

Growth of the herbivorous juvenile COTS phase and potential for adult-juvenile semiochemical communication

Maria Byrne, David Beale, Scott Cummins, Symon Dworjanyn, Benjamin Mos and Cherie Mottie



Great Barrier
Reef Foundation



Growth of the herbivorous juvenile crown-of-thorns starfish (COTS) phase and potential for adult-juvenile semiochemical communication

Maria Byrne¹, David Beale², Scott Cummins^{3,4}, Symon Dworjanyn⁵, Benjamin Mos⁶, Cherie Mottie⁷

1. School of Life and Environmental Sciences, The University of Sydney, Camperdown, NSW 2006, Australia
2. Environment, Commonwealth Scientific and Industrial Research Organisation, Ecosciences Precinct, Dutton Park, QLD 4102, Australia
3. Centre for Bioinnovation, University of the Sunshine Coast, Maroochydore, QLD 4556, Australia
4. School of Science, Technology and Engineering, University of the Sunshine Coast, Maroochydore, QLD 4556, Australia
5. National Marine Science Centre, Southern Cross University, PO Box 4321, Coffs Harbour, NSW 2450, Australia
6. Moreton Bay Research Station, School of the Environment, The University of Queensland, Dunwich, Minjerribah, QLD 4183, Australia
7. The Australian Institute of Marine Science, Townsville, QLD 4810, Australia

COTS Control Innovation Program | A research and development partnership to better predict, detect and respond to crown-of-thorns starfish outbreaks



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Reef Foundation



Inquiries should be addressed to:

Maria Byrne

School of Life and Environmental Sciences, The University of Sydney, Camperdown, NSW 2006, Australia

maria.byrne@sydney.edu.au

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The COTS Control Innovation Program extends its deepest respect and recognition to all Traditional Owners of the Great Barrier Reef and its Catchments, as First Nations Peoples holding the hopes, dreams, traditions and cultures of the Reef.

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Acronyms and Abbreviations

AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
CCA	Crustose Coralline Algae
CCIP	Crown-of-thorns starfish Control Innovation Program
COTS	Crown-of-thorns starfish
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CT	Constant Temperature
edf	Estimated Degrees of Freedom
FSW	Filtered SeaWater
GAMM	Generalised Additive Mixed effect Model
GBR	Great Barrier Reef
HOV	Homogeneity of Variance
LMM	Linear Mixed Model
ML	Maximum Likelihood
MS	Mass Spectrometry
QToF	Quadrupole Time-of-Flight
RNA	RiboNucleic Acid
SE	Standard Error
SIMS	Sydney Institute of Marine Science
TFA	Trifluoroacetic Acid
uHPLC	ultra-High Performance Liquid Chromatography
USC	University of the Sunshine Coast
USyd	University of Sydney
UV	Ultraviolet
VIF	Variance Inflation Factor

EXECUTIVE SUMMARY

The crown-of-thorns starfish (COTS, *Acanthaster cf. solaris*) is a coral predator that, in population outbreaks, causes major coral loss in Indo-Pacific reefs. Current paradigms to explain the cause of outbreaks focus on larval and adult stages. The early herbivorous juvenile stage of COTS and its influence on outbreaks remain poorly understood in this species. The biology and ecology of early juvenile COTS are crucial to understand because they are resilient to food scarcity, exhibit marked growth plasticity and can survive as algae-eating juveniles for years. This is the basis of the juveniles in waiting hypothesis as a contributing explanation of COTS outbreaks and provides the context for this project (CCIP-P-03) as part of the COTS Control Innovation Program.

We investigated the biology of the herbivorous juvenile stage of COTS in three main research areas: 1) growth and ontogenetic change in juvenile morphology from settlement to determine if morphometrics can be used as an aging tool – essential to model COTS population dynamics, 2) juvenile behaviour with a focus on the potential for adult-juvenile semiochemical communication, and 3) the application of ‘omics technologies (transcriptomics, proteomics, lipidomics and metabolomics) across juvenile ontogeny to characterise the biochemistry and molecular biology of their chemosensory biology and potential identification of agents as new avenues for chemical control of COTS.

Juvenile COTS have distinct morphological traits yet a detailed understanding of the ontogenetic changes in these traits is lacking. Given the potential to remain as herbivores for years at growth stasis, a more detailed assessment of their age and growth dynamics is needed. An ability to age juvenile COTS in the field is key to understanding the contribution of cohorts of juveniles to outbreaks. In a model test approach, we addressed the hypothesis that a suite of morphological traits might be useful to determine the age of the juveniles. We asked if specific features can be linked to their age. We documented growth and trait change in a large population of juveniles from settlement to 10 months of age. Ontogenetic changes in eight traits over time were quantified, including those used previously with COTS (total diameter, arm number) and traits not previously quantified (e.g. spine number and type). Combinations of traits were modelled against age to explore their potential to be used as indicators of age. Total diameter exhibited a strong association with age, as did covarying traits: central disk diameter, mouth diameter and arm length. Our results indicate that a combination of morphological traits has potential as an indicator of juvenile up to 10 months of age. In models using traits that did not covary, the number of pointed spines exhibited a strong linear effect on age. This trait has potential as an age marker for field caught juveniles. Our results will inform age modelling of juvenile COTS using morphological traits, contributing to the understanding of the biology and ecology of this cryptic life stage.

We addressed major knowledge gaps in our understanding of the sensory abilities of juvenile COTS. This is important because intraspecific and habitat-mediated chemical cues can play major roles in structuring marine populations especially for species like COTS where juvenile and adult habitats and diets differ. Juvenile COTS live in coral rubble-crustose coralline algae (CCA) which serves as their nursery habitat providing food and protection as they grow to

become competent coral predators. The juvenile nursery habitat is separated spatially from the adult habitat. As for other predatory sea stars, there may be a feedback, whereby adult COTS suppress juvenile transition to the adult phase. We investigated the behaviour of herbivorous juvenile COTS in flow-through choice chambers to determine if chemical cues from their habitat influences movement and their transition to become coral predators. Juveniles at the diet transition stage were exposed to cues from their nursery habitat (coral rubble covered with CCA), live coral and adult COTS to determine if waterborne cues influence movement. In response to CCA and coral as sole cues the juveniles moved towards the cue source and, when these cues were presented in combination, they exhibited a preference for coral. The juveniles moved away from adult COTS cues indicating the presence of an avoidance semiochemical, communication between adults and juveniles. Exposure to food cues (coral, CCA) in the presence of adult cues resulted in variable responses. Our results show the strong chemosensory ability of juvenile COTS and their innate behavioural responses to cues that they had not previously experienced in their life and suggest an adult-juvenile feedback mechanism mediated by chemical communication. Cues from the adult population may deter juveniles from the switch to corallivory. As outbreaks wane, juveniles released from competition may serve as a proximate source of outbreaks.

Intraspecific communication mediated by chemical cues is well known to occur between adult COTS (e.g. feeding attractants, reproductive pheromones) and the results of our behavioural study provided evidence for adult-juvenile semiochemical communication. The trait-age study indicated the potential ability to age juvenile morphologically, but they may also have age-related biochemistry. These observations point to the need to better understand the sensory abilities and biochemistry/molecular biology of the juveniles. In an integrative omics investigation, the transcriptome, proteome, lipidome, and metabolome of juvenile COTS from very early life stages (2–4 months) to older (up to 2.5 years) juveniles are being characterised with a focus on the small biochemical molecules and proteins that may be associated with their sensory/communication biology. We will also determine when the juveniles start producing toxins and thereby have enhanced protection from predation. The latter is likely to be related to age and along with other chemical markers may be useful as age markers. The semiochemical communication between the adults and juveniles indicated the presence of agents produced by the adult that deters the juveniles from making the ontogenetic switch to corallivory, and that the juveniles have the sensory capacity to detect these agents. This points to the potential to develop new avenues for push-pull semiochemical control of COTS to investigate prospective control agents.

1. INTRODUCTION

The crown-of-thorns starfish (COTS, *Acanthaster cf. solaris*) is a coral predator that, in population outbreaks, causes major coral loss in the Great Barrier Reef (GBR). Current paradigms to explain the causes of outbreaks focus on larval and adult stages (Pratchett et al. 2014). Recent research points to the potential role of reservoirs of juveniles that may accumulate on the reef infrastructure over several years in driving outbreaks (Deaker et al. 2020a). This is the basis of the ‘juveniles in waiting’ hypothesis. Over multiple recruitment events, cohorts of juveniles have the potential to accumulate as a ‘hidden army’ until suitable conditions manifest, serving as a proximate source of outbreaks.

For predatory sea stars, intraspecific and habitat-mediated chemical cues can play a major role in structuring populations especially for species like COTS where juvenile and adult habitats and diets differ (Nauen 1978; Barker 1979; Byrne et al. 2021). Juveniles of several sea star species have been shown to remain in their recruitment-nursery habitat for some time at growth stasis while they subsist on local resources before migrating to the adult habitat where they switch to a new diet and grow to maturity (Nauen 1978; Byrne et al. 2021). Juvenile COTS live in coral rubble-crustose coralline algae (CCA) habitat which serves as their nursery habitat providing them with food and protection as they grow to become competent coral predators. They feed on CCA as their preferred food but can also subsist on algal films in controlled laboratory conditions (Deaker et al. 2020b). As found for other predatory sea stars (Nauen 1978; Byrne et al. 2021), COTS juveniles may wait for prey to become available and potentially a reduction in competition from adults before they make the switch to a coral diet. This delay is suggested to indicate a negative density dependent feedback control of population dynamics that may involve adult-juvenile semiochemical communication (Byrne et al. 2021, 2023).

Juvenile COTS are cryptic in the reef infrastructure and so are difficult to detect and study (Wilmes et al. 2020a). We have a poor understanding of the spatial distribution of juvenile COTS in nature and the conditions that they experience. Despite extensive searches including destructive sampling efforts, herbivorous juvenile COTS are rarely seen (Moran 1986; Doherty and Davidson 1988), although a large population of juveniles was observed on a reef flat in Fiji (Zann et al. 1987) and a recent study on the GBR located juveniles on rubble in forereef habitat (Wilmes et al. 2020b).

The biology and ecology of juvenile COTS are crucial to understand, and the research undertaken in this project (CCIP-P-03) focused on this life stage. We investigated three main research areas: 1) growth and ontogenetic change in juvenile morphology to determine if morphometrics can be used as an aging tool and to inform models of COTS population dynamics, 2) juvenile behaviour with a focus on the potential for adult-juvenile semiochemical communication, and 3) the first application of multi-omics technologies across juvenile ontogeny to characterise the biochemistry and molecular biology of their chemosensory and communication biology and potential identification of agents as new avenues for biocontrol of COTS.

1.1 Ontogenetic change in juvenile morphology

The timing and size at which juveniles undergo the ontogenetic shift from herbivory to corallivory varies depending on the availability of preferred coral prey and other factors (e.g. sublethal predation) (Johansson et al. 2016; Wilmes et al. 2020a; Deaker et al. 2021; Neil et al. 2022). Juvenile COTS can transition to a coral diet as early as four to six months of age and 8–10 mm diameter (Yamaguchi 1973; Lucas 1984). On an algal diet in the absence of coral prey, juveniles reach a maximum size of ~20 mm diameter which can be followed by a prolonged period of growth stasis that can last for years (Lucas 1984; Deaker et al. 2020a). In nature, juvenile size at the diet switch to corals is highly variable (Zann et al. 1987; Wilmes et al. 2020a). A study on the GBR found juvenile COTS as large as ~24 mm diameter living on CCA and a tracking study in Fiji found large juveniles (10–32 mm diameter) in CCA habitat (Zann et al. 1987; Wilmes et al. 2020a). These juveniles may have been several years old. The age-size disconnect, where the age of individual juveniles cannot be discerned from their size, presents a challenge to modelling growth in COTS.

A detailed understanding of the ontogenetic changes in the morphology of juvenile COTS is lacking. The morphological development of juvenile COTS has been followed in laboratory studies, two of which documented growth from metamorphosis in a small number (6–14) of juveniles (Yamaguchi 1973; Lucas 1984). These studies used total diameter and the number of arms to quantify size and growth. In this project, we documented development of a large laboratory population of juveniles from settlement to the advanced herbivorous juvenile (300 days post-settlement) and quantified change in eight morphological traits. These traits included previously used parameters (e.g. diameter, arm number) and those not previously quantified (e.g. spine type and number). We sought to identify markers/traits either individually or in combination that vary regularly over time for potential use as age markers. An ability to age juvenile COTS using morphological features would greatly enhance our understanding of their biology and post-settlement processes, as well as the potential of juveniles that have accumulated in their coral rubble habitat to generate outbreaks (Wolfe and Byrne 2024).

In a model test approach, we addressed the hypothesis that a suite of combined traits might be useful as an indicator of juvenile age. Addressing knowledge gaps on juvenile COTS to inform age modelling using morphological traits, contributes to the understanding of the biology and ecology of this cryptic life stage.

1.2 Juvenile behaviour

The sensory biology and chemical ecology of larval and adult COTS are well studied (Pratchett et al. 2014; Caballes and Pratchett 2017; Motti et al. 2018). Adult COTS can respond to chemical cues originating from a distance without direct contact with the source (Caballes and Pratchett 2017; Motti et al. 2018). There is strong evidence that the population dynamics of COTS is mediated by intraspecific semiochemical communication, where signals released by individuals convey information that elicits a behavioural change in conspecifics (Motti et al. 2018). In contrast, the sensory biology and chemical ecology of the

juvenile stage are poorly known. This gap in knowledge is addressed in this project (CCIP-P-03) with a focus on the environmental cues underlying habitat recognition and the transition from herbivory to corallivory.

There is considerable interest in the development of targeted mitigation strategies, beyond current adult culling approaches, to address the environmental and economic impacts of COTS, including the potential use of semiochemicals (Motti et al. 2018; Høj et al. 2020). Thus, it is important to understand the sensory ability of juvenile COTS and role of chemical cues in the diet transition to coral. Responses to environmental olfactory cues has applications in the design of pest management strategies to disrupt nuisance species (Sorensen and Johnson 2016; Kamio et al. 2022). There is interest in identifying species-specific biochemical agents that could be used in COTS control (Høj et al. 2020; Harris et al. 2025).

We investigated the behaviour of juvenile COTS in response to a range of waterborne chemical cues that they had not previously experienced to determine if innate behaviour may be involved in habitat 'recognition'. These experiments involved juveniles at the size/age for transition to corallivory. It is important to understand what promotes the herbivorous juveniles to become coral predators and we investigated the potential that chemical ecology is involved. Using flow-through choice chamber experiments we provided the juveniles with cues from coral rubble covered with CCA (nursery habitat), live coral and adult COTS in various combinations to determine their ability to respond to waterborne chemical stimuli. We assessed if these cues can be detected by the juveniles and influence their 'decision' with respect to movement, direction, and locomotory speed towards or away from the source of the cue. Juvenile COTS can detect the presence of algae and live coral (Johansson et al. 2016) and so we expected that they would be attracted to olfactory information from these sources. With respect to adult COTS, on one hand, we expected that the juveniles would be attracted to the adults as a signpost to the presence of coral prey, and on the other hand, that they may be repelled by the adults to avoid competition, indicating density dependent feedback in migration to the adult habitat and diet. The research is novel in characterising juvenile-adult interactions.

1.3 Ontogenetic change in juvenile biochemical profile

The crown-of-thorns starfish is a highly specialised sea star known for the unique sensory biology, morphology, biochemistry and molecular biology of the adult stage (Garm et al. 2017; Mendoza-Porras et al 2023; Smith et al. 2023). There is a strong interest in the biochemistry of COTS throughout their life cycle, particularly in relation to bioactive and semiochemical sensing molecules. Research on the sensory behaviour of adult COTS and characterisation of the genome, metabolome, lipidome and transcriptome has provided important insight into their biology and potential products that may be useful as control agents of COTS (Hall et al. 2017; Harris et al. 2025). In contrast to the adult stage there are no studies on the biochemical profile of juvenile COTS. In the first multi-omics study of juvenile COTS we are detailing their metabolome, lipidome, proteome and transcriptome.

