Quantifying Shedding and Degradation Rates of Environmental DNA (eDNA) from Pacific Crown-of-thorns Seastar (Acanthaster cf. Solaris)

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Abstract

Population outbreaks of the corallivorous crown-of-thorns seastar (CoTS; *Acanthaster* spp.) are significant threats to the Indo-Pacific reefs. Although recent research demonstrated that environmental DNA (eDNA) techniques could improve CoTS monitoring and management, the interpretation of surveillance results has been limited by uncertainties about eDNA dynamics in aquatic environments. Here, we aimed to identify biotic and abiotic factors affecting the shedding and degradation rates of CoTS eDNA. Using laboratory-raised 8-month-old juvenile CoTS, aquarium experiments were conducted to test the effect of two temperatures (24 and 28°C) and three feeding treatments (no food, food available and food inaccessible) on eDNA shedding rate. Additionally, CoTS eDNA degradation rate was quantified under three temperatures (24, 26 and 28°C). We found that eDNA shedding rate was affected by feeding treatment ($p < 0.0001$) but not temperature. Specifically, the shedding rate under food accessible treatment was about 7 times higher than that of food inaccessible treatment ($p < 0.0001$), whereas the presence of coral reduced the shedding rate by half (food inaccessible vs no food, $p = 0.0249$). Degradation of CoTS eDNA was rapid (half-life = 14h) and not affected by temperature. Our results demonstrated that feeding activity increased eDNA release, but some of the released DNA was lost potentially due to binding to coral surface mucus layer or skeleton. The rapid degradation rate indicated that results of eDNA surveillance likely reflects recent and local occurrence of CoTS. Although further testing is needed, this study provided support for using eDNA as a novel detection tool for early life stages of CoTS on coral reefs.

Introduction

In recent decades, coral reefs around the world have been declining rapidly (Hughes et al. 2017). While recurrent climate-induced bleaching is the leading cause of coral mortality globally (Hughes et al. 2018), reef degradation in the Indo-Pacific region is exacerbated by population outbreaks of the corallivorous crown-of-thorns seastar (CoTS; *Acanthaster* spp.) (Baird et al. 2013; Nakamura et al. 2014; Saponari et al. 2014). On Australia's Great Barrier Reef (GBR), De'ath et al. (2012) documented a 50% decline in coral cover between 1985 and 2012, almost half of which was attributed to irruptions of CoTS. Despite considerable research effort over the past three decades, development of effective management strategies to control CoTS outbreaks on the GBR remains constrained by the uncertainty in the underlying causes (Pratchett et al. 2017). In addition, the failure to effectively detect early life stages of CoTS has prevented timely intervention and hindered our ability to answer critical research questions (Pratchett and Cumming 2019; but see Wilmes et al. 2016, 2019, 2020a, b). Recent research, however, has demonstrated that environmental DNA (eDNA) techniques may be a promising path forward for the improvement of CoTS detection and management (Doyle et al. 2017; Uthicke et al. 2018; Doyle and Uthicke 2020).

Environmental DNA is defined as DNA extracted from environmental samples (e.g., water, soil, air), which is released by organisms in the form of urine, feces, mucus, shed cells, hair, or any other secretions and discharges (Taberlet et al. 2012). In the past decade, eDNA has rapidly emerged as a promising tool for target species detection (species-specific DNA) and biodiversity assessments (metabarcoding) (Thomsen

and Willerslev 2015). The eDNA technique can potentially transform environmental management because it is more cost- and time-efficient, as well as equally or more sensitive than traditional survey methods (Rees et al. 2014). Realizing the potential of eDNA as a novel tool for CoTS management, recent research has developed a CoTS-specific quantitative PCR (qPCR) assay based on mitochondrial DNA (cytochrome oxidase subunit 1, COI) to identify, quantify, and describe distribution patterns of planktonic CoTS larvae on the GBR (Uthicke et al. 2015a; Doyle et al. 2017; Uthicke et al. 2019). Subsequently, the qPCR assay was adapted for digital droplet PCR (ddPCR) and used for the sensitive detection of post-settlement CoTS eDNA (Uthicke et al. 2018). More recently, a lateral flow assay (dipstick) method has been developed to allow rapid in-field detection of CoTS eDNA (Doyle and Uthicke 2020). These recent breakthroughs hold promise that the eDNA technique could supplement existing survey methods to improve monitoring of CoTS populations and allow early detection of outbreaks.

Before eDNA can be applied as a reliable tool for CoTS management, however, there are several knowledge gaps to be addressed. In particular, like most other eDNA studies (Yates et al. 2019), the relationship between the amount of eDNA detected and its source (i.e., abundance and location of CoTS) is not yet fully understood. This is because the production, degradation, and transportation of eDNA are influenced by a number of abiotic and biotic factors, resulting in variable and complex eDNA dynamics in aquatic environments (Harrison et al. 2019), especially under tropical conditions (Huerlimann et al. 2020). To establish robust survey designs and accurate interpretation of surveillance results, it is critical to identify major factors affecting the amount of eDNA detected.

