Validation of eDNA as a viable method of detection for dangerous cubozoan jellyfish

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June 18, 2020

Abstract

Stings from certain species of cubozoan jellyfish are dangerous to humans and their seasonal presence in tropical marine waters poses a significant risk to coastal communities. The detection of cubozoans is difficult due to high spatial and temporal variation in their occurrence and abundance. Environmental DNA (eDNA) has the potential to detect rare species and therefore offers potential to detect cubozoans, not only pelagic medusae, but presence of cryptic polyp life-stages. The objective of this study was to validate the use of eDNA as a viable detection method for four cubozoan species (Chironex fleckeri, Copula sivickisi, Carybdea xaymacana and Carukia barnesi). Species-specific primers were developed for each of these four cubozoans and an eDNA approach validated utilising both laboratory and field trials. Laboratory DNA degradation experiments demonstrated that C. sivickisi DNA degraded quickly but could still be detected in sea water for up to 9 days post-jellyfish removal. Positive detection was found for C. fleckeri, C. xaymacana and C. sivickisi medusae in the waters surrounding Magnetic Island, Queensland, in the Austral spring/summer (September-January). Based on visual surveys there was a poor relationship between concentration of eDNA and abundance of jellyfish. Positive eDNA amplification was also shown near the substratum when C. sivickisi medusae were absent. This can only be explained by the detection of polyps. Consequently, eDNA is an effective tool to detect both the medusae and polyps of cubozoans. This approach provides the means to reduce the risk of envenomation to swimmers and enhance our knowledge of cubozoan ecology.

Keywords: cubozoan, environmental DNA, PCR, Irukandji, jellyfish

Introduction:

The Cubozoa (box jellyfishes) are a class of jellyfish with relatively low species diversity (˜50 species; Kingsford, Michael J. & Mooney, 2014). Many cubozoans are regarded as dangerous and even life-threatening venomous animals that pose challenges to managers of coastal resources in tropical and subtropical regions (Kingsford*et al.* 2018). One species, *Chironex fleckeri,* is considered one of the world's most venomous marine species and has been responsible in Australia for at least 70 deaths in the past 50 years, with mortalities now being increasingly recognised and reported from other Indo-Pacific countries (Seymour, 2002; Kingsford *et al.*2012). Furthermore, envenomation from another 10 cubozoan species can result in the debilitating condition 'Irukandji syndrome' (Ponce et al., 2015). Envenomations and Irukandji syndrome can lead to death, or serious injury, with symptoms that include lower back pain, muscle cramps, vomiting/sweating, vasoconstriction, prostration, possible hypertension, feeling of impending doom and acute heart failure or death (Tibballs *et al.* 2012). Though these jellyfishes pose major health risks to humans, their spatial rareness and elusiveness makes detection in marine waters difficult and as a result their ecology and distribution is presently poorly understood (Tibballs *et al.*2012; Kingsford & Mooney 2014)

Jellyfish have polymorphic lifecycles, where the pelagic adult medusa are the most conspicuous form. The small benthic polyp stages (asexual stages) are usually cryptic, with *Chironex fleckeri* the only cubozoan

species where polyps have been detected *in situ*(Hartwick 1991). This is because benthic cubozoan polyps are minute (< 2 mm) and even the adult medusoid (sexual phases) of some taxa can be very small. This alone has contributed to the many challenges of studying cubozoans (Kingsford & Mooney 2014).

The biogeographic range of a species generally corresponds to a 'metapopulation' made up of relatively autonomous 'stocks' with little connectivity among them. Moreover, within stocks small geographic areas such as bays may be considered 'local populations' that can have a high level of connectivity (Kingsford & Mooney 2014). Recent research has suggested that even small local populations of some cubozoans may have little exchange with other populations (Schlaefer *et al.* 2020); but critical to understanding levels of connectivity is a knowledge of the movements of medusa and the sources of polyps. The accurate detection of cubozoans is critical for minimising the risk of envenomation to humans and to better understand their ecology (Kingsford*et al.* 2018). Another justification for understanding population structure and the dispersal potential of jellyfish is related to climate change. It has been predicted that jellyfish blooms will intensify in occurrence and the geographic range of deadly jellyfish will expand (van Walraven *et al.* 2016). Given the potential changes in level of risk and the related threat to tourism, it is important to develop technologies that improve the detection of cubozoans as they expand their ranges.

