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Developing an effective marine eDNA monitoring: eDNA detection at pre-outbreak densities of corallivorous seastar (*Acanthaster* cf. *solaris*)

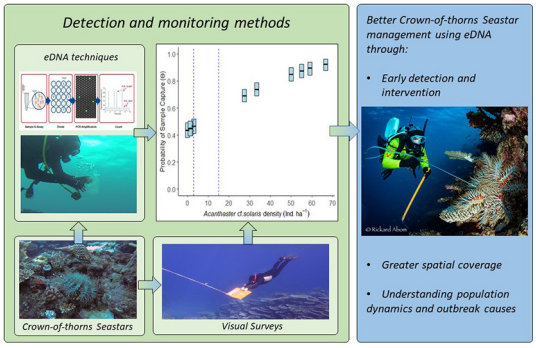
Sven Uthicke [a,*], Barbara Robson [a], Jason R. Doyle [a], Murray Logan [a], Morgan S. Pratchett [b], Miles Lamare [c]

[a] Australian Institute of Marine Science, PMB No. 3, Townsville MC, QLD 4810, Australia
[b] ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD 4811, Australia
[c] Department of Marine Science, University of Otago, Dunedin 9016, New Zealand

HIGHLIGHTS

- eDNA can detect the Crown of Thorns Seastar (CoTS), an important coral predator.
- eDNA detection was not improved by taking into consideration water age, tides or and temperature.
- Occupancy can detect low densities with a low number of replicates.
- Occupancy per reef is correlated with CoTS densities but saturates at higher densities.
- eDNA detects below outbreak levels and is a fast alternative to traditional surveys.

GRAPHICAL ABSTRACT



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ABSTRACT

Outbreaks of the corallivorous Crown-of-Thorns Seastar (CoTS) *Acanthaster* cf. *solaris* contribute significantly to coral reef loss. Control of outbreaks is hampered because standard monitoring techniques do not detect outbreaks at early (low density) stages, thus preventing early intervention. We previously demonstrated that eDNA monitoring can detect CoTS at intermediate densities. Here, we test whether detection probability can be improved by (i) targeted site selection or collection at specific times and (ii) moving from an average eDNA copy number approach (based on the limit of quantification) to a presence/absence approach (based on the limit of detection). Using a dataset collected over three years and multiple reef sites, we demonstrated that adding water residence age, sea surface level and temperature into generalized linear models explained low amounts of variance of eDNA copy numbers. Site specific CoTS density, by contrast, was a significant predictor for eDNA copy numbers. Bayesian multi-scale occupancy modelling of the presence/absence data demonstrated that the probability of sample capture (θ) on most reefs with intermediate or high CoTS densities was >0.8. Thus, confirming CoTS presence on these reefs would only require 2–3 samples. Sample capture decreased with decreasing CoTS density. Collecting ten filters was sufficient to reliably (based on the lower 95 % Credibility Interval) detect CoTS below nominal outbreak levels (3 Ind. ha^{-1}). Copy number-based estimates may be more relevant to quantify CoTS at higher densities. Although water residence age did contribute little to our models, sites with higher residence times may serve as sentinel sites accumulating eDNA. The approach based on presence or absence of eDNA facilitates eDNA monitoring to detect CoTS densities below outbreak thresholds and we continue to further develop this method for quantification.

* Corresponding author.
E-mail address: suthicke@aims.gov.au (S. Uthicke).

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1. Introduction

In addition to climate change (e.g., Lough et al., 2018), population explosions ('outbreaks', 'irruptions') of Crown-of-Thorns Seastars (CoTS, *Acanthaster* sp.) are contributing to reduced coral cover on coral reefs in the Indo-Pacific region including French Polynesia (Kayal et al., 2012), Indonesia (Baird et al., 2013) and Okinawa, Japan (Nakamura et al., 2014). The Great Barrier Reef (GBR) is experiencing its fourth major outbreak of the Pacific CoTS (*A.* cf. *solaris*) since the 1960s (Pratchett et al., 2014; Pratchett et al., 2017), with distinct signs of a 5th outbreak building up.

Knowledge of the timing and location of outbreaks is required to facilitate an understanding of their causes and controls, while early detection would enable early intervention (culling). Routine monitoring for CoTS on the GBR is conducted visually using manta tow surveys since the mid-1980s. While this technique is important and has been used to document CoTS outbreaks over the last three decades (Vanhatalo et al., 2017), it only detects 5–10 % of the individuals present due to low visibility and cryptic behaviour of juveniles and adults (Fernandes et al., 1990). Thus, to better understand when, where and why outbreaks start, there is an urgent need to develop and implement more resolved detection methods (Pratchett et al., 2017), to better understand when, where and why outbreaks start.

Environmental DNA (eDNA) is defined as genetic material obtained directly from environmental samples (i.e., soil, sediment, water column) without 'first isolating any target organisms' (Taberlet et al., 2012). The sources of eDNA in marine and terrestrial samples may be shed skin cells, and excretion of mucus, urine or faeces (Rees et al., 2014). Proof of concept for the feasibility of measuring eDNA in aquatic samples was originally illustrated by detecting introduced species in freshwater ponds (Ficetola et al., 2008) and other freshwater systems (Jerde et al., 2013; Rees et al., 2014; Fukumoto et al., 2015; Hunter et al., 2017). In the marine realm, detection and quantification of eDNA can be more challenging due to much larger dilution factors, currents, and a generally harsh physical environment. In the last 5 years, however, several studies successfully extended the eDNA approach to work on marine crustaceans (Forsström and Vasemägi, 2016), molluscs (Mauvisseau, 2017), echinoderms (Uthicke et al., 2018), cnidarians (Minamoto et al., 2017), teleosts (Thomsen et al., 2016; Yamamoto et al., 2017), or whole communities (Jeunen et al., 2019). More recently, this approach has also been applied to detect invasive or nuisance species in the marine realm (LeBlanc et al., 2020; Kim et al., 2021).