While all organisms release DNA fragments into the environment continuously, the rate of shedding is largely variable depending on the characteristics of the organism and its responses under different environmental conditions (Stewart 2019). Although previous research has demonstrated high correlation between eDNA concentration and biomass of post-settlement CoTS in both mesocosm and field studies (Uthicke et al. 2018), such relationships can be confounded or obscured by changes in environmental conditions. For example, previous research on marine fish (Jo et al. 2019) and freshwater fish (Lacoursiere-Roussel et al. 2016) found increased eDNA shedding rates at higher water temperatures. This is possibly due to increases in metabolic activities of ectotherms in warmer water (Schulte 2015), which promotes the release of genetic material in the form of metabolic waste into the environment. It is likely that such effects may also be observed in CoTS, as their metabolic rate increases with water temperature (Birkeland and Lucas 1990). Apart from temperature, eDNA shedding rate is also affected by the behavior of the target organism. Klymus et al. (2015) found that feeding increases the eDNA shedding rate of freshwater fish by ten-fold, leading to the conclusion that gut cells shed via feces could be a major source of eDNA. Although the mechanism of eDNA shedding may be different for CoTS, it is important to establish whether feeding behavior of CoTS affects the release of genetic material into the water column, as it is known to be a voracious feeder which spends almost half of its time feeding (De'ath and Moran 1998).

After genetic materials are shed from organisms, eDNA concentration at the site of release decreases over time due to degradation and transportation (Harrison et al. 2019). Establishing the degradation rate

of target eDNA is essential for understanding detection errors and for relating results of eDNA surveys to species distribution. For example, false positive detection can occur if eDNA remains detectable after local extinction of target species, or if eDNA sustains long enough to be transported to locations where the organism has not been present (Barnes and Turner 2015). It is likely that the degradation of CoTS eDNA, like most other eDNA, would follow a first order exponential decay model, and would be accelerated by increased temperature (Strickler et al. 2015; Lance et al. 2017; Jo et al. 2019). Another process contributing to the removal of eDNA from the water column is the binding to particulate matter or incorporation into substrates (Harrison et al. 2019). Previous studies demonstrate that binding of eDNA to inorganic or organic particles can protect it from degradation by nucleases or microbes (Levy-Booth et al. 2007), and thus further complicates the dynamics of eDNA in aquatic environments through retention in benthic substrate and resuspension into the water column. While incorporation and preservation of eDNA in biofilm (Wood et al. 2020) and sediment (Turner et al. 2015) have been reported previously, such effect has not been tested on coral reefs. If CoTS eDNA can bind to the carbonate skeleton or biofilm on the surface mucus layer of coral, it would affect eDNA detectability on reefs, and thus bias the interpretation of results.

To further extend the use of eDNA as a management tool, its ability to detect early life stages of CoTS needs to be assessed. Due to the small size, highly cryptic nature and nocturnal behavior, detection and study of newly settled individuals (0 + year class) in the field has been largely limited (Pratchett et al. 2017; but see Wilmes et al. 2016, 2019, 2020a, b). As such, critical questions around settlement patterns and stock-recruitment relationships remain unresolved, constraining the understanding and management of CoTS outbreaks (Pratchett et al. 2017). In addition, it is unclear how laboratory-based hypotheses regarding early development and predation of juvenile CoTS can be applied in the natural environment (Deaker et al. 2020; Balu et al. 2021). It is likely that eDNA can be used as a novel tool to complement existing methods in the detection of newly settled and juveniles CoTS in the field, providing new opportunities to tackle previously intractable research questions.

The purpose of this study was to identify major factors affecting the shedding and degradation of CoTS eDNA. Furthermore, this study set out to assess the suitability of eDNA technique for the detection of early life stages of CoTS. A series of aquarium experiments was conducted to quantify the eDNA shedding rate of juvenile CoTS (~ 8 months post-settlement) under a range of treatments. Specifically, the effect of two temperature regimes (24°C and 28°C, approximate winter and summer temperatures in the Central Section of the GBR), and three feeding treatments (no food present, food available and food inaccessible) on eDNA shedding rate was tested. The comparisons between “food available” and “food inaccessible” treatments provided insights into the effect of feeding on eDNA shedding rate; while “food inaccessible” treatment was included to make comparisons with the “no food” treatment to account for any loss of eDNA due to presence of coral. In addition, the degradation rate of CoTS eDNA was quantified under three different temperature regimes (24, 26 and 28°C) in laboratory environment. The results of this study will help to inform the use of eDNA as a novel tool for the management of CoTS outbreaks on the GBR.

Materials And Methods

Experimental design: quantification of eDNA shedding rates

All experiments on eDNA shedding rates were conducted at the Australian Institute of Marine Science (AIMS) National Sea Simulator between 27 July 2020 and 25 August 2020. Laboratory-reared juvenile CoTS settled in December 2019 according to method described by Uthicke et al. (2015b) were used as study subjects (⌀ 10–15mm). All CoTS were fed only with corals once individuals had switched diet from crustose coralline algae to coral. The experimental set-up comprised of 21 aquaria of 4 L capacity, 18 of which were assigned as treatment, and three as negative control (containing seawater only) to monitor for contamination. Each aquarium had a separate flow-through system. Seawater used in the system was filtered to 0.02µm.

Four rounds of experimental runs were conducted in series to quantify for eDNA shedding rates under different temperature and feeding treatments (Fig. 1). The first two rounds were conducted at the Central GBR summer average temperature (28°C) and the second two were conducted at winter average (24°C) (Australian Institute of Marine Science 2017). Each round of experimental run had three feeding treatments (six replicates each), which were randomly assigned to aquaria: 1) no food, 2) food available (a ~ 4–5 cm fragment of coral *Acropora millepora* was provided for feeding), and 3) food inaccessible (the coral fragment was surrounded by a 1 mm mesh to restrict access by CoTS). The food inaccessible treatment was included as a proxy for eDNA loss in the system due to presence of coral. Individual CoTS (n = 18) were randomly introduced into one aquarium each for 24 hours before water samples (1 L) were collected. The experiment was repeated with another 18 individuals under the same temperature to obtain a total of 12 replicates per feeding treatment per temperature. Water temperature was then gradually adjusted to 24°C and CoTS allowed to acclimatize for one week before another two rounds of experimental runs were conducted.