It is important to characterise the biochemistry and molecular biology of the juveniles as they age to better understand the interaction with the adults with respect to sensory reception and semiochemical sensing molecules. There is intense interest in COTS toxin biochemistry and at what stage the juveniles start producing toxins and thereby experience increased protection from predation. The presence of metabolites, lipids and proteins associated with asterosaponin and plancitoxin (sea star toxins) production (Shiomi et al. 2004; Stonik et al. 2020; Mendoza-Porras et al. 2023) are being investigated.

Research on juvenile biochemical profile in collaboration with the CCIP-R-11 project (Motti et al. 2025) may lead to the discovery of novel semiochemicals as new avenues for control of COTS, with potential to interrupt the juvenile diet transition to corallivory. This research is also enabling a comparative study with the biochemical profile of the adults for which an extensive data set is available (e.g. Motti et al. 2025). As adult COTS have a highly specialised biochemistry, we expect that juveniles will also have unique and interesting biochemical features. It will be important to know which chemicals act on juveniles, which act on adults, and which act on both life stages.

1.4 CCIP Program, impact pathway and expected benefits.

Project CCIP-P-03 in the Prediction Subprogram of CCIP (**Figure 1**) is the most comprehensive study to date on the herbivorous juvenile stage of COTS. Our work contributes to the fascinating story of these small starfish and has attracted considerable interest across the full range of stakeholders and the public through diverse communication tools.

The challenge for COTS management is to obtain the best information possible to guide actions and interventions and to communicate these initiatives to government, stakeholders and the interested public. In CCIP-P-03 we have generated new data on the biology of the juvenile herbivorous stage of COTS addressing gaps in our understanding of the very early benthic life stage.

Outcomes from this project provide new data to reassess paradigms used in COTS management and can be used to inform models of COTS population dynamics that guide approaches to suppression. New knowledge on the sensory behaviour and biochemical profile of juveniles points to new avenues for innovative biochemical control. While more research is needed to understand their behaviour in nature, our findings indicates that adult-derived semiochemical agents may suppress the transition of juveniles from herbivory to corallivory. These agents may be of interest for the COTS control program as potential 'push' agents.

Knowledge of the juvenile stage is crucial because controlling populations at this vulnerable point in the life cycle could be transformative for outbreak management. The research affirms the need for incorporation of the population dynamics of the juvenile stage in the modelling programs that are being used to inform the integrated pest management program for COTS. The findings of the growth analysis may inform modelling of the age of juveniles in year one. Trait analysis of juveniles maintained for years (e.g. 6+ years, Deaker et al. 2020a) is needed

to have a better understanding as to how changes in the first year are expressed in older herbivorous juveniles that may reside in reef rubble habitat (Wolfe and Byrne 2024). Overall, outcomes of CCIP-P-03 may inform current approaches to monitoring and surveillance with respect to the juvenile stage.

The research undertaken in CCIP-P-03 had the following major aims:

1. Assemble time series images of juvenile COTS as they grow to identify age/size related morphological traits from the newly settled larva to the advanced juvenile.
2. Contribute to a trait-based understanding of the biology and ecology of juvenile COTS by quantifying a broad range of morphological traits.
3. To identify morphological traits that, either individually or in combination, can be used as an indicator of juvenile age independent of juvenile size to inform COTS control models.
4. To determine if juvenile COTS have innate behavioural responses mediated by chemosensory reception.
5. To determine if the diet and habitat transitions in juvenile COTS are influenced by chemical cues.
6. Characterise juvenile-adult interactions to determine if cues from adults influence juvenile behaviour (the possibility of semiochemical communication).
7. To characterise the biochemical profile of juvenile COTS to determine if there is an age-specific biochemical profile thereby complementing the morphological trait study.
8. To interrogate the juvenile COTS 'omics array data for potential agents that may be useful for COTS biocontrol.

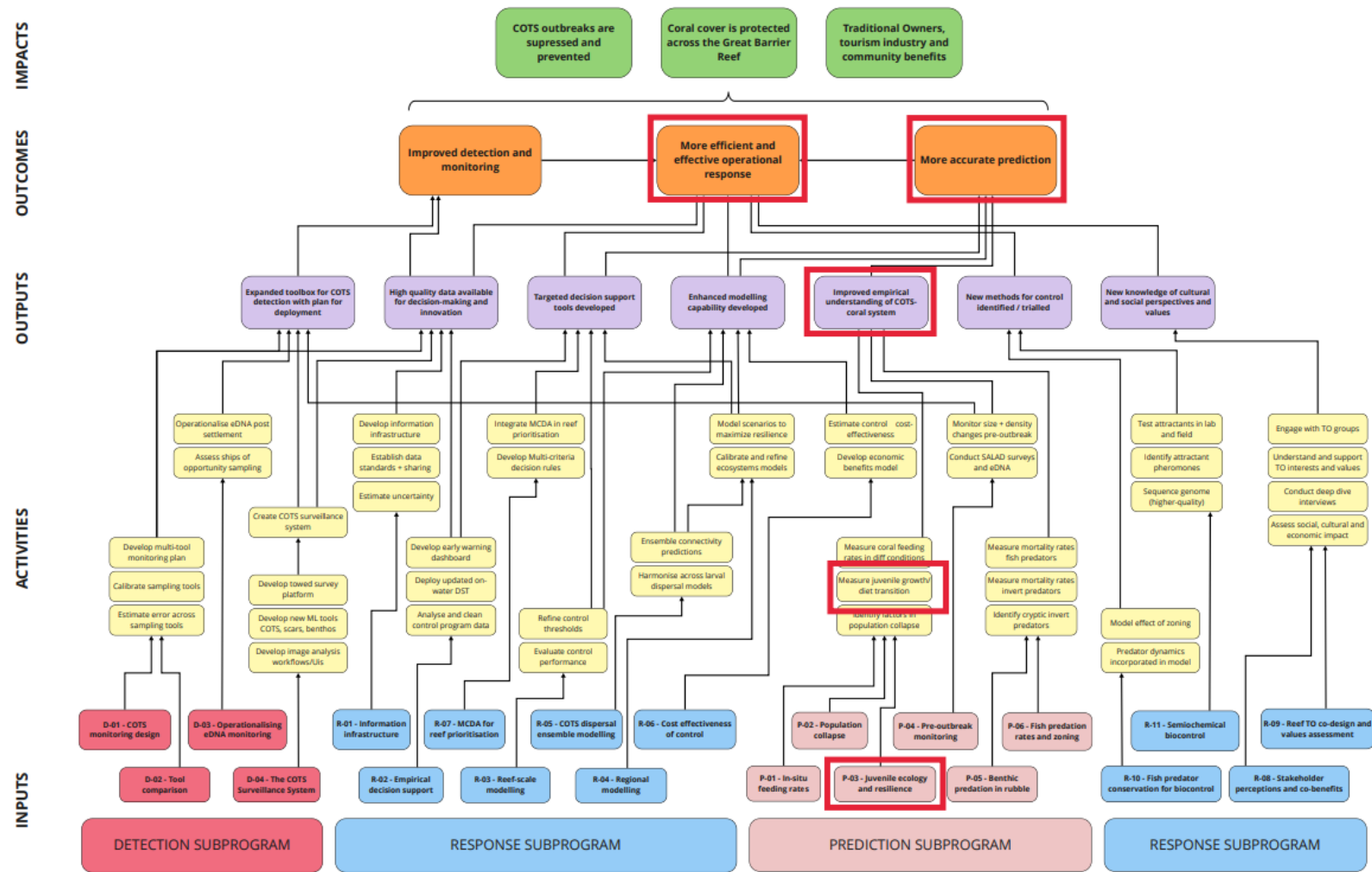


Figure 1. CCIP-P-03 program logic model detailing impact pathway.

CCIP-P-03

2. METHODS

Mature COTS, collected by the COTS Control team were shipped to Sydney and maintained as broodstock at the Sydney Institute of Marine Sciences. They were used to obtain gametes to establish larval cultures following routine methods (Clements et al. 2022). Larvae were reared in aerated 1 L beakers of ultraviolet (UV) sterilised 1 μm filtered seawater (FSW) at 26°C in a constant temperature (CT) room and fed *Proteomonas sulcata* (20,000 cells mL^{-1}) every two days. When they reached the competent brachiolaria larval stage (16 days post-fertilisation), settlement was induced using coralline algae, *Amphiroa* sp. The juveniles were reared in the CT room at 25–26°C in UV sterilised 1 μm FSW and fed small pieces of *Amphiroa* sp. They were initially placed in individual wells (5 mL) in 12-well culture dishes where they were reared up to (~3 mm diameter) and then moved to wells (10 mL) in 6-well dishes. As the juveniles grew, they were moved into small (10–20 mL) and then large (150–250 mL) dishes. By 6–8 months, juveniles were placed into 100–200 mL glass culture dishes. Food and FSW were renewed every three days, while culture dishes were replaced weekly.

All data analyses (see below) were carried out using R version 4.3.3 (R Core Team 2024).

2.1 Ontogenetic change in juvenile morphology

We maintained a population of 110–150 juveniles for over 10 months. The juveniles were randomly selected ($n = 25\text{--}35$ per ~30 days, 7–300 days) for photography using an Olympus dissecting microscope with an attached DP23 digital microscope camera and Olympus CellSens software. In total, ~1,250 photographs were used for this study. The photos were analysed using ImageJ (v1.53k) with eight traits measured (**Figure 2**): 1) total diameter (arm tip to arm tip), 2) disk diameter, 3) mouth diameter, 4) arm length (from the oral side) and the number of 5) arms, 6) arm buds, 7) pointed spines, and 8) marginal spines). Arm length and the number of pointed and marginal spines were measured using the three longest arms and the mean datum was used for analysis. Data for some traits (pointed spines, marginal spines, mouth diameter, disk diameter, arm length) were not assessed in the smallest juveniles (≤ 60 days age) as they were not present or were difficult to resolve (e.g. pointed spines, disk diameter) and to reduce handling of the very small juveniles.

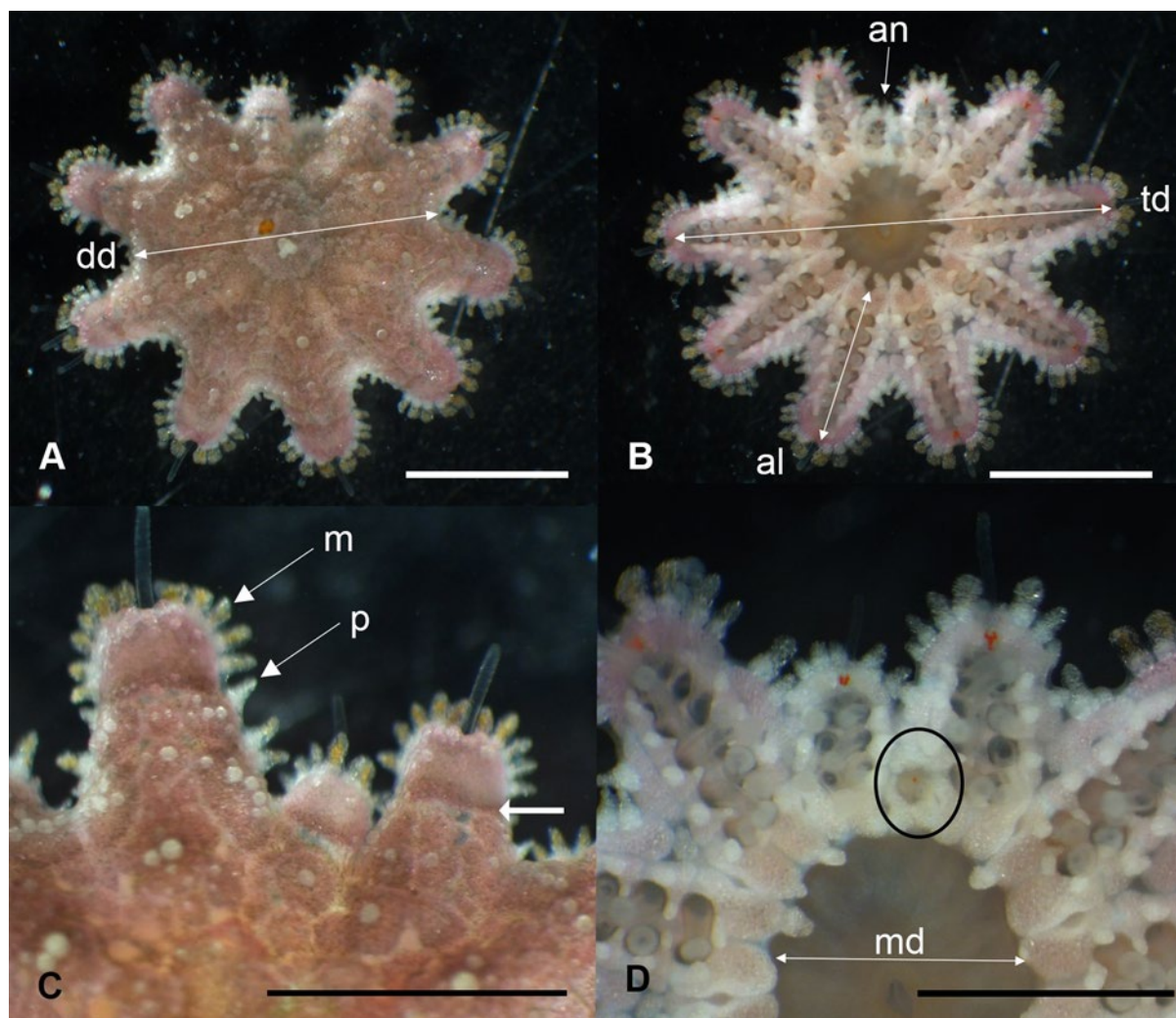


Figure 2. Morphological traits measured shown in 180-day-old juveniles. **A, C**, Aboral surface. **B, D**, Oral surface. Al = arm length; an = new arm; dd = disc diameter; m = marginal spine; md = mouth diameter; p = pointed spine; td = total diameter. Bold white arrow (Figure 2C) indicates the border of the growth zone (growth zone band) on the arm. Black circle (Figure 2D) indicates arm bud. Scale bars = 2 mm.

In the first approach, the data on trait change over time were analysed for individual traits. Then, we explored if changes in combinations of multiple traits over time could be used to model the age of COTS juveniles. Univariate generalised additive mixed models (GAMMs) were fit using the ‘mgcv’ package 1.8.41 (Wood 2011) to explore the effect of time (the single smooth term predictor) on each of the eight morphological traits individually. These models do not constrain age-trait relationships to the shapes of pre-specified parametric classes (Yee and Mitchell 1991), allowing the data to determine the shape of the response curve. Where traits exhibited a linear relationship with time (indicated by GAMM-estimated effective degrees of freedom ~ 1) linear mixed effect models (LMMs) were instead fit to the data with the morphological traits as the response variable.

The global dataset contained some repeated measures. Thus, juvenile ID was modelled as a random effect in both the GAMMs and LMMs (random intercept). For all models, parameter estimates were obtained using the maximum likelihood (ML) approach, as estimates obtained using this method are more precise and bias is trivial at large sample sizes (Quinn and Keough

2002). Smoothing was conducted using the penalised thin plate spline method (Wood 2003). The basis dimension value for each trait (k) was selected such that k was minimised whilst still encapsulating the necessary dimensions of the underlying curve (Wood 2003). To avoid overfitting, the maximum k value was set to 4.

Initial data exploration revealed that the number of arms exhibited under-dispersion. Thus, this trait was modelled using a quasi-Poisson error distribution with a log link function. In quasi-Poisson models, variance is a linear function of the mean (Ver Hoef and Boveng 2007), allowing for modelling of under-dispersed data. All other traits were modelled using a Gaussian error distribution as the data were continuous.

The predicted values of GAMMs were plotted using the 'ggeffects' v1.2.2 (Lüdtke 2018) and 'ggplot2' v3.4.1 packages (Wickham 2016). Assumptions of normality of residuals and homoscedasticity were verified using the 'mgcv' package v1.8.41 (Wood 2011). LMMs were also plotted using 'ggplot2' v3.4.1 (Wickham 2016). Assumptions of linearity, homoscedasticity, normality of residuals and normality of random effects were confirmed using the 'performance' package v0.10.2 (Lüdtke et al. 2021).

To explore if morphological change could be used to indicate juvenile age, we used data for the eight traits as predictors against age in multivariate LMMs. Trait predictors were first examined using a matrix of pairwise scatterplots. The relationships between several traits were non-linear but monotonic. Further, some traits contained multiple observations of the same value. Hence, correlations between traits were examined using Kendall's τ -b, a correlation measure that does not assume the relationship between variables is linear (Quinn and Keough 2002) and can account for ties in rankings arising from multiple observations of identical values (Togashi and Sakai 2019).