For echinoderms, specific primers have been developed to detect larvae of the invasive sea star species *Asterias amurensis* (Richardson et al., 2016; Ellis et al., 2021). A CoTS specific primer set was originally developed to identify and quantify larvae in the plankton (Uthicke et al., 2015; Doyle et al., 2017). Subsequently, the same set of primers was used to detect and quantify post-settlement CoTS in intermediate to high densities using droplet digital (dd) PCR (Uthicke et al., 2018; Kwong et al., 2021) or lateral flow devices (Doyle and Uthicke, 2020). More recently, this method has also been applied to detect CoTS recruits in settlement traps (Doll et al., 2021).

Appropriate individual density thresholds for management action in CoTS outbreaks are debated. A density of 10–15 Ind. ha^{-1} was initially proposed (Keesing, 1990; Moran and De'ath, 1992) based on the maximum density of CoTS that can be sustained without net coral loss. A more conservative threshold of 3 Ind. ha^{-1} was suggested because models indicated that reproductive success of CoTS disproportionally increases above that density (Rogers et al., 2017). Whether densities exceed either of those thresholds is difficult to measure accurately using traditional in water surveys and require enormous sampling effort and replication because standard transect based techniques cover relatively small areas (~tens to hundreds of m^2). As a compromise, CoTS on the GBR are counted using manta tows, which cover a larger area (i.e. ~2400 m^2), but have low detection probability CoTS (see above).

Here, we are testing if monitoring of CoTS using eDNA can be extended to low densities and to the sub-reef level. For the best-possible sample detection we used ddPCR, which can achieve a much lower limit of quantification that the commonly used qPCR (Uthicke et al., 2018). Over three years, several sites within six reefs on the GBR were sampled several times per trip. To better understand if physical environmental variables influence eDNA measurements at each site, we evaluated if three key variables associated with reef flushing, tidal exchange and sea temperature can improve prediction of eDNA concentration. For this, we utilised the eReefs marine modelling suite, which has been developed to provide information and predictions regarding hydrodynamic and biogeochemical conditions on the GBR (Steven et al., 2019b). The suite is based on modelling software that, among other components, includes a 3D hydrodynamic model, and a biogeochemical models that together comprise the CSIRO Environmental Modelling Suite (CSIRO-EMS) (Baird et al., 2020). While these provide model output on nominal 1 km and 4 km grid scales over the entire GBR, an additional component of the eReefs suite, RECOM, allows users to nest local models on a finer scale within the GBR-wide models to get more detailed spatial and temporal resolution for individual reefs. Specifically, we used RECOM to extract values of water residence age (age of water over reefs), temperature and tide level (sea surface elevation) for each site at each sampling time and included these into statistical models predicting eDNA concentrations. Using our field data, we also applied Bayesian multiscale occupancy modelling (Dorazio and Erickson, 2018) of presence/absence of CoTS eDNA to test whether low concentrations of CoTS can be detected using this methodology in conjunction with occupancy modelling, and to determine the amount of sampling effort needed to detect CoTS in pre-outbreak (< 3 Ind. ha^{-1}) levels to allow for early detection and intervention. Our study demonstrates that early detection monitoring of CoTS using eDNA is feasible and has potential advantages in efficiency (field time required, financial) over traditional survey techniques.

2. Materials and methods

2.1. Field collection

Water samples for eDNA analysis were collected from mid-shelf reefs in the Central section of the GBR in three consecutive sampling years during one vessel-based field trip per year (2018: 06/08–11/08, 2019: 7/05–13/05, 2020: 12/05–17/05). In that section of the GBR, we collected samples at 2–3 sites per reef (Table 1), which were between 460 and 1880 m apart. Samples were also collected from five sites at Lizard Island, which is located in the Northern section of the GBR. These sites were separated by distances between 1080 and 4600 m and collected during two trips to Lizard Island Research Station in 2019 (26/08–29/08) and 2020 (26/8–30/8).

During each trip, sample collections occurred two to three times per site over a few days (see Table 1). In addition, at each sampling site we conducted three to six, 50 m × 4 m underwater transects once per trip. Due to the patchy and cryptic distribution of CoTS at low densities (~<10 Ind. ha^{-1}), underwater survey methods covering small areas are unreliable. Thus, we complemented the two low density reefs (Lizard Island, Elizabeth Rf., in the Northern and Central Section of the GBR, respectively) with additional information. On Elizabeth Rf. we conducted 30 2 min manta tows in 2018 and observed zero CoTS. Similarly, no CoTS were observed in surveys by the AIMS Long Term Monitoring Program (LTMP) (https://apps.aims.gov.au/reef-monitoring/reefs) in 2015 and 2021 (36 manta tows during each survey) at this reef. Based on this, we assigned 'zero' for the CoTS density on Elizabeth Rf. Similarly, near zero or zero CoTS were observed on Lizard Island during LTMP surveys (2017: 88 transects, zero CoTS; 2018 80 transects, zero CoTS, 2021: 90 transects, 1 CoTS) or our own surveys (Table 1). However, scooter-assisted large area diver-based visual surveys at Lizard Island. detected an average 1.53 Ind. ha^{-1} in 2019 and 1.25 Ind. ha^{-1} in 2020 (M. Pratchett, unpublished data). These densities were used for modelling.