Because the aquaria were maintained at two turnovers per hour, it was assumed that an equilibrium state was achieved after CoTS were introduced into the aquarium for 24 hours (48 turnovers). An equilibrium state was defined as the time period when eDNA concentration in the water column did not change over time, as eDNA shedding was in equilibrium with eDNA dilution. The exact flow rate (L h^{-1}) of each aquarium was measured immediately before water sampling. At the end of each experimental run, weight and diameter of CoTS was individually measured to the nearest mg and mm, respectively. Water temperature and dissolved oxygen were monitored daily to ensure water quality. The aquaria were bleached in 10% sodium dichloroisocyanurate (NaDCC) solution and rinsed with freshwater between experiments to prevent cross-contamination.

Experimental design: quantification of eDNA degradation rates

Experiment to assess the degradation rate of CoTS eDNA was conducted from 21 to 26 September 2020 at AIMS. Seawater from a 10,000 L tank (flow rate ~ 14 L min^{-1}) containing five adult CoTS (diameter ~ 25 cm) was used in this experiment, as it has shown to provide sufficient copies of CoTS eDNA in the water column (~ 1,000,000 DNA copies L^{-1}, Uthicke et al. 2018). A total of 50 L of seawater was transferred to a water drum, which was installed with a circulation pump to ensure that the water sample was well-mixed. The water drum was transported immediately to the laboratory where 81 glass reagent bottles (previously bleached in 10% NaDCC solution and rinsed with freshwater) were filled with 500 mL water sample. To determine the decay rate constants of CoTS eDNA at different temperatures, reagent bottles were randomly and evenly allocated to three incubators which were set at 24, 26 and 28°C, respectively. Water samples were collected immediately after the bottles were filled (time 0), and then after 3, 6, 12, 24, 48, 72, 96 and 120 hours. At each time point, three bottles from each incubator were randomly chosen and filtered. Two negative controls, which contained filtered seawater without CoTS eDNA, were placed in each incubator and filtered at the end of the experiment to control for contamination. An additional reagent bottle containing water sample was placed in each incubator, which was used for daily monitoring of water temperature and dissolved oxygen. Two HOBO pendant temp-light data loggers (UA-002-64, Onset Computer Corp., Bourne, MA) were placed in each incubator to ensure the consistency of temperature throughout the experiment.

Environmental DNA sampling and extractions

All water samples were filtered using a filtration cartridge (sourced from Smith-Root Inc.) and an eDNA sampling device Grover-Pro™ (Grover Scientific Pty Ltd) through sterile cellulose nitrate membrane filters (1.2 µm pore size, 47 mm diameter). All equipment used for filtration (i.e., filtration cartridges, forceps and tubes) were bleached in 10% NaDCC solution and rinsed with freshwater after every use. Filters were folded and stored at room temperature in 1.5 mL screw cap microtubes filled with Qiagen buffer ATL until extraction, which was conducted within a month.

Extraction of eDNA from filters was conducted using Qiagen DNeasy Blood and Tissue Kit with slight modification of the manufacturer's protocol as per Doyle and Uthicke (2020). The final elution step was repeated to increase overall DNA yield.

Droplet digital PCR

Droplet digital PCR (ddPCR) was conducted using the Bio-Rad QX200 ddPCR system. Copy numbers of the target COI gene were measured in all samples using previously developed CoTS-specific primers (CoTS-COI-F-1321 and CoTS-COI-R-1446) following the method described in Uthicke et al. (2018). The limit of quantification (LOQ) for ddPCR is > 4 positive droplets (~ 8 DNA copies) per PCR reaction. Samples were analyzed in technical duplicates which were then combined as a single data point (Uthicke et al. 2018).

Data analysis

Environmental DNA shedding rate per individual (DNA copies h^{-1}) was calculated by converting the eDNA concentration from copies per ddPCR reaction to copies per 1 L water sample, multiplied by aquaria flow rate (L h^{-1}). This calculation was based on the assumption that eDNA shedding was in equilibrium with eDNA dilution when water samples were collected. Environmental DNA loss in the system due to degradation was assumed to be negligible due to the relatively fast turnover (two turnovers per hour). As the calculation was based on the amount of eDNA detected in the water column, which was under the potential influence of the presence of coral, the shedding rates documented here are apparent (or net) shedding rates. For the ease of reading, the apparent shedding rates are referred to as “shedding rates” from here after. To allow comparison with previous studies on adult CoTS and other taxa, the shedding rates were also calculated and presented as DNA copies h^{-1} g^{-1}. All average shedding rates reported here were estimated marginal means ± standard error, derived using the R package emmeans (Lenth 2020).

