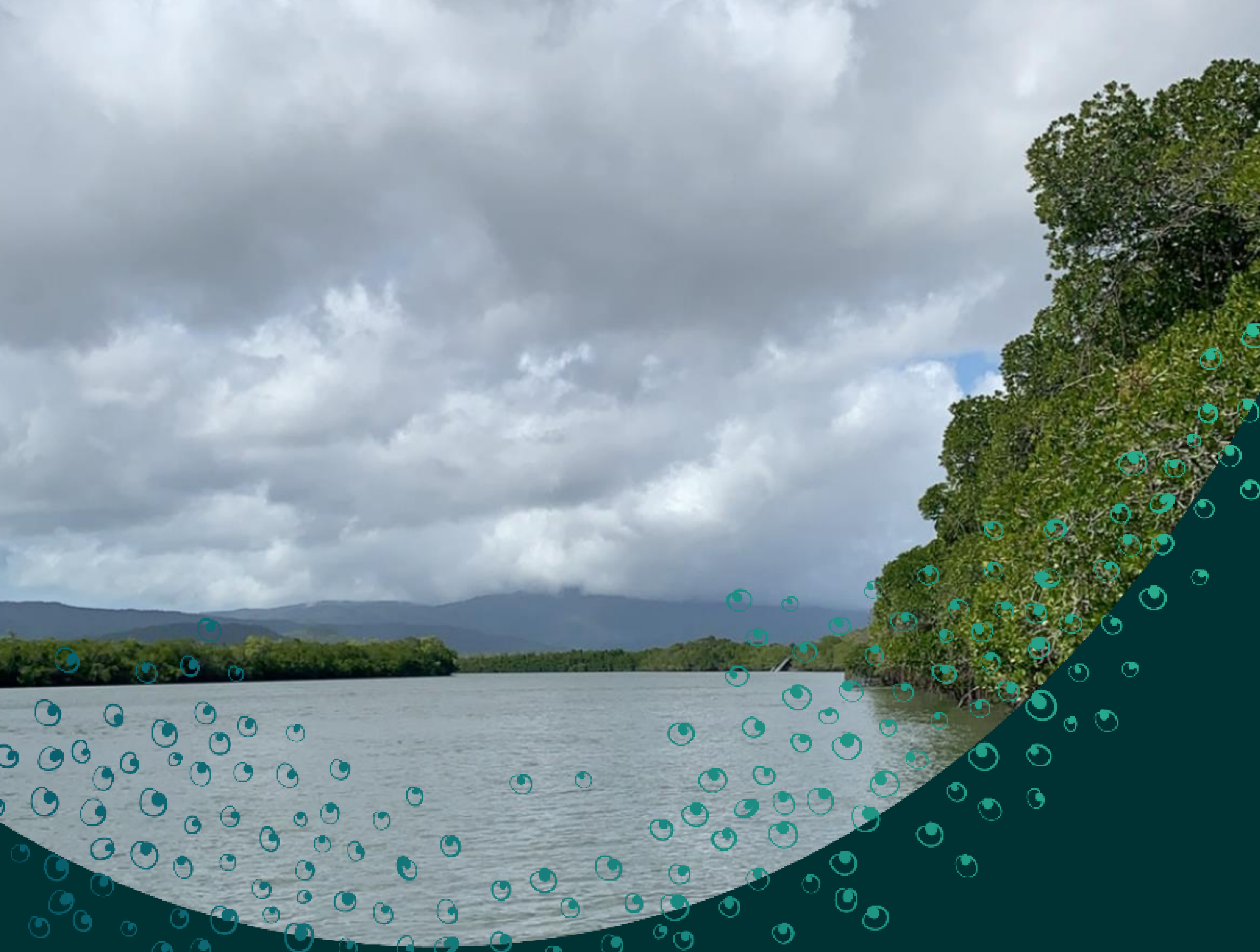

Stable isotope indicators of aquaculture discharge

Pilot study: June 2021

Queensland Government

Prepared by: Aquatic Ecosystem Health, Science Division, Department of the Environment, Tourism, Science and Innovation

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29th November 2024

Contents

Executive Summary

This pilot study trialled the use of stable isotope indicators in mangrove leaves, primarily ^{15}N enrichment, to complement water quality monitoring of tidal estuaries receiving aquaculture discharge. Water quality and stable isotope samples were collected from five estuaries in the Proserpine and O'Connell basins, in the Mackay Whitsunday Natural Resource Management (NRM) region. Proserpine Prawn Farm (De Costi Seafoods) is an existing prawn farm that currently discharges to Sandfly Creek, which flows into Eden Lassie Creek, the water intake for the farm is located in the Gregory River, within the Proserpine Basin. Hervey Creek and Dempster Creek are located in the O'Connell Basin (Exmoor ADA), have yet to be developed for aquaculture, and served as additional reference estuaries.

At locations subject to existing aquaculture discharge (Sandfly Creek, Eden Lassie Creek; Proserpine Basin), elevated $\delta^{15}N$ values (5.01‰) were observed indicating significant anthropogenic input of excess nutrients, relative to reference streams (2.92‰) (Gregory River; Proserpine Basin; and Hervey Creek, Dempster Creek; O'Connell Basin). The observed effects were greatest (7.82‰) within Sandfly Creek, at the site of the aquaculture discharge. Within Eden Lassie Creek, the highest values (6.13‰) were observed downstream of the confluence with Sandfly Creek. Within the Gregory River at which the farm intake is located, $\delta^{15}N$ enrichment was highest at upstream sites (3.78‰), relative to those further downstream at the mouth (2.88‰). At reference locations in the O'Connell Basin, a degree of human influence was also apparent from current land use with enrichment at midstream sites, Dempster Creek (3.59‰) and Hervey Creek (3.07‰), relative to the creek mouths (2.43‰ and 2.13‰ respectively).

Ambient water quality data largely supported spatial trends in $\delta^{15}N$ enrichment. In Sandfly Creek, consistently elevated nutrient and chlorophyl-a (chl-a) levels, coupled to reduced water clarity (secchi depth), and dissolved oxygen (DO), were observed over the monitoring period between October 2020 and November 2022. Median values for these indicators failed to meet relevant Water Quality Objectives (WQO) for Mackay Whitsunday moderately disturbed, mid estuary water types (Environmental Protection (Water) Policy 2009). In the tidal, Eden Lassie Creek, the greatest water quality effects were observed upstream of the confluence with Sandfly Creek (as opposed to downstream for ^{15}N enrichment), with elevated levels of chl-a and ammonia (NH_3), exceeding relevant WQO. Levels of chl-a exceeding WQO were consistently observed across all sites. One major water quality event was observed in Sandfly Creek over the course of monitoring in March 2022, which occurred after sampling for isotope analysis in June 2021. The event was characterised by high DO saturation (216.3%), with elevated levels of oxidised nitrogen (NO_x), total nitrogen (TN), total phosphorus (TP), and NH_3. Elevated values for TN, and TP were also observed at sites within Eden Lassie Creek, upstream of the confluence with Sandfly Creek, and to a lesser extent at the site downstream. These pilot results highlight the utility of stable isotope tools to investigating the spatial extent of nitrogen assimilation in biota from aquaculture discharge loads. This study has generated opportunities for further study, with the following recommendations should further detailed insights wish to be generated:

- Repeat isotope analysis with increased replication and sampling power at the leaf, tree, and/or site level to further elucidate the spatial extent of effects and any change associated with recent water quality.
- Establish any seasonal variation in $\delta^{15}N$, e.g. associated with summer, wet season.
- Re-visit sites to assess any change in $\delta^{15}N$ and water quality indicators, subsequent to any aquaculture development in the O'Connell Basin.

Introduction

The Queensland Government identified six land-based Aquaculture Development Areas (ADA) in 2019 to promote sustainable aquaculture in Queensland (details can be viewed at: Aquaculture development areas | Business Queensland). These land parcels were located adjacent to estuarine waters and considered suitable for saltwater aquaculture. Development of the ADA, which are located in Great Barrier Reef (GBR) catchments will likely involve discharging wastewater into Queensland waters, which typically constitutes an Environmentally Relevant Activity (ERA) under the Environmental Protection Act 1994. An Environmental Authority (EA) issued by the Queensland Government, Department of the Environment, Tourism, Science and Innovation (DETSI) is required to operate an ERA. In preparation for future development of these sites, and with support from the Department of Primary Industries (DPI), DETSI has undertaken a program to gather baseline water quality data in waterways adjacent to the proposed ADA areas. Baseline water quality data assists investors and government in identifying environmental values relevant to EA assessments and informs environmental quality objectives. The monthly water quality monitoring program was initiated in October 2020 and continued until April 2023. A detailed description of the program and the water quality results for the first year can be found in the "Queensland Aquaculture Development Area (ADA) Pilot Project: Water Quality Monitoring Program 2020-2021" report (2021). Whilst the program provided a substantial baseline of physicochemical data in these estuaries, the inclusion of additional indicators relevant to biological endpoints is also valuable.