LMM assumptions of linearity, homoscedasticity, non-multicollinearity and normality of residuals and random effects were checked using the 'performance' package v0.10.2 (Lüdtke et al. 2021). As there can be multicollinearity between three or more variables even if no pair has a particularly high correlation (James et al. 2013), the variance inflation factor (VIF) was used to assess collinearity. The VIF threshold used to indicate collinearity varies, with studies considering $VIF > 2.5$ (Johnston et al. 2018), > 5 (James et al. 2013) or > 10 (Quinn and Keough 2002) as violating this assumption. We used $VIF \geq 5$ as the multicollinearity threshold to balance the stability of model parameter estimates with the risk of excluding variables of interest due to correlation.

Total diameter, central disk diameter, mouth diameter and arm length were highly correlated ($\sim \tau > .80$; **Table A 1**) and exhibited multicollinearity ($VIF \geq 5$). As the total diameter trait (r) incorporates the central disk, mouth opening and arm length, we used total diameter as the proxy measure of individual size. Thus, we ultimately used five traits as predictors of age: total diameter, number of pointed spines, number of marginal spines, number of arm buds and number of arms. However, models containing both pointed spine and total diameter exhibited multicollinearity and so only one of these traits could be used in any one model. We fit models using the pointed spine trait as this trait has not been previously studied and holds promise as a useful trait.

Using the pointed spines, marginal spines, number of arms, and number of arm buds traits as fixed effect predictors, multivariate linear mixed models were fit using the 'lmerTest' package

v3.1.3 (Kuznetsova et al. 2017). Juvenile ID was modelled as a random effect and parameter estimates were obtained using ML estimation. Candidate models containing all subset combinations of the trait predictors were constructed and compared using Akaike information criterion (AIC) (in 'MuMIn' v1.47.5; Barton 2023).

A small-sample modification of AIC (AIC_C), appropriate when the number of observations is less than 40 times the number of estimated parameters (Burnham and Anderson 2002) was used for comparison between models. Lower AIC_C scores indicate a candidate model is a better fit for the dataset relative to competing models in the set. Traits from the best-ranked model of each trait group were standardised to estimate the relative strength of effects of predictors on the outcome variable (age; Schielzeth 2010; Siegel and Wagner 2022).

2.2 Juvenile behaviour

The behaviour experiments were conducted using a suite of cues including live coral, coral rubble encrusted with CCA, and adult COTS. The CCA covered rubble was collected from One Tree Island, GBR and maintained in a tropical aquarium at 26°C. Corals (*Acropora* sp.) were sourced from aquarium suppliers. Adult COTS were maintained in a 160 L tropical aquarium system at 26°C. Prior to the start of the coral and CCA rubble experiments these cues were maintained for 2 h in the header tank (25 L) source water before turning on the flow. The amount of rubble and live coral used covered the base of the header tank as similarly as possible between trials to emulate a layer of habitat in nature (**Figure A 2**). Adult COTS were placed in the header source for 30 min prior to the start of the experiment. During this time water circulation was maintained using a water pump.

The behavioural responses of the juveniles were tested in the CT room (26°C) using a two-channel choice flume (**Figure A 2**) starting when they were 11 months old. Over the duration of the replicate runs the mean diameter of the juveniles ranged between 9.4–12.8 mm diameter (Webb et al. 2024). Steady gravity-driven flow (8.3 cm³/s) in the flume was controlled by flow meters (Dwyer MMA series) and laminar flow was established at the inflow point by a filter pad lined with a mesh screen. To ensure that behaviour was not influenced by vision, the experiments were done under red light 625 nm (Ocean Optics, Spectrophotometer USB4000; Ocean View 2.0 Software) which exceeds the photoreception range of COTS (Hall et al. 2017). Prior to use in trials the juveniles were placed in clean containers of 1.0 µm UV FSW with no food for 24 hrs.

The water flow on each side of the flume was segregated by a divider which ensured parallel flow into the main chamber supporting a distinct distribution between the cue source flows and eliminated backflow (**Figure A 2**). Each side of the flume was supplied with water from a 25 L header tank, that contained a treatment cue. A single juvenile was placed in the centre of the main chamber where it would receive even flow from both sides of the flume. At the point of water outflow, a gate was installed to allow the juvenile to acclimate for one min in static water. The gate was then removed, and flow was turned on. Juveniles were allocated 20 min to respond as demonstrated by upstream movement towards the cue source or downstream movement. The treatment cues — live coral, CCA reef rubble and COTS — were tested individually against the FSW flow (no cue) and against each other (CCA/live coral; CCA/COTS; COTS/live coral). Each juvenile was used only once per treatment. Treatment cues were

rotated between left and right sides of the flume to remove a side bias. Images taken at 20 sec intervals after the acclimation period were captured with an Olympus Tough TG-6 (Olympus) camera and were then autogenerated into time lapse videos. The camera was mounted above the choice flume with the entire arena and scale in view.

Juvenile movement in the time-lapse files was analysed using Tracker (Version 6.0.1). Each file was calibrated in Tracker to display at a framerate of 1.5/s. Path length (m) was manually calculated from the starting point until the juvenile moved towards or away from the direction of flow. Tracking was used for single and two-cue treatments for the juveniles that remained in the field of view for the entire time and so could be completely followed (n: coral = 11, CCA = 14, COTS = 20, FSW = 8, CCA/Coral = 13, CCA/COTS = 12, Coral/COTS = 12). The paths taken by the juveniles were traced (**Figure A 3**).

A Chi-squared goodness of fit test was used to analyse the percentage frequency of the juvenile choice responses with equal expected proportions. Choice response data were analysed with 25%:25%:25%:25% probability of upstream movement to either treatment cue, downstream, or no movement. For the FSW only (control) the data were analysed with 33%:33%:33% probability of upstream movement, downstream movement, or no movement. The choice data were illustrated as the percentage of individuals in each response (e.g. to cue, downstream, no movement).

Tracking data on speed (mm/min), distance moved (mm) and time to make a choice (min) were analysed. The speed data were analysed by a one-way analysis of variance (ANOVA) with treatment as a fixed factor with four levels (attraction to coral, attraction to CCA, upstream movement in FSW only, avoidance to adult COTS) with the FSW as the control. Homogeneity of variance (HOV) and normality were confirmed by Levene's and Shapiro-Wilk tests, respectively (significance $\alpha = p < 0.05$). Tukey's HSD post hoc-pairwise comparison was performed to identify treatments that differed. Data on distance moved and time to make a choice did not meet the assumption of HOV, and so these data were analysed using Kruskal-Wallis non-parametric ANOVA with treatment type as a fixed factor. Dunn Test (R Package FSA) with Bonferroni corrected p-values was used post hoc to identify significant pairwise treatment effects.

For the two-cue experiments, data on distance moved, time to make a choice and speed were analysed by a one-way analysis of variance (ANOVA) with treatment as a fixed factor with three levels (CCA/live coral, CCA/COTS, live coral/COTS). Assumptions of HOV and normality were confirmed as above, except for normality of the distance moved data. As ANOVA is robust to this the analysis was undertaken and a Tukey HSD post hoc-pairwise comparison was performed.

2.3 Ontogenetic change in juvenile biochemistry and molecular biology

For metabolomics, lipidomics and proteomics, juveniles reared as above were snap frozen in liquid nitrogen, stored at -80°C, and shipped on dry ice to Dr David Beale (CSIRO) and Dr. Scott Cummins (University of the Sunshine Coast, USC). For transcriptomics, juveniles were preserved in RNAlater and shipped to Cummins, followed by RNA isolation using Trizol reagent (Thermo-Fisher) and RNA-seq using the Novogene (Singapore) RNA-seq Illumina sequencing

pipeline. Gene expression was analysed by mapping RNA-seq reads to the COTS genome. In addition, a *de novo* assembled transcriptome was prepared from juvenile RNA-seq reads using the CLC Genomics Workbench. For metabolomics and lipidomics, the tissues were freeze-dried and extracted, and the workflow followed as described in Mendoza-Porras et al. (2023) using liquid chromatography coupled with high-resolution mass spectrometry (e.g. LC-Triple Quadrupole Mass Spectrometer (LC-QQQ-MS) and LC-Quadrupole Time-of-Flight Mass Spectrometer (LC-QToF-MS) utilising a combination of targeted and untargeted profiles of polar metabolites and non-polar lipids.

Raw spectral datasets of metabolites and lipids were processed and analysed using MassHunter Profinder and relative abundance was determined using multivariate statistical analysis approaches. Significant metabolites and lipids will be mapped onto a metabolic network using the KEGG mapper. Asterosaponin analyses were independently carried out using an in-house asterosaponin database on the acquired QToF data (see Mendoza-Porras et al. 2023) and a new comprehensive asterosaponin database (Cummins and Motti, unpublished).

Protein pellets produced by the CSIRO team were sent to Cummins for proteomics (for detailed methods see Smith et al. 2023). COTS tissue extracts containing proteins were freeze-dried for 1 h. Following freeze-drying, 30 ml of 8M urea was added, vortexed and centrifuged at 4,000 xg for 25 mins. The supernatants were collected and divided into >10 kDa and <10 kDa samples using a Amicon® Ultra-15 Centrifugal Filter Unit (Merck). The <10 kDa and >10 kDa supernatants were stored at -80°C prior to lyophilisation using a Savant SpeedVac Concentrator (Thermo Fisher Scientific, MA, USA). The lyophilised <10 kDa and >10 kDa proteins were resuspended in 1 mL of 0.1% trifluoroacetic acid (TFA) and desalted using a Sep-Pak Plus C18 cartridge (Waters) to concentrate the samples and remove small molecules. An 8 mL aliquot of 0.5% acetic acid in 70% acetonitrile and 29.5% Milli-Q water was run through the column and the solutions were frozen at -80°C before lyophilisation. The lyophilised proteins were resuspended in 6M urea and the protein concentration was determined using UV spectrophotometry (NanoDrop ND-2000). Trypsin digestion of the samples was conducted and ultra-high performance liquid chromatography (uHPLC) tandem mass spectrometry (MS) was performed following trypsin digestion to identify proteins. Juvenile COTS proteins were identified against both genome- and transcriptome-derived protein models. Ongoing work is comparing metabolites, lipids and proteins across the COTS juvenile ages, and against the existing adult data.

2.4 Stakeholder Engagement

This project is the most comprehensive study to date on the development and behaviour of the herbivorous juvenile stage of COTS and our findings have attracted considerable attention across a range of stakeholders who we have interacted with. With respect to engagement with managers, we have liaised with the Great Barrier Reef Marine Park Authority (Reef Authority) throughout the project, largely through our main contact Dr. Jessica Stella. In direct communications with Dr. Stella, we have kept the Reef Authority up to date with our findings and provided access to information before finalising publications. Through our research on COTS, we have very good rapport with the Reef Authority.

We have been in close communication and worked closely with the COTS Control teams (INLOC and Blue Planet Marine). This has involved many email exchanges regarding collection of COTS and other queries. We have also met Control team members at CCIP meetings and on the Reef during a trip after the Reef Resilience Symposium. In 2023 under the Byrne permit and with the permission of the Reef Authority we arranged for the Blue Planet Marine team to do a sweep around the entire One Tree Reef. This was an important opportunity to generate key data for this highly protected reef and to confirm that COTS densities are low in this region. This was appreciated by all parties.

Our research provides a fascinating story about the hard to study juvenile COTS and has attracted considerable interest among stakeholders and the public. We have presented our findings at several forums in addition to the CCIP communication workshops. Press releases through the University of Sydney (USyd) and Sydney Institute of Marine Science media offices foreshadowing publication of our findings and popular science communication tools such as our article on the Conversation (<https://theconversation.com/young-crown-of-thorns-starfish-can-survive-heatwaves-thats-yet-more-bad-news-for-the-great-barrier-reef-215543>) have prompted enquiries by interested persons and the media. These products have generated extensive follow up communications in traditional media (TV and radio) including the Science Show with Robyn Williams (ABC Radio National; May 2024). We have also disseminated our findings in digital media (social media, online publishers) facilitating communications of research outcomes to the general community, managers and a broad range of stakeholders interested in the GBR. Our communication products are accessible and easy to grasp for public audiences.

Our CCIP research on juvenile COTS was presented at the Australian Coral Reef Society Conference (2023), the European Echinoderm Conference in Lyon (2023), the Reef Resilience Symposium (2024), International Echinoderm Conference (2024), Australian Marine Science Conference (2024) and in seminars (USyd).

3. RESULTS

3.1 Ontogenetic change in juvenile morphology

The relationship between total diameter and age was approximately sigmoidal (estimated degrees of freedom (edf) = 2.62; **Figure 3A**; **Table A 2**). At the beginning of the study, the week-old juveniles had a mean diameter of 0.63 mm (SE = 0.02, n = 29), increasing to 8.82 mm (SE = 0.14, n = 30) by 300 days age. The mean diameter of the juveniles at 90, 121, 137, 180 and 215 days post settlement was: 2.46 mm (SE = 0.10, n = 34, range 1.36–3.67 mm), 3.53 mm (SE = 0.12, n = 29, range 2.44–4.82 mm), 3.78 mm (SE = 0.15, n = 25, range 2.23–4.92 mm), 5.68 mm (SE = 0.16, n = 30, range 3.76–7.68 mm), 6.69 mm (SE = 0.18, n = 30, range 4.32–8.56 mm), respectively (**Figure 3A**). The first juveniles reached the lower limit of the threshold to transition to corallivory (~8 mm diameter) at ~215 days. At 272 days post-settlement, the mean diameter was 8.11 mm (SE = 0.15, n = 30). In the initial months, total diameter did not vary much across individuals, but with time became more variable (**Figure 3A**).

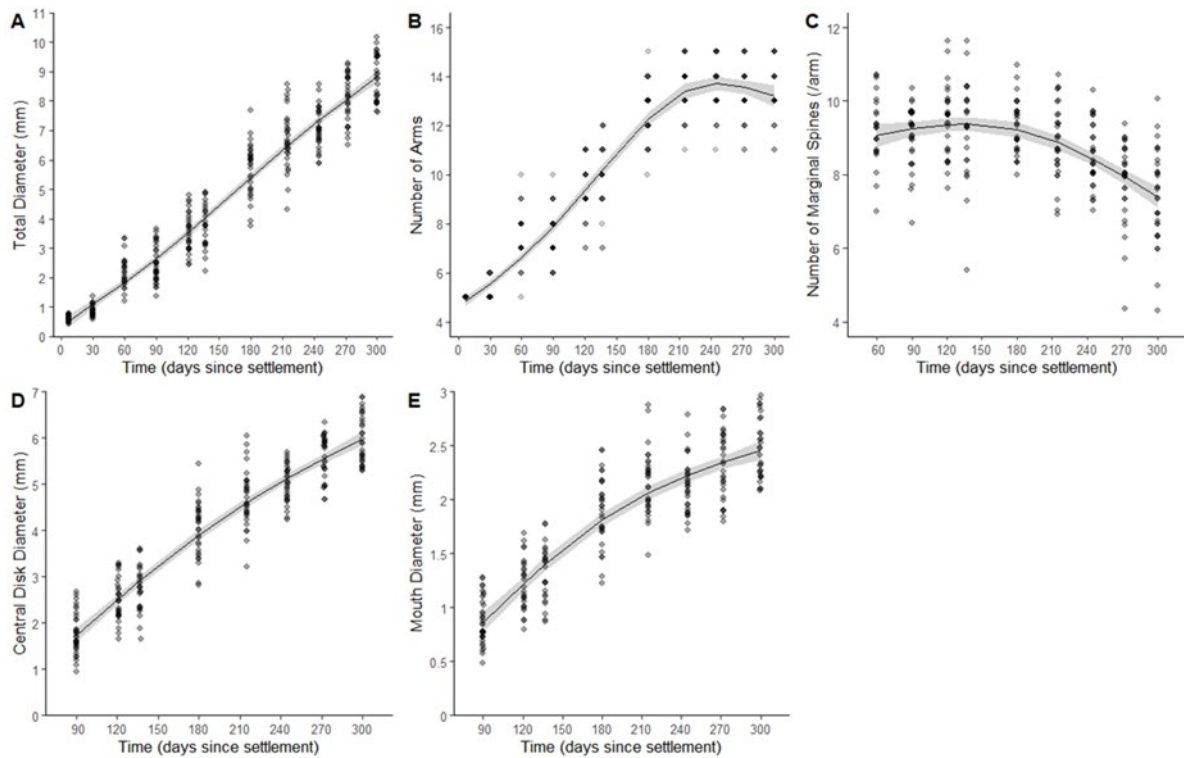


Figure 3. GAMM plots displaying relationships between **A.** total diameter, **B.** the number of arms, **C.** number of marginal spines, **D.** central disk diameter, **E.** mouth diameter and time in juveniles over 300 days post-settlement. Data points represent individual observations ($n = 317, 317, 263, 237$ and 235 for total diameter, the number of arms, marginal spines, central disk and mouth diameter, respectively). The grey shaded region indicates the 95% confidence interval. Data points are 'jittered' to avoid overplotting (width = 0.15, height = 0).