Water samples (2.5 l) were collected using Smith-Root eDNA filter housings (Cat # 10966) containing a 1.2 μm mixed cellulose ester (MCE) 47 mm filter membrane. Water was pumped directly from the ocean through the MCE filter using an eDNA sampling device (Grover-Pro™) sourced from Grover Scientific Pty Ltd. Within 1–2 h after collection, filters were removed from the filter housings and carefully folded into eighths and

Table 1
Sample sites for eDNA sampling of *Acanthaster* cf. *solaris* and numbers of visits per year (N-visit) and number of 2.5 l water samples taken in total (N-filters). With 1 exception, samples were included in the analysis using eDNA copy numbers based on LOQ and environmental parameters derived from eReefs. The exception was one site on Lodestone Rf. where only 6 samples were collected during one visit. Thus, a total of 737 individual samples at 65 year/site/visit combinations were included in the statistical analysis using based on eDNA copy number quantification. *Occupancy* indicates the number of samples per year included in the occupancy analysis. On Lizard Island, only samples from Clam Gardens, Big Vicki's Reef and Lizard Island Lagoon were included in the occupancy analysis (sample numbers given in italics). Thus, a total of 678 water filters were included into the occupancy analysis. CoTS densities on the Reef level and site level used for both analyses are presented in Supplementary Tables 1 and 2.

Reef	Site	Latitude	Longitude	N-Visit (18/19/20)	N-filters (18/19/20)
Lizard Island	Mermaid Cove	−14.646	145.4536	0/0/2	0/0/35
	Clam Gardens	−14.6616	145.4489	0/0/2	0/30/30
	Casuarina Beach	−14.6744	145.4409	0/0/2	0/0/30
	Big Vicki's Rf.	−14.6838	145.4394	0/1/2	0/40/31
	Lizard Island Lagoon	−14.6875	145.455	0/2/2	0/30/29
	Occupancy				*0/100/90*
Lodestone Rf.	LR1	−18.6881	147.1000	3/0/0	18/0/0
	LR2	−18.6999	147.1093	3/0/0	18/0/0
	LR3	−18.689	147.0956		(6/0/0)
	Occupancy				*42/0/0*
Davies Rf.	DR1	−18.8263	147.6294	4/4/2	24/39/31
	DR2	−18.8309	147.6355	5/4/2	30/40/29
	Occupancy				*54/79/60*
Bowden Rf.	BR1	−19.0381	147.9263	4/3/2	24/30/30
	BR2	−19.0308	147.9363	5/3/2	30/30/30
	Occupancy				*54/60/60*
Elizabeth Rf.	ER1	−19.3294	149.0536	0/1/2	0/10/30
	ER2	−19.3343	149.0505	0/1/2	0/8/31
	Occupancy				*0/18/61*

placed in a 1.5 ml screw cap vial. Qiagen buffer ATL (540 μl) was then added to preserve the DNA until extraction (Majaneva et al., 2018). All equipment was cleaned between uses by soaking for 30 min in a concentrated chlorine solution (100 g dichloroisocyanuric acid per 20 l, equivalent to 0.275 % *w/v* available chlorine).

2.2. DNA extraction and digital droplet PCR assay

Filters were extracted on a Qiacube automated nucleic extraction instrument using modified version of the Qiagen DNeasy Blood and Tissue extraction kit (Doyle and Uthicke, 2020). Elution was in 150 μl (2018/2019) or 50 μl $TE_{0.1}$ (2020). Subsequently, digital droplet PCR (ddPCR) analysis was conducted as described in Uthicke et al. (2018) in the absence of restriction enzyme. Briefly, PCR conditions were 1 cycle at 95 °C for 10 min, 40 cycles of 95 °C for 30s and 60 °C for 1 min; followed by 1 cycle at 98 °C for 10 min and a 10 °C infinite hold. Droplets were subsequently counted on a QX200 droplet reader (Bio-Rad). For presence/absence testing, a positive detection the limit of detection, LOD was defined as one that contains a positive droplet count greater than the no template controls (NTC) and field blank controls (Hunter et al., 2015). For calculations determining the number of CoTS eDNA copies, the limit of quantification (LOQ) was defined as per Uthicke et al. (2018) at 5 positive droplets per reaction.

2.3. eReefs parameters

In the present study, we applied RECOM models with a horizontal resolution of approximately 250 m × 250 m, nested within the 1 km grid-scale eReefs hydrodynamic model (Steven et al., 2019a), to obtain estimates of water temperature (°C), sea surface elevation (m above mean sea level) and "water residence age" (hours) at our sampling sites at the time of sampling. Water residence age, is calculated as described by Mongin and Baird (2014) following the precedent of Monsen et al. (2002). The Water residence age tracer is a simulated dissolved substance that increases at a rate of $1\ h^{-1}$ when over a reef and decays at a rate of $0.2 d^{-1}$ when not over a reef. It is advected and dispersed with the movement of the water. Hence, it is a tracer that reflects how much time a typical parcel of water within a given model grid-cell has spent over reefs, potentially exposed to adult CoTS.

Water residence age and water temperature model outputs are given at hourly intervals, and for each sampling occasion, we extracted the values nearest in time at each sampling site. eReefs are open-access from https://github.com/csiro-coasts/EMS, and model outputs for the GBR over the period December 2010 to July 2021 are available from https://dapds00.nci.org.au/thredds/catalogs/fx3/catalog.html or in a processed format from https://ereefs.aims.gov.au/ereefs-aims. To extract time-series from the model outputs, we used the "ereefs" package for R which is available open access (https://github.com/open-AIMS/ereefs).