Quantification of stable isotopes enables the identification of primary producers (e.g. using $\delta^{13}C$) and trophic position (e.g. using $\delta^{15}N$) (Souza et al. 2018). Delta ^{15}N markers also enable the detection of aquaculture and sewage discharge effects in sediments, primary producers, and consumers (as reviewed in Medina-Contreras and Arenas 2023). Elevated $\delta^{15}N$ values from anthropogenic influences are associated with excess nutrients, generating increased isotope fractionation via volatilisation of ammonia and/or microbial processing (Gritcan et al. 2016). In particular under increased P loading, primary producers respond with rapid nitrogen assimilation, thereby resulting in elevated ^{15}N values (Wang et al. 2015; Zheng et al. 2019). This increased assimilation can negatively affect mangrove composition and function (Reis et al. 2017; Erazo and Bowman 2021). Enriched $\delta^{15}N$ values up to ≈10‰ have previously been detected in seagrass (*Zostera capricorni*), macroalgae (*Gracilaria edulis, Catenella nipae*) and mangrove leaves (*Avicennia marina*) in proximity to sewage outfalls around Moreton Bay (Costanzo et al. 2001). Whereas $\delta^{15}N$ values of oceanic influenced samples in the same study were between 1.6‰ - 3.0‰, enrichment of $\delta^{15}N$ increasing towards the source of the sewage outfalls. Enrichment of ^{15}N was also observed in mangrove leaves and macroalgae downstream of the discharge point of a FNQ prawn farm (Burford et al. 2003). Enrichment was greatest during harvesting, and within the first 1-2km of the discharge in mangrove leaves (mean 7.5‰ ± 2.8 SD) and macroalgae (mean 5.5‰ ± 0.2 SD), relative to reference locations (mean 3.2‰ and 2.8‰ respectively) (Burford et al. 2003). Analysis of $\delta^{15}N$ from macrophytes sampled in more remote estuarine locations along the eastern shore of the Gulf of Carpenteria (Skardon, Wenlock, Hay-Embly, Archer-Watson), revealed differences in overall $\delta^{15}N$ values according to macrophyte type but not species. Saltmarsh (*Sporobolus virginicus, Sarcocornia quinqueflora*) showed the greatest level of $\delta^{15}N$ enrichment (2.8‰ ± 0.3 SEM), with seagrass (*Halodule* spp., *Halophila* spp.; 1.6‰ ± 0.1) and mangrove leaves (*Rhizophora* spp., *Avicennia* spp., *Ceriops* spp; 1.4‰ ± 0.2) exhibiting consistent and depleted values relative to the other groups (Gorman et al. 2023).

The use of $\delta^{15}N$ stable isotope values in mangrove (*Rhizophora stylosa*) leaves to detect effects associated with aquaculture discharge and excess nutrients was piloted in June 2021. The Proserpine Prawn Farm intakes water from the Gregory River estuary and discharges wastewater into Sandfly Creek, a small tidal creek, which then flows into the Eden Lassie Creek estuary, located within the Proserpine River drainage basin. Land use within the Gregory sub-catchment surrounding the prawn farm includes grazing, forestry, farming, and conservation. Within the Eden Lassie sub-catchment, the primary land use surrounding the mid-lower estuarine reaches is marsh and wetlands, with grazing, conservation, and farming in the upper reaches. As such $\delta^{15}N$ values of the wider region are expected to reflect a relatively low level of human use and nutrient input. Sites in two additional estuaries were sampled to provide reference sites and baseline information, Dempster Creek, and Hervey Creek, located within in the O'Connell River drainage basin. Both creeks drain the Exmoor Station, which is proposed for aquaculture development under the Exmoor ADA (Figure S1). All estuaries are subject to hyper salinisation during reduced rainfall periods coinciding with neap tide, with the Mackay region subject to high tidal ranges (Aquaculture development areas | Business Queensland).

Objectives

Pilot objectives were to:

- Quantify $\delta^{15}N$ and associated variability in mangrove leaves (*Rhizophora stylosa*) at a site of aquaculture effluent output at Proserpine Prawn Farm and reference sites;
- Quantify baseline $\delta^{15}N$ and associated variability in the Exmoor ADA prior to aquaculture development;
- Compare stable isotope values at aquaculture sites to reference locations; and
- Relate stable isotope values to standard water quality indicators.

Methods

Stable isotope sampling

Stable isotope sampling sites were spread over two basins: Proserpine and the O'Connell. The existing aquaculture facility is located within the Proserpine Basin within Sandfly Creek (SFC; n=4), the discharge being located at site SFC3. Sandfly Creek drains to Eden Lassie Creek (EDC; n=8), EDC sites therefore comprise upstream (US) and downstream (DS) locations in relation to Sandfly Creek (Figure 1). Proserpine reference sites were located within the Gregory River (GRG; n=4), where the farm water intake is located. In addition, two reference estuaries were sampled in the O'Connell River drainage basin (Exmoor ADA), Dempster Creek (DMP; n=12) and Hervey Creek (HVY; n=12). Both estuaries have yet to be developed for aquaculture. In each creek, sampling locations included upstream (US), midstream (MS) and downstream (DS) sites (Figure 2).

Between 23rd - 24th June 2021, at each site, leaves of the mangrove *Rhizophora stylosa* were collected for stable isotope analysis. The second youngest leaf of a rosette above the highest water level was removed for analysis from between three to five trees. Samples were stored in individual zip-lock bags and frozen until analysis. Sample preparation and stable isotope analysis were conducted by Griffith Stable Isotope Laboratory, Queensland. Leaves were rinsed with distilled water and then dried in a convection oven at 60ºC for 48 hours. Once dried, samples were ground to a homogenous powder using a Retsch MM400 mixer mill and 5.13 - 7.07mg, placed into tin capsules and pelletised for isotope-ratio mass spectrometry analysis (IRMS). IRMS was conducted using a Sercon Hydra 20-22 with a Sercon Europa EA-GSL sample preparation system. Total elemental carbon and nitrogen (%), carbon to nitrogen ratio, and isotopic delta ^{15}N and ^{13}C (‰) were quantified.

Figure 1 Stable isotope sampling locations surrounding Proserpine Prawn Farm (test sites in red, reference sites in blue), the water in-take in the Gregory River (GRG) and discharge point to Sandfly Creek (SFC) and Eden Lassie Creek (EDC).

Figure 2 Stable isotope sampling locations in Dempster Creek (DMP) and Hervey Creek (HVY) (O'Connell River Basin; Exmoor/ Bloomsbury ADA)

Water quality sampling

Water quality data was collected monthly between October 2020 and November 2022 as per the "*Monitoring and Sampling Manual: Environmental Protection (Water) Policy*" [PDF 9.5 MB] (DES 2018). Physicochemical parameters measured were specific conductivity, temperature, pH, dissolved oxygen, turbidity, chlorophyll-a (chl-a), secchi depth, and total suspended solids (TSS). A YSI EXO2 or EXO3 multi-parameter sonde was used to measure specific conductivity, temperature, pH, dissolved oxygen, and turbidity *in-situ* at the surface (0.2m) and at discrete depths (1.0m increments). Surface water samples were collected for chl-a, total and dissolved nutrients, and TSS. To reduce the effect of tidal variation and provide water quality data representative of the waterway, sampling was conducted on an outgoing (ebb) tide.

The Proserpine Basin ambient water quality sites are summarised in Figure 4. Sites in the O'Connell Basin and Exmoor ADA are summarised in

Figure 3. Sampling sites extend across a variety of water types and management intents, relevant water quality objectives (WQO) are summarised in Table 1, Figure S2, and Figure S3.

Figure 3 Proserpine Prawn Farm water quality sampling locations along Eden Lassie Creek (EDC), Sandfly Creek (SFC) and the Gregory River (GRG), Proserpine Basin.

Figure 4 Exmoor/Bloomsbury ADA. Water quality sampling locations in Dempster Creek (DMP) and Hervey Creek (HVY), in the O'Connell Basin.

Table 1 Water Quality Objectives (WQOs) relevant to the study area (Environmental Protection (Water) Policy 2009)

Water Area	Water Type	Intent/ level of protection	Ammonia N (µg/L)	Oxidised N (µg/L)	Total N (µg/L)	FRP (µg/L)	Total P (µg/L)	Chl-a (µg/L)	DO (% sat)	Turbidity (NTU)	Secchi depth (m)	pH
Mackay Whitsunday	Mid-estuary	SD and HEV	2 – 5 – 10	1 – 7 – 30	190 – 240 – 380	8 – 15 – 25	15 – 25 – 40	0.7 – 1.1 – 1.8	70 – 105	3 – 5 – 8	1 – 1.3 – 1.7	7 – 8.4
		MD	10	30	400	30	40	2	70 – 105	10	≥1	7 – 8.4
SD2381	Enclosed coastal/ lower estuary waters	HEV	7 – 10 – 15	2 – 4 – 10	110 – 120 – 160	2 – 3 – 5	10 – 15 – 20	0.8 – 1.3 – 2.0	85 – 90 – 105	ND	ND	8.1 – 8.3 – 8.4
Edgecumbe Bay	Lower estuary/ enclosed coastal waters in GBR Marine Park outside core port water (s1, s2)	MD	2	4	90	1	12	1	90 – 100	3 (dry) 4 (wet)	≥1.5	8 – 8.4

*Note: WQOs for indicators are shown as a range of 20th, 50th and 80th percentiles to be maintained or achieved (e.g. 3–4–5), lower and upper limits (e.g. pH: 7.2–8.2), or as a single value (e.g. 15). For single value WQOs, medians (or means where specified) of test data should be less than or equal to the WQO, unless otherwise indicated. Except where otherwise stated, HEV/SD WQOs are an annual range, and MD WQOs are an annual median.

HEV – high ecological value; SD – slightly disturbed; MD – moderately disturbed.

Data analysis

Comparison was made of stable isotope and water quality indicators between sites exposed to aquaculture discharge (aquaculture; Sandfly Creek, Eden Lassie Creek) and reference sites (Proserpine Basin: Gregory River; O'Connell Basin: Dempster Creek, Hervey Creek) (aquaculture v reference), at the stream, and site level.

Total elemental nitrogen and carbon, and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were compared via non-parametric univariate tests, due to the unbalanced sampling design and associated data spread. Univariate tests for two groups, (aquaculture v reference; Proserpine v O'Connell) comprising Mann-Whitney t-test (two-tailed), and for multiple comparisons with greater than two groups (streams; sites), using Kruskal-Wallis, with the statistical package GraphPad Prism (v9.4.1).

Multivariate tests were conducted to explore similarity between sites and key explanatory water quality indicators with PRIMER/PERMANOVA (v7.0.18) following data pre-treatment (square root transformation and normalisation). Similarity between samples, sites, and basins, was then visualised by metric MDS, with vector overlay of the most correlated variables.