A generalized linear mixed model with a gamma distribution and log link was used to assess the significance of temperature and feeding treatment on eDNA shedding rate per CoTS. The generalized linear mixed model was generated using the glmer function in the R package lme4 (Bates et al. 2015). Temperature, feeding treatment, and weight were regarded as fixed factors. Weight was included as a covariate in the model to account for small differences in size between individuals. To account for the dependency of the data due to conduction of experiment in series, round of experiment was included as a random factor. Thus, the model is akin to a split-plot design with round nested within temperature. An initial model was built with all the fixed and random factors, as well as interaction between temperature and feeding treatment. Subsequent models were built with elimination of non-significant terms. The model with the lowest Akaike Information Criterion (AIC) value was chosen as the best-fitting model (Burnham and Anderson 2002). Pairwise post-hoc tests were performed using the “Tukey” adjustment in the R package emmeans (Lenth 2020) to assess differences between the three feeding treatments. To ensure assumptions of the model were met, the variances were checked to ensure homoscedasticity across categories (Bolker et al. 2009). The fit of each model was assessed by residual diagnostics performed with the R package DHARMa (Hartig 2017). These tests indicated that assumptions were met and that models using gamma distribution with the log link provided a good fit.

To estimate the decay rate constants of CoTS eDNA under different temperatures, an exponential decay model was fit to the raw data from the degradation experiment:

$$N_t = N_0 e^{-\lambda t}$$

where N_t is the eDNA concentration at time t (DNA copies L^{-1}), N_0 is the eDNA concentration at time 0, and λ is the decay rate constant (h^{-1}). Decay rate models were fitted for each temperature treatment using the nls function in R. Environmental DNA half-life and time to 99% decay were then calculated using the estimated decay rate constant obtained from model fitting. To determine whether the decay rate constant differed significantly among temperature treatments, eDNA concentration was log transformed to linearize the data. A linear model was then fitted for all data points from three temperature treatments, using time, temperature, and interaction between time and temperature as factors. Analysis of variance

(ANOVA) was conducted to determine the significance of the interaction between time and temperature. A significant interaction would indicate differences in slopes among groups (temperatures). All statistical analyses were conducted in R version 4.0.2 (R Core Team 2020).

Results

Quantification of eDNA shedding rates

Four experimental runs were conducted to test the effect of temperature and feeding treatment on eDNA shedding rates. Water temperature and dissolved oxygen remained relatively constant throughout all the experiments (Table S1). Average readings (± SD) of the two temperature regimes were 27.7 ± 0.1 and 23.8 ± 0.05°C, respectively. The overall average dissolved oxygen level (± SD) was 8.4 ± 0.3 mg L^{-1} (equivalent to oxygen saturation levels above 98%). The flow rates (± SD) were maintained at an average of 6.95 ± 0.92 L h^{-1}, equating to approximately 1.7 turnover per hour. The average weight and diameter (± SD) of CoTS used in the shedding experiments was 86.1 ± 24.5 mg and 12.4 ± 1.2 mm, respectively. CoTS eDNA was not detected in any negative controls (n = 15), indicating no cross-contamination throughout all experiments.

To find the best fitting model, comparisons were made among several models that were built with different combinations of factors. After removing the non-significant factors (i.e., temperature, weight, and interaction between feeding treatment and temperature), the best model (based on AIC values) included only feeding treatment as a fixed effect and round of experiment as a random effect (Table 1). Feeding treatment was highly significant in the model (ANOVA, $F_{2,72} = 16.72$, $p < 0.0001$). Tukey's pairwise post-hoc test indicated that eDNA shedding rate was significantly different among all three treatments (Fig. 2). The average shedding rate (± SE) was the highest when food was accessible, which was almost seven times higher than when food was inaccessible (post-hoc test, $p < 0.0001$). When no food was provided, the average shedding rate was approximately two times higher than "food inaccessible" treatment (post-hoc test, $p = 0.0249$).

Table 1
Analysis of variance (ANOVA) table of the generalized linear mixed models examining factors affecting environmental DNA shedding rate of crown-of-thorns seastar (CoTS; *Acanthaster* cf. *solaris*). Values in bold indicate significant factors.

Fixed effects	Degrees of freedom	Sum of squares	Mean Square	F-statistic	p-value
Initial model (AIC = 1282.6)					
Feeding treatment	2	45.505	22.753	17.201	**< 0.0001**
Temperature	1	0.146	0.146	0.110	0.7097
Weight	1	0.062	0.062	0.047	0.7556
Feeding treatment*Temperature	2	0.409	0.204	0.155	0.8368
Final model (AIC = 1275.2)					
Feeding treatment	2	45.204	22.602	16.728	**< 0.0001**

Quantification of eDNA degradation rates

Crown-of-thorns seastar eDNA from one large tank was used in a 120-hour degradation experiment. Daily monitoring of water quality demonstrated that temperatures were consistent and dissolved oxygen levels were near saturation throughout the experiment (Fig. S1, Table S2). CoTS eDNA was not detected in any negative controls (n = 6), indicating no cross-contamination throughout the experiment. CoTS eDNA exhibited good fit ($R^2 > 0.90$) to a monophasic exponential decay model for all temperature treatments (Fig. 3). The decay rate constants were estimated as 0.034, 0.050 and 0.068 h^{-1} at 24, 26 and 28°C, respectively. The interaction between time and temperature in a linear model for all three temperatures was highly insignificant (ANOVA, $F_{2,75} = 0.503$, $p = 0.6064$), indicating that the slopes among temperature groups were not statistically different. Thus, there was no detectable temperature effect on eDNA degradation rate. Combining all data points, the overall decay rate constant was 0.048 h^{-1}. The corresponding half-life was 14.48 hours and the time to 99% decay was 96.18 hours.

Discussion

Recent research has demonstrated the applicability of eDNA techniques in the management of CoTS outbreaks (Doyle et al. 2017; Uthicke et al. 2018; Doyle and Uthicke 2020). However, the interpretation of surveillance results has been limited by the uncertainties in the dynamics of eDNA in aquatic environments. The present study tested if the amount of CoTS eDNA detected was influenced by

temperature, feeding, and presence of coral. This study also quantified CoTS eDNA degradation rates under different temperature regimes.