2.4. Statistical analyses

All statistical analysis were conducted in R 4.1.0 (R-Core-Team, 2018). We used generalized linear mixed models to test for the effects of eReefs derived parameters (water residence age, surface elevation and temperature) and CoTS density at the site x visit level (Table 1, 65 datapoints each with independent estimates for eReef parameters) on the number of CoTS eDNA copies per l l of water. For optimum fit to model assumptions and to warrant slopes are comparable, predictors were first log-transformed and subsequently standardised to a mean of zero and standard deviation of one. Initial data exploration indicated data fitting against a negative binomial with a log link was most suitable for our data. The sum of eDNA copies on the site x visit level was used as the response. Site was used as a random factor to account for the dependency structure. Models also included the (log-transformed) number of filters as an offset. As a result, the models effectively estimated counts per filter, yet the inclusion of an offset permits the models to be fit against a family well suited to the observed count data (negative binomial), rather than attempt to apply a conforming, yet less suitable family (gamma) to densities. Models were run using the BFGS optimiser function. The glmmTMB (Magnusson et al., 2016) and DHARMA (Hartig, 2020) packages were used to fit and perform model validation, respectively.

To identify parameters of importance affecting eDNA concentrations, we ran five models of declining complexity, and subsequently identified the best model using Akaike Information Criterion (Akaike, 1974). Model 1 included all variables (water residence age, surface elevation, temperature, CoTS density) with all possible interactions, thus representing a complex interplay among measured covariates, whereas Model 2 included all variables without interactions. Model 3 and 4 dropped temperature and surface elevation, respectively, until only CoTS density remained in Model 5 (Table 2A lists all models).

Table 2
Generalized linear models testing the effect of eReefs parameters and CoTS density on *Acanthaster* cf. *solaris* eDNA. (A) Models selection using Akaike Information Criterion (AIC), and (B) details of the two models finding most support. K: the number of parameters in the model.

A)	K	AIC	ΔAIC
Model 4: water residence age + CoTS density	5	619.17	0
Model 5: CoTS density	4	620.34	1.17
Model 3: water residence age + surface elevation + CoTS density	6	621.51	2.34
Model 2: temperature + water residence age + surface elevation + CoTS density	7	621.67	2.5
Model 1: temperature * water residence age * surface elevation * CoTS density	18	644.09	24.92

B)	Estimate	SE	z	Pr(>|z|)
Model 4				
Intercept	3.8825	0.448	8.666	$<2.00 \times 10^{-16}$
scale(water residence age_log)	0.4507	0.3702	1.218	0.223
scale(CoTS_site_log)	2.4753	0.4714	5.251	1.51×10^{-07}
Model 5				
Intercept	2.5711	0.4326	5.943	2.80×10^{-09}
scale(CoTS_log)	4.0012	0.4539	8.814	$< 2.00 \times 10^{-16}$

To determine if presence/absence data (based on the limit of detection) allows for detection of CoTS at low densities, we used Bayesian occupancy models, following parameter definitions given by Erickson et al. (2019). The probability of sample capture (θ), is the probability of CoTS eDNA being present on a filter sample if CoTS eDNA is present in the water. The probability of detection (p) expresses the probability of a single technical replicate (all samples were run in 2 technical replicates per filter) to detect CoTS eDNA if present. The probability of site occurrence (ψ) expresses how likely it is that any CoTS DNA is at a site. Bayesian Multiscale Occupancy analysis was conducted using the R library eDNAOccupancy (Dorazio and Erickson, 2018). The latter analyses were conducted on an aggregated dataset for each reef at any given year by ignoring the site level. This was done because CoTS density estimates on the site level are not sufficiently accurate for low densities and the spatial scale not appropriate. This yielded 12 unique samples with high numbers of filter replicates (42–100, see Table 1). Based on the limit of detection, CoTS eDNA for each technical replicate from each filter was scored as present or absent. We calculated the probability of sample capture (θ) for each year on each reef, keeping both site occurrence and probability of detection constant. This was achieved by calculating posterior distributions of model parameters using a Markov chain with 10,000 iterations. The estimated values of the model parameters were used to derive the posterior mean and 95 % credibility interval of p, ψ and θ using a burnin period of 1000. Convergence of the models was tested with trace plots, and autocorrelation with autocorrelation plots.

Based on the θ for each reef and year combination, we calculate the probability of detection (P_N) of CoTS eDNA for a given number of filters with:

$P_N = 1 - (1 - \theta)^N$ with N = the hypothetical number of filters collected (from 1 to 30). These calculations were conducted for the posterior mean, and lower and upper credibility intervals. In a second multiscale occupancy analysis we used a similar model as above but added CoTS density as a co-variate for each reef/year combination.

3. Results

3.1. Site specific data and analysis based on copy numbers and limit of quantification (LOQ)

The eReefs-derived parameters, water residence age, surface elevation (tide level) and temperature vary among sites, and within sites depending on sampling time (Fig. 1). Supplementary Fig. 1 illustrates the difference in water residence age at the sites on Lizard Isl. at two separate days under different conditions. Within sites, water residence age (the main parameter of interest) varies as a function of tide and wind-driven currents. At most sites water residence age was relatively stable, with the largest variance observed in Lizard Island Lagoon. Overall, the residence age of the reef water at the sites was relatively short, with maximum values of ~6 h, and most values <1 h. Surface elevation varied between −1.4 and 0.79 m above mean sea level (negative values indicate heights below mean sea level). As we collect eDNA only after summer to avoid contamination with larval DNA, water temperatures varied between 23.4 and 26.8 °C.

Site specific *Acanthaster* cf. *solaris* densities ranged between zero and 125 ind. ha^{-1}. At the site level, transect sampling indicated CoTS were undetectable at Lizard Island and on Elizabeth Rf. In contrast, high and variable numbers on the mid-shelf reefs near Townsville (Lodestone Rf., Davies Rf., Bowden Rf.) reflect the fact that this was near the front of the ongoing outbreak.