Results

Stable isotope indicators

Total elemental nitrogen and carbon

Comparison of aquaculture influenced (Eden Lassie Creek, Sandfly Creek; Proserpine Basin) to reference sites (Gregory River; Proserpine Basin; Hervey Creek, Dempster Creek; O'Connell River Basin) revealed significant differences in mangrove leaf total elemental carbon and nitrogen (aquaculture n=38; reference n=88; Mann-Whitney $P<0.05$; Figure 5). Elemental nitrogen was approximately 16% higher in leaves sampled from aquaculture effluent exposed sites (mean = 1.41% ± 0.03 SEM), to reference sites (mean = 1.2% ± 0.03 SEM). C:N ratios were also approximately 14% lower in leaves from aquaculture exposed sites (mean = 33.88 ± 0.78 SEM; reference mean = 39.71 ± 1.03 SEM). To test for any differences at the basin scale which may have contributed to this effect, comparison was also made between samples from reference sites only in each basin (Proserpine n=12; O'Connell n=76), with all tests returning a non-significant result.

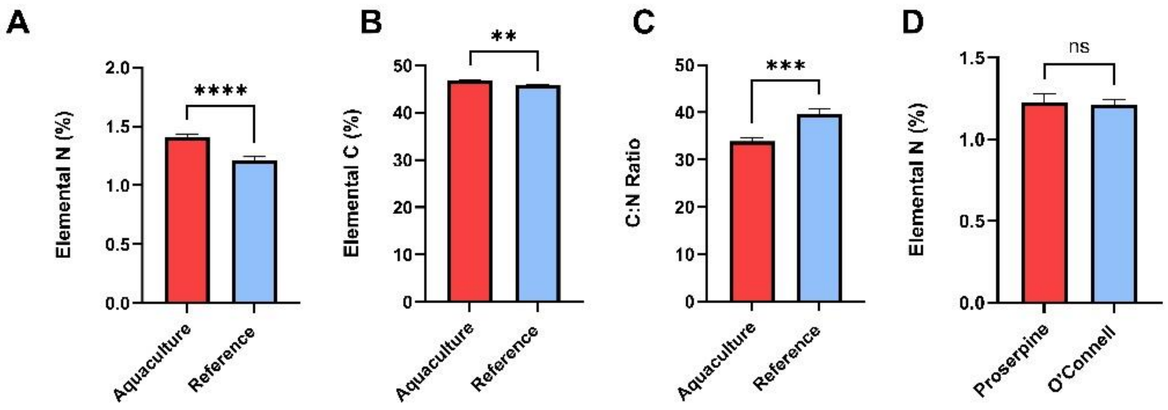

*Figure 5 Mangrove leaf carbon and nitrogen indicators A) elemental N (%); B) elemental C (%); C) C:N ratio; at aquaculture (Eden Lassie Creek; Sandfly Creek) and reference (Gregory River, Proserpine; Hervey Creek and Dempster Creek; O'Connell River); and D) elemental N (%) at O'Connell reference locations only, values presented are means with S.E.M.. * Indicate a significant pairwise test result (** P<0.005, ***P<0.001, ****P<0.0001), ns indicates no significant test result (Mann-Whitney t test, two tailed).*

At the stream level, differences in total elemental carbon and nitrogen were detected in samples from Eden Lassie Creek and Sandfly Creek (Proserpine Basin), relative to reference locations in Hervey Creek and Dempster Creek (O'Connell Basin; Figure 6). Elemental differences were greatest where the aquaculture discharge is located in Sandfly Creek, for elemental nitrogen (mean = 1.49% ± 0.04 SEM), and C:N ratio (mean = 31.9 ± 1.23 SEM).

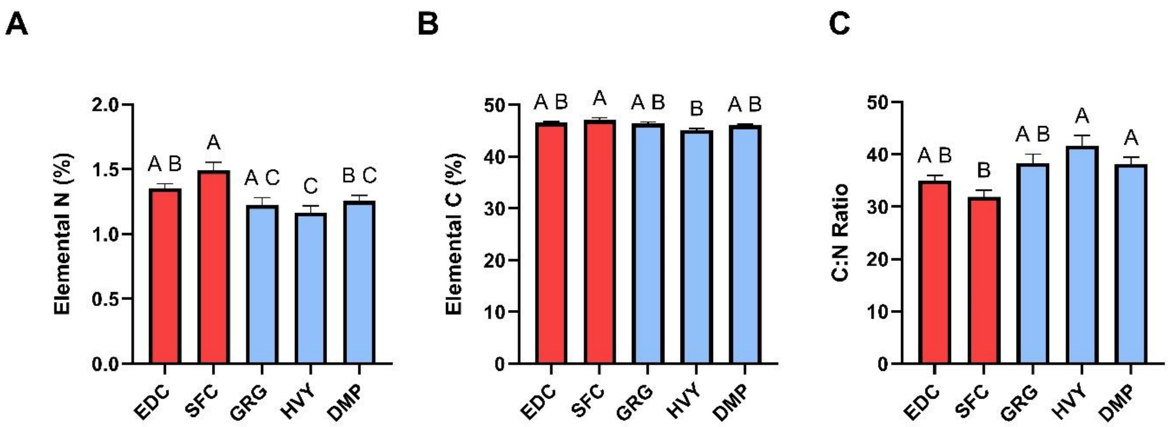

Figure 6 Mangrove leaf carbon and nitrogen indicators by stream A) elemental N (%); B) elemental C (%); C) C:N ratio; at aquaculture (red: Eden Lassie Creek, EDC; Sandfly Creek, SFC) and reference (blue: Gregory River, GRG, Hervey Creek, HVY, and Dempster Creek, DMP); values presented are means with S.E.M.. Letters indicate significant test groups (P<0.05, Kruskal-Wallis).

Isotopic delta ^{15}N

A 70% increase in $\delta^{15}N$ enrichment was observed at aquaculture discharge exposed sites, relative to reference sites (Figure 7). The highest $\delta^{15}N$ enrichment was observed in Sandfly Creek (stream mean = 5.73‰ ± 0.62 SEM), at the aquaculture discharge site (SFC3; site mean = 7.82‰ ± 0.95 SEM). In contrast, within Eden Lassie Creek, the highest degree of $\delta^{15}N$ enrichment was observed at the site downstream of the confluence with Sandfly Creek (EDC DS1; site mean = 6.13‰ ± 0.47 SEM). However, within Eden Lassie Creek, no clear spatial trend was apparent across sites (2.47 – 6.13‰). The $\delta^{15}N$ of leaves collected from reference stream locations ranged from 2.65‰ ± 0.18 SEM in Hervey Creek, 3.06‰ ± 0.13 SEM in Dempster Creek, to 3.33‰ ± 0.20 SEM in the Gregory River.

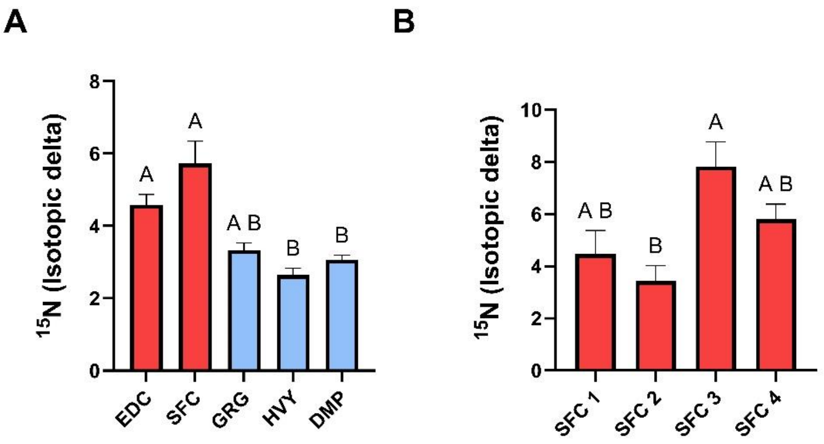

Figure 7 Mangrove leaf isotopic delta ^{15}N; A) within streams; (red: Eden Lassie Creek, EDC; Sandfly Creek, SFC) and reference (blue: Gregory River, GRG, Hervey Creek, HVY, and Dempster Creek, DMP); B) Sandfly Creek sites (SFC); values presented are means with S.E.M.. Letters indicate significant test groups (P<0.05, Kruskal-Wallis).

Within the Gregory River where the farm intake is located, $\delta^{15}N$ enrichment was highest at upstream sites (GRG 3 and GRG 4; mean 3.78‰ ± 0.20 SEM), relative to those further downstream (GRG 1 and GRG 2; mean 2.88‰ ± 0.23 SEM). Within the O'Connell Basin, variability was also apparent in $\delta^{15}N$ enrichment between sites, but not between creeks. Enrichment of $\delta^{15}N$ was observed at midstream sites relative to downstream sites; a 44% increase in Hervey Creek (mean $\delta^{15}N$ of 3.07‰ ± 0.35 SEM), and a 47% increase in Dempster Creek (mean $\delta^{15}N$ of 3.59‰ ± 0.21 SEM) (Figure 8).

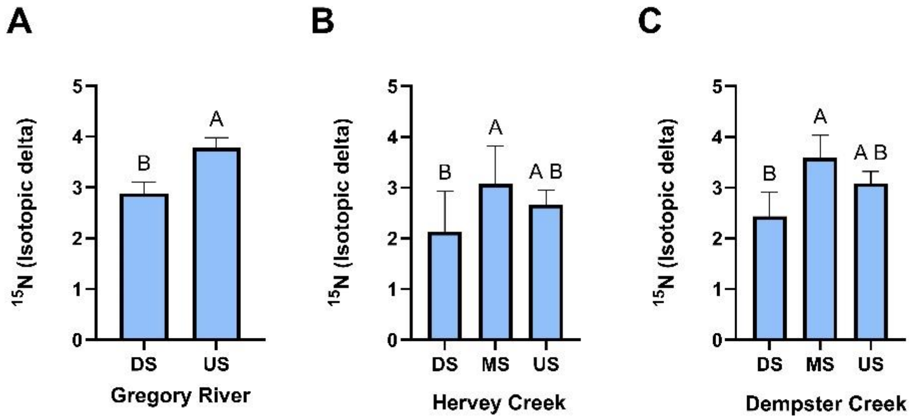

Figure 8 Mangrove leaf isotopic delta ^{15}N at reference sites in A) Gregory River; B) Hervey Creek, and C) Dempster Creek (DS downstream, MS midstream, US upstream); values presented are means with S.E.M.. Letters indicate significant test groups (P<0.05, Kruskal-Wallis).

