Supplementary Materials

Use of eDNA to Determine Source Locations of Deadly Jellyfish (Cubozoa) in a Tourism Hotspot

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Table S1. Species-specific *Chironex fleckeri* and generic fish endogenous control assay information (adapted from Morrissey et al. (2022)).

Assay	Label	Sequence (5′ – 3′)	Reference
Chironex fleckeri (species-specific)	Forward	ATCTTCCACTGTCTCAGCTTTACC	Morrissey et al. (2022)
	Reverse	CCTCAGTACTCGTGTCTCCCTA	
	TaqMan MGB Probe	(FAM)-CTCGTCCTTCCAAGTATAAG-(MGB)	
Generic fish - Endogenous Control	Forward	GACCTCGATGTTGGATCA	Furlan & Gleeson (2016)
	Reverse	CTCAGATCACGTAGGACTTTA	
	TaqMan MGB Probe	(VIC)-ACATCCTAWTGGTGC-(MGB)	

Table S2. eDNA sample collection sites with depth, depth-integrated temperature and salinity, number of positive technical replicates and eDNA concentrations (copies L^{-1}) during the December 2020 and February 2021 sampling periods.

Sampling Time	Site no.	Depth (m)	Temperature (°C)	Salinity (ppt)	No. of positive detections / 12	Copies per L
December 2020	5	2.5	29.5	36.2	1	32
	6	0.4	30.9	36.4	2	45.8
	16	2.2	29.5	36.2	5	97.9
	19	2.4	29.4	36.2	3	275.6
	22	1	31.1	36.2	0	0
	23	0.4	29	36.2	0	0
Average	–	–	29.9	36.2	–	–
February 2021	5	2.8	30.3	33.8	0	0
	6	2.1	29.9	32.4	2	63.4
	16	2.2	29.8	33.6	2	36.5
	19	3.1	30.3	33.8	2	92.4
	22	0.8	30.2	33.6	2	17.8
	23	0.7	30	33.4	1	26.8
Average	–	–	30.1	33.4	–	–

Table S3. eDNA sample collection sites with depth (m), depth-integrated temperature and salinity, number of positive technical replicates and eDNA concentrations (copies L^{-1}) during the March and December 2021 sampling periods. * indicates sites where data were not collected.

Sampling Time	Site no.	Depth (m)	Temperature (°C)	Salinity (ppt)	No. of positive detections / 12	Copies per L
March 2021	1	8.1	28.1	32.8	0	0
	3	5.1	28.8	32.9	0	0
	5	0.5	29.5	32.8	0	0
	6	0.8	27.2	31.4	4	134.8
	14	10.7	28.4	32.9	0	0
	15	8.2	28.5	32.9	0	0
	16	1.4	29.6	31.4	3	58.2
	17	11.4	28.3	32.9	0	0
	18	8.9	28.6	32.9	0	0
	19	1.7	*	*	0	0
	20	11.2	28.3	32.9	0	0
	21	7.8	28.3	32.9	0	0
	22	1.2	27.9	32.8	0	0
	23	1.2	28.2	31.9	1	18.8
Average	–	–	28.4	32.6	–	–
December 2021	1	11.7	30	35.3	0	0
	3	8.8	30.1	35.2	0	0
	5	0.5	31.2	35.2	0	0
	6	0.4	30.2	35.5	1	33.5
	14	12.3	30.1	35.3	0	0
	15	8.9	30.1	35.2	0	0
	16	3	30.4	35.3	0	0
	17	11.8	30	35.2	0	0
	18	8.7	30.3	35.2	0	0
	19	4.2	30.6	35.3	0	0
	20	13	30.1	35.3	0	0
	21	8.1	30.2	35.3	0	0
	22	0.9	30.7	35.3	0	0
	23	0.4	30.6	35.3	1	22.5
Average	–	–	30.3	35.3	–	–

Table S4. eDNA sample collection sites with depth (m), number of positive technical replicates and eDNA concentrations (copies L^{-1}) during the July 2020 sampling period. Note, temperatures and salinities were not measured during that sampling period.