Between 30- and 215-days post-settlement, one arm was on average added every 21.6 days (0.046 days/new arm). Arm addition plateaued after 215 days post-settlement with a maximum of 15 arms observed (**Figure 3B**). The relationship between time and the number of arms was approximately sigmoidal (edf = 2.96; **Table A 2**).

The formation of a new arm was signalled by the appearance of an arm bud. After 30 days, the mean number of arm buds was 0.24 ($SE = 0.09$, $n = 25$). Only one arm bud was generally present at any one time (**Figure 2**). As the bud grew to form a new arm, a new arm bud appeared between the two most recently formed arms. By 90 days post-settlement, the mean number of arm buds increased to 0.82 ($SE = 0.07$, $n = 34$), remaining steady through 137 days ($\bar{x} = 0.83$, $SE = 0.08$, $n = 24$). The number of arm buds declined as the maximum arm number was reached around ~215 days. No arm buds were observed from 245 days post-settlement.

At 90 days, the average arm length was 0.81 mm (SE = 0.04, $n = 34$, range 0.45–1.19 mm) with little variation among individuals (**Figure 4A**). Arm length increased linearly with age (est. = 0.01 ± 0.0003 , $df = 163.7$, $t = 36.663$, $p < .001$, $n = 235$, $R^2_{\text{Marginal}} = 0.865$; **Figure 4A**), reaching a mean of 3.19 mm by 300 days post-settlement (SE = 0.07, $n = 30$, range 2.49–3.87 mm). Overall, variation in arm length was low in the early months and gradually increased with age (**Figure 4A**).

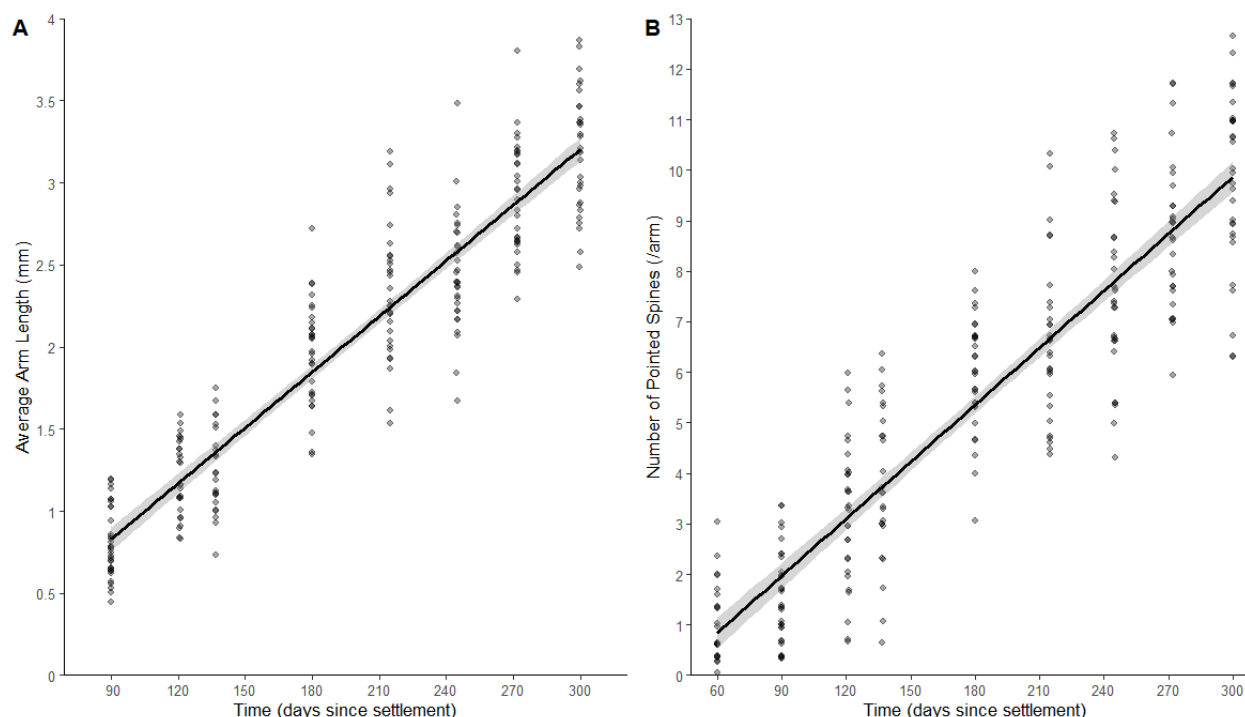


Figure 4. Linear mixed effects models showing relationships between **A.** average arm length ($n = 235$) and **B.** number of pointed spines per arm ($n = 263$) and time in COTS juveniles over 300 days post-settlement. The grey shaded region indicates the 95% confidence interval. Data points are 'jittered' to avoid overplotting (width = 0.15, height = 0).

The first spines that formed after metamorphosis were the fan-shaped (marginal) spines on the tips of arms (**Figure 5**). These function in attachment to the substratum. The pointed spines appeared later and eventually dominated the surface. In the first ~245 days the marginal spines outnumbered the pointed spines and were the dominant spine type present in early juveniles. Marginal spines were occasionally observed on the sides of arms, growing off fleshy nodules on proximal regions of the arm. In juveniles ~3.5 mm diameter, the marginal spines began to take on a yellow-orange hue as the colour of the juvenile changed from translucent to pink. The number of marginal spines remained relatively unchanged in the early months before decreasing past ~215 days post-settlement (**Figure 3C**). 60-day-old juveniles had an average of 9.22 (SE = 0.18, $n = 25$, range 7–10.67) marginal spines per arm and this was similar at 215 days ($\bar{x} = 8.91$, SE = 0.18, $n = 30$, range 7–10.67). These spines decreased by 300 days ($\bar{x} = 7.4$, SE = 0.23, $n = 30$, range 4.33–10).

From approximately 180 days post-settlement, the pointed spines became more prominent and so the juveniles increasingly resembled the thorny appearance of adults as they aged (**Figure 5**). The transition to a dominance of pointed spines was gradual and variable among individuals of similar sizes and ages. These spines were located around the arms, along the edges and

towards the ambulacral groove (**Figure 5**). The largest pointed spines were those along the sides of arms. By 90 days post-settlement, the juveniles had an average of 1.53 (SE = 0.15, n = 34) pointed spines per arm, increasing linearly with age (est. = 0.038 ± 0.01 SE, $df = 218.28$, $t = 32.984$, $p = <0.001$, $n = 263$, $R^2_{\text{Marginal}} = 0.822$; **Figure 4B**) to a mean of 9.81 (SE = 0.31, n = 30) by 300 days.

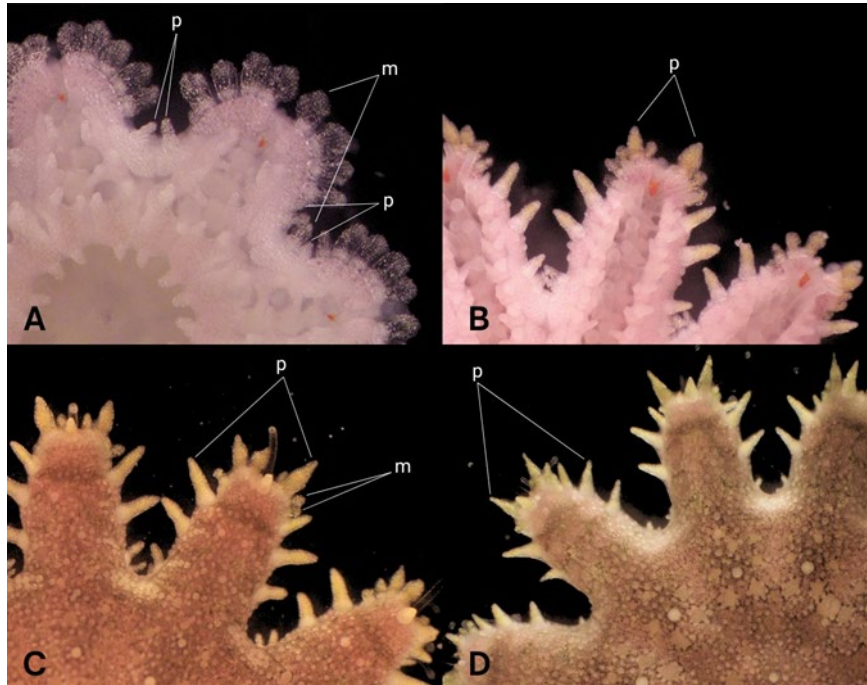


Figure 5. Spine development. **A.** Arm tip of an early juvenile showing the abundant marginal (m) and developing (p) pointed spines. **B.** Aboral view of the tip of the arms where two pointed spines (p) develop. **C.** Tip of the arm of an older juvenile with marginal (m) spines but now dominated by pointed (p) spines. **D.** With growth and age, the pointed spines (p) elongate and are prominent along the sides of the arm. Scale bars = 1 mm.

The transition to adult-like spines began with the lengthening of pointed spines on the sides of arms. The two marginal spines closest to the terminal tentacle groove became opaque and began to lengthen, transitioning to a diamond-like shape. These spines continued to lengthen as the juveniles aged, converging on the appearance of the pointed spines and conveying a two horn-like appearance at the arm tip (**Figure 5**). It seemed that these marginal spines transitioned to the structure of a pointed spine, although it is also possible that they dropped off and were replaced. As the pair of pointed spines at the tip of the arms developed, more pointed spines appeared on the sides of arms and around the growth zone, and those along the sides of the arms lengthened. Pointed spines also began to form on the aboral surface of the arm toward the end of the study. Variation in the number of pointed spines increased with age (**Figure 4B**), ranging from 6.33 to 12.67 per arm by 300 days post-settlement.

At 90 days post-settlement, the mean diameters of the central disk and mouth were 1.78 mm (SE = 0.08, n = 33, range 0.94–2.68 mm) and 0.87 mm (SE = 0.04, n = 34, range 0.49–1.28 mm), respectively. The growth patterns of the central disk (edf = 1.94; **Figure 3D**, **Table A 2**) and mouth (edf = 2.63; **Figure 3E**, **Table A 2**) diameters were monotonic positive but decreased marginally in growth rate toward the end of the study. Variation was lowest in the earlier months for both traits (**Figure 3D,E**). Central disk diameter increased to an average of

5.96 mm (SE = 0.09, n = 30, range 5.30–6.88 mm) by 300 days post-settlement, while mouth diameter averaged 2.47 mm (SE = 0.05, n = 30, range 2.08–2.97 mm).

Combinations of traits (pointed spines, marginal spines, arms, arm buds) were used to explore their use as an aging tool for the juveniles (**Figure A 1**). As pointed spines, total diameter, central disk diameter, mouth diameter and arm length exhibited multicollinearity, these traits could not be included as predictors in a single model (see methods). The first model (model 1) contained four traits (the number of pointed spines, marginal spines, arms and arm buds) and explained the largest percentage of total variance with respect to age ($R^2_{\text{marg}} = 0.870$; **Table 1**) of all candidate models. This model also exhibited a high AIC_{wt} (0.944), suggesting this was the best candidate model as indicative of age (**Table A 3**). The second ranked model (model 2) contained pointed spines, marginal spines and the number of arms and explained a high percentage of total variance with respect to age ($R^2_{\text{marg}} = 0.865$), but the AIC_{wt} was considerably lower (0.056; **Table A 3**). There was no support for all other candidate models (AIC_{wt} ~ 0). Standardised regression coefficients of model 1 indicated that the number of pointed spines was the strongest single predictor of age, followed by the number of arms, number of marginal spines, and number of arm buds, respectively (**Table 1B**).

Table 1. Model 1 parameters for linear mixed models testing the effects of morphological traits on age of herbivorous juveniles. A. Unstandardised and B. standardised model coefficients are presented. Using Akaike Information Criterion (AIC), this model was selected as the best of all candidate models constructed from the number of pointed spines per arm (PSpines), number of marginal spines per arm (MaSpines), number of arms (nArms) and number of arm buds (nBuds) traits. As spine data were collected from 60 days (see methods), the model incorporates data from 60 to 300 days post-settlement. Estimates, standard errors (SE), t- and z-statistics, and associated p-values are depicted for each model parameter. n = 261.

A				
Parameter	Estimate	SE	t	Pr(> t)
Intercept	74.1273	15.9842	4.638	<0.001
PSpines	12.2098	0.8969	13.613	<0.001
MaSpines	-7.5539	1.5449	-4.890	<0.001
nArms	10.0116	1.0657	9.395	<0.001
nBuds	-11.5409	4.1076	-2.810	0.006
B				
Parameter	Estimate	SE	z	Pr(> z)
Intercept	182.672	1.902	96.035	<0.001
PSpines	40.092	2.945	13.613	<0.001
MaSpines	-9.012	1.843	-4.890	<0.001
nArms	27.297	2.906	9.395	<0.001
nBuds	-5.632	2.004	-2.810	0.006

3.2 Juvenile behaviour

In the FSW only treatments, the juveniles that did move (55%, n = 42) showed a preference for upstream movement with 21 individuals moving upstream and only 2 moving downstream. A significant number of juveniles did not move ($\chi^2 = 15.57$, $p < 0.05$; **Figure 6A**). In the single cue experiments (vs FSW), the juveniles demonstrated significant attraction to live coral and CCA-coral rubble ($\chi^2 = 25.2$, $p < 0.005$; $\chi^2 = 18.2$, $p < 0.005$; respectively) and avoidance to cues from adults COTS ($\chi^2 = 9.84$, $p < 0.05$; **Figure 6B,D,F**). For CCA rubble (n = 27) and coral (n = 30) presented as single cues, ~60% of the juveniles moved towards these cues with movement downstream or into the FSW flow being far less prevalent. In these treatments 22% and 30% of juveniles remained stationary, respectively. Of the 50 juveniles that were exposed to cues from adult COTS, 34% moved away from the cue upstream to FSW, 30% moved downstream, 30% did not move and a significantly lower number (6%) moved towards the adult cue (**Figure 6D**).