Stable isotope ratios and delta ^{13}C carbon

Altered stable isotope ratios ($\delta^{13}C$: $\delta^{15}N$; Figure 9) were also observed at aquaculture influenced streams relative to reference. Stream specific differences in $\delta^{13}C$ were also apparent, with Eden Lassie Creek (-28.13‰) and Dempster Creek (-28.26‰) samples more enriched, relative to Gregory River (-29.41‰), but not to Dempster Creek (-28.26‰) and Hervey Creek (-28.86‰) (Kruskal-Wallis, P<0.05).

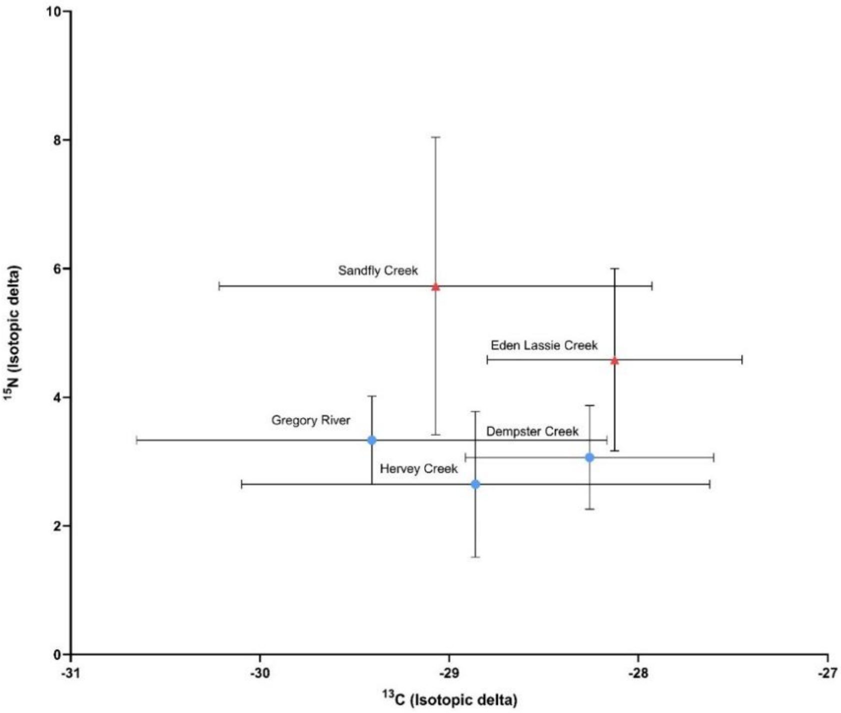

Figure 9 Stable isotope ratios (‰) of mangrove leaves collected from aquaculture (red; Sandfly Creek, Eden Lassie Creek) and reference stream locations (blue; Gregory River, Hervey Creek, Dempster Creek) (values are means, with SD)

Water quality indicators

Differences in water quality indicators were observed at the basin level over the monitoring period, noting that all aquaculture sites are within the Proserpine Basin (PERMANOVA, F=10.4, P=0.001). Highly correlated contributing variables (Pearson r >0.75) to basin differences, included total nitrogen (TN) and total phosphorus (TP), secchi depth, pH, turbidity, and dissolved oxygen (DO) (Figure 10).

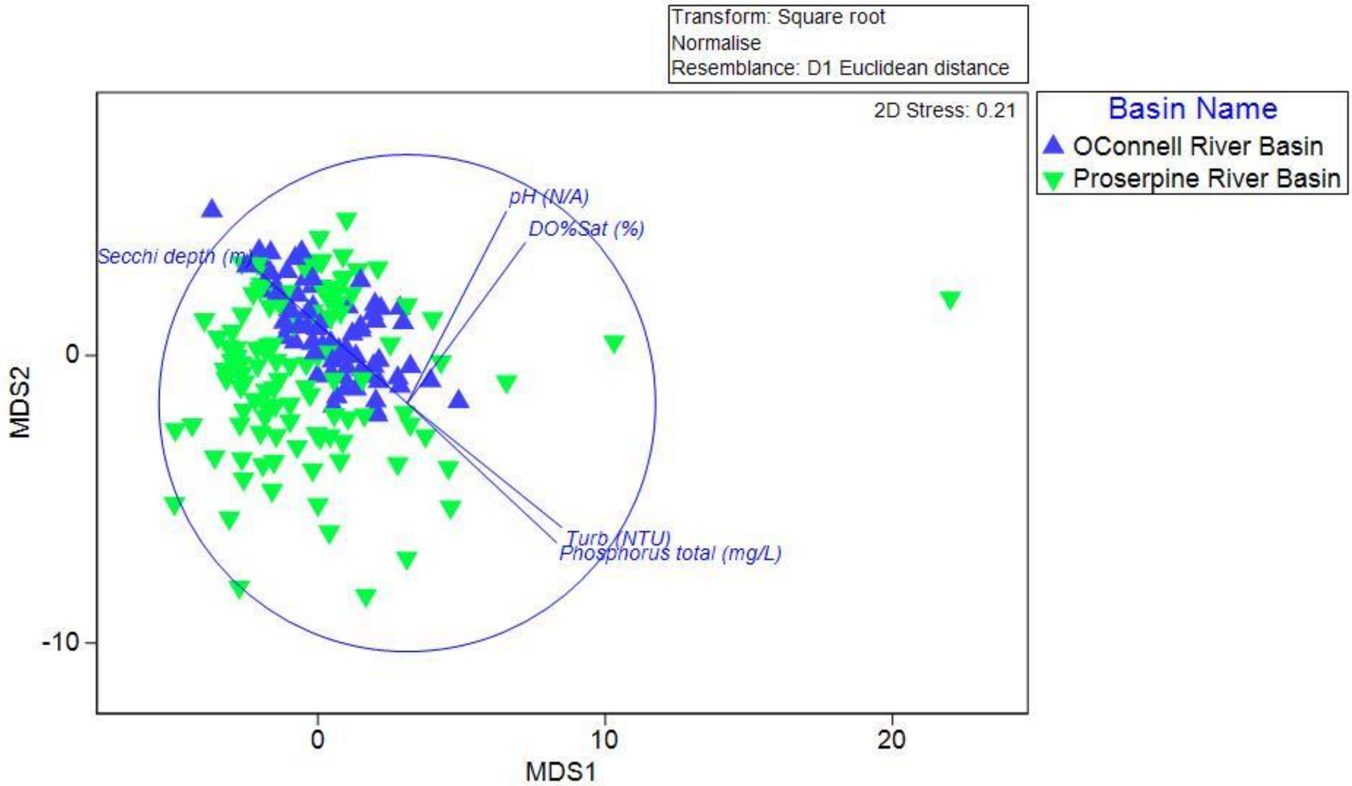

Figure 10 Metric MDS plot of ambient water quality data, points represent sampling events and are coloured according to basin, with overlay of key correlated variables (Pearson r >0.75), total nitrogen and total phosphorus were highly correlated and therefore overlap (only total phosphorus is shown for clarity).

At the stream level, differences in water quality indicators were apparent at the site of aquaculture discharge, Sandfly Creek, and to a lesser extent at Eden Lassie Creek, relative to reference streams (Gregory River, Dempster Creek, Hervey Creek) (Figure 11). At Sandfly Creek, median values observed over the monitoring period were most elevated relative to reference nutrient indicators, TP (0.08 mg/L), FRP (0.018 mg/L), TN (0.55 mg/L), NH_3 (0.02 mg/L), and NO_x (0.015 mg/L). However, differences were also observed for turbidity, DO, and secchi depth relative to reference streams. At Eden Lassie Creek, median values over the course of monitoring were also elevated for the nutrients, NH_3 (0.005 mg/L), and NO_x (0.009 mg/L).