Environmental DNA (eDNA) has the potential to greatly enhance research on the ecology of cubozoans and to assist managers of coastal resources to minimise the risks of envenomation (Kingsford *et al.* 2018). Environmental DNA technology, which is the detection of DNA shed in the environment by an organism as a part of normal metabolism, excretion, death and reproduction. This technique has been used to detect rare (Jerde *et al.* 2011; Keskin *et al.* 2016; Simpfendorfer*et al.* 2016), invasive (Robson *et al.* 2016) and sporadically distributed species, including scyphozoan jellyfish. For example, (Minamoto *et al.* 2017) used an eDNA approach to detect presence of medusae of the Japanese sea nettle (*Chrysaora pacifica*) in Mazizuru Bay, Kyoto, while (Gaynor *et al.* 2017) looked at the applicability of eDNA to detect early life-stages of*Chrysaora quinquecirrha* in Barnegat Bay, New Jersey. However, to date there has been no published works on the use of an eDNA approach to detect occurrence of venomous cubozoans and the focus has only been on ephyrae and medusa, not the benthic polyps.

The objective of this study was to develop and validate species-specific PCR primers that could be used to detect four cubozoan species that occur in marine waters along the east coast of tropical Australia; namely, *Chironex fleckeri, Copula sivickisi, Carybdea xaymacana*and *Carukia barnesi.* Of the species selected the chirodropid*C. fleckeri* is considered highly venomous and deadly to humans, and victims of envenomation by the carybdeids C. *xaymacana* and*C. barnesi* often exhibit symptoms of Irukandji syndrome;*C . sivickisi* is harmless to humans. Our specific aims were as follows: (1) develop species-specific primers to detect presence of each of these cubozoans; (2) experimentally determine the degradation rate of cubozoan eDNA; (3) determine if eDNA could detect cubozoan medusae in the field and ascertain if the amount of eDNA correlated with abundance as estimated from visual surveys; (4) determine if presence of cryptic cubozoan polyps can be detected with eDNA at times when medusa are absent using *Copula sivickisi* as the model organism.

Methods:

Primer Design

Specimens of *C. fleckeri* , were collected from Horseshoe Bay (19.1333° S, 146.8500° E), Magnetic Island, Queensland (1 November 2018), with other species having been collected either off the coast of Magnetic Island, or Cairns, Queensland (Supplementary materials). Genomic DNA (gDNA) was extracted using a Qiagen DNeasy Blood and Tissue Kit (Venlo, The Netherlands). A 584 bp fragment of the mitochondrial 16S rRNA gene was amplified via PCR using the "universal jellyfish primers", Forward-16SL, (Bayha *et al* . 2010) Reverse-Aa_H16S_15141H (Ender & Schierwater 2003) and thermocycling conditions reported for the forward and reverse primers respectively. Purified PCR product was then sent to the Australian Genome Research Facility (AGRF, Brisbane, Australia) to be Sanger sequenced. Returned sequences were then edited and aligned in Geneious (Biomatters, Auckland, New Zealand) and put into a n-Blast search downloaded

from the NCBI website to confirm their taxonomy and to identify likely polymorphic sites among species suitable for the design of species-specific eDNA primers (Appendix S1, Supporting information).

Species-specific eDNA primers were designed for *C. fleckeri, C. sivickisi* , *C. xaymacana* and *C. barnesi* using Geneious (Biomatters, Auckland, New Zealand) based on alignment of sequences and identification of unique primer sequences for each species within the mtDNA 16s rRNA gene. The specificity of species-specific primers was then assessed against the genomic DNA of all other cubozoan taxa from waters surrounding Magnetic Island, Queensland.

Using Real-Time PCR, technical replicates for each species were performed. Amplicons were visualised on a 1.5% agarose electrophoresis gel at 80 V for 40 min against other cubozoan species (Appendix S2, Supporting information). The triplicate technical replicates of each species were repeated for qPCR reactions. To deem specificity of primers, exclusive amplification of the target species was completed.