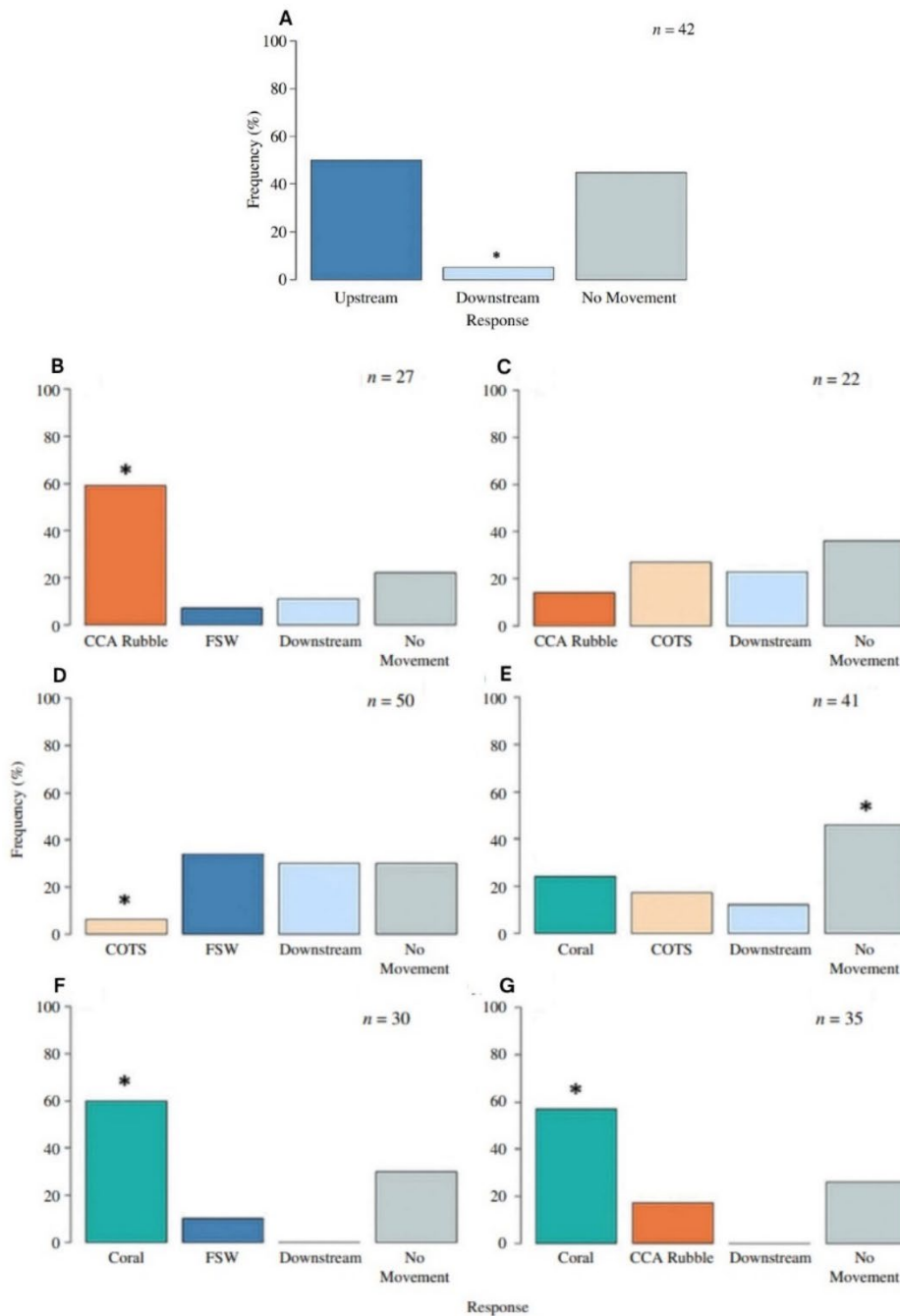


Figure 6. The percentage frequency (%) of juvenile choice responses analysed against an expected distribution (equal probability; A: 33%, B-G: 25%) of movement to cue, upstream (FSW), downstream, or no movement (Chi-squared goodness of fit test, * = significance). A. In response to filtered sea water only, juveniles avoided moving downstream. B. In response to CCA rubble as a single cue, most juveniles moved to CCA rubble. C. When two cues are present, CCA rubble and COTS there was no significant choice. D. In response to adult COTS as a single cue, most juveniles appeared to avoid this cue. E. When two cues are present, coral, and adult COTS, most juveniles did not move. F. In response to coral as a single cue, most juveniles moved to coral. G. When two cues are present, coral and CCA rubble, most juveniles moved to coral.

In the CCA rubble-live coral two-cue experiment, the choice response for coral was 3-fold greater than that for CCA rubble ($\chi^2 = 24.1$, $p < .005$; **Figure 6G**). Overall, 57% ($n = 35$) of the juveniles moved towards coral, as for the coral only runs, and 26% juveniles remained stationary. In the coral/COTS trials, the no movement (46%) category was significant ($\chi^2 = 11.2$, $p < 0.05$). In the two-cue experiments where one of the cues was adult COTS, direct observation indicated that the juveniles had a meandering movement, and their responses were variable (**Figure 6C,E**). When simultaneously presented with adult COTS and the CCA rubble cue there was no significant choice (**Figure 6C**). Across all combinations of cues, a similar percentage ($\bar{x} = 34\%$, $SE = 0.35$, $n = 7$ trial types) of juveniles remained stationary with their arm tips raised sensing the environment.

There was a significant effect of cue type on juvenile movement (**Figure 7A,B,C**). There was a significant difference in the speed of juveniles in the single cue experiments with the movement to the coral cue being the slowest ($\bar{x} = 6.8$ mm/min, $SE = 0.7$, $n = 11$) (ANOVA, $F_{(3,49)} = 9.68$, $p = < 0.01$) while the other treatments did not differ (**Figure 7C**). In the FSW controls and CCA coral rubble treatments the juveniles moved upstream at an average rate of 14.2 mm/min ($SE = 1.5$, $n = 8$) and 12.6 mm/min ($SE = 0.8$, $n = 14$), respectively. While it is difficult to separate the contributions of the physical stimulus from flow and olfactory information in the upstream movement towards CCA-coral rubble and live coral, the dominant movement was into the arm of the flume from which flow plus cue was delivered. The juveniles moved away (downstream and into FSW flow; pathways that the juveniles took are illustrated in the **Figure A 3.**) from adult COTS cue source at an average rate of 14.5 mm/min ($SE = 1.1$, $n = 20$).

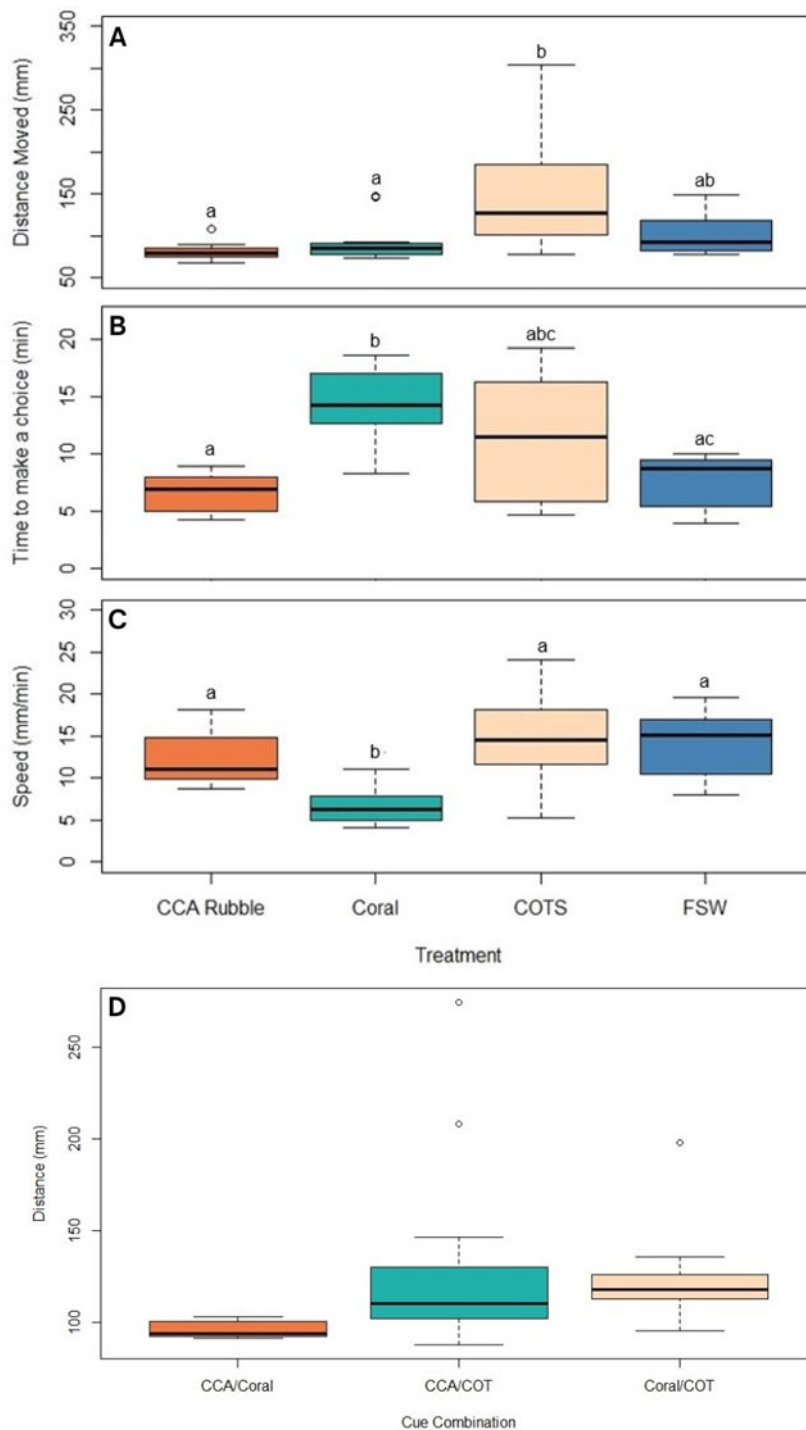


Figure 7. Tracking data on A-C single-cue experiments showing **A.** total distance moved (mm), **B.** time to make a choice (min), and **C.** speed (mm min⁻¹) of juvenile COTS to single treatments: CCA-covered coral rubble (n = 14), live coral (n = 11), adult COTS (n = 20) and FSW (n = 8). **D.** Two-cue experiments showing total distance moved (mm) in response to CCA-covered coral rubble/live coral (n = 13), CCA/adult COT (n = 12) and live coral/COT (n = 12). Letters indicate treatments that differed significantly (Tukey's HSD test (C); Kruskal-Wallis/Dunn's test (A, B)).

The time to make a choice was also influenced by treatment cue (Kruskal-Wallis $\chi^2 = 17.94$, df = 3, $p < 0.01$) with the time to move towards the coral cue being the slowest ($\bar{x} = 14.5$ min, SE = 0.9, n = 11), while the other treatments did not differ (**Figure 7B**). Time to make a choice was

fastest towards the CCA-coral rubble ($\bar{x}$ = 6.8 min, SE = 0.4, n = 14). The slowest response recorded was for juveniles moving away from the adult COTS cue ($\bar{x}$ = 11.3 min, SE = 1.2, n = 20), but note this was also the most variable (**Figure 7B**).

The total distance moved by the juveniles also differed in the single cue experiments (Kruskal-Wallis χ^2 = 24.35, df = 3, p < 0.01). The shortest and most direct paths taken were in response to the CCA-coral rubble, live coral and FSW treatments ($\bar{x}$ = 80.9 mm, SE = 2.7, n = 14; 94 mm, SE = 8.11, n = 11; $\bar{x}$ = 101.9 mm, SE = 8.7, n = 8; respectively), which did not differ. The total distance moved by the juveniles in response to the adult COTS was significantly longer ($\bar{x}$ = 144.7 mm, SE = 12.9, n = 20; **Figure 7A**) due to the meandering path they took moving away (downstream or upstream into the FSW only flow) from the cue.

Similarly, in the two-cue experiments the distance moved by juveniles was greater in the presence of the adult COTS cue (together with CCA-coral rubble or live coral) due to their meandering movement. This contrasted with the shorter more direct movement in the CCA-coral rubble/live coral cue combination (ANOVA, $F_{(2,34)} = 3.529$, p < 0.05). In the CCA-coral rubble/COTS and live coral/COTS experiments the mean distance moved was 131.0 mm (SE = 15.9, n = 12) and 124.1 mm (SE = 7.3, n = 12), respectively, while the distance moved in response to CCA-coral rubble/live coral was $\bar{x}$ = 96.4 mm (SE = 1.4, n = 13; **Figure 7D**). There was no significant difference in the time to make a choice (ANOVA, $F_{(2,34)} = 0.6$, p = 0.554) or speed (ANOVA, $F_{(2,34)} = 0.48$, p = 0.623) in the two cue experiments.

3.3 Ontogenetic change in juvenile biochemical profile

There were 3,114 metabolites found in the three age groups of juvenile COTS that were analysed (12, 15 and 27 months old). The biomolecules show different biochemical processes related to COTS age. Out of these, 822 had significant changes (p < 0.05) in their levels across the three groups. These polar metabolites mainly belonged to pathways such as beta oxidation of very long chain fatty acids, fatty acid creation, citric acid cycle, mitochondrial electron transport chain, Warburg effect, amino sugar metabolism, androgen and estrogen metabolism, and purine metabolism. **Figure 8A** gives a general overview of these metabolites (identified) and metabolite-like (unknown) molecules.

Out of 601 lipids that were analysed across the three age groups, 239 showed significant changes in their levels (p < 0.05) across the three age groups. These lipids mainly belonged to the lipid superclasses of lipids and lipid-like molecules, sterol lipids, fatty acyls lipids, glycerophospholipids and sphingolipid. **Figure 8B** gives a general overview of these lipids (identified) and lipid-like (unknown) molecules in PLS-DA plots of the metabolite (**Figure 8C**) and lipid (**Figure 8D**) data for the age groups.

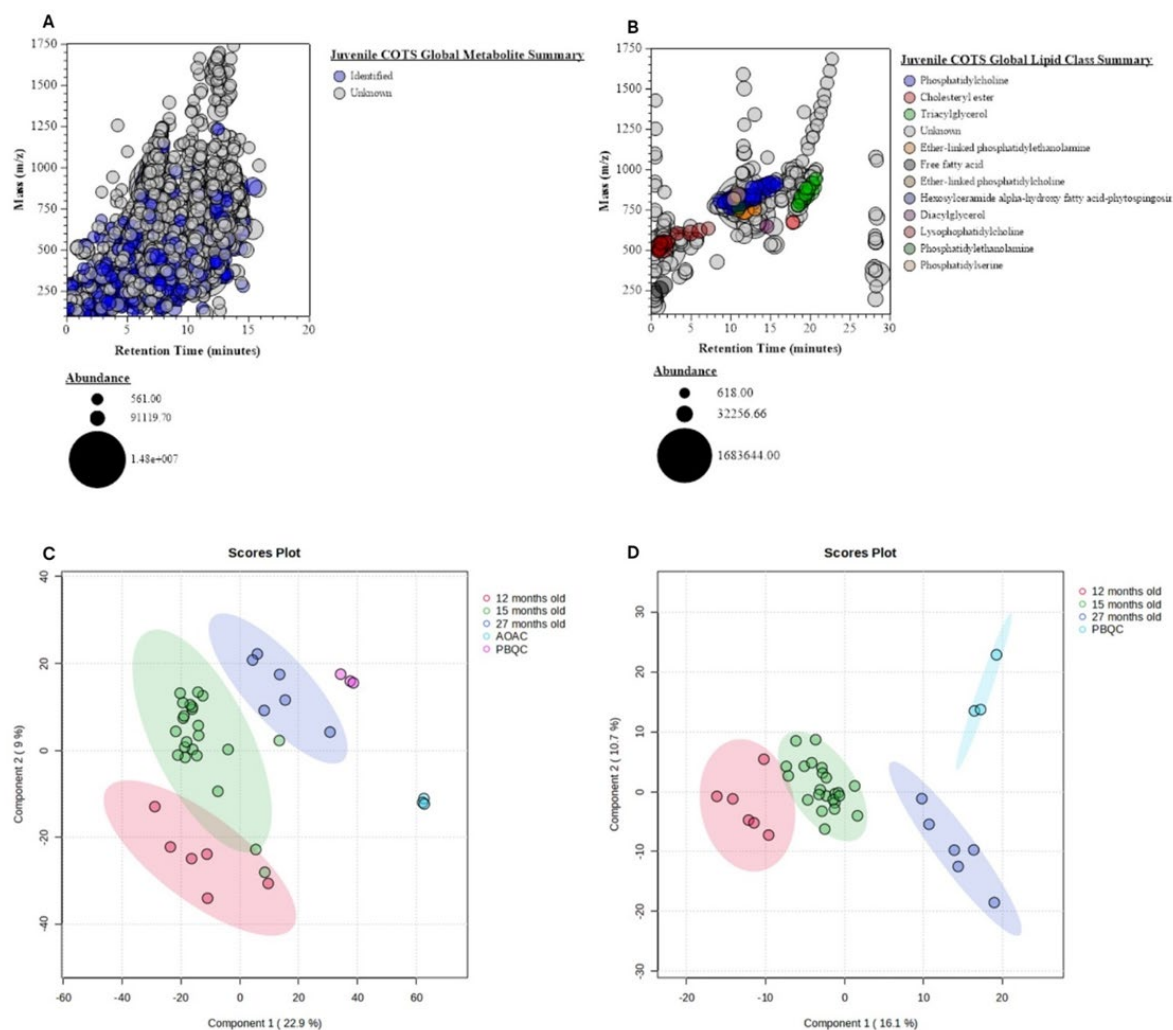


Figure 8. A. Global polar metabolite profile (untargeted metabolomics assay). B. Global non-polar metabolite (lipid) profile (untargeted lipidomic assay). C. Metabolite and D. lipid PLS-DA plot for the analysed COTS age groups.