Environmental DNA shedding rate

Shedding rate was found to be seven times higher when food was accessible compared to when food was inaccessible, indicating that feeding behavior of CoTS promotes eDNA release into the environment. This result is consistent with previous findings in freshwater fish, where feeding increases eDNA shedding rate by ten-fold (Klymus et al. 2015). CoTS are voracious coral predators which feed by distending their stomach through the oral cavity and spreading it over the surface of the coral (Brauer et al. 1970). Enzymes are secreted from the stomach to digest coral tissue, which is then harvested as the stomach is retracted (Brahimi-Horn et al. 1989). During this extra-oral feeding process, eDNA is likely to be shed from the digestive system exposed to the environment. While the physiological origins of eDNA in most cases remain uncertain, our results suggest that feeding behavior might be a major driver of eDNA shedding in CoTS. As such, the timing of feeding activities should be considered in the design of eDNA surveys. For example, during evening and nighttime when adult CoTS are more actively feeding (Burn et al. 2020; Ling et al. 2020), the eDNA concentration in the water column might be higher, introducing bias to the surveillance results.

Comparison of two non-feeding treatments (food inaccessible vs no food treatment) indicated that the amount of eDNA detected was significantly reduced by the presence of coral. Since eDNA can be readily incorporated into biofilm on the surfaces of aquaria (Wood et al. 2020), we hypothesize that CoTS eDNA was bound to the biofilm on the surface mucus layer of coral in this experiment. This is supported by the fact that coral mucus is an efficient trap for suspended particulate matter (Brown and Bythell 2005). Alternatively, eDNA could be adsorbed to the carbonate skeleton of coral via electrostatic interaction (Hou et al. 2014). While incorporation of eDNA into sediments (Turner et al. 2015; Buxton et al. 2017) and biofilms (Wood et al. 2020) have been documented previously, this study is the first to report possible binding of eDNA to coral. Further investigation, such as determining presence of CoTS eDNA in coral biofilm, tissue, or skeleton, is needed to test this hypothesis. If the effect of binding to coral is significant, it might imply that eDNA concentration measured in the field can be affected by coral cover or the presence of biofilms on other substrate surfaces.

No relationship between water temperature and eDNA shedding rate was found in the present study. Existing literature shows that the effect of temperature on eDNA shedding rate is species- and ecosystem-dependent. For example, within ambient temperature range, eDNA shedding rate of freshwater fish was not affected by water temperature (Takahara et al. 2012; Klymus et al. 2015), while cane toads were hypothesized to shed more eDNA in warmer water (Villacorta-Rath et al. 2020). When tested under extended temperature ranges, tropical freshwater fish exhibited higher eDNA shedding rate at extreme temperature (35°C) (Robson et al. 2016). As such, it is difficult to draw a general conclusion about relationship between temperature and eDNA shedding rate. Although this study demonstrated that water temperature does not directly influence eDNA shedding rate, seasonal changes in CoTS behavior should

be considered when interpreting eDNA surveillance results. For example, the overall feeding rate of adult CoTS was found to be higher in summer followed by a significant reduction after spawning (Keesing and Lucas 1992). Additionally, CoTS are known to aggregate for spawning when water temperature is about 28°C (Lucas 2013). These behavioral changes might introduce variations in eDNA signal, and thus incorporating knowledge of biology and ecology of CoTS into eDNA studies is necessary.

The average eDNA shedding rates per juvenile CoTS found in this study (10^2 to 10^3 DNA copies h^{-1} $individual^{-1}$) were substantially below those documented for adult CoTS (10^8 DNA copies h^{-1} $individual^{-1}$) (Uthicke et al. 2018). This difference in shedding rate per individual can be attributed to the vast disparity in biomass of CoTS (< 0.1 g in the current study; ~1 kg in Uthicke et al. 2018). However, even when compared as weight specific rates, the eDNA shedding rate of juvenile CoTS (~ 10^4 DNA copies h^{-1} g^{-1}) is tenfold less than that of their adult conspecifics (~ 10^5 DNA copies h^{-1} g^{-1}). By contrast, Maruyama et al. (2014), found approximately four times higher eDNA shedding rate per unit biomass in juvenile fish than in adult fish. The authors suggested that such difference arose due to higher metabolic rate in juveniles. In our case here, the lower skeleton to wet weight ratio in larger CoTS (Kettle and Lucas 1987) might be a possible explanation for the higher per biomass shedding rate in adults. Nonetheless, comparison of eDNA shedding rate between juvenile and adult CoTS here needs to be interpreted with caution. This is because the two studies were conducted in different experimental systems (mesocosm vs aquarium) and under different conditions (e.g., water temperature, food availability). Further research is needed to validate the eDNA shedding rate of CoTS during different life stages. Ideally, quantification of shedding rate should be conducted on a range of life stages under standardized protocols.