Quantitative PCR

For *C. sivickisi* , the RT-PCR analysis was based on a TaqMan hydrolysis probe (Cop_siv_16S_P - 5'-AACTATTCGCCTCAC-3') assay (Table 1). Each qPCR reaction was run on a QuantStudio 5 Real-Time PCR machine (Thermo Fisher Scientific) and consisted of 5.0 µL 1x TaqPath, 0.9 µM Copula_16S_F (Forward), 0.9 µM Copula_16S_R (Reverse), 0.25 µM Copula_16S_P probe, 2.5 µL sample and 0.7 µL of MilliQ for a final reaction volume of 10 µL. Results were obtained using optimised thermocycling for 40 cycles for 5 min at 95° C, 30 s at 95° C, 15 s at 60° C.

For all other species (*C. fleckeri, C. xaymacana* and *C. barnesi*), eDNA detection was via a SYBR Green Power Up assay, with each reaction consisting of 1.4 µL MilliQ, 10 µL 2x PowerUp SYBR Green, 0.9 µM Forward primer, 0.9 µM Reverse primer, 5 µL sample for a final reaction volume of 20 µL. This reaction volume was chosen due to the lower specificity when compared to the hydrolysis probe and ran at optimised thermocycling for 50 cycles of 2 min at 50 °C, 2 min at 95 °C , 15 s at 95 °C, 1 min at 65 °C, utilising a Melt Curve of 15 s at 95 °C, 1 min at 60 °C, 15 s at 95 °C. Each sample was run in triplicate. Water samples that produced a PCR product were sent for Sanger sequencing (AGRF, Brisbane, Australia) to confirm the species-specificity of the resulting PCR product. For all species, amplification of a single technical replicate deemed the sample replicate as a positive detection.

Water sampling and eDNA preservation

In the field, seawater was collected in sterile 2 L plastic bottles based on volumes used in previous studies in our lab and other studies on the detection of rare and invasive species (Robson *et al.*2016; Simpfendorfer *et al.* 2016; Uthicke *et al.* 2018). For all field experiments, prior to sampling, all equipment was soaked in 10% bleach for 2 hr and then rinsed with reagent-grade water (x3) to eliminate the risk of contaminants. Equipment controls, where 500 mL of sterilised water was passed through the water pump and filters before placed into Longmire's solution, were taken before sampling at each of the sites to detect for possible cross contamination of equipment.

Environmental DNA was extracted from sea water samples using a workflow process (Preserve, Precipitate, Lyse, Precipitate, Purify (PPLPP)), Edmunds and Burrows, (unpublished) (Appendix S3, Supporting information). Once precipitated through the PPLPP process, DNA was purified using the Zymo One Step PCR Inhibitor Removal Kit (Irvine, California, USA).

Experiment 1: DNA Degradation

An DNA degradation experiment was conducted using *C. sivickisi* to determine how quickly eDNA may degrade once the jellyfish is removed. Decay to $< 1\%$ of the original eDNA concentration has been found in some studies to take anywhere from 48 hr to 10 days (Thomsen*et al.* 2012; Pilliod *et al.* 2014); however, there have been no such determinations for cubozoans. In this trial, *C. sivickisi* medusae were collected at night off the coast of Magnetic Island, North Queensland (24/09/2019). Once collected, a single adult jellyfish (inter-pedalia distance 2-3 cm; n = 16) was placed in a 10 L bucket (n = 16) filled with 7 L of artificial

saltwater. After 12 h the medusa was removed. Each bucket (n = 4) was sampled once so that all samples were independent with the full 7 L of water being filtered through 5 um nylon filters. The filtering of water (n = 4 buckets per time) was undertaken on day 0, 3, 6 and 9 post the removal of jellyfish to determine the concentration of eDNA remaining in the water column. Filter papers were placed in sterilised 2 mL tubes containing 1 mL of Longmire solution (Renshaw *et al.* 2015; Williams *et al.*2016) and stored at 4 °C for future extraction and analysis.