3.4 Transcriptomic reference for juveniles

Juvenile COTS RNA at 12, 15 and 27 months old were sequenced, and genome reference-guided assembly provided a total of 79,949 transcripts with a mean length of 838 bp. KEGG classification analysis (to assist with potential gene function) determined that the majority of total expressed genes across 12–27 months represented associated functions in genetic information processing, metabolism and signalling (**Figure 9**). A comparison of the different juvenile age groups predicted significant differences within certain genes based on hierarchical gene expression clustering (**Figure 10**). While comparison of gene function enrichment indicated that between COTS 12–15, 15–27 and 12–27 months, there were more genes involved in kinase activity, peptidase activity and cellular nitrogen metabolism, respectively (**Figure 11**).

KEGG Classification

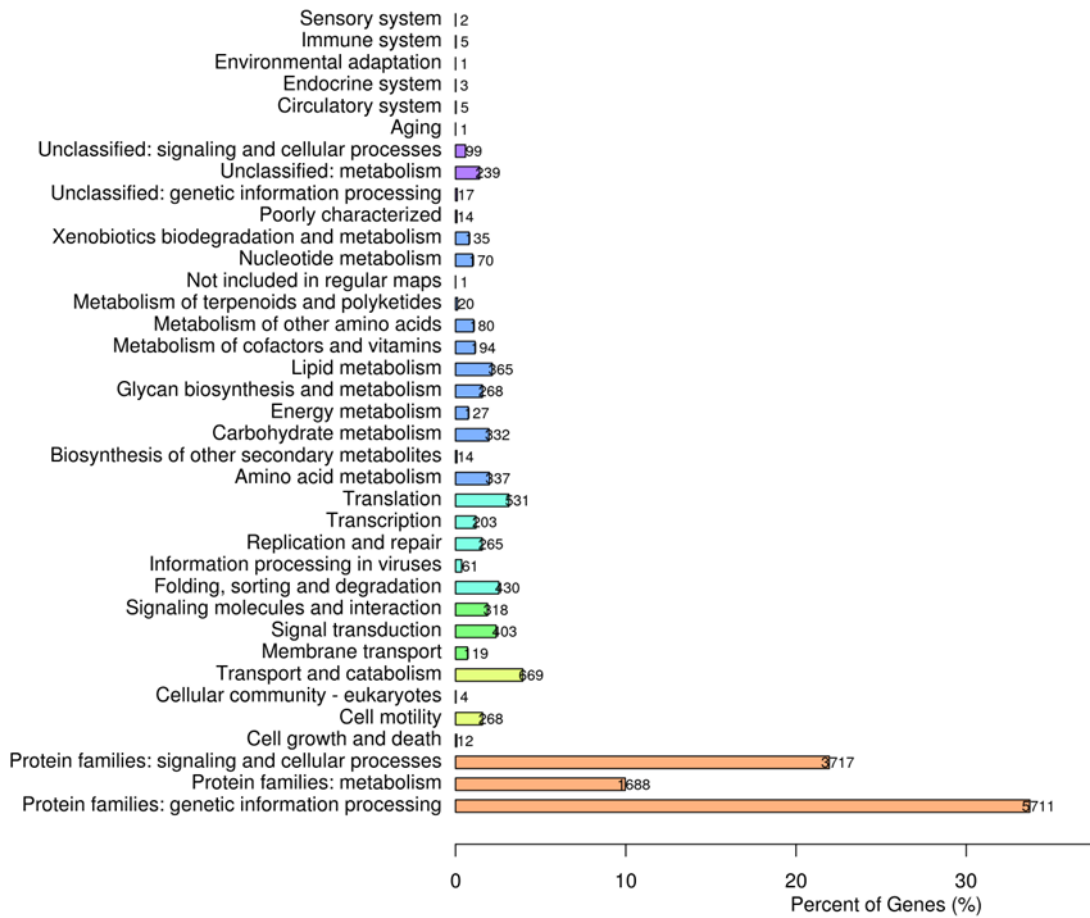


Figure 9. KEGG functional classification of total juvenile genes identified.

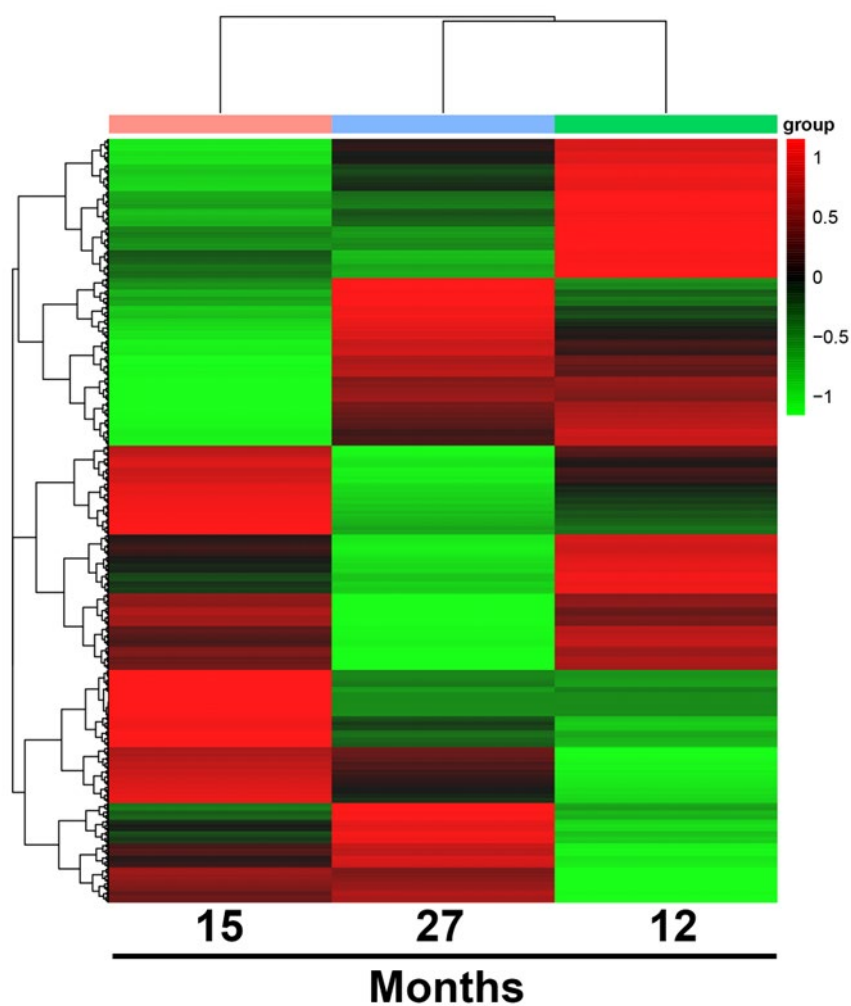


Figure 10. Heatmap with hierarchical clustering of COTS months (x axis) and genes (y axis), where red indicates high gene expression and green relatively low gene expression. Clustering indicates those genes and COTS months that have relatively similar gene expression.

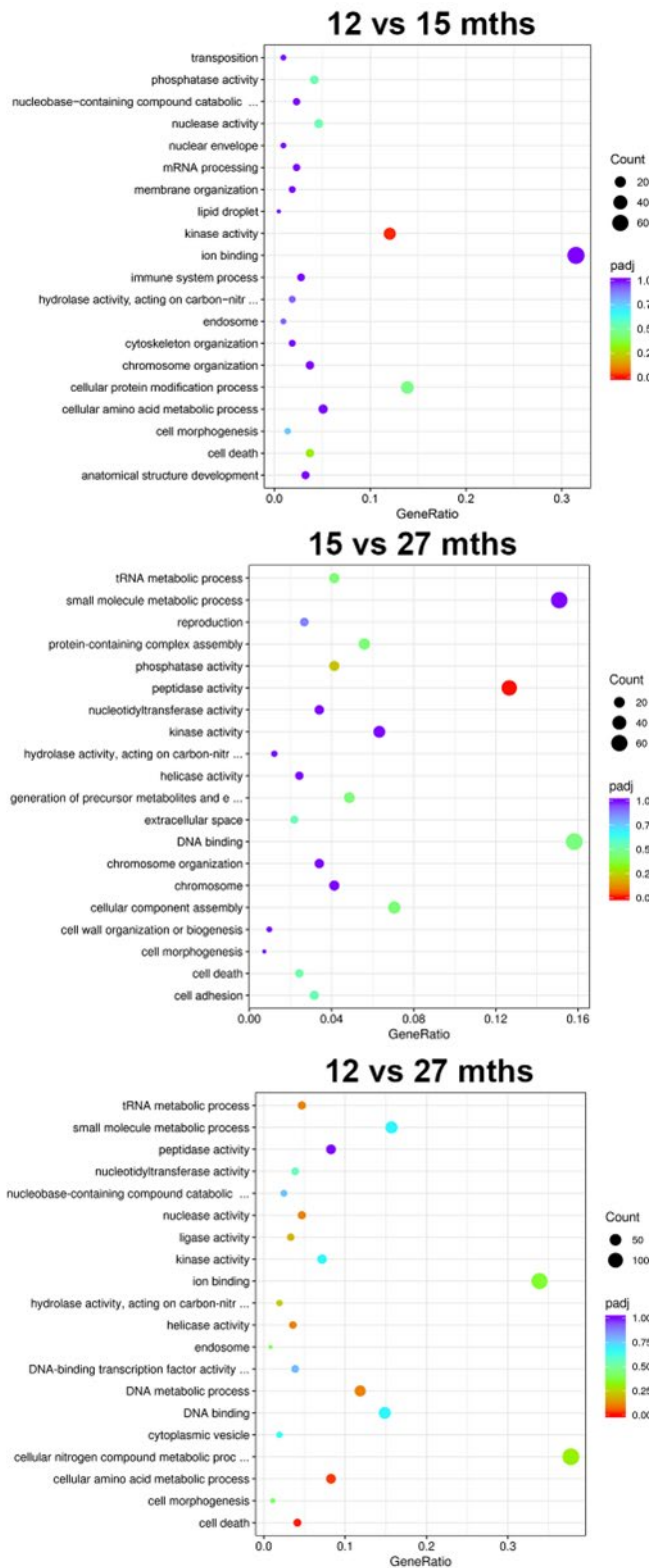


Figure 11. Enrichment analysis of gene upregulation between juvenile COTS stages.

4. DISCUSSION AND OUTPUTS

4.1 Ontogenetic change in juvenile morphology

The early juvenile stages of *Acanthaster cf. solaris* are difficult to study as they live in cryptic habitats (Wilmes et al. 2020a). This life stage is important to understand because juvenile survival and migration to adult habitat are critical demographic processes that regulate species' population dynamics (Gosselin & Qian 1997; Hunt & Scheibling 1997; Gillanders et al. 2003). For *Acanthaster cf. solaris*, the juvenile nursery habitat and diet is very different from those of the adults and there can be considerable spatial separation between the habitats. Juvenile sea stars can remain in their nursery habitat with growth stasis for some time, as seen for *Marthasterias glacialis* and *Asterias rubens* in nature (Nauen 1978; Byrne et al. 2021) and COTS in captivity (6+ years; Deaker et al. 2020a). While it is unknown if this 'juveniles in waiting' phenomenon is common for COTS in the wild where they would be subject to higher mortality compared to controlled laboratory conditions, the potential for an age-size disconnect presents a challenge for modelling population dynamics of these ecologically important echinoderms.

In the only tracking study of juvenile COTS, Zann et al. (1987) followed a population over two years on a reef flat in Fiji. When first encountered the juveniles ranged in size from 10–32 mm diameter and were estimated to be 7 months old. Most of them were < 20 mm diameter and were found living on coralline algae in an area with low coral cover (Zann et al. 1987). Given the size range of the juveniles, the population probably included individuals of mixed diet histories and from multiple cohorts. In a GBR study, juveniles 2–64 mm total diameter (only 2 > 50 mm) were sampled over 8 months (Wilmes et al. 2020a). With the assumption that the juveniles were a single cohort from a summer recruitment date, age (post spawning) was estimated and used for growth analysis. More than one year class may have been present (Wilmes et al. 2020a). In both field studies, the large size range of the herbivorous stage juveniles present and the presence of large juveniles (~20 mm diameter) living in CCA-coral rubble habitat shows that the size/age of juveniles at the transition to corallivory can be much larger than the ~8 mm diameter minimum (Yamaguchi 1973) and likely to be older than the < 1 year estimate used in growth models (Wilmes et al. 2017). Several studies suggest or show that herbivorous juvenile COTS in nature may be several years old (see review Birkeland and Lucas 1990 review).

Unless juvenile populations are monitored from settlement, as in serendipitous encounters in nature (e.g. Sewell and Watson 1993; Byrne et al. 2021) or through ex situ rearing as in the present study, it is difficult to quantify cohort age and model growth dynamics, especially due to the growth plasticity of juvenile sea stars and their potential for growth stasis. We documented growth of a large population of juvenile COTS from the beginning of their benthic life to 300 days (10 months) post-settlement and so were able to quantify growth characteristics in individuals of known age. We undertook a new approach to quantify ontogenetic change in juvenile COTS from settlement, using a suite of traits, including previously unexplored ones (e.g. spine type and number). Eight traits were explored to assess their potential as indicators of age. Avoiding multicollinearity, we identified two groups of traits for analysis. Within their respective groups, diameter and the number of pointed spines were the strongest linear

predictors of age across all traits. As diameter and the number of pointed spines could not be included in the same model, direct comparison of their standardised model coefficients was not possible. Our results indicate that use of a combination of morphological traits that do not covary, is a promising approach to model juvenile age in *Acanthaser cf. solaris*. The ability to track and model the changes in growth in juveniles of known age would complement the models generated for growth of juveniles collected in the field where age was estimated (Wilmes et al. 2020a).

In the first trait group, the number of pointed spines trait had the strongest linear effect on age followed by the number of arms, marginal spines and arm buds. The pointed spines trait as a predictor of age had a standardised slope almost double the magnitude of the number of arms. The strength of this trait was likely due to the consistent formation of new spines as the arms grew, as well as their appearance on the aboral surface with growth. These spines are a major feature of COTS and may be a good indicator of age as they appear to be added in a regular pattern. The effect of age on this trait was generally homogenous between individuals. In contrast the effect of age on diameter and the number of arms traits was not homogenous between individuals. In early juveniles, pointed spines grew on the sides of arms and began to appear on the aboral body wall of the juveniles toward the end of our study. In the second trait group, diameter had the strongest linear effect on age, followed by the number of marginal spines and number of arm buds. Total diameter was the driving component of each of the best five models from this trait group.

It is not known if growth stasis impacts juvenile trait development. The pointed spines continue to be added at the same rate, even following the decline in growth. If they are added regularly through periods of growth stasis, they would have potential as an age marker irrespective of size and food conditions. The growth of pointed spines through periods of growth stasis is an important topic for future research as its application as an age marker in the field depends on its independence with size growth. In addition, exploration of other traits (e.g. number of madreporites, disc granules) would be useful to determine their potential to be used as indicators of age.

Although the juveniles were reared in similar conditions, there was considerable variability in their development and pace of growth. Our measurements were taken in randomly sampled juveniles from two genetically different sources (parents) and so we cannot attribute variability to genotypic or phenotypic differences among individuals. Variable growth may have been influenced by size at metamorphosis. Some of the newly metamorphosed COTS we reared were almost twice the size of their clutch mates (Byrne, pers obs). This variation is important in nature where growth rates and survival are often determined by the size of the post-larval juvenile (Gosselin and Qian 1997). The importance of size/age dependent mortality has been shown for COTS (Keesing and Halford 1992; Keesing et al. 2018).