When compared on a per biomass basis, eDNA shedding rate of juvenile CoTS found in the present study was approximately one to two orders of magnitude less than marine fish (~ 10^6 DNA copies h^{-1} g^{-1}) (Maruyama et al. 2014) and freshwater fish (~ 10^5 DNA copies h^{-1} g^{-1}) (Jo et al. 2019). Previous research suggests that eDNA shedding rate is dependent on the characteristics and behavior of the target organism (Spear et al. 2015; Wood et al. 2020). It is possible that CoTS (both juvenile and adult) exhibit lower eDNA shedding rates per biomass as they are relatively sedentary when compared with fish which swim constantly in the water column and shed scales or mucus as the main source of eDNA (Sassoubre et al. 2016). Comparison between studies remains limited as eDNA shedding rates have been reported differently (e.g., accumulated eDNA concentration vs per unit time vs per unit time per individual/biomass). Reporting shedding rate per unit time per individual as well as per unit time per biomass in future studies would allow direct comparison and improve understanding of factors driving eDNA shedding in different organisms.

Environmental DNA degradation rate

No effect of water temperature on eDNA decay rate was found in this study. While temperature-dependent degradation of eDNA has been reported frequently, in most cases the range of temperatures examined are wider than the present study. For example, Eichmiller et al. (2016) conducted experiments at 5, 15, 25 and 35°C and found significantly lower eDNA degradation rate at 5°C. Similarly, Strickler et al. (2015) found

no effect of temperature at 25 and 35°C but significantly lower degradation rate at 5°C. According to these results, it appears that differences in eDNA degradation rate arise when there is a substantial change in temperature. As such, it can be concluded that within the natural temperature range of the GBR, water temperature might not be the major factor affecting eDNA degradation rate. To improve understanding of seasonal dynamics of eDNA degradation, future studies should continue to examine the effect of other environmental factors. High UV radiation, low salinity and low pH, for example, have been shown to promote DNA degradation (Strickler et al. 2015; Collins et al. 2018). Furthermore, microbial and exogenous enzymatic activities as the biological causes of degradation are still major knowledge gaps to be addressed.

The decay rate constant (0.048 h^{-1}) found in this study is in the same range as marine fish (0.055–0.101 h^{-1}) (Sassoubre et al. 2016), freshwater fish (0.015–0.100 h^{-1}) (Eichmiller et al. 2016) and amphibians (0.076 h^{-1}) (Villacorta-Rath et al. 2020) under similar environment. The good fit to a monophasic exponential decay model and the comparable decay rate constants across taxa supports the hypothesis that the mechanism of eDNA degradation is consistent despite potentially different sources (Sassoubre et al. 2016). The half-life documented in this study (14.48 hours) aligns with previous observations that most marine eDNA half-lives fall between 10–50 hours, supporting the subsequent conclusion that in general, eDNA degrades slower in marine environments than in freshwater (Collins et al. 2018). Given the rapid time to 99% decay (96 hour) in a laboratory environment, together with other forms of eDNA removal that are present in the field (e.g., diffusion, adsorption to sediments or substrate) (Harrison et al. 2019), it is likely that the results of eDNA surveillance would reflect recent and local occurrence of CoTS.

Implications for eDNA as a monitoring tool for early life stages of CoTS

This study demonstrated the possibility to detect the relatively weak eDNA signal from juvenile CoTS, indicating that eDNA can potentially be developed into a novel tool for the detection and monitoring of early life stages of CoTS on coral reefs. The results of this study will likely be applicable to early outbreak reefs where the number of adult CoTS is minimal. This is because adult CoTS exhibit five orders of magnitude higher eDNA shedding rate (Uthicke et al. 2018) which could mask the eDNA signal from juveniles. However, given the high mortality rates of juveniles (e.g., 2.6% per day for ⌀ 3mm individuals [Keesing et al. 2018]), it is likely that juveniles are many orders of magnitude more abundant that adults which may compensate for the lower eDNA release rates. Detecting and potentially quantifying early life stages of CoTS with eDNA can provide us with a forecast for population outbreaks, adding to the toolbox for an early warning system together with larval monitoring (Doyle et al. 2017) and rapid in-field testing (Doyle and Uthicke 2020). Furthermore, detection of newly settled individuals (0 + year old) via comparison of eDNA concentration pre- and post-spawning can improve our knowledge in recruitment events and the translation of larval density into settlement. However, further investigations are required before eDNA can be applied in the field for juvenile CoTS monitoring. For example, pilot studies are needed to achieve appropriate methodologies for the accurate detection of eDNA released from juveniles

in natural environment. It is likely that water samples will need to be collected close to the substrate due to the relatively low eDNA shedding rate.

After refinement and validation of methodology, eDNA techniques would open new opportunities for field-based studies on early life stages of CoTS, which was largely constrained previously due to the difficulty in detection of individuals. For example, eDNA techniques can be deployed in the field to validate laboratory-based hypotheses. In particular, Deaker et al. (2020) recently proposed that juvenile CoTS have the ability to prolong their herbivorous phase and delay growth for at least six years while waiting for favorable conditions (e.g., availability of coral food) to arise (known as the “Peter Pan effect”, or “juveniles in waiting”). This hypothesis can be tested in the field by collecting regular eDNA samples from early outbreak reefs or reefs between outbreaks to monitor the changes in abundance of juvenile CoTS. Together with larval monitoring and knowledge of the timing of spawning events, we can understand whether the presence and build-up of juvenile CoTS on certain reefs before outbreaks is maintained by the “Peter Pan effect” or by new recruitment each year.

In conclusion, our study clarified important aspects of the CoTS eDNA dynamics. First, we demonstrated that feeding behavior of CoTS increases eDNA shedding rate, while presence of coral reduces the amount of eDNA detected. Second, rapid degradation of CoTS eDNA indicated that results of eDNA survey likely reflect recent and local occurrence of CoTS in the field. Finally, our study also provided support that eDNA can become a complementary tool for the detection of early life stages of CoTS on coral reefs, opening new opportunities to tackle critical research questions that were previously intractable. However, further research and validation is needed to achieve accurate inferences from eDNA surveillance results.