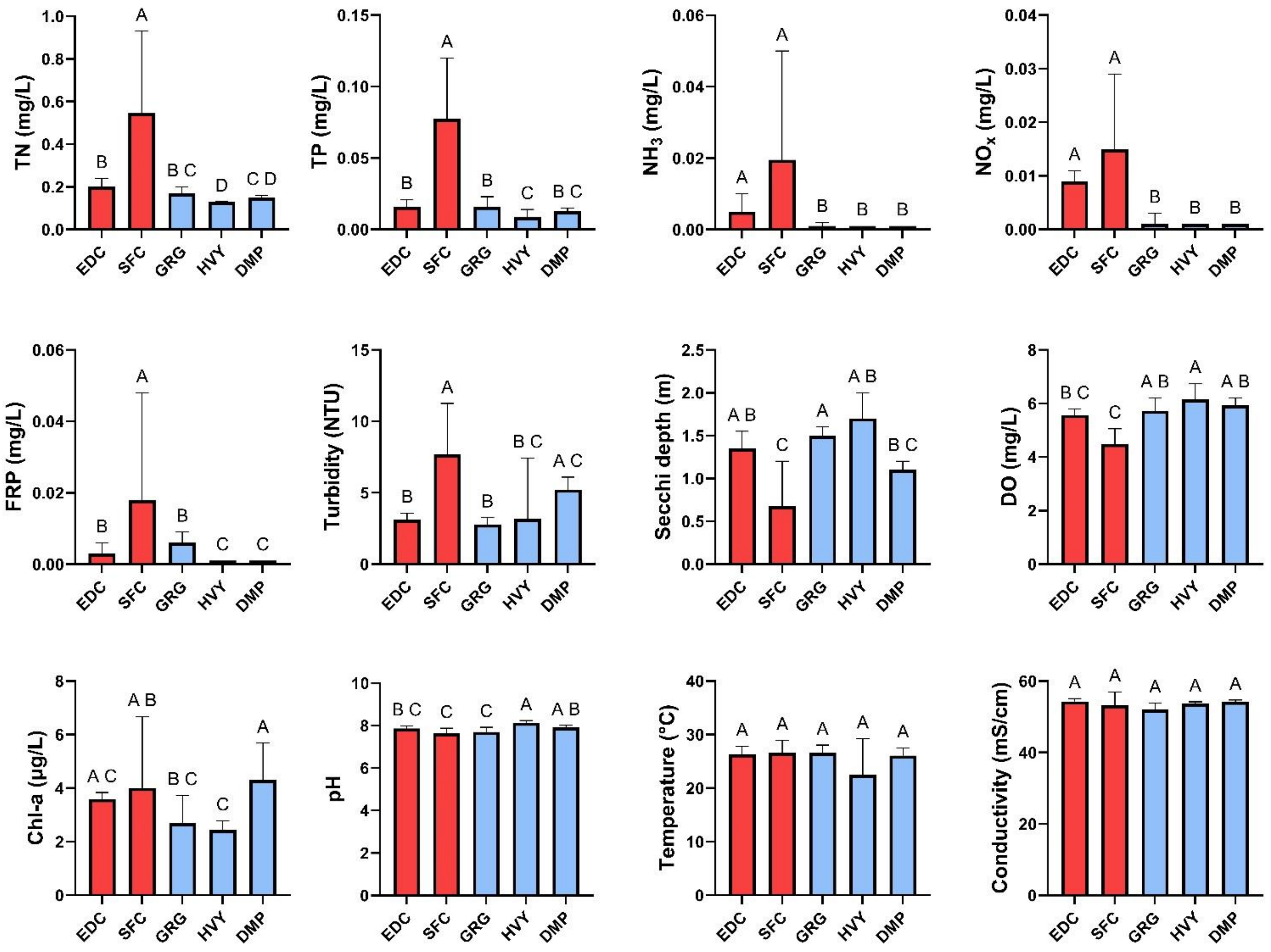

Figure 11 Summary of key ambient water quality indicator data collected at aquaculture stream (red: Eden Lassie Creek, EDC; Sandfly Creek, SFC) and reference (blue: Gregory River, GRG, Hervey Creek, HVY, and Dempster Creek, DMP) monitoring locations (values presented are medians with 95% CI) between May 2021 and November 2022. Kruskal-Wallis test between streams, letters indicate significant test groups (P<0.05).

At the site level, the greatest water quality effects were observed at the discharge site (SFC 1.3) in Sandfly Creek as discussed above, and to a lesser extent at Eden Lassie Creek sites immediately upstream (EDC 3.2), and downstream (EDC 2.1) for the nutrients NH_3 and NO_x (Figure 12).

Relating median site values to the relevant WQO (Table 2) major exceedances were observed at the discharge site (SFC 1.3) for chl-a, DO, NH_3, TN, TP, and secchi depth. Similarly at the site upstream of the confluence with Sandfly Creek (EDC 3.2), WQO were exceed by median values of chl-a and NH_3. Further downstream in Eden Lassie Creek (EDC 2.1), more stringent WQO objectives were exceeded for chl-a, DO, NH_3, NOx, TN, pH, TP, secchi depth, and TSS. WQO were widely exceeded for chl-a, with no site median meeting WQO over the period, with the highest values observed in Dempster Creek.

Table 2 Water quality data summary by stream and site AMTD (km) between 05/2021 to 11/2022. Orange shading indicates exceedance of WQO (MD) and/or 80th percentile (SD/HEV).

Stream Name	Site		Chl-a (µg/L)	DO% Sat (%)	DO (mg/L)	NH_3 (µg/L)	NO_x (µg/L)	TN (µg/L)	pH	FRP (µg/L)	TP (µg/L)	Secchi depth (m)	TSS (mg/L)	SpCond (mS/cm)	Temp (°C)	Turb (NTU)
Eden Lassie Creek	-0.3	Number	13	14	14	13	13	13	14	13	13	11	13	14	14	14
		Median	2.46	91.75	6.25	1	1	140	8.06	1	10	1.55	11	54.28	26.41	2.87
		Minimum	1.06	82.2	5.2	1	1	110	7.99	1	6	1.15	1	47.76	19.33	1.15
		Maximum	4.39	102.1	7.33	7	2	230	8.18	5	14	1.8	19	55.55	29.05	4.08
	WQO	Edgecumbe Bay SD HEV	1	90-100		2-2-5	1-4-8	80-90-125	8-8.4	1-1-3	10-12-13	>1.5	2			2-4-14
Eden Lassie Creek	2.1	Number	13	14	14	13	13	13	14	13	13	13	13	14	14	14
		Median	3.47	78.85	5.39	8	10	230	7.83	3	20	1.2	13	54.63	26.3	3.13
		Minimum	1.94	58.7	3.72	1	1	160	7.68	1	13	0.6	7	42.95	19.65	1.93
		Maximum	4.89	84.3	5.94	20	76	630	7.92	14	47	1.9	36	57.29	28.71	18.78
	WQO	Edgecumbe Bay SD HEV	1	90-100		2-2-5	1-4-8	80-90-125	8-8.4	1-1-3	10-12-13	>1.5	2			2-4-14
Eden Lassie Creek	3.2	Number	13	14	14	13	13	13	14	13	13	14	13	14	14	14
		Median	4.28	72.8	5.12	11	14	250	7.71	6	24	1.23	16	54.43	26.36	3.87
		Minimum	2.10	52	3.28	1	1	170	7.58	1	16	0.6	7	36.67	20.2	2.33
		Maximum	8.85	223.9	14.69	49	87	1600	8.43	18	170	1.9	35	57.46	29.44	12.6
	WQO	Mackay Whitsunday Mid estuary MD	2	70-105		10	30	400	7-8.4	30	40	>1				10
Sandfly Creek	1.3	Number	13	14	14	14	14	14	14	14	14	14	12	14	14	14
		Median	4.01	64.25	4.49	19.5	15	545	7.65	18	77.5	0.68	19	53.15	26.6	7.69
		Minimum	1.40	39.1	2.54	1	1	280	7.41	2	22	0.3	11	33.44	19.31	2.78

Stream Name	Site		Chl-a (µg/L)	DO% Sat (%)	DO (mg/L)	NH_3 (µg/L)	NO_x (µg/L)	TN (µg/L)	pH	FRP (µg/L)	TP (µg/L)	Secchi depth (m)	TSS (mg/L)	SpCond (mS/cm)	Temp (°C)	Turb (NTU)
		Maximum	9.98	216.3	13.8	620	580	4300	8.77	160	570	1.7	67	57.84	30.26	29.13
	WQO	Mackay Whitsunday Mid estuary MD	2	70-105		10	30	400	7-8.4	30	40	>1				10
Gregory River	-1.6	Number	15	16	16	15	15	15	16	15	15	14	1	16	16	16
		Median	2.70	95.6	6.57	1	1	120	8.19	1	7	1.68	16	54.12	26.75	2.35
		Minimum	1.14	91.3	5.69	1	1	90	7.96	1	4	1.2	16	50.84	19.46	0.96
		Maximum	9.51	101.5	7.4	2	1	170	8.26	2	15	2.4	16	56.89	29.05	7.19
	WQO	Mackay Whitsunday Mid estuary MD	2	70-105		10	30	400	7-8.4	30	40	>1				10
Gregory River	3.6	Number	15	16	16	15	15	15	16	15	15	16	2	16	16	16
		Median	3.15	76.4	5.05	1	1	170	7.7	7	16	1.58	13.5	50.65	26.48	2.79
		Minimum	0.70	60.5	3.83	1	1	130	7.49	1	10	0.6	12	32.29	20.56	1.07
		Maximum	8.57	81.4	6.15	22	21	390	7.92	19	34	2.4	15	58.2	30.44	15.55
	WQO	Mackay Whitsunday Mid estuary MD	2	70-105		10	30	400	7-8.4	30	40	>1				10
Gregory River	8.4	Number	15	16	16	15	15	15	16	15	15	15	2	16	16	16
		Median	2.70	77.25	5.36	3	5	210	7.58	14	26	1.35	15.5	45.84	27.12	3.59
		Minimum	0.54	52.8	3.76	1	1	170	7.41	6	17	0.9	11	18.36	20.54	1.6
		Maximum	8.55	87.3	7.08	56	45	550	7.71	41	60	1.8	20	56.86	29.9	6.8
	WQO	Mackay Whitsunday Mid estuary MD	2	70-105		10	30	400	7-8.4	30	40	>1				10
Hervey Creek	-0.5	Number	4	4	4	4	4	4	4	4	4	4	4	4	4	4
		Median	2.61	95.45	6.65	1	1	120	8.20	1	7	1.55	12	53.9	22.12	3.25
		Minimum	1.74	92.2	6.01	1	1	100	8.11	1	4	0.7	9	53.62	21.7	0.34
		Maximum	3.14	96.2	6.81	1	1	130	8.26	1	14	2.4	15	54.51	29.79	7.51