Sampling Time	Site no.	No. of positive detections / 12	Copies per L
July 2020	1	0	0
	3	1	62.5
	5	0	0
	6	1	63.3
	14	0	0
	15	0	0
	16	0	0
	17	0	0
	18	0	0
	19	0	0
	20	0	0
	21	0	0
	22	0	0
	23	0	0

Table S5. eDNA sample collection sites with depth (m), depth-integrated temperature and salinity, number of positive technical replicates and eDNA concentrations (copies L^{-1}) during the July 2022 sampling period. * indicates sites where data were not collected.

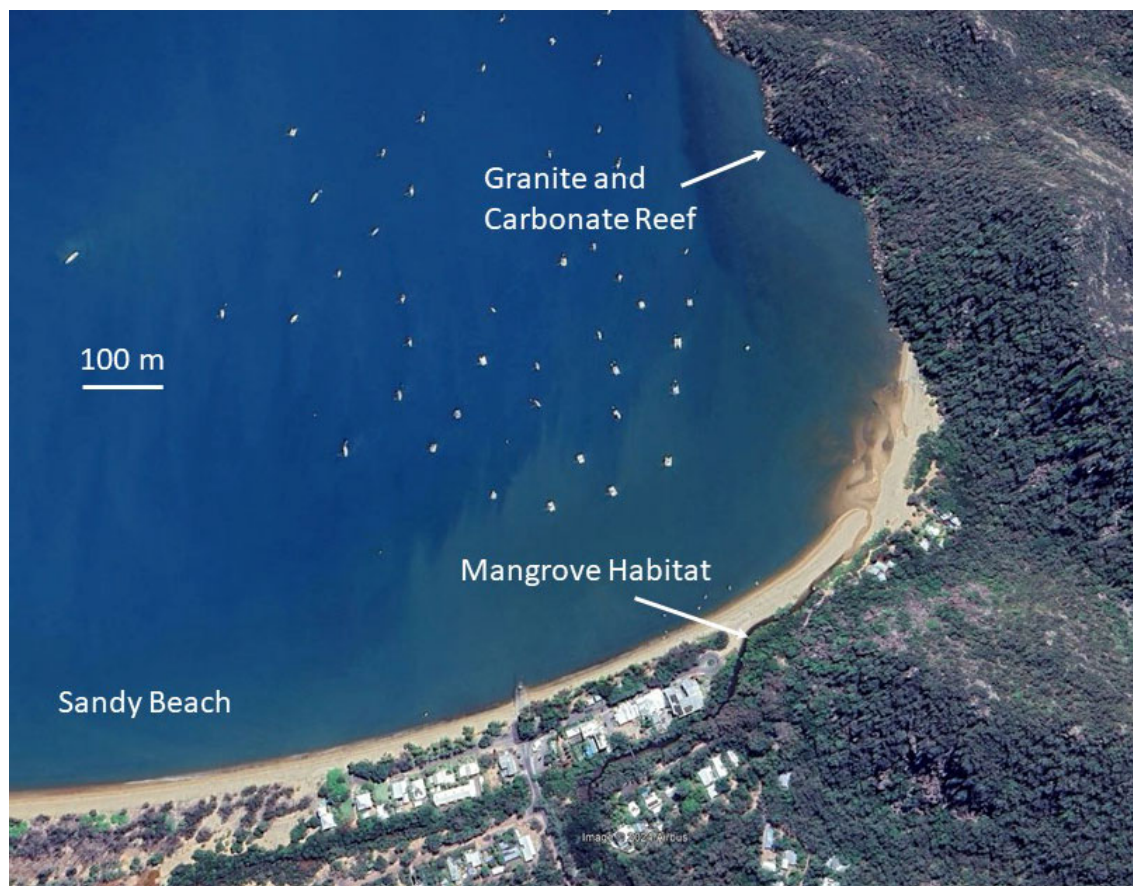| Sampling Time	Site no.	Depth (m)	Temperature (°C)	Salinity (ppt)	No. of positive detections / 12	Copies per L
July 2022	2	0.5	20.3	34.3	0	0
	3	0.7	20.2	34.2	0	0
	4	0.3	21.2	34.1	0	0
	6	0.8	20.2	34.1	0	0
	7	0.4	21	34.3	0	0
	8	0.8	22.7	7.6	0	0
	10	0.8	20.5	2.5	0	0
	9	0.8	20.8	11.3	1	73.6
	11	0.8	20.5	2.5	0	0
	12	0.7	20.5	34.4	0	0
	13	1	*	*	0	0
	16	0.6	20.7	34.3	0	0
	19	0.6	21	34.2	0	0
	22	0.1	*	*	0	0
	23	0.4	20.2	34.2	2	82.3
	14	10.1	20	31.5	0	0
Average	–	–	20.7	25.96	–	–