Declarations

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Collections were conducted under a permit from the Great Barrier Reef Marine Park Authority (Permit No. G38062.1). Ethical approval under Australian legislation is not required for invertebrates used in this study.

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Figures

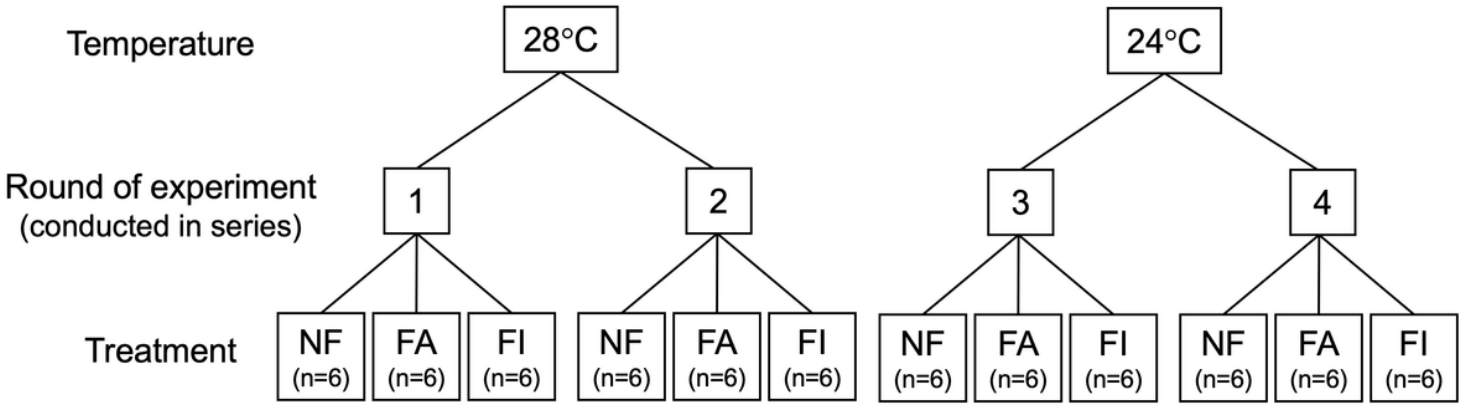

Figure 1

Experimental split-plot design to investigate the effect of temperature, feeding, and presence of coral on environmental DNA shedding rate of crown-of-thorns seastar. Treatment NF = no food; FA = food available; FI = food inaccessible. Each round of experiment also included three seawater only controls to test for potential contamination.