We analysed five different models to test whether those including eReefs derived parameters performed better in explaining eDNA concentrations than a model containing CoTS densities alone. AIC comparison of the models identified the model containing both CoTS density and water residence age with CoTS density only (Model 4) best explained observed eDNA measurements and was closely followed by a model containing CoTS only (Table 2). However, in the former case, water age was not statistically significant, with the slope more than five times smaller than for CoTS density (Table 2B). Plotting Model 5 supports a good relationship between eDNA copy numbers and CoTS densities (Fig. 2), although we occasionally observed zero CoTS eDNA above LOQ at high CoTS densities in some time * site combinations. The total dataset used in these analyses consisted of 65 site × visit combinations with individual e-Reef parameters. Overall, 39 of these had copy numbers below detection limit (scored as zero), 20 of which were in locations without CoTS being observed. Although this is of no concern for the model fits, these numbers emphasize that the sample size taken for this analysis (6–8) would be insufficient for quantification using the limit of quantification if only taken at a single occasion. There were no incidents of positive detections without CoTS being observed at the respective site.

3.2. Reef specific data based on limit of detection (LOD) and occupancy modelling

We used Bayesian occupancy modelling for the analysis of presence/absence data based on LOD and tested whether data thus analysed can detect CoTS at low densities. The mean probability of occurrence (ψ) of CoTS eDNA held constant over all site/reef combinations was 0.924 (95 % Credibility Interval: 0.732–0.998). The probability of detection per technical replicate was 0.810 (95 % CI: 0.780–0.839). Thus (based on the mean) with 2 technical replicates per filter the probability of detection CoTS eDNA (if present on the filter) was 0.964. The mean of the probability of sample capture (θ) varied between 0.08 (Elizabeth Rf. 2020) and 0.99 (Davies Rf. 2019) (Fig. 3).

Based on the lower credible estimate (a conservative approach), only two eDNA filter samples would have been sufficient to reliable (i.e., $P_N > 0.95$) detect the presence of CoTS on 50 % of the reef/time combinations tested (Fig. 4). Even on Lizard Island, where densities were below nominal outbreak levels (<3 Ind. ha^{-1}), 9 filter samples were required to reliably detect CoTS. On Elizabeth Rf. (where no CoTS were detected in surveys), θ was low and its credibility interval large. Thus, on the latter reef, 30 samples would be insufficient to reliably detect CoTS.

We also ran a model using CoTS densities as a co-variate to predict sample capture. Capture in that model increases roughly linearly until CoTS densities of about 30–40 ha^{-1} (Fig. 5). Thereafter the curve saturated resembling an asymptote. Similar to the model illustrated in Fig. 4, the model with co-variate suggested that (based on the lower CI) 7 samples would be sufficient to for a positive CoTS DNA detection with density as low as those detected on Lizard Island in both years ($P_7 = 1\text{-}((1\text{-}0.391)^7) = 0.969$).

4. Discussion

The present study confirms that both *Acanthaster* cf. *solaris* (CoTS) eDNA abundance (average copy numbers) and the percentage of filters with positive detections reflects variation in local CoTS density on the GBR

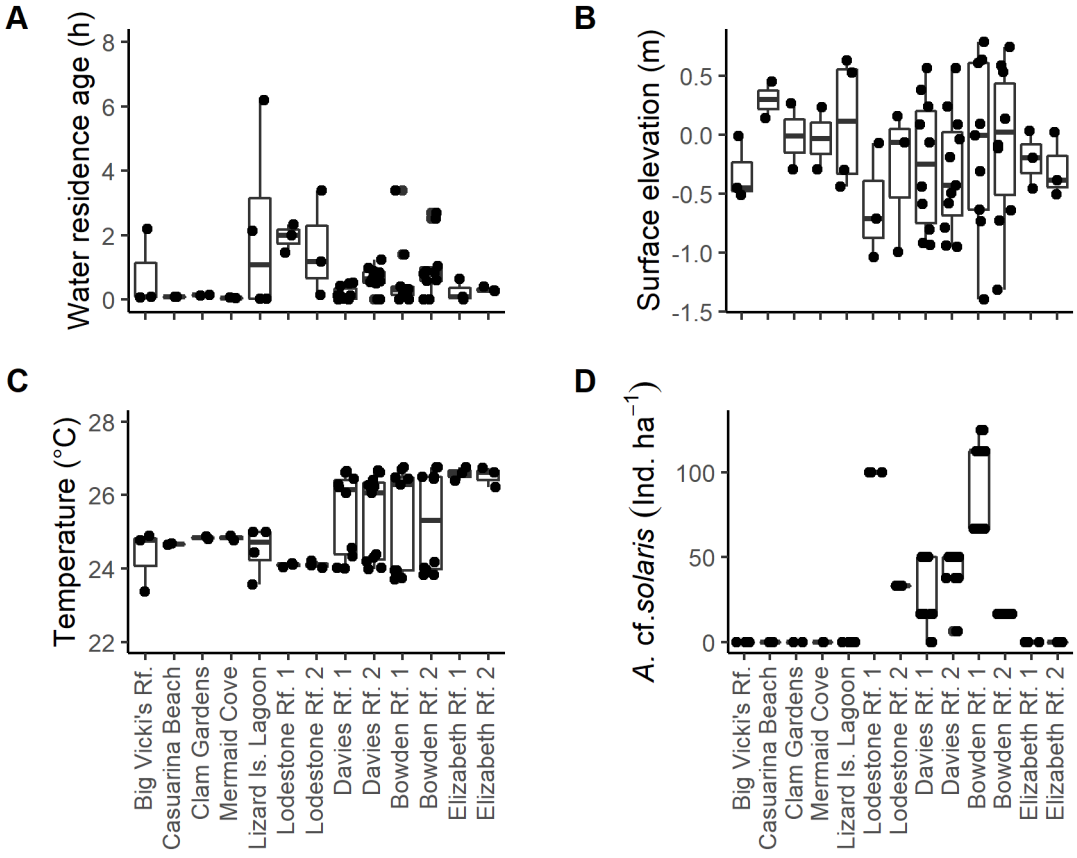

Fig. 1. Variation among study sites over the course of the study for eReefs derived parameters and CoTS densities measured in underwater transects: (A) water residence age, (B) surface elevation or tides, (C) sea temperature, and (D) observed CoTS densities.