Experiment 2: Field experiment

Abundance estimates: Over the course of 5 nights, medusae were collected in known jellyfish 'hotspots' (Schlaefer *et al.* 2020) between September andNovember for *C. sivickisi* and one night for *C. xaymacana.* Sites were separated by tens to hundreds of metres in shallow waters near Magnetic Island, North Queensland (19.1359º S, 146.842º E) (Table 3). The medusae of *C. sivickisi* and *C. xaymacana* are photopositive and were attracted to lights using 'JellyCams' (Schlaefer *et al.* 2020); in this way it was possible to estimate the presence and abundance of jellyfishes. Each 'JellyCam' had an LED torch (2000 lumens) attached to weighted crates. The devices were lowered at each of the sites in 2-5 m of water and left for 30 min to attract cubomedusae for sampling. A snorkeler obtained an estimate of jellyfish abundance within a depth of 3 m from the surface and within a 2 m radius of the light over a course of 2 minutes. b.) Water sampling: For *C. fleckeri,* water samples were taken during daylight hours in the Australian summer (January) in Horseshoe Bay, Magnetic Island, Queensland, from four sites separated by several hundreds of meters (creek, boat ramp, inside the stinger net and northeast side of the bay). Horseshoe Bay was chosen due to historical data from Surf Life Saving Queensland (SLSQ) indicating that medusae were commonly present in the bay during the summer months (Dec-Feb). For *C. sivickisi, C. xaymacana* and *C. barnesi* , Nelly, Geoffrey, Arthur and Florence Bays, Magnetic Island, were chosen for sampling due to historical evidence for containing adult medusae in the Austral spring/summer (September-January) (Schlaefer *et al.*2020). Water samples were taken using sterilised 2 L containers collecting water within 0.5 m of the surface and within a 2 m radius of the JCAM light. Samples were stored on ice and taken back to the lab where they were pumped through either a 20 µm (*C. fleckeri*samples due to high turbidity of sample), or 5 µm (*C. sivickisi, C. xaymacana* and *C. barnesi*) nylon filter within 12 h of collection. Once filtered, filters were removed using sterilised forceps and placed into Longmire's preservative solution (Renshaw*et al.* 2015). All environmental samples were stored at 4ºC.

Experiment 3: Detection of life stages

The presence of *C. sivickisi* medusa in waters surrounding Magnetic Island is highly seasonal (Schlaefer *et al.* 2020), restricted to the spring-summer period. This species, therefore, provided a good model to determine if eDNA could detect the presence of cubozoan polyps when adults were absent. Water for eDNA analysis was collected from nearshore waters surrounding Magnetic Island, Queensland, at three sites separated by 300 - 400 m during the Austral winter (June 2019). Depth stratified water samples were collected into 1L General Oceanics Niskin near the surface and just above the substratum; the total water column was 3 – 6 m deep. Sampling was targeted over coral reef largely covered by the macro alga *Sargassum* and dead coral matrix, as adult medusae are abundant over this habitat in the Austral spring and generally stay close to this preferred habitat (Schlaefer*et al.* 2020). Accordingly, this habitat it is where embryo bundles would likely be released following copulation (García-Rodriguez*et al.* 2018). Water samples (2 L) were taken from 'jellyfish hot spots' at Geoffrey Bay (Site 1, 19.15.332º S & 146.86479 º E; Site 2 - 19.15.486 ºS, 146.86.183º E) and Nelly Bay (Site 3, -19.17.102º S, 146.84.831º E), Magnetic Island. Samples collected during the winter were also taken after dark (1830-1930 h as they were during the medusa season (Sept-Nov). Water was taken in sterile 2 L containers and placed on ice which were then filtered within 12 h of collection. Filter holders were changed for each replicate to minimize the chances of contamination among sites and replicates. Individual filter holders were loaded with 5 µm nylon filters. After filtration of each sample and equipment blanks, the nylon filters were placed in 1 mL of Longmire solution and stored at 4 ºC for DNA extraction.

It was possible that stratification of the water column would influence level of detection of eDNA. Accordingly, conductivity (PSU), temperature and depth (CTD), measurements were taken at each site during the jellyfish

season. These data were used to identify the presence/absence of thermoclines and haloclines in the water column, which could potentially inhibit the vertical dispersal of DNA through the water column.

Results:

Primers

Species-specific primers were successfully developed for each target cubozoan. Each primer set was tested using exclusion PCR against all other cubozoan species found in the waters surrounding Townsville, North Queensland, and only amplified PCR product from the target species. No cross-amplification was observed.

Degradation experiment

DNA (0.08 ± 55.30 ng of eDNA/μL) was detected in buckets from Day 0 of removal of jellyfish and observed to rapidly decay by Day 3 to about 12 % of initial levels (48.2 ± 13.9 ng/uL) where they plateaued (Figure 1). By Day 9, eDNA levels were <1% of initial concentrations and in some buckets undetectable. A nested ANOVA indicated significant difference among days. There were significant differences between containers (replicates) within days and this explained 65.7% of the variation. Variation at the subsampling level of each replicate bucket was small (<1%).