Stream Name	Site		Chl-a (µg/L)	DO% Sat (%)	DO (mg/L)	NH_3 (µg/L)	NO_x (µg/L)	TN (µg/L)	pH	FRP (µg/L)	TP (µg/L)	Secchi depth (m)	TSS (mg/L)	SpCond (mS/cm)	Temp (°C)	Turb (NTU)
	WQO	SD2381 lower estuary, HEV	0.8-1.3-2	85-90-105		7-10-15	2-4-10	110-120-160		2-3-5	10-15-20					
Hervey Creek	-1	Number	4	4	4	4	4	4	4	4	4	4	3	4	4	4
		Median	2.14	97.35	6.87	1	1	100	8.21	1	4.5	2	10.5	53.78	22.42	1.35
		Minimum	0.52	94.7	5.9	1	1	90	8.15	1	1.5	0.65	8	53.52	21.96	0.53
		Maximum	2.93	99.9	7.09	1	1	130	8.29	1	15	2.7	20	54.41	29.67	11.61
	WQO	SD2381 lower estuary, HEV	0.8-1.3-2	85-90-105		7-10-15	2-4-10	110-120-160		2-3-5	10-15-20					
Hervey Creek	1.9	Number	4	4	4	4	4	4	4	4	4	4	4	4	4	4
		Median	1.84	90.1	6.16	1	1	125	8.08	1	10.5	1.33	9.5	53.99	22.49	3.77
		Minimum	1.50	85.6	5.9	1	1	120	7.99	1	7	0.9	7	51.99	21.41	1.77
		Maximum	2.41	94	6.57	1	3	130	8.16	2	14	2.25	23	54.26	29.41	7.56
	WQO	Mackay Whitsunday Mid estuary SD, HEV	0.7-1.1-1.8	70-105		2-5-10	1-7-30	190-240-380	7-8.4	8-15-25	15-25-40	1-1.3-1.7				3-5-8
Hervey Creek	2.6	Number	4	4	4	4	4	4	4	4	4	4	4	4	4	4
		Median	2.76	85.05	5.94	1	1	145	7.93	1	12	1.3	16.5	53.71	23.03	4.77
		Minimum	2.47	83.8	5.82	1	1	130	7.85	1	8	0.9	10	50.91	22.27	2.13
		Maximum	3.07	92.5	6	3	5	160	8.11	3	16	2	21	53.97	29.22	7.43
	WQO	Mackay Whitsunday Mid estuary SD, HEV	0.7-1.1-1.8	70-105		2-5-10	1-7-30	190-240-380	7-8.4	8-15-25	15-25-40	1-1.3-1.7				3-5-8
Dempster Creek	-1.2	Number	13	14	14	14	14	14	14	14	14	14	12	14	14	14
		Median	3.34	95.1	6.34	1	1	115	8.19	1	9.5	1.45	12	54.47	26.31	3.33
		Minimum	1.09	81.1	5.32	1	1	90	7.91	1	4	0.6	0.5	52.51	20.99	0.31
		Maximum	8.28	105.1	7.09	7	6	220	8.23	2	25	4.5	31	55.62	29.85	12.05
	WQO	SD2381 lower	0.8-1.3-2	85-90-105		7-10-15	2-4-10	110-120-160		2-3-5	10-15-20					

Stream Name	Site		Chl-a (µg/L)	DO% Sat (%)	DO (mg/L)	NH_3 (µg/L)	NO_x (µg/L)	TN (µg/L)	pH	FRP (µg/L)	TP (µg/L)	Secchi depth (m)	TSS (mg/L)	SpCond (mS/cm)	Temp (°C)	Turb (NTU)
		estuary, HEV														
Dempster Creek	0.7	Number	14	16	16	16	16	16	16	16	16	14	14	16	16	16
		Median	4.78	89.35	6.14	1	1	150	8.03	1	13.5	0.98	17	54.12	25.08	6.07
		Minimum	2.00	76.9	5.03	1	1	90	7.8	1	8	0.5	10	50.84	20.69	1.27
		Maximum	11.72	103.5	7.12	8	8	230	8.23	3	25	2.5	47	55.99	29.46	22.64
	WQO	SD2381 lower estuary, HEV	0.8-1.3-2	85-90-105		7-10-15	2-4-10	110-120-160		2-3-5	10-15-20					
Dempster Creek	2.6	Number	13	14	14	14	14	14	14	14	14	14	12	14	14	14
		Median	6.09	86.15	5.80	1	1	145	7.88	1	12	1.15	13.5	54.41	26.27	3.97
		Minimum	1.87	74.1	4.83	1	1	110	7.7	1	8	0.9	6	48.66	20.68	2.49
		Maximum	9.35	90.5	6.53	11	9	290	8.07	5	20	1.7	27	56.56	29.61	7.41
	WQO	Mackay Whitsunday Mid estuary SD, HEV	0.7-1.1-1.8	70-105		2-5-10	1-7-30	190-240-380	7-8.4	8-15-25	15-25-40	1-1.3-1.7				3-5-8
Dempster Creek	3.8	Number	13	14	14	14	14	14	14	14	14	14	12	14	14	14
		Median	3.87	83.35	5.66	1	1	175	7.76	1	15.5	0.9	16.5	54.25	26.28	6.77
		Minimum	1.36	71.7	4.66	1	1	130	7.58	1	12	0.8	13	46.47	20.91	4.23
		Maximum	8.84	89	6.31	9	8	400	7.96	5	29	1.4	70	56.73	29.63	10.26
	WQO	Mackay Whitsunday Mid estuary SD, HEV	0.7-1.1-1.8	70-105		2-5-10	1-7-30	190-240-380	7-8.4	8-15-25	15-25-40	1-1.3-1.7				3-5-8

Over the course of monthly water quality monitoring at the site adjacent to the aquaculture discharge in Sandfly Creek (SFC 1.3), water quality indicators demonstrated temporal differences with one major outlier, an event in March 2022 (Figure 13, point 7). The event was characterised by high DO saturation (216.3%), with highly correlated and elevated variables of NO_x (0.58 mg/L), TN (4.3mg/L), TP (0.57 mg/L), and NH_3 (0.62 mg/L). Elevated values were also observed at sites within Eden Lassie Creek, upstream of the confluence with Sandfly Creek (ELC 3.2) for TN (1.6mg/L), and TP (0.17mg/L), and to a lesser extent at the site downstream (ELC 2.1) for TN (0.63mg/L), and TP (0.047mg/L).

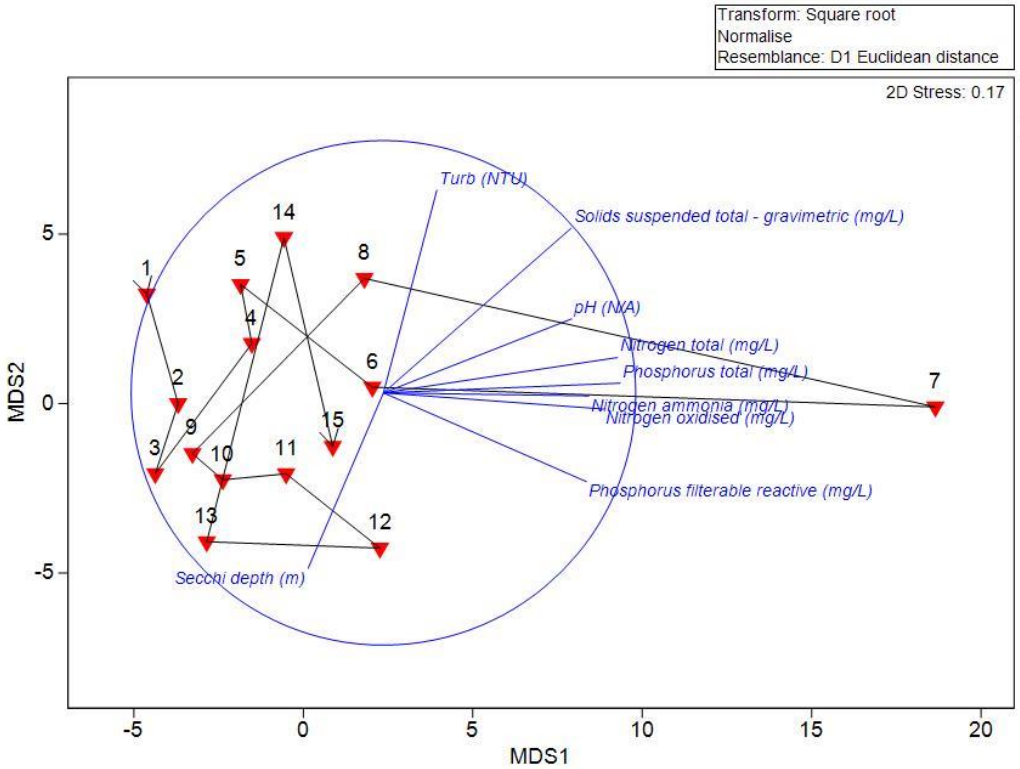

Figure 12 Metric MDS of water quality indicators at the Sandfly Creek site (1.3 AMTD), with time trajectory of monthly sampling events (1-15) and vector overlay of highly correlated variables (Pearson r >0.75).

Discussion and conclusions

This pilot trialled the use of stable isotope indicators, primarily $\delta^{15}N$, in mangrove leaves, *Rhizophora stylosa*, to monitor the effects of aquaculture discharge to surrounding estuarine environments. This study also generated baseline stable isotope information at estuarine sites in the Exmoor ADA (O'Connell Basin) prior to aquaculture development.

At locations subject to existing aquaculture discharge in the Proserpine Basin, in Sandfly Creek and Eden Lassie Creek, enrichment of $\delta^{15}N$ was observed in mangrove leaves, relative to reference sites in the O'Connell Basin. Delta ^{15}N values (7.82‰) showed the greatest enrichment at the site of the Proserpine Prawn Farm discharge, within Sandfly Creek. Values within Eden Lassie also differed to reference, the highest values (6.13‰) being observed downstream of the confluence of Sandfly Creek, but otherwise with no clear spatial trend across sites. Within the Gregory River where the farm water intake is located, $\delta^{15}N$ enrichment was highest at upstream sites (3.78‰), relative to those further downstream (2.88‰). Within the O'Connell Basin, $\delta^{15}N$ values were lower overall at 2.65‰ in Hervey Creek, and 3.06‰ in Dempster Creek.

A similar degree of ^{15}N enrichment to that observed in Sandfly Creek was also observed in mangrove leaves downstream of the discharge point of a 30 hectare FNQ prawn farm (Burford et al. 2003). The main daily pond exchange rate over the growth season for this farm was 3.9%, and net nitrogen discharged was 0.99 kg/ha pond area (Burford et al. 2003). Enrichment was greatest during harvesting, and within the first 1-2km of the discharge (mean 7.5 ± 2.8 SD), relative to reference locations (mean 3.2 ± 0.8 SD), and greater than 2km downstream (mean 3.7 ± 1.3 SD) (Burford et al. 2003). In contrast, ^{15}N of mangrove leaves sampled from remote locations with minimal human impact in FNQ have been observed at 1.4‰ (Gorman et al. 2023). Elevated $\delta^{15}N$ values in Sandfly Creek and to a lesser extent in Eden Lassie Creek indicate significant anthropogenic input of excess nutrients, resulting in increased nitrogen assimilation and isotope fractionation in the receiving environment. This increased assimilation can negatively affect mangrove composition and function (Reis et al. 2017; Erazo and Bowman 2021). A degree of human influence is also apparent within the O'Connell Basin associated with current land use. These results highlight the utility of isotope tools to mapping the spatial extent of both low-level, and more significant long-term inputs, via a single sampling event.

