feeding solely on CCA
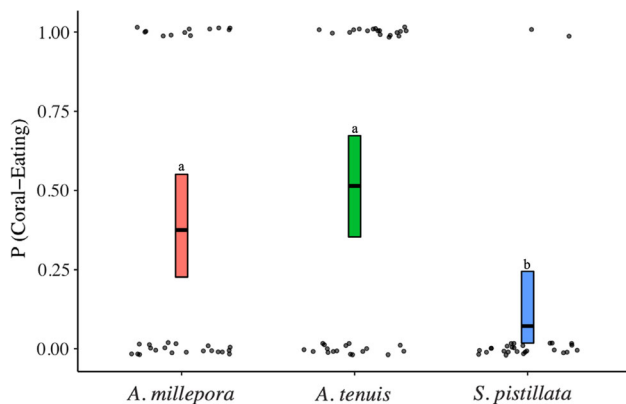

Fig. 4 Probability of juvenile COTS transitioning to feeding on different coral species at 175 days. Bars represent 95% confidence intervals, letters are significance levels from Tukey's HSD and dots represent status of individual COTS, '0' for non-coral eating, '1' for coral eating

number of nematocysts reported for *Acropora* and *Stylophora* is similar ($\sim 20^4$ cm^{-1}), though the haemolytic activity of *Stylophora* is 600-fold higher than that of *Acropora* (Ben-Ari et al. 2018), and it has been suggested that this haemolytic activity is associated with defence against predators (Ben-Ari et al. 2018). This offers a hypothesis as to why COTS prefer certain coral species over others, which should be investigated in future experiments.

Here we have demonstrated that COTS must reach a size of 7.5 – 8.5 mm in diameter before transitioning to coral diets, and that this observation was consistent across two distinct larval cohorts and previous aquarium studies (Yamaguchi 1974). Under laboratory conditions, the

probability of transition equals zero for animals < 136d, independent of food supply, while with preferred food available juveniles have a 0.5 probability of transitioning after 175–192 d, and 1 after 236 d. We observed that transition was delayed when less attractive food was available, providing data needed for population dynamic modelling. Future research could test transition probabilities along gradients of coral density and different species composition, and relate this to field data. This could be used to test for a potential undescribed prey/predator feedback mechanism and elucidate the likelihood of a 'hidden army' of long-term CCA feeding COTS standing by to seed the next outbreaks (Deaker et al. 2020b).

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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