Detection in the field:

In general, where the four species of jellyfish were known to occur, their eDNA was detected. *C. sivickisi* was detected in 100% of replicates at sites 1, 3 and 4. At some sites no lights were used to attract jellyfish ('blind' samples), but eDNA from these sites was still detected (sites 10, 11). There was no correlation between abundance of*C. sivickisi* medusa in the field and quantity of DNA (Figure 2, r = - 0.04, df = 23; P > 0.05).

C. xaymacana and *C. fleckeri* were relatively rare at the time of sampling and *C. fleckeri* was not detected visually, although they were known to be in the area due to observations and collection in tows from Surf Lifesaving Queensland. *Carybdea xaymacana* was detected by both JCAMS, as well as with snorkelling surveys; however, only at two sites and with low species abundance. That being said, this species was also still detected using an eDNA approach.*Carukia barnesi* was never visually detected during sampling and this concurred with zero detection with eDNA (Table 2).

A reverse thermocline was detected at 2 m with no significant halocline being detected.

When the adult medusae of *C. sivickisi* were not present (i.e. in winter), an eDNA signature of this species was detected in water samples. Positive detection was only made in water samples collected within 0.5 m of the substratum. The eDNA of *C. sivickisi* was not detected at the surface (Figure 3). Given medusae of this species are not present at the time of sampling, the eDNA signature can only be explained by the presence of polyps hidden within the coral substratum. It is likely that a shallow thermocline constrained the eDNA signature below the surface (Figure 3a).

Discussion:

This study is the first to report on the detection of small cubozoan jellyfish in marine systems using an eDNA approach. Species-specific primers were designed for four cubozoans and eDNA analyses confirmed detection in the field against visual observations or known presence of jellyfish in proximate locations at the time of water sampling. The study is also the first to highlight the potential for eDNA to detect cubozoan polyps based on *C. sivickisi* as a model, thus providing a genetic tool to locate source reefs that provide recruits to adult medusae populations. This particular finding is significant, as many cubozoans are dangerous to humans and currently there is a paucity of knowledge on the source areas for medusae and therefore how to monitor and better potentially manage jellyfish envenomation risks.

Understanding the rate at which DNA degrades in the aquatic environment can help identify if a species has been present within a certain time. Based on a simple laboratory trial, which only focused on microbial decay on eDNA, our findings indicated a fast degradation of jellyfish DNA within the first 3 days of the animal being removed from the treatment, with only a residual signature left after 9 days in some replicates.

Our findings were similar to studies on other taxa where it is has been concluded that DNA can remain in aquatic environments for 2 to 10 days, depending on dilution and aquatic factors (Thomsen *et al.* 2012; Pilliod *et al.* 2014). In the oceanic environment, currents, UV radiation, dilution and microbial decay all affect the degradation of DNA further confirming close proximity of an organism if eDNA is detected. These findings suggest that due to the rapid decay of DNA the target organisms we sought to detect in the field were, or had been in the area recently, and/or eDNA has been carried by currents from proximate locations. Furthermore, our data is consistent with the findings from Minamoto *et al.* (2017), suggesting the rate of DNA degradation in jellyfish is rapid, with fastest decline in the first 3 days and that oceanic forces act on the eDNA of jellyfishes and rapidly disperse and eliminate DNA. To determine the true effect currents have on eDNA dispersal, modelling is required to determine the distance that eDNA can be advected from a source.

An environmental DNA approach was successful in the detection of jellyfish DNA in both the laboratory and the field. For most jellyfish taxa we knew the target species was present in the field before sampling. *Copula sivickisi* , *C. xaymacana* and *C. fleckeri* were detected at multiple sites where they had been visually observed. The poor relationship between quantity of DNA and abundance of those species where we were able to obtain visual countswas probably due to the following: (1) the heterogenous distribution of DNA in the water column due to the clumping nature of eDNA (Furlan *et al.* 2016); (2) the source(s) of all eDNA in samples is not known, be that from organisms nearby or some distance away;

(3) where samples have been taken some distance from source organisms, this leaves more time for microbial and physical decay; though this may not be the case in the current study as jellyfish were observed close to where the samples were taken; (4) the speed of dilution of eDNA from the source (Gargan *et al.* 2017); (5) small scale oceanography may have a role in concentration and dispersion; (6) vertical stratification of the water column preventing the eDNA from being transported through the thermocline; (7) sea water temperature, as detectability from the source may increase with temperature (Lacoursière-Roussel *et al.*2016). The clumped nature of eDNA (Furlan *et al* 2016) also emphasises the importance of replication at multiple levels, among locations, sites within location and replicates within sites.