Growth of juvenile COTS is strongly influenced by temperature and diet (Yamaguchi 1973; Lucas 1984; Deaker et al. 2020b). Growth is faster on a diet of CCA than on *Amphiroa* (Deaker et al. 2020b). The seven juveniles reared at 27–28 °C on a CCA diet by Yamaguchi (1973, 1974) reached the 8 mm size limit to transition to coral at 4 months old and reached a terminal number of 16–18 arms by 4.5 months. A slower growth was recorded by Lucas (1984) in nine juveniles reared at 21–27 °C (mean 24.5 °C) on a CCA diet. These juveniles achieved 8 mm diameter at ~ 7 months. A subsequent study of juveniles reared in similar conditions revealed

that at one, four, and seven months in age they had mean diameters of 1.0, 2.8 and 4.9 mm, respectively (Keesing and Halford 1992). The size and age of juveniles in these studies (Lucas 1984; Keesing and Halford 1992) are similar to those determined here. The juveniles that we reared at 25–26°C and fed *Amphiroa* sp. reached 8 mm diameter at seven months, although this was variable (4.3–8.6 TD) and a population wide mean of 8 mm diameter was not reached until nine months with a maximum of 15 arms. Our juveniles did not reach the growth stasis size plateau (Deaker et al. 2020a) but may have done so if we extended our rearing for a few months.

The number of arm buds and marginal spines were negatively associated with age as these are early juvenile traits with the spines used for attachment to the substratum. Arm addition decreased by seven months. The fate of the marginal spines was not clear. Their decrease with age indicates that some marginal spines were lost or resorbed with some evidence that they may transition to become pointed spines. In older juveniles, from around 6 months in age, pointed spines appeared where some of the original marginal spines had been located.

As the juveniles aged, they changed from stellate five and multiarmed stars to a disk shape as the central disk became dominant. The disk grew to extend over the basal region of the aboral side of the arms. As a result, only a portion of the actual arm length was evident from an aboral view. Thus, we used oral arm length as a better reflection of true arm length. We recommend use of the oral side arm measure for COTS as arm length is a key trait used to characterise growth in sea stars. Other studies of COTS have measured the arms from an aboral view (e.g. Deaker et al. 2021).

Toxic spines are a distinct feature of COTS compared to other sea stars and indicates that there has been selection for protection against predators. This defensive capacity undoubtedly contributes to their success. As a unique feature of COTS, the biology of the spines is of interest to explore. Our results suggest that the pointed spines trait may serve as an age marker for field collected juveniles. The spines have also been used to age adult COTS using the pigment bands that develop along the shaft and which appear to be formed periodically (Stump and Lucas 1990). Thus, the spines may provide insight into the biology and ecology of COTS as an age marker across multiple life stages. The importance of pointed spine development as a potential age marker for juvenile and adult COTS deserves attention.

Understanding of COTS outbreaks is impeded by a lack of data on juvenile recruitment, settlement, mortality and juvenile growth rates (Wilmes et al. 2018). A method to age juveniles is needed to inform population models seeking to understand post-settlement processes and the role of the early post-settlement stages in driving COTS outbreaks. Our results show that morphometric trait modelling analysis has potential as a new approach to age juvenile COTS to inform age estimates of juveniles on the reef (Wilmes et al. 2017, 2020a). It would be of interest to follow changes in particular traits, particularly the pointed spines in the herbivorous stage of COTS over years. In laboratory flow through aquaria these juveniles exhibited minimal to no size increase between 1 and 6.5 years in age when the study stopped (Deaker et al. 2020). Investigation of telomere length analysis, age-related telomere attrition in juveniles across a range of known ages, as trialled with adult COTS (Kwong et al. 2023), is also a candidate aging method to explore with the juveniles.

4.2 Juvenile behaviour

Waterborne cues convey environmental information to aquatic organisms that elicit behavioural change with semiochemicals being important in intraspecific communication. For COTS, this is best known for chemical cues that mediate behaviour of the adults (Motti et al. 2018). Far less is known about the role of chemical cues in olfactory communication between the adult and juvenile life stages which is important in the context of spatially segregated juvenile and adult habitats (Gillanders et al. 2003). To help tease out potential responses to reef chemistry we presented herbivorous-stage juvenile COTS with olfactory cues from their environment in various combinations. We show that they have distinct chemosensory capabilities and are able to respond to novel olfactory cues not previously encountered delivered from a distance in flowing water. Our findings indicate the presence of innate behaviour in the juveniles in responses to a range of cues.

The juveniles responded to cues that signpost the location of their food/nursery area (CCA covered coral rubble), the presence of coral prey and adults. In response to rubble-CCA and coral as single cues, they moved towards the source of these cues and, when these two cues were presented in combination, they exhibited a preference for coral. As nursery habitats are important for juvenile survival and the switch to coral prey is an essential step in transitioning to maturity in COTS, these responses were expected. In contrast, there was no clear indication of a 'decision' among juveniles that moved in the two-cue CCA-rubble/COTS and live coral/COTS trials. The variable responses observed in these trials may reflect the situation in nature where the juveniles need to integrate multiple cues together with semiochemicals from adults. Interestingly, across all cue combinations a similar percentage of juveniles remained stationary with their arm tips raised sensing the environment. This may indicate that a proportion of juvenile COTS have a cautious immobile response potentially as a defence against being detected by predators.

Reef rubble covered by CCA attracts larval settlement, placing post-settlement juvenile COTS in their nursery habitat which provides them with food and shelter (Wilmes et al. 2020b). Juvenile attraction to the reef rubble cue was very strong despite months of rearing in isolation. The juveniles had the shortest path length and fastest time to make a positive choice in response to the reef rubble cue. The source of this cue, rubble collected from the reef likely reflects the habitat of the juveniles in nature (Wilmes et al. 2020b). In addition to CCA, this habitat supports a diversity of encrusting biota, meiofauna, algal biofilm and microbes, to name a few sources of habitat chemistry. The attraction of the juveniles towards coral rubble is likely due to the detection of a chemical mixture that conveys information as to the location of the nursery habitat.

The response of juveniles to their first exposure to coral indicates an innate attraction to coral prey, as indicated in other studies (Johansson et al. 2016; Neil et al. 2022). While there was a definite attraction towards live coral (shortest distance moved), the time that the juveniles took to make this choice was the longest of any treatment and with the slowest average speed, indicating cautious attraction. This cautious approach is warranted. The transition to a coral diet is perilous for juvenile COTS as they can be damaged or killed by coral defences (Deaker et al. 2021).

We were uncertain whether the juveniles would respond to adult chemistry with attraction or avoidance. When exposed to olfactory information from adults as a single cue source, 94% of the juveniles moved downstream away from the source of the cue, into the no cue FSW flow or remained still. These results indicate avoidance of adults. The reasons why juveniles appeared to flee from adult cues is unknown, but our results indicate that olfactory information from adults, at least in isolation or close proximity may impede the ontogenetic transition to corallivory. These results contrast with the mutual attraction between adult COTS where individuals move towards feeding or spawning conspecifics, an attraction promoted by pheromones (Caballes and Pratchett 2017; Harris et al. 2025). The spatial distance at which semiochemical communication between juveniles and adults may occur will be important to determine.

We were interested in how the juveniles would respond to cues from live coral together with adult cues given that they were at the diet transition stage and their strong positive and negative responses to these cues as individual stimuli, respectively. In the coral/adult COTS experiments the juveniles exhibited variable responses. While more juveniles moved towards the coral than the adult cue, downstream movement also occurred, and no movement was significant. Variable responses may also be expected in nature due to the plethora of olfactory information that the juveniles would be exposed to. There may also be interindividual variability in juvenile behaviour at the diet transition stage potentially related to juvenile size, but this is not known. It will be important to understand how juvenile COTS respond to a range of reef olfactory cues in nature such as those from crabs that prey on them (Desbiens et al. 2023) and chemical cues that act across life stages (juveniles and adults).

When juvenile COTS achieve competence to transition to a coral diet, they may have to navigate to find corals at a distance. In a tracking study, juveniles (15–40 mm diameter) migrated 800 m from their back reef nursery habitat towards the reef crest over ~2 years (Zann et al. 1987). This migration was likely driven by waterborne cues from coral on the reef crest. On the reef, juvenile COTS experience a complex suite of cues, and as radial animals, can receive olfactory information from many directions. It would be of interest to investigate the responses of the juveniles to the predatory crabs that occur with them in the rubble (Desbiens et al. 2023).

On an algal diet the juveniles exhibited growth stasis with the maximum size being < 20 mm diameter, as found in other studies (Lucas 1984; Deaker et al. 2020a,b). Juveniles are resilient to food scarcity, being able to delay their transition to corallivory until favourable conditions arise with no influence of a prolonged algal diet on their growth once coral prey is available (Deaker et al. 2020a,b). Juvenile transition to corallivory is influenced by the availability of coral prey (Neil et al. 2022), which would be unpredictable following COTS outbreaks, cyclones, and bleaching events. The collapse of the reef to coral rubble following a bleaching mortality is followed by the overgrowth of algae. Thus, herbivorous juvenile COTS living in the reef infrastructure may benefit from climate-change driven coral mortality and increase in their nursery habitat and algal food (see models in Wolfe and Byrne 2024). Juveniles persisting in the rubble habitat post bleaching would be well positioned to transition to corallivory in parallel with coral growth, impeding reef recovery.

The appearance of new coral eating juveniles (as small as 3 cm diameter) after a COTS outbreak has passed (Moran et al. 1985) points to the emergence of juveniles that were in the

reef infrastructure. Our study provides insight into juvenile behaviour that may underlie these observations. The size range of COTS in outbreaks indicates the presence of multiple cohorts (Chandler et al. 2023). This may be due, at least in part, to the waiting stage phenomenon. The behavioural ecology of juvenile COTS at the transition to corallivory may be important in understanding and predicting outbreaks. An important question is what drives herbivorous juvenile COTS to become coral predators.

As is characteristic of marine species, density dependent processes across life stages (settler, juvenile and adult density) and life stage interactions (e.g. competition, mortality, emigration) (Gillanders et al. 2003; Moksnes 2004) undoubtedly influence COTS population dynamics. Conspecific interference competition can be a selective force in migration of juvenile marine invertebrates from their nursery habitat (Ratchford and Eggleston 1988; Moksnes 2004). Juvenile avoidance of adult cues indicates the presence of negative density dependent feedback, as known for other predatory sea stars (Nauen 1978; Byrne et al. 2021) where the presence of adults appears to deter juvenile recruitment into the adult habitat and diet. Juveniles may delay the transition to corallivory to avoid competition for food, especially if adult density is high (Wolfe and Byrne 2024). Density dependent feedback is likely to be key to the outbreak phenomenon. The potential accumulation of juveniles within the reef infrastructure over multiple spawning years (depending on survival) (Deaker et al. 2020a) is a potentially underappreciated driver of COTS outbreaks.

4.3 Ontogenetic change in juvenile biochemical profile

The metabolomics and lipidomics research is ongoing to incorporate recently generated younger juveniles. Together with data from the proteomics research, we will integrate the biochemical profile of the juveniles across ages from early (4 months old) to advanced (27 months old) herbivorous juveniles with respect to juvenile physiology, sensory molecules, the onset of toxicity (e.g. production of saponins) and application for management.

4.4 Context with CCIP research and technology tool development

An important implication of juvenile COTS avoidance to adult cues is that the physical removal of adult COTS may release juveniles residing in the reef infrastructure from competition interference thereby promoting their transition to corallivory potentially extending an outbreak. Our findings lend support to the ‘juvenile in waiting’ hypothesis (Deaker et al. 2020a). Chemical ecology and the potential for cross-life stage communication is likely to be a key facet in the periodic emergence of large numbers of COTS. That said, in the real world of the reef juveniles would experience a complex mixture of many simultaneous and interacting chemosensory cues as they transition to be coral predators. This complex mixture is also likely to differ between reefs, for instance with respect to the predatory guild present. Teasing out the responses of juvenile COTS to diverse reef chemistry remains a key challenge.

The results of the behaviour study, together with characterisation of the biochemistry of the juveniles as well as that of adult COTS (Hall et al. 2017; Mendoza-Porras et al. 2023; Smith et al. 2023), contributes to knowledge to develop strategies as to how semiochemicals may be used as a management tool for COTS. For instance, chemical agents isolated from adults

might be used to reduce the number of juveniles making the transition to become coral predators. While there is great complexity and ethical considerations with respect to the use of biocontrol agents in the marine environment, the ability to manipulate population dynamics through the use of influential chemical agents has been successfully used as a management tool for nuisance aquatic species (Sorensen et al. 2016; Kamio et al. 2022).

4.5 Output descriptions and type

4.5.1 Output descriptions

- New knowledge on trait-based indicators of age/growth in juvenile COTS.
- New knowledge on behavioural responses of juveniles to adult pheromones.
- New knowledge of biochemistry of juvenile COTS (ongoing).
- Datasets:
 - Quantitative data on eight morphological traits through juvenile development to 10 months post settlement
 - Webb M. 2024 Data from: Chemosensory behaviour of juvenile crown-of-thorns sea stars (*Acanthaster* sp.), attraction to algal and coral food, and avoidance of adult conspecifics [Dataset]. Dryad (doi:10.5061/dryad.0k6djhb6k)
 - Data on the biochemical profile of juvenile COTS.

4.5.2 Output type

- Knowledge - Publication/manuscripts
 - Byrne M, Clements MJ, Hill RT, Selvakumaraswamy P, Webb MS, Wilson LJ, (2024) Body form transition in the juvenile crown-of-thorns sea star from the 5-armed metamorphosed juvenile to the multi-armed body dominated by the central disk. *Cah. Biol. Mar.*65: 307-317.
 - Webb M, Clements M, Selvakumaraswamy P, McLaren E, Byrne M (2024) Chemosensory behaviour of juvenile crown-of-thorns sea stars (*Acanthaster* sp.), attraction to algal and coral food and avoidance of adult conspecifics. *Proceedings of the Royal Society B* 291: 20240623. <https://doi.org/10.1098/rspb.2024.0623>
 - Wilson LJ, White T, Lamare M, Selvakumaraswamy P, Byrne M (2025) Growth and ontogenetic change in juvenile crown-of-thorns sea star (*Acanthaster* sp.) morphology: can morphometrics be used as an aging tool? *Coral Reefs* 44: 701-719.
 - Juvenile biochemistry (ongoing)

5. RESEARCH SYNERGIES AND NEXT STEPS

Characterisation of the innate sensory abilities of juvenile COTS and how they respond to chemical cues from their environment is of direct relevance to the CCIP-R-11 Semiochemical Biocontrol project (Motti et al. 2025). In particular, the ability of the juveniles to ‘recognise’ and respond to chemical cues from adult COTS points to the possibility that adult to juvenile communication through olfactory agents and receptors to sense these agents influences recruitment/population dynamics. Through this project we have stayed in close contact with the CCIP-R-11 team in meeting with Cherie Motti (AIMS) and Scott Cummins (USC). As the data from the juvenile-adult interaction experiments unfolded they were shared with these colleagues. The outcomes of this project (CCIP-P-03) point to the need for follow up investigations of the biochemical and molecular basis for communication between these life stages. The work done in CCIP-P-03 and CCIP-R-11 sets the foundation for the development of the value-add application with the establishment of a new collaboration with David Beale (CSIRO). This is a substantial research synergy that may lead to the discovery of novel semiochemicals as new avenues for COTS control, with the potential to interrupt the juvenile diet transition to corallivory. Moreover, as we have established methods and protocols to characterise juvenile behaviour in CCIP-P-03 this along with the biochemical characterisation of the juveniles in the value add provides the basis for future bioassay trials testing the efficacy of candidate semiochemicals for COTS control. This research on the natural products chemistry of juvenile COTS is novel and will provide important insights into the biology and ecology of the juvenile stage.

Our findings on the juvenile stage along with data from other CCIP projects will be important to inform modelling of the population dynamics of COTS, especially to evaluate the conditions where the juvenile in waiting phenomenon may be important.

5.1 Research priorities

1. Establishing a method to age juvenile and adult COTS to inform estimates of the age of populations on the reef remains an urgent priority. Targeted research is needed to explore the application of a suite of tools, trait modelling, spine banding and telomere analysis and other potential molecular avenues with individual COTS where the age is known from settlement to determine the potential application of these methods to age juvenile and adult COTS.
2. Investigate the response of juveniles to multiple simultaneous interactive cues from the environment with respect to behavioural decision making. It is likely that the influence of the juveniles in waiting phenomenon differs among reefs.
3. Explore the biochemistry of juvenile and adult COTS to develop strategies as to how semiochemicals may be used as a management tool for COTS. Establish a juvenile-adult behavioural bioassay system to test semiochemical communication between these life stages.
4. Integrate the juveniles state in models of the population dynamics of COTS generated from data obtained across the CCIP program.