Figure S1. Labelled satellite image displaying the flora and landform of Horseshoe Bay, Magnetic Island.
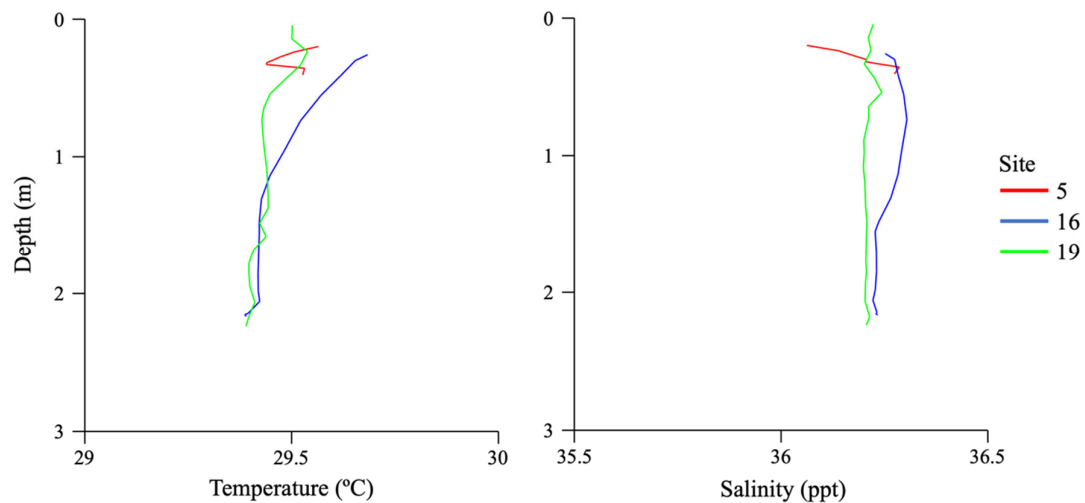
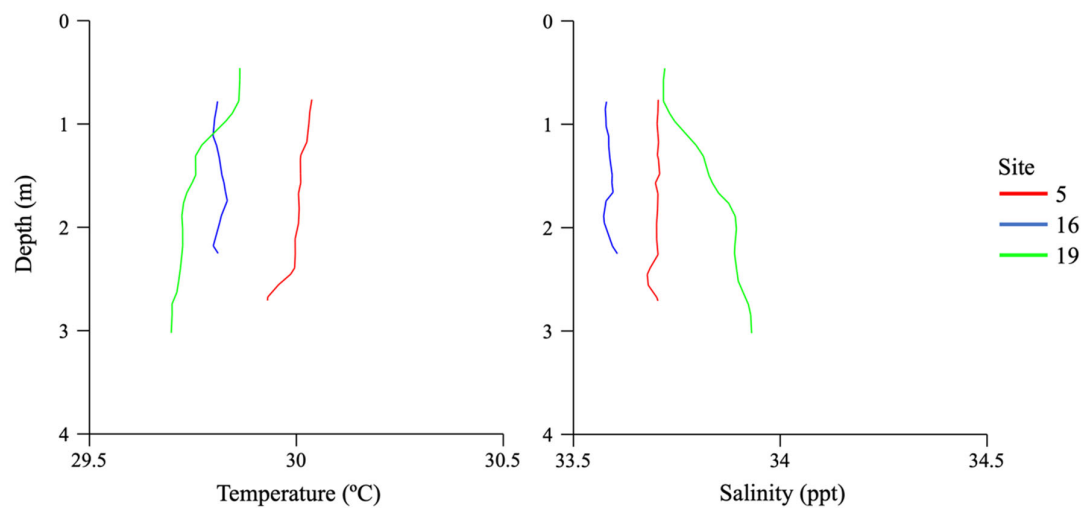**Figure S2.** Depth profiles of temperature and salinity at nearshore sample sites for the December 2020 sampling time.