JellyCams (JCAMS) (Schlaefer *et al.* 2020) and counts of jellyfish within a few metres of the lights were useful for determining the presence/absence of jellyfish, as well as abundance. Clearly the JCAMs provided a more accurate estimate of relative abundance than eDNA, but the two techniques combined are useful, particularly when jellyfish are rare and are less likely to be detected in lights. For example, *C. xaymacana* was relatively rare in JCAMS imagery and visual counts, but positive detections were revealed using eDNA techniques, even without visual detection. Samples where jellyfish were known to inhabit a site provided a true positive value of detection.

An eDNA approach was shown to have utility to detect the proximate presence of *C. sivickisi* polyps. We were able to detect an eDNA signature in water samples taken close to the substratum during winter, when adult medusae were absent. At all of the sites we sampled for polyps, adult medusae were present during the previous jellyfish season (Sept-Nov). Following the mating of adults, females drop a bundle of embryos on the substratum (Garm *et al.* 2015) as their home ranges are in the tens of metres (Schlaefer *et al.* 2020) and we assume they release planulae at the time. Accordingly, any eDNA of *C. sivickisi* that was detected could only be explained by the proximate presence of polyps of the species. Critically, the benthic polyps could only be detected in depth stratified sampling that including samples taken within 0.5 m of the substratum. It is likely the thermocline acted as a barrier reducing or preventing small amounts of DNA from the substratum reaching the surface, as thermoclines are well known for blocking the vertical passage of particles (Gray & Kingsford 2003) and therefore may inhibit the movement of DNA particles through the water column. Location of polyp beds *in situ* for most cubozoans has eluded scientists to date, due to their cryptic nature, small size, complex habitats and low water visibility. Our study is the first to detect the likely presence of polyps *in situ* and thus shows eDNA provides a strong predictor for where polyps will be located. This in turn allows for the identification of polyp reefs that serve as sources for adult medusae in the summer season. This ability to now detect all life history stages will provide new opportunities to

understanding the ecology and habitat use of cubozoans.

In conclusion, in the current study is was demonstrated that an eDNA approach is an effective technology to detect cubozoans in marine systems, both medusae and putatively the cryptic polyp life-stage. Environmental DNA provides a cost-effective and less labour-intensive way to detect jellyfish that have a broad spatial and temporal variation. A major finding was that we could detect eDNA of *C. sivickisi* polyps *in situ* . Environmental DNA therefore can be used to detect the source locations seeding adult cubozoans and potentially fast-track our understanding of jellyfish ecology. This approach will also be of high utility to detect potential range shifts of cubozoans as a result of climate change.

Acknowledgments:

The authors would like to thank Cecilia Villacorta Rath, Madie Cooper and Alyssa Budd for sharing protocols throughout the project. We are also grateful for the assistance in the field from volunteers.

Table 1: Species-specific mtDNA 16S rRNA primer sequences for four cubozoan species, showing the length (bp) of final PCR fragment, as well as the assay type (SYBR or TaqMan).

Species	Primer	Sequence	Length PCR Fragment (bp)	Assay Ty
	Cop_siv_16S_P	5'-AACTATTCGCCTCAC-3'	199	
C. sivickisi	Cop_siv_16S_F	5'-CTGTCGAGCTTAATTGGTATC-3'		TaqMan
	Cop_siv_16S_R	5'-CACAATTATGGGGCGGAA-3'		
C. fleckeri	Chi_flec_16S_F	5'-GACTGTTTACCAAAAACATA-3'	180	SYBR
	Chi_flec_16S_R	5'-CGATTCAATATCACTGGGAGGCAAG-3'		
C. xaymacana	Car_xay_16S_F	5'-TCTATCTGTTGCAACAAAGGTCC-3'	127	SYBR
	Car_xay_16S_R	5'-GACCCACAGATTTCGTGACTG-3'		
C. barnesi	Car_bar_16S_F	5'-TGAGGCCTGCTCACTGATTC-3'	280	SYBR
	Car_bar_16S_R	5'-CTTCTTTCCCCGATCAAACCAAC-3'		