6. MANAGEMENT IMPLICATIONS AND IMPACT

6.1 Integrated pest management framework

While the results of CCIP-P-03 do not immediately inform COTS management policy and practice, the potential that morphological traits may be useful as markers to age juvenile COTS has applications in modelling the population dynamics of COTS. The findings of the growth analysis may inform modelling of the age of juveniles in nature. However, research with field-caught juveniles remains a challenge. This project has provided new data to reassess the paradigms used to guide COTS management and to inform models of COTS population dynamics used to guide approaches to suppression. Outcomes of CCIP-P-03 affirm the importance of incorporating the population dynamics of the juvenile stage in the CCIP modelling programs that are being used to inform the integrated pest management program for COTS.

6.2 Outcomes and Impacts

The research in CCIP-P-03 contributes directly to the CCIP **Output**: improved empirical understanding of COTS coral system. Our results add to recent published work by the Byrne group (e.g. Deaker et al. 2020a; Mendoza-Porras et al. 2023) and other researchers (e.g. Wilmes et al. 2020a; Neil et al. 2022) building on the pioneering work of Lucas (1984) and Yamaguchi (1973). We have significantly increased the knowledge of the herbivorous stage of juvenile COTS, specifically growth in their first year and their behavioural responses to reef cues. The data will help to inform models of population dynamics. The challenge for management is to obtain the best information possible to guide actions and interventions. In CCIP-P-03 we have generated comprehensive new data on the growth and the sensory abilities and behaviour of juvenile COTS which may contribute to the success of this life stage.

Project outcomes contribute to the major CCIP **Impact**: COTS outbreaks are suppressed in providing new data to reassess paradigms used in management. New knowledge on the sensory behaviour and biochemical profile of the juveniles point to new avenues for innovative biochemical control. Juvenile behaviour suggests that adult-derived semiochemical agents may suppress the transition of juveniles from herbivory to corallivory. Our data points to the possibility that at least for some reefs, the cryptic juvenile population may be key to understanding the emergence of waves of outbreaks. Ongoing research is likely to identify agents that may be of interest for the COTS Control Program as potential ‘push’ agents.

Overall, outcomes of this project (CCIP-P-03) may lead to fine-tuning current monitoring and surveillance approaches with respect to the juvenile stage.

7. ACKNOWLEDGEMENTS

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assisted with modelling juvenile traits. Natasha Rae, Emily McLaren and Natasha Garner assisted with juvenile rearing and staff of the Sydney Institute of Marine Science (SIMS) helped maintain the adults. The COTS Control Team are thanked for collecting the adults. We thank Professor Michael Kingsford (James Cook University) for providing the choice chambers.

8. DATA ACCESSIBILITY

Data used in the construction of this report can be obtained from:

- Quantitative data on eight morphological traits during juvenile development up to 300 days post-settlement is available at: <http://doi.org/10.25910/3fwp-yy62>
- Webb M (2024) Data from: Chemosensory behaviour of juvenile crown-of-thorns sea stars (*Acanthaster* sp.), attraction to algal and coral food, and avoidance of adult conspecifics [Dataset]. Dryad (doi:10.5061/dryad.0k6djhb6k)
- Data on the biochemical profile of juvenile COTS (ongoing) available via request from David Beale (david.beale@csiro.au) and Scott Cummins (scummins@usc.edu.au).

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10. APPENDIX

Table A 1. Kendall's τ -b correlation matrix for the eight morphological traits and age. TDiam: total diameter; MDiam: mouth diameter; DDiam: central disk diameter; ArmLen: average arm length; nArms: number of arms; PSpines: number of pointed spines (/arm); MaSpines: number of marginal spines (/arm); nBuds: number of arm buds; Age: age (days post-settlement). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Variable	TDiam	MDiam	DDiam	ArmLen	nArms	PSpines	MaSpines	nBuds	Age
TDiam	—								
MDiam	0.8025***	—							
DDiam	0.9397***	0.8320***	—						
ArmLen	0.9262***	0.7553***	0.8820***	—					
nArms	0.7997***	0.7973***	0.7249***	0.6389***	—				
PSpines	0.7859***	0.6562***	0.7368***	0.7643***	0.6378***	—			
MaSpines	-0.3048***	-0.2588***	-0.3301***	-0.3335***	-0.2026*	-0.2987***	—		
nBuds	-0.2444***	-0.5368***	-0.5606***	-0.5582***	-0.2577***	-0.5066***	0.3480***	—	
Age	0.8608***	0.6983***	0.8024***	0.7921***	0.7667***	0.7668***	-0.3622***	-0.2619***	—

Table A 2. Model specifications and statistics for GAMMs exploring relationships between morphological traits and time as a smooth predictor. *k*: basis dimension; *edf*: effective degrees of freedom; *F*: F-statistic; R^2_{adj} : adjusted R^2 . Note: *edf* = reference *edf* in all cases. Traits with *edf* ~ 1 were subsequently modelled using linear mixed effects models.

Trait	<i>k</i>	Response Distribution	<i>edf</i>	<i>F</i>	<i>n</i>	<i>p</i> -value
Total Diameter (mm)	4	Gaussian	2.61	1746	317	<0.001
Number of Arms	4	Quasipoisson	2.96	956.4	317	<0.001
Number of Pointed Spines (/arm)	3	Gaussian	1.40	759.5	263	<0.001
Number of Marginal Spines (/arm)	3	Gaussian	1.97	60.44	263	<0.001
Average Arm length (mm)	3	Gaussian	1.04	1288	235	<0.001
Central Disk Diameter (mm)	3	Gaussian	1.94	882.4	237	<0.001
Mouth Diameter (mm)	4	Gaussian	2.63	309.1	235	<0.001

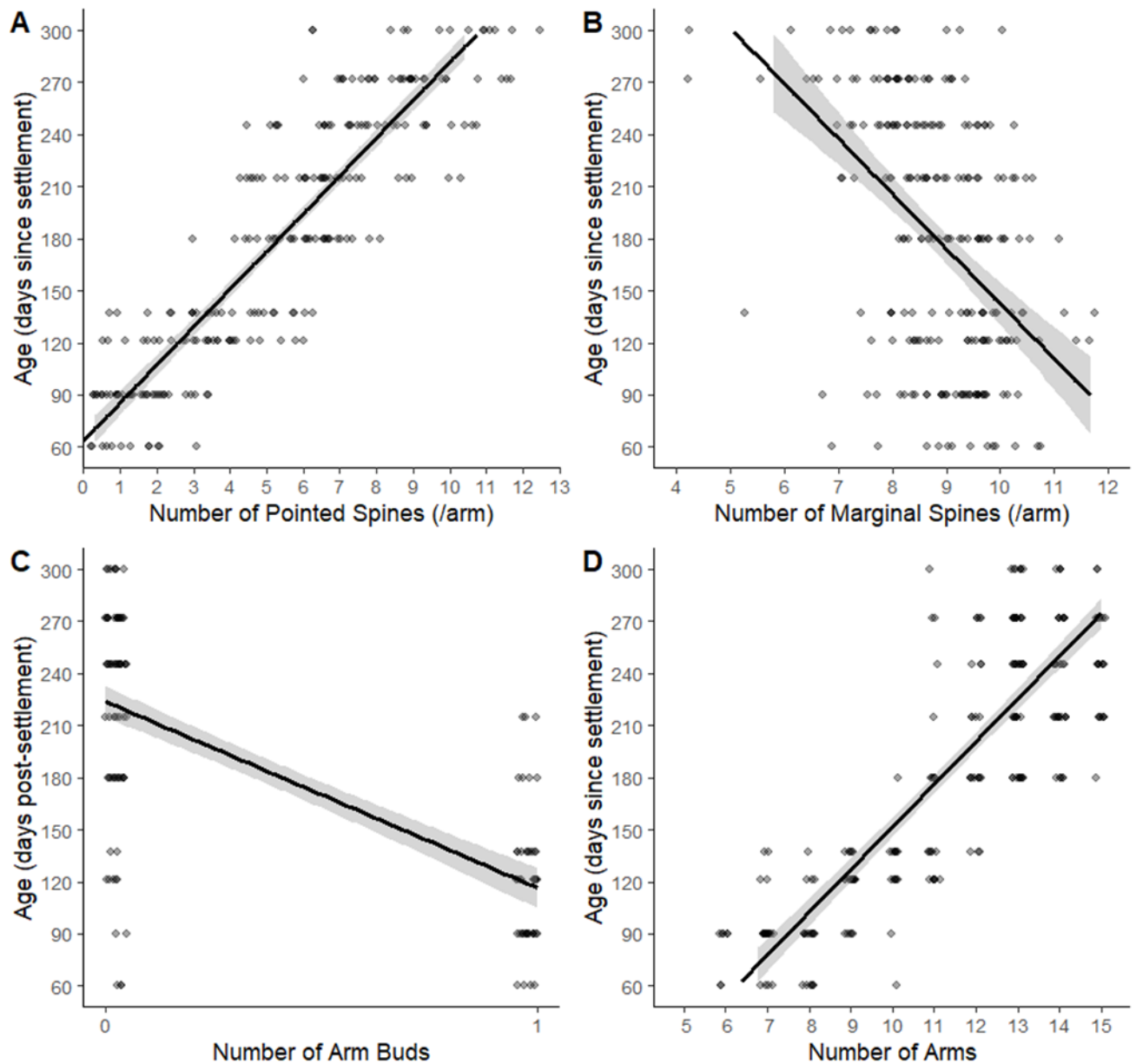


Figure A 1. Linear mixed model displaying the relationships between **A.** the number of pointed spines (/arm), **B.** the number of marginal spines (/arm), **C.** the number of arm buds and **D.** the number of arms and age of herbivorous crown-of-thorns starfish juveniles. As spine data could only be collected from 60 days, the model incorporates data from 60 to 300 days post-settlement. Data points represent individual observations ($n = 261$). The grey shaded region indicates the 95% confidence interval. Data points are 'jittered' to avoid overplotting (pointed spines, marginal spines, arms: width = 0.15, height = 0.15; arm buds: width = 0.05, height = 0.15).

Table A 3. Model selection table to compare morphological trait models used to predict the age of herbivorous juvenile COTS. The traits included the average number of pointed spines (/arm; PSpines), average number of marginal spines (/arm; MaSpines), number of arms (nArms) and number of arm buds (nBuds). As spine data could only be collected from 60 days, models incorporate data from 60 to 300 post-settlement. 'Predictors' refers to the combination of explanatory variables included in each model. ΔAIC is the difference in AIC_c between the candidate model and the model with the lowest AIC_c . AIC_{wt} is the AIC weight, the probability that the model represents the best of all candidate models obtained from this dataset (according to AIC). $R^2_{conditional}$ describes the variance explained by both fixed and random factors, whereas $R^2_{marginal}$ describes variance explained by fixed effects alone. Parameters with a significant slope are highlighted in bold. The five best models are presented. $n = 261$.

Model Number	Predictors	AIC_c	ΔAIC	AIC_{wt}	$R^2_{conditional}$	$R^2_{marginal}$
1	PSpines, MaSpines, nArms, nBuds	2456.6	0.00	0.944	0.961	0.870
2	PSpines, MaSpines, nArms	2462.2	5.66	0.056	0.960	0.865
3	PSpines, nArms, nBuds	2477.0	20.45	0.000	0.961	0.852
4	PSpines, nArms	2486.3	29.77	0.000	0.961	0.842
5	PSpines, nBuds, MaSpines	2527.7	71.08	0.000	0.929	0.837
6	PSpines, nBuds	2532.4	75.83	0.000	0.930	0.830
7	PSpines, MaSpines	2552.2	95.59	0.000	0.923	0.816
8	PSpines	2559.3	102.71	0.000	0.926	0.804

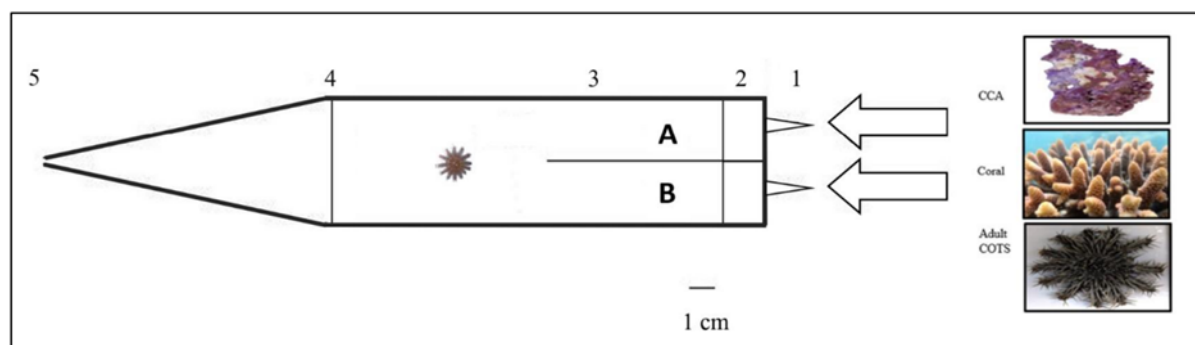


Figure A 2. Illustration of the straight y-maze flow-through choice chamber used. 1) Inflow point; 2) Mesh for laminar flow; 3) Choice flumes (A&B); 4) Acclimation gate; 5) Outflow point (drain). Image adapted from Gerlach et al. (2007).

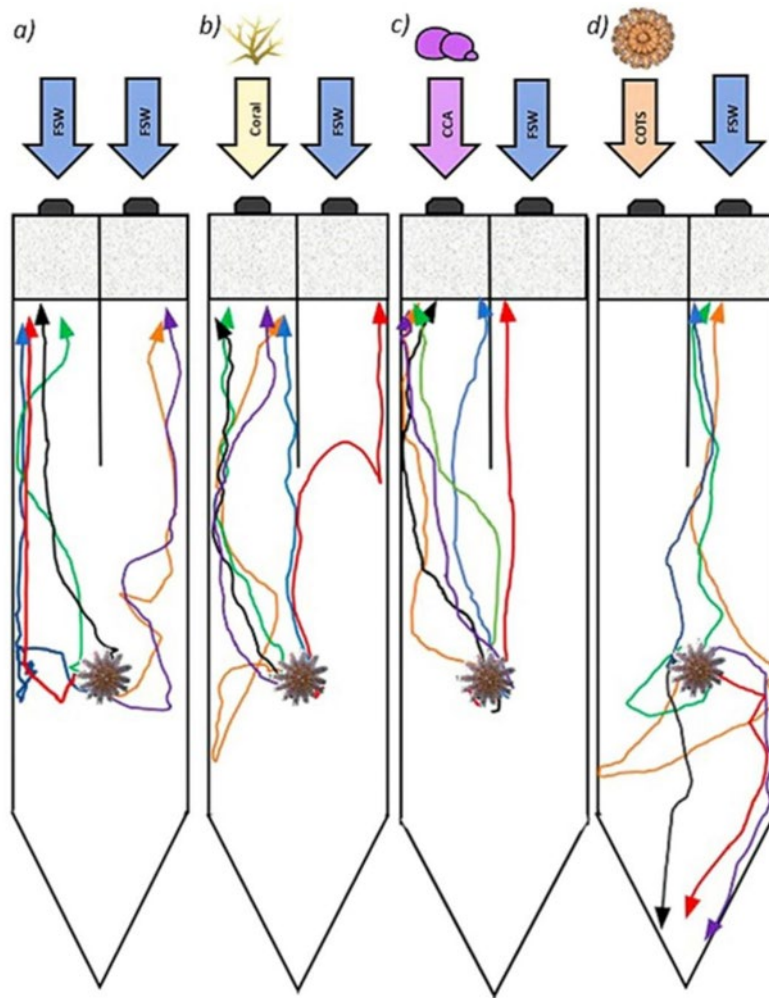


Figure A 3. Examples of tracked pathways that the juveniles took with the direction of movement indicated by the arrows in response to single cues from: a) FSW only, b) live coral, c) CCA covered coral rubble, and d) adult COTS.

Maria Byrne

School of Life and Environmental Sciences, The University of
Sydney, Camperdown, NSW 2006, Australia
maria.byrne@sydney.edu.au

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