Figure S3. Depth profiles of temperature and salinity at nearshore sample sites for the February 2020 sampling time.
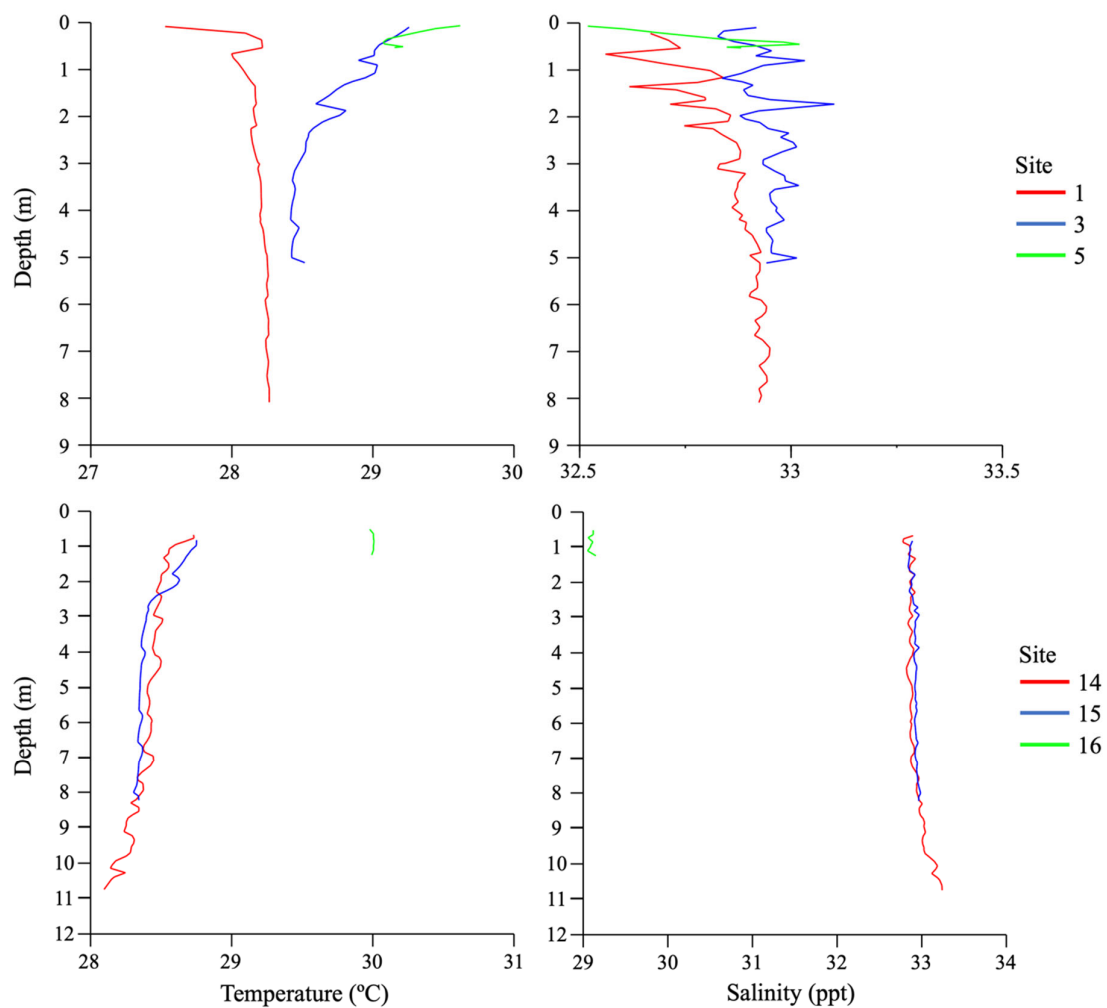
Figure S4. Depth profiles of temperature and salinity at offshore (sites 1 and 14), mid-shore (sites 3 and 15) and nearshore (sites 