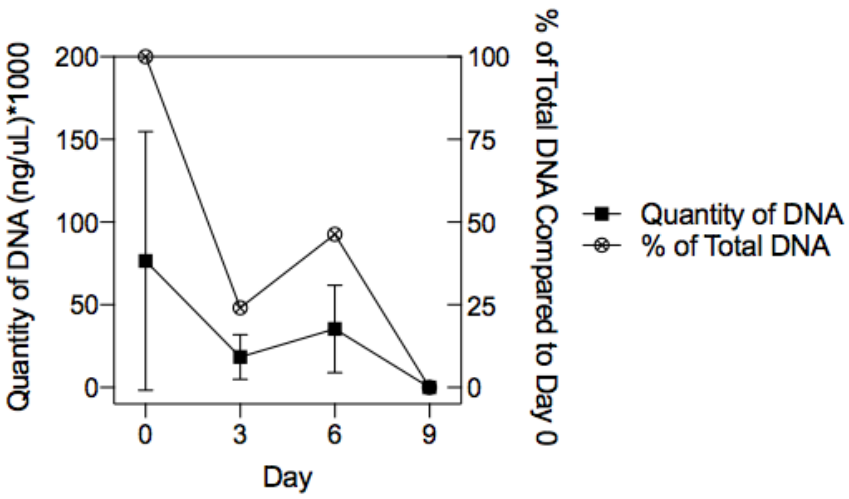

Figure 1: Quantity of DNA (ng/µL) after filtering 7 L of water from four independent tanks over a course of 9 days. Filtering was undertaken 0, 3, 6, 9 days post removal of jellyfish to determine the rate of DNA degradation. Standard errors are shown as bars for level of eDNA at each day.

Table 2: Field validation including visual counts of cubozoan jellyfish species at each location with average concentration of DNA (ng/µL) from filtered 2 L samples. All field replicates were collected from marine areas

surrounding Magnetic Island, North Queensland. All sites, excluding *Chironex fleckeri* samples and 'blind' sites utilised JellyCams. Note: no visual counts were attempted when comparing eDNA for *C. fleckeri* .

Species	**Location**	**Collection date**	**Site Name**	**Visual Count of jellyfish**	**No.**
Copula sivickisi		(1/11/18)	Site 1	100	2
		(15/11/18)	Site 2	125	2
		(15/11/18)	Site 3	150	2
	Geoffrey Bay	(24/09/19)	Site 4	21	3
		(24/09/19)	Site 5	115	3
		(07/10/19)	Site 6	32	3
		(07/10/19)	Site 7	15	3
		(07/10/19)	Site 8	15	3
	Nelly Bay	(17/10/19)	Site 9	630	3
Blind		(15/11/18)	Site 10	0	2
		(07/10/19)	Site 11	0	3
		(17/01/19)	Creek	0	2
	Horseshoe Bay	(17/01/19)	Stinger Net	0	2
		(17/01/19)	Boat Ramp	0	2
Chironex fleckeri		(17/01/19)	Northeast side of Bay	0	2
		(24/09/19)	Site 1	0	2
		(24/09/19)	Site 2	0	3
	Geoffrey Bay	(24/09/19)	Site 3	0	3
		(24/09/19)	Site 4	0	3
		(07/10/19)	Site 5	0	3
		(24/09/19)	Site 1	0	2
		(24/09/19)	Site 2	0	3
	Geoffrey Bay	(24/09/19)	Site 3	0	3
		(24/09/19)	Site 4	0	3
		(07/10/19)	Site 5	0	3
Carybdea xaymacana		(07/10/19)	Site 6	0	3
		(07/10/19)	Site 7	0	3
	Florence Bay	(14/11/19)	Site 8	1	3
		(14/11/19)	Site 9	1	3
	Arthur Bay	(14/11/19)	Site 10	1	3
		(14/11/19)	Site 11	0	3
		(24/09/19)	Site 12	0	2
		(24/09/19)	Site 13	0	3
	Geoffrey Bay	(24/09/19)	Site 1	0	3
		(24/09/19)	Site 2	0	3
		(07/10/19)	Site 3	0	3
Carukia barnesi		(07/10/19)	Site 4	0	3
		(07/10/19)	Site 5	0	3
	Florence Bay	(14/11/19)	Site 6	0	3
		(14/11/19)	Site 7	0	3
	Arthur Bay	(14/11/19)	Site 8	0	3
		(14/11/19)	Site 9	0	3