reefs sampled, as shown by Uthicke et al. (2018). Moreover, with the possible exception of water residence age we found no evidence that including other environmental parameters improve the model. Bayesian occupancy modelling provide important insights into sample sizes needed to detect CoTS at below outbreak densities. Given very moderate sample sizes required (<10), early detection of CoTS outbreaks is feasible using eDNA analyses on the presence/absence level and would be markedly faster than under water surveys. However, it should also be stated that under

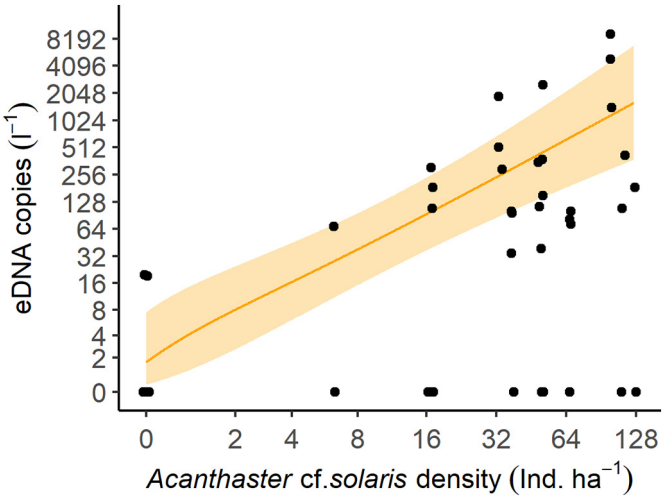

Fig. 2. Model fit (Model 5) and raw data (black dots) illustrating the relationship between CoTS densities and CoTS eDNA copy numbers.

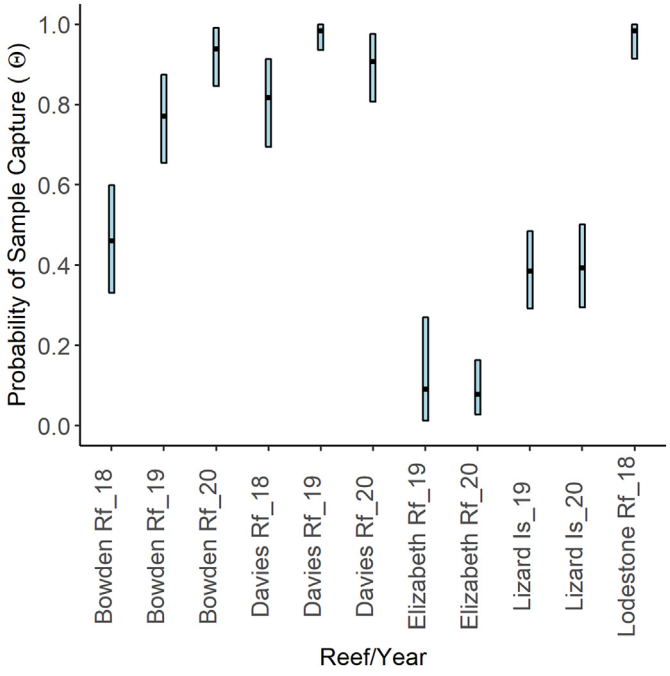

Fig. 3. Occupancy models. θ (black bar: median, blue bar indicates the 95% credibility range) denotes the probability of detection for a single water sample at respective reef/year combinations.