Ambient water quality data largely supported trends in $\delta^{15}N$, in Sandfly Creek, elevated nutrient (NH_3, TN, TP) and chl-a levels, coupled to reduced water clarity (secchi depth) and DO were observed, failing to meet relevant WQO for moderately disturbed mid estuary water types (Environmental Protection (Water) Policy 2009). Prawn aquaculture discharges typically contain high concentrations of suspended solids and nutrients, particularly nitrogen, relative to intake levels (Burford et al. 2003). These high loads of nutrients and suspended solids have the potential to have adverse effects on the receiving waters, including stimulating algal blooms and creating anoxic conditions. Interestingly, the greatest effects observed to water quality in Eden Lassie Creek, differed to that of the ^{15}N data, and were upstream of the confluence with Sandfly Creek (which is tidally influenced), with elevated levels of chl-a and NH_3, exceeding WQO. Levels of chl-a exceeding WQO were also consistently observed across all sites. As point data, water quality indicators showed variability within and between streams, as a result of water type, seasonal drivers, and acute events over the course of monitoring. One major event was observed over the course of water quality monitoring in March 2022, this event occurred after the sampling for isotope analysis which was conducted in June 2021.

Recommendations

This pilot study has generated a number of interesting opportunities for further study, with the following recommendations should further detailed insights wish to be generated:

- Repeat isotope analysis with increased replication and sampling power at the leaf, tree, and/or site level to further elucidate variability and spatial extent of effects of the existing aquaculture discharge and any change associated with recent water quality, including major events as observed in March 2022.
- Establish any seasonal variation in $\delta^{15}N$, e.g. associated with summer, wet season.
- Re-visit Exmoor ADA sites to assess any change in $\delta^{15}N$ and water quality indicators, subsequent to any aquaculture development.

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Supplementary Information

Table S1 Delta ^{15}N sampling site details.

ADA site	Creek	Site name	Latitude	Longitude	Description	Samples
Exmoor	Dempster Creek	DMP_DS4	-20.68797	148.73198	Dempster Creek 2.9km D/S from mouth on South Bank	3
Exmoor	Dempster Creek	DMP_DS3	-20.67922	148.72040	Dempster Creek 1.2km D/S from mouth on South Bank	3
Exmoor	Dempster Creek	DMP_DS2	-20.67501	148.71201	Dempster Creek 0.1km D/S from mouth on South Bank	3
Exmoor	Dempster Creek	DMP_DS1	-20.67917	148.70795	Dempster Creek 0.6km from mouth on South Bank	3
Exmoor	Dempster Creek	DMP_MS4	-20.68415	148.70384	Dempster Creek 1.35km from mouth on South Bank	3
Exmoor	Dempster Creek	DMP_MS3	-20.68839	148.69843	Dempster Creek 2.1km from mouth on North Bank	5
Exmoor	Dempster Creek	DMP_MS2	-20.69031	148.69285	Dempster Creek 2.8km from mouth on North Bank	3
Exmoor	Dempster Creek Tributary	DMT_MS1	-20.69688	148.69230	Dempster Creek Tributary 0.7km from junction with Dempster Creek on North Bank	3
Exmoor	Dempster Creek	DMP_US4	-20.68805	148.68649	Dempster Creek 3.5km from mouth on North Bank	3
Exmoor	Dempster Creek	DMP_US3	-20.69200	148.68186	Dempster Creek 4.2km from mouth on North Bank	3
Exmoor	Dempster Creek	DMP_US2	-20.69677	148.68205	Dempster Creek 4.9km from mouth on North Bank	3
Exmoor	Dempster Creek	DMP_US1	-20.69886	148.67824	Dempster Creek 5.6km from mouth on South Bank	3
Exmoor	Hervey Creek	HVY_DS4	-20.71927	148.73918	Hervey Creek 1.3km D/S from mouth on south Bank	3
Exmoor	Hervey Creek	HVY_DS3	-20.71176	148.73783	Hervey Creek 0.0km at mouth on south bank	3
Exmoor	Hervey Creek	HVY_DS2	-20.71552	148.73400	Hervey Creek 0.4km from mouth on south bank	3
Exmoor	Hervey Creek	HVY_DS1	-20.71487	148.72939	Hervey Creek 0.9km from mouth on north bank	3
Exmoor	Hervey Creek	HVY_MS4	-20.71585	148.72956	Hervey Creek 1.4km from mouth on north bank	3
Exmoor	Hervey Creek	HVY_MS3	-20.71461	148.72555	Hervey Creek 1.9km from mouth on north bank	5
Exmoor	Hervey Creek	HVY_MS2	-20.71264	148.72558	Hervey Creek 2.4km from mouth on north bank	3
Exmoor	Hervey Creek	HVY_MS1	-20.71000	148.72598	Hervey Creek 2.9km from mouth on North Bank	3
Exmoor	Hervey Creek	HVY_US4	-20.70665	148.72237	Hervey Creek 3.4km from mouth on North Bank	3
Exmoor	Hervey Creek	HVY_US3	-20.70840	148.72001	Hervey Creek 3.9km from mouth on south bank	3
Exmoor	Hervey Creek	HVY_US2	-20.70816	148.72006	Hervey Creek 4.4km from mouth on north bank	3
Exmoor	Hervey Creek	HVY_US1	-20.71257	148.72034	Hervey Creek 4.9km from mouth on North Bank	3
Proserpine	Eden Lassie Creek	EDC_DS4	-20.17629	148.41007	Eden Lassie Creek 0.8km from mouth on South Bank	3
Proserpine	Eden Lassie Creek	EDC_DS3	-20.17947	148.40727	Eden Lassie Creek 1.3km from mouth on North Bank	3
Proserpine	Eden Lassie Creek	EDC_DS2	-20.18255	148.41089	Eden Lassie Creek 1.7km from mouth on North Bank	3
Proserpine	Eden Lassie Creek	EDC_DS1	-20.18154	148.41500	AMTD 000210	3
Proserpine	Eden Lassie	EDC_US4	-20.18458	148.41360	Eden Lassie Creek 3.2km from	3

ADA site	Creek	Site name	Latitude	Longitude	Description	Samples
	Creek				mouth on North Bank	
Proserpine	Eden Lassie Creek	EDC_US3	-20.18604	148.41051	Eden Lassie Creek 3.7km from mouth on South Bank	3
Proserpine	Eden Lassie Creek	EDC_US2	-20.18915	148.41287	Eden Lassie Creek 4.2km from mouth on South Bank	3
Proserpine	Eden Lassie Creek	EDC_US1	-20.19049	148.41651	Eden Lassie Creek 4.7km from mouth on North Bank	3
Proserpine	Sandfly Creek	SFC_MS1	-20.18613	148.41890	Sandfly Creek 0.1km from junction with Eden Lassie Creek on South Bank	3
Proserpine	Sandfly Creek	SFC_MS2	-20.18706	148.42042	Sandfly Creek 0.9km from junction with Eden Lassie Creek on South Bank	3
Proserpine	Sandfly Creek	SFC_MS3	-20.19165	148.42121	Sandfly Creek 1.5km from junction with Eden Lassie Creek on South Bank adjacent to discharge	5
Proserpine	Sandfly Creek	SFC_MS4	-20.18905	148.42440	Sandfly Creek 2.1km from junction with Eden Lassie Creek on South Bank	3
Proserpine	Gregory River	GRG_1	-20.17140	148.44294	Gregory River 1.0km from mouth on North Bank	3
Proserpine	Gregory River	GRG_2	-20.17471	148.44620	Gregory River 1.5km from mouth on North Bank	3
Proserpine	Gregory River	GRG_3	-20.17423	148.45372	Gregory River 2.5km from mouth on South Bank	3
Proserpine	Gregory River	GRG_4	-20.17236	148.45816	Gregory River 3.0km from mouth on South Bank	3