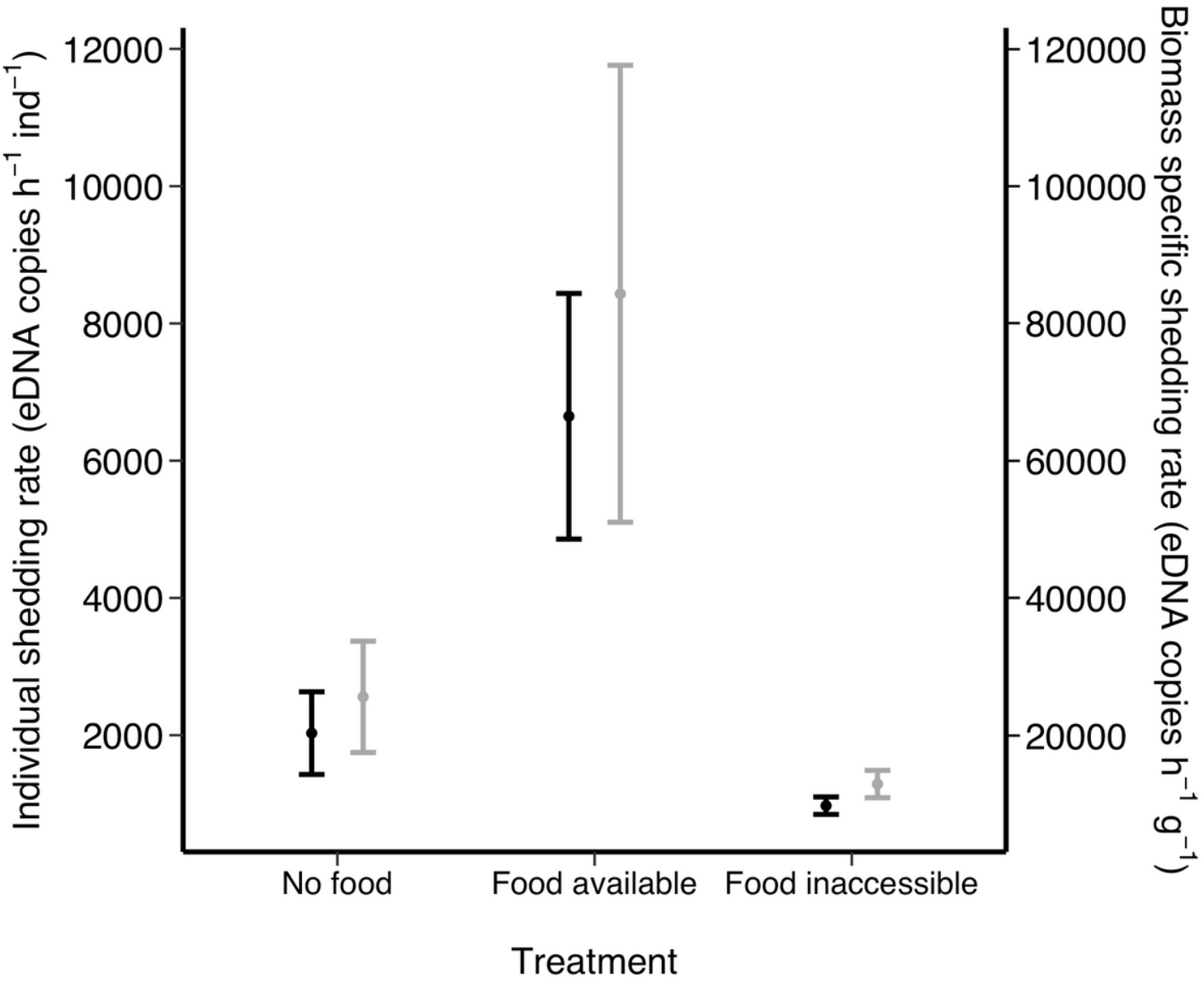

Figure 2

Average environmental DNA shedding rate (± SE) of ~8 months post-settlement juvenile crown-of-thorns seastar (CoTS; Acanthaster cf. solaris) under different feeding treatments. Black and grey bar represent shedding rate per individual (left-hand y-axis) and per unit biomass (right-hand y-axis), respectively.

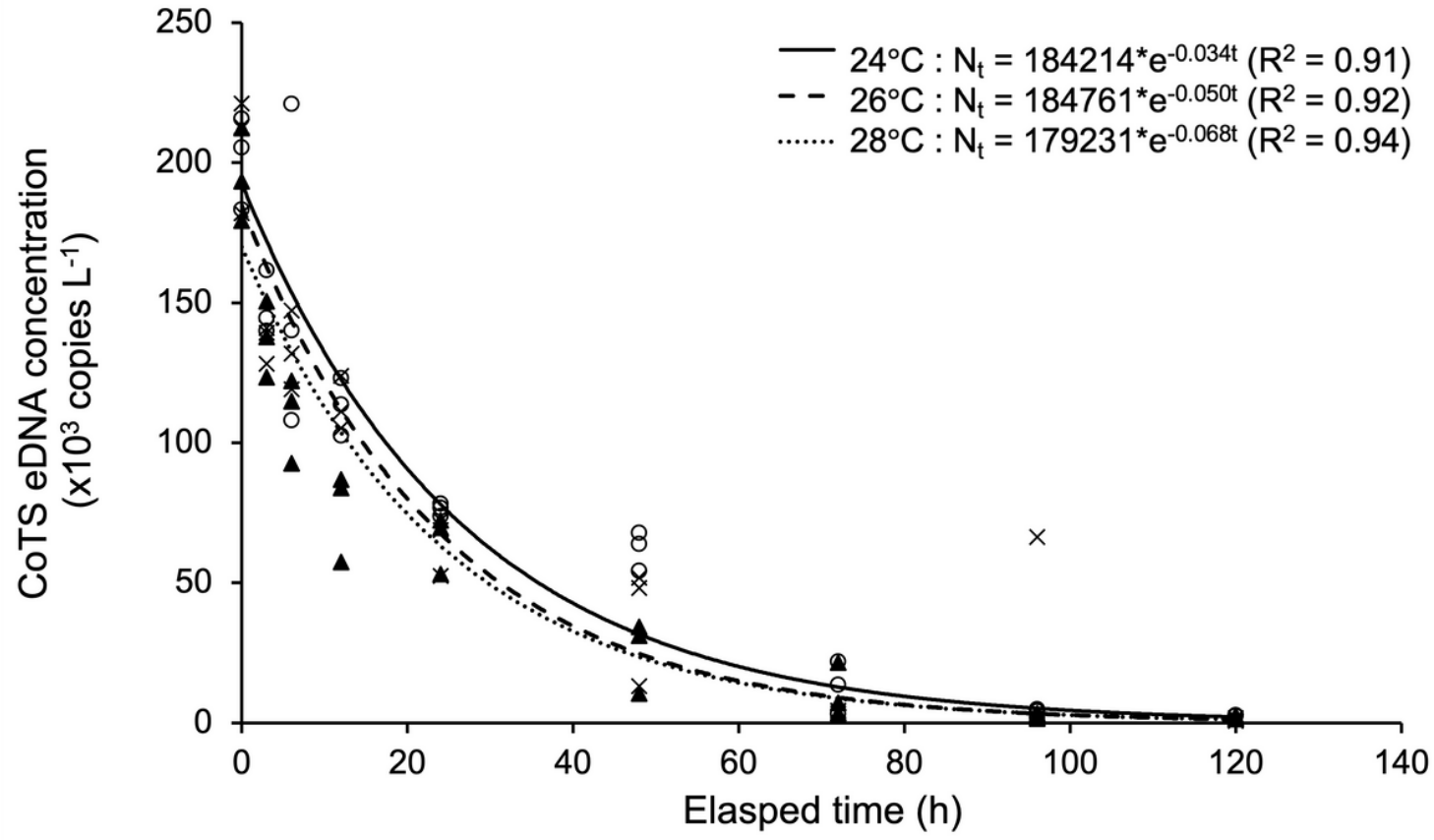

Figure 3

Concentration of crown-of-thorns seastar (CoTS; Acanthaster cf. solaris) environmental DNA (eDNA) through time under different temperature regimes. Circles, crosses and triangles represent eDNA concentration at 24, 26, and 28°C, respectively.

Supplementary Files

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