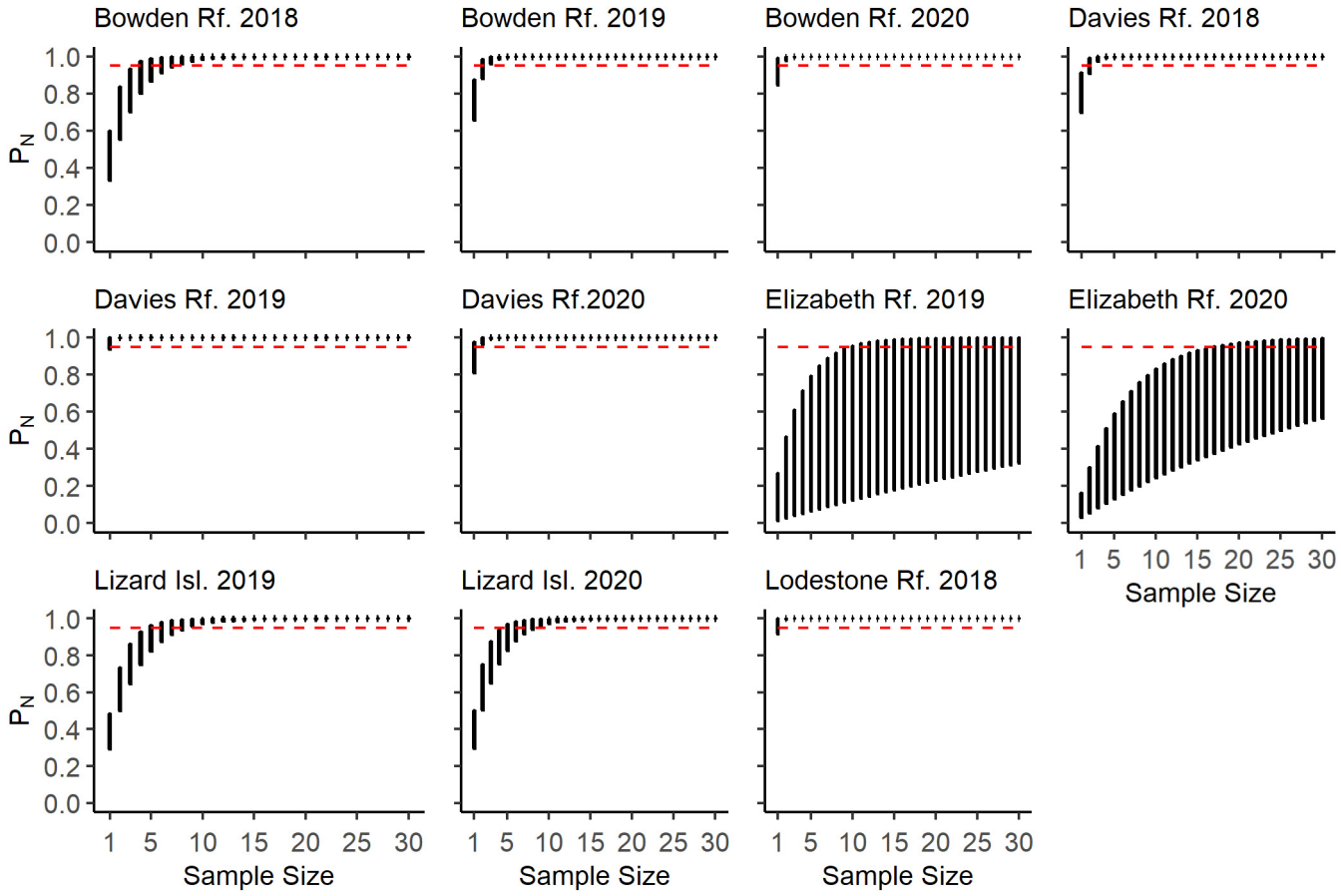

Fig. 4. The probability of detecting *Acanthaster* cf. *solaris* given a specific number of filters tested (P_N) on individual reefs and years depending on sampling size.

water methods can have other advantages, such as providing the ability to investigate population structure (e.g., thought size measurements) or collect tissues, e.g., for sex determination or genetics, at the same time. In addition, eDNA approaches can also have limitations. For instance, it is currently unresolved how far eDNA on coral reefs can be transported between sites which could lead to false positive detections. Because the method is highly sensitive, sample fixation and storage without contamination with CoTS DNA may not be possible under all circumstances.

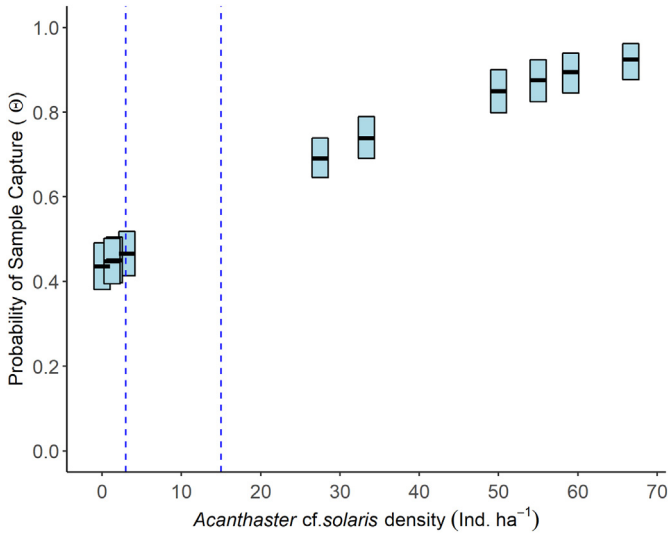

Fig. 5. θ (mean value: black line) and 95 % credibility intervals (blue bars) as a function of *Acanthaster* cf. *solaris* density. Vertical dashed lines indicate 3 Ind. ha^{-1} and 15 Ind. ha^{-1} outbreak thresholds.

We could not find support for models containing sea surface elevation or temperature in our analyses. About 50 % of CoTS eDNA degrades withing 14 h, but effects of temperature on CoTS eDNA shedding and degradation was negligible at temperatures between 24 and 28 °C (Kwong et al., 2021). Although eDNA degradation and production can be temperature dependent (Jo et al., 2019), other studies in aquatic environments have only found reduced degradation at temperatures as cold as 5 °C (Strickler et al., 2015; Eichmiller et al., 2016). We restricted our post-settlement eDNA assays to the winter period to avoid potential contamination by larval eDNA present during the spawning season in summer (Uthicke et al., 2019). A potentially slower eDNA degradation during colder winter months may be an additional advantage for sampling in winter.

We considered testing the effects of tide levels important because it is conceivable that eDNA is more concentrated during low tide, when eDNA released in a smaller volume of water over reefs. However, apart from during actual slack tides, lower water over the reef is typically associated with higher currents (Bernoulli's principle). Thus, to some extent, the possible effects of surface elevation are also captured by the water residence age parameter. We did not find surface elevation to be a significant predictor of post settled CoTS eDNA.