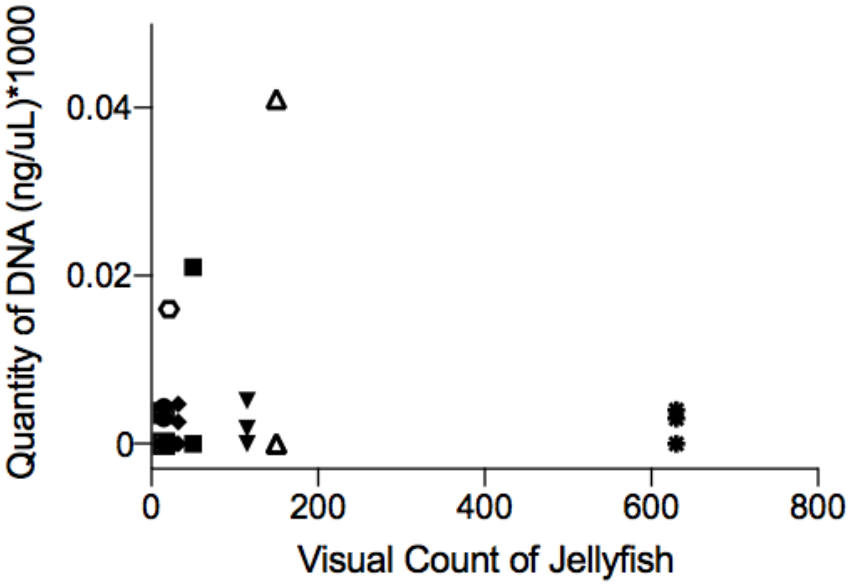

Figure 2: Correlation between visual abundance counts of*Copula sivickisi* and average quantity of DNA (ng/µL) at the respective site within Geoffrey and Nelly Bay, Magnetic Island. Sites 1-11 are represented by a different symbol.

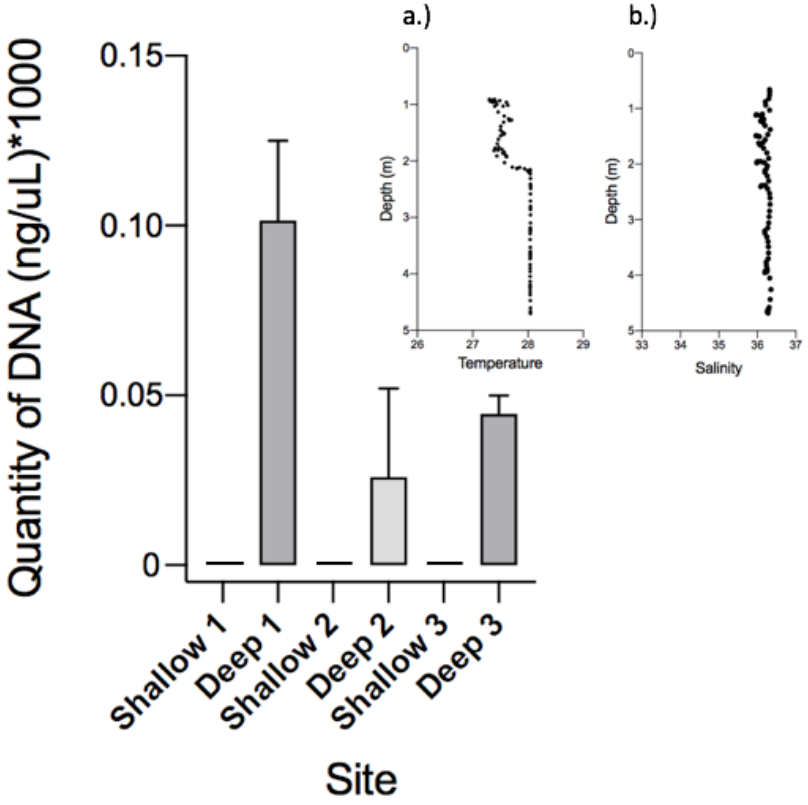

Figure 3: (Left) Quantity of *Copula sivickisi* eDNA (ng/μL) when sampled from surface (Shallow) or benthic (Deep) waters surrounding Magnetic Island, along with (a) temperature and (b) salinity (PSU) profiles of the water column at the time of sampling.

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