5 and 16) sample sites for the March 2021 sampling time.
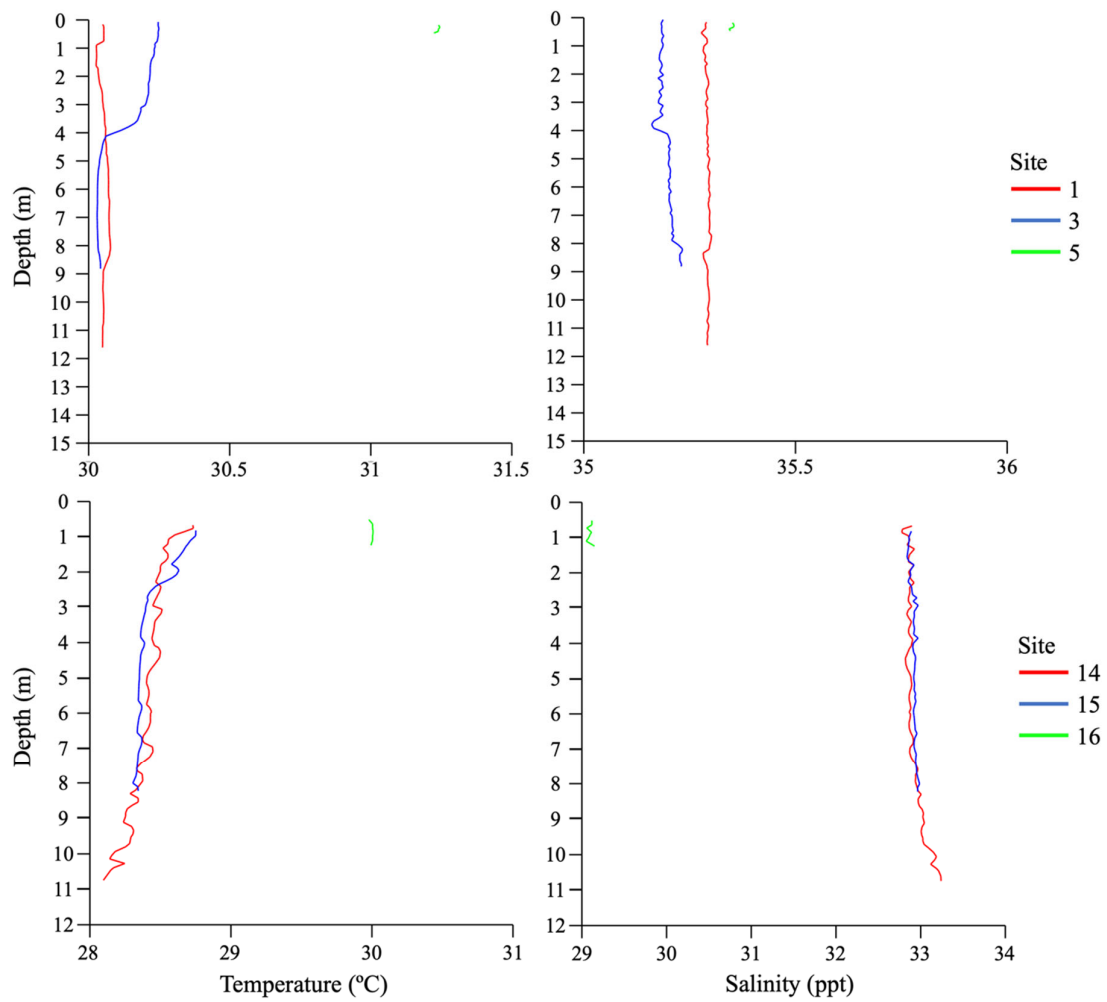
Figure S5. Depth profiles of temperature and salinity at offshore (sites 1 and 14), mid-shore (sites 3 and 15) and nearshore (sites 5 and 16) sample sites for the December 2021 sampling time.