Where water residence age is high, a parcel of water has spent more time over a reef, potentially accumulating eDNA from target organisms associated with the reef. Hence, we hypothesised that increased water residence age could increase eDNA detection probability. Although a model including water residence age was the best performing model, the variable itself was not significant and the (standardised) slope much smaller than for CoTS densities. A longer time of water on reefs should

still increase the eDNA concentration. Thus, it is likely residence time remains an important factor to consider in sampling design. One possibility is that the relatively course (250 m × 250 m) spatial resolution of our hydrodynamic model has reduced water residence age gradients in the model data and limited our ability to capture any such effect in the statistical models. Future work will include a targeted in silico search for reef locations and times with higher retention times than covered here, and in situ testing for eDNA concentrations. To illustrate the importance of the time of sampling, consider the difference for one of our main sites (Lizard Island) at two different scenarios (Supplementary Fig. 1). At a day with strong winds and intermediate tidal exchange, water age at very few sites is above 0.25d. By contrast, on a calm day during neap tides large areas on the south and west of the island have values >0.5 d.

In addition, biotic factors were not directly investigated here, but may interact with water flow and retention on the reef to influence eDNA detection. In particular, reef flats are characterised by large numbers of suspension and filter feeders, such as sponges, which have been shown to accumulate eDNA (Mariani et al., 2019) from the water column and could therefore possibly reduce an eDNA signal under certain conditions such as low water exchange.

Consistent with observations seen here that tides and water flow did not determine the eDNA signal, previous work in a temperate coastal environment found that even under high water exchange eDNA signals are conserved across small spatial scales. For example, Jeunen et al. (2019) found eDNA could differentiate adjacent community types (i.e. rocky reef, open water, mudflats) within a 5 km area despite strong tide-driven currents (>1.6 m s^{-1}) and wave-driven mixing.

Several studies have recently suggested eDNA could be used to monitor marine species (e.g., fished or nuisance species). For example, studies on the fished *Octopus vulgaris* found a positive correlation between biomass and eDNA concentration (Mauvisseau, 2017) and jellyfish eDNA in Japan matched actual distribution patterns (Minamoto et al., 2017). Recent studies developed methods to quantify marine fish or describe fish diversity (Thomsen et al., 2016; Yamamoto et al., 2017), and in temperate regions to differentiate communities across small spatial scales, both horizontally (<5 km, Jeunen et al., 2019) and with depth (< 50 m, Jeunen et al., 2020). Uptake of eDNA methods in tropical marine environments has been slower, but some metabarcoding studies on diversity of fish or other reef life exist (DiBattista et al., 2017; Stat et al., 2017) demonstrating applicability of eDNA methods for biomonitoring.

Multiscale occupancy modelling is considered a useful framework to analyse eDNA data (Dorazio and Erickson, 2018). In aquatic environments, this method has been used, e.g., to analyse data on introduced pythons (Hunter et al., 2015), goby (Schmelzle and Kinziger, 2016), Manatees (Hunter et al., 2018) or an amphibian pathogen (Schmidt et al., 2013). We demonstrated here that presence/absence data can be used to detect very low levels of a marine invertebrate of interest, and multiscale occupancy analysis was a robust way to analyse the data. Using presence/absence data was superior in detecting low CoTS densities because the limit of detection can be used, whereas the limit of quantification is applied when quantifying eDNA copy numbers. However, this does not render the latter method redundant given that the occupancy started to saturate (i.e., most filters have eDNA copies) at higher CoTS concentrations, meaning copy number-based estimates density will be more accurate at higher densities.

The strategy proposed here (repeated field samples followed by the highly sensitive ddPCR assay and occupancy analysis) for the early detection of CoTS outbreaks can also resolve ecological questions important to understand drivers of outbreaks. For instance, the location and timing of primary outbreaks is still not known (Pratchett et al., 2021), largely due to logistical constraints and limited spatiotemporal resolution of visual surveys. eDNA tools would greatly aid in resolving the specific timing and location of initial population increases, especially when incorporated into an integrated monitoring program. In addition, eDNA techniques are being considered as population genetic markers (Adams et al., 2019). Thus, these techniques could be developed for better understanding the nature of CoTS outbreaks by adding to understanding connectivity. For example, while mitochondrial markers used in this study are suitable for broad identification of CoTS eDNA due to the high copy number per individual (Doyle et al., 2017), the development of nuclear markers may lead to indicators of diversity and population size of CoTS on reefs, as well as connectivity between reefs.

5. Conclusions

We showed that detecting whether CoTS on a reef are above threshold levels can be achieved with small amount of sampling effort. If only detection of a threshold level was needed, other non-quantitative eDNA detection methods could also be applied. For instance, CoTS eDNA detection using lateral flow is equally as sensitive as ddPCR (Doyle and Uthicke, 2020), and binomial data (presence/absence) produced by that method are amenable to occupancy analysis. Given the low sample number required for a detection of a threshold level, several reefs could be surveyed per day even if a conservative approach is chosen and several sites per reef are surveyed. Full quantification would need collections of larger samples size and application of both analytical methods described here, and inclusion of biotic parameters might improve model performance. Either way, a suite of eDNA sampling and analysis tools for surveying CoTS populations is now available for application in early detection monitoring.

CRediT authorship contribution statement

Sven Uthicke: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original draft, Funding acquisition **Barbara Robson:** Software, Writing – Original draft, Formal analysis, Visualisation, **Murray Logan**: Software, Writing – Original draft, Formal analysis, Visualisation, **Jason Doyle:** conceptualization, Methodology, Investigation, Writing – Original draft, **Morgan Pratchett**: Investigation, Writing – Original draft, **Miles Lamare**: Conceptualisation, Investigation, Writing – Original draft.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sven Uthicke reports financial support was provided by Ian Potter Foundation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.158143.

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