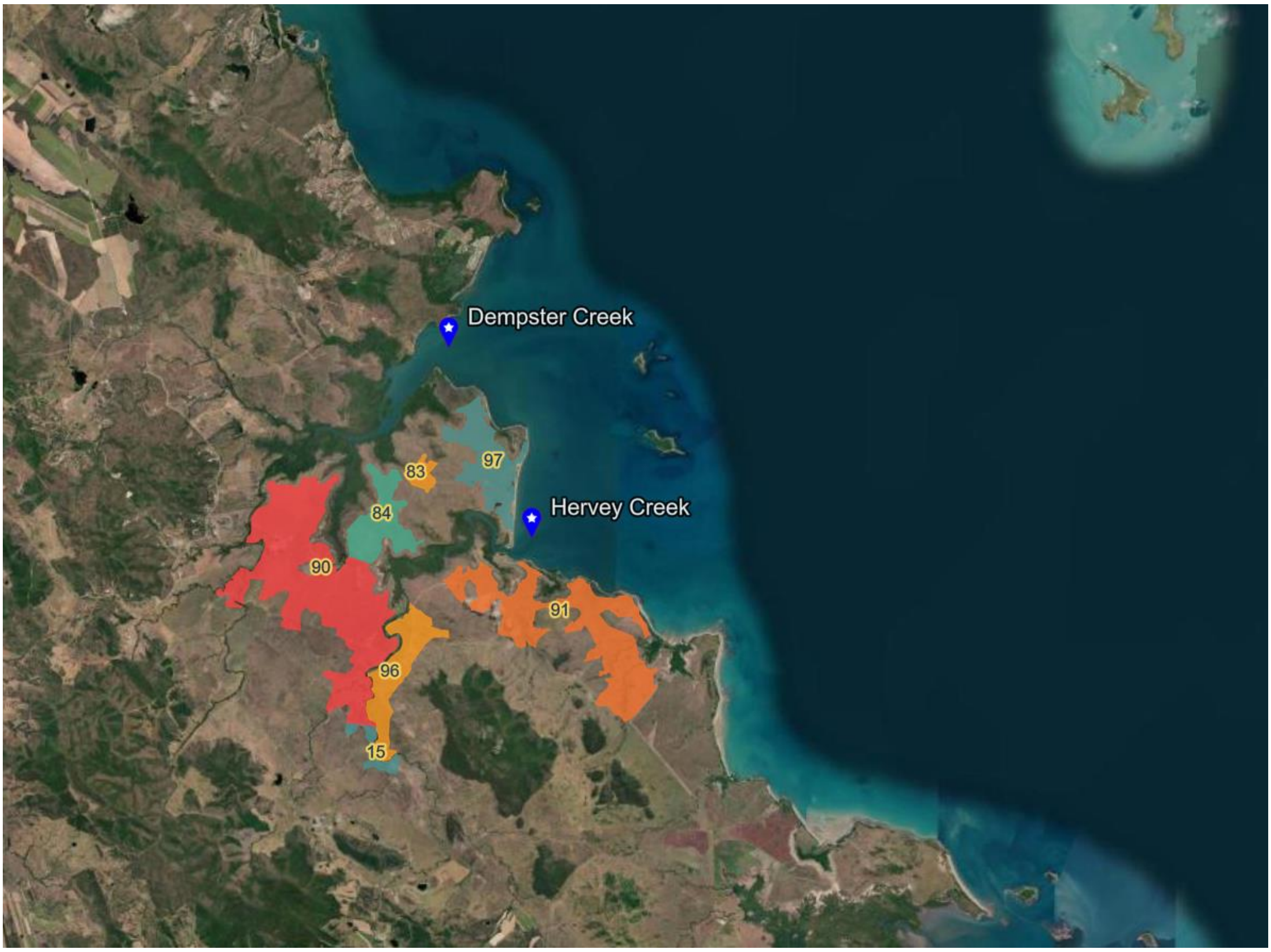

Figure S1 Dempster Creek and Hervey Creek in the O'Connell River Basin; Exmoor/ Bloomsbury ADA

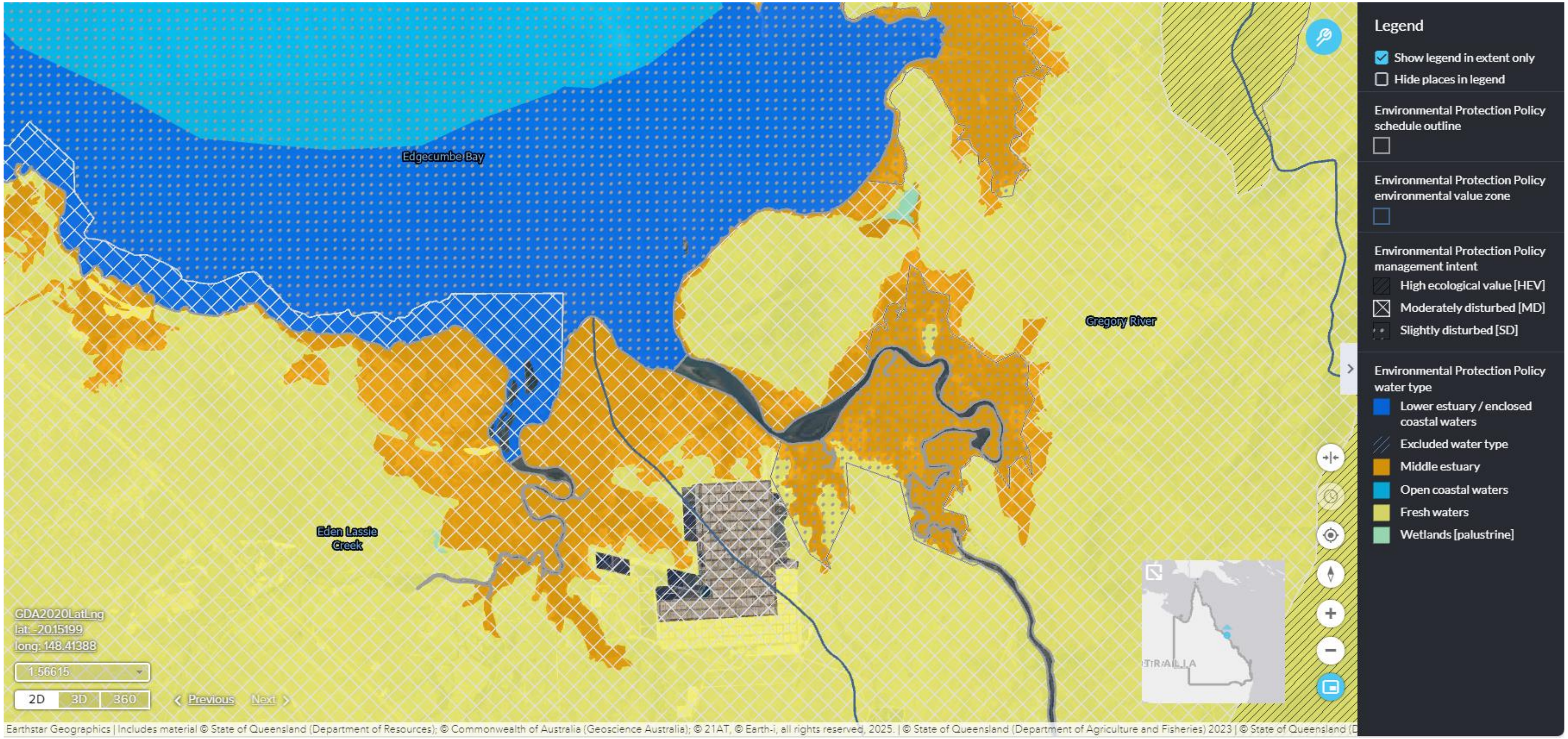

Figure S2 Environmental Protection Policy management intent and water types around Proserpine Prawn Farm

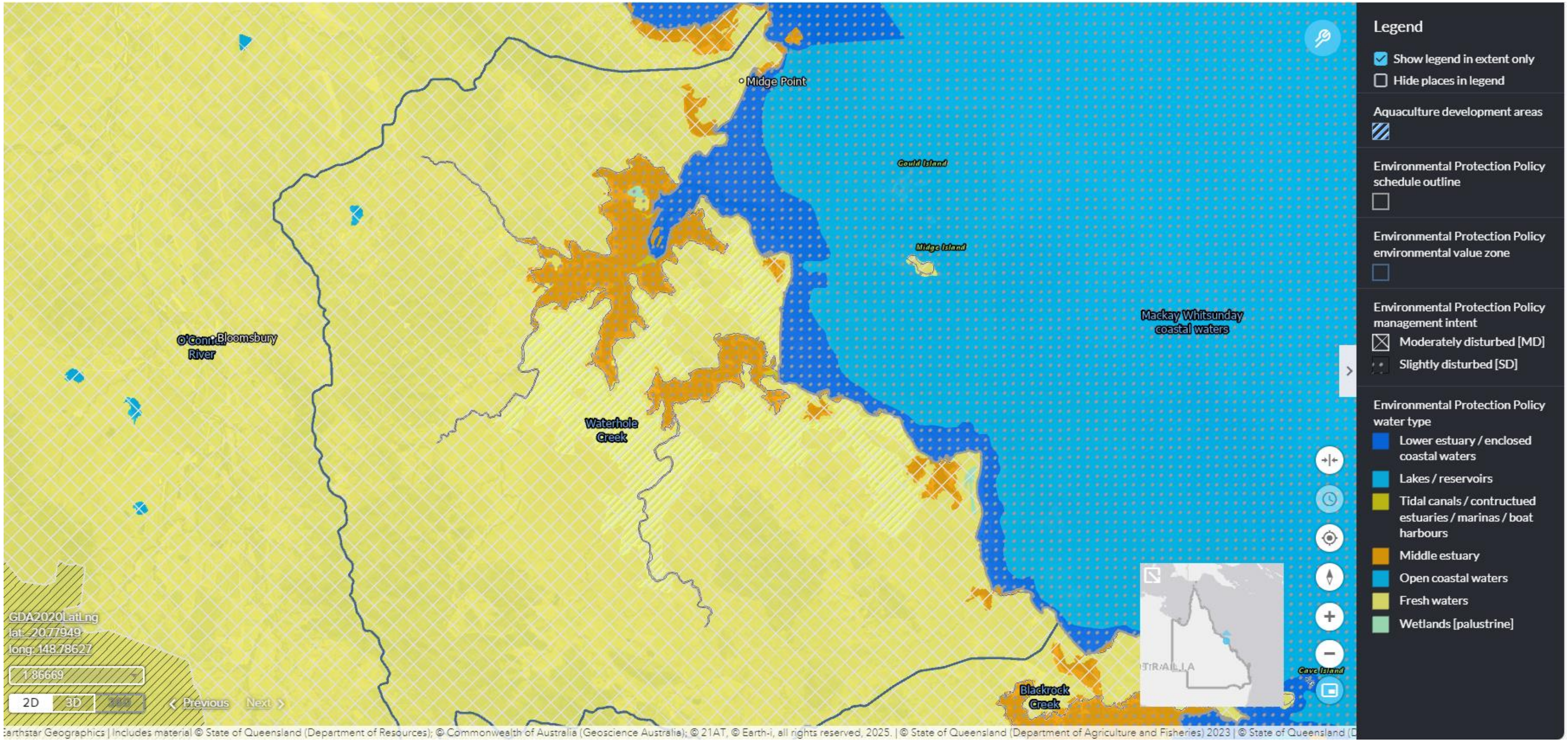

Figure S3 Environmental Protection Policy management intent and water types around Exmoor/ Bloomsbury ADA