

Environmental Impacts of Sea Cage Aquaculture in a Queensland Context – Hinchinbrook Channel Case Study (SD576/06)

Project Leader: Dr David McKinnon

FINAL REPORT

David McKinnon, Lindsay Trott, Samantha Duggan,
Richard Brinkman, Dan Alongi, Sarah Castine & Frances Patel



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Executive Summary

This document is the final report of a project entitled *Environmental Impacts of Sea Cage Aquaculture in a Queensland Context – Hinchinbrook Channel Case Study (SD576/06)* conducted by the Australian Institute of Marine Science (AIMS) as part of a research agreement with the Queensland Department of Tourism, Regional Development & Industry (DTRDI), the Queensland Department of Primary Industries and Fisheries (DPI & F) and Lyntune Pty Ltd trading as Bluewater Barramundi.

The Bluewater Barramundi farm is located in an extensive mangrove ecosystem within the Great Barrier Reef World Heritage Area (GBRWHA). This area is a Habitat Protection Zone of the Queensland Great Barrier Reef Coast Marine Park, and is within the Wet Tropics World Heritage Area. The farm is comprised of 32 synthetic mesh cages permanently moored in the main channel of Conn Creek, a side branch of Hinchinbrook Channel. It is approved to hold a maximum tonnage of 450t of barramundi *Lates calcarifer*, but usually holds less than 250t. Standard guidelines for sea cage farm operations and the appropriate monitoring strategies to track environmental impacts are yet to be defined in tropical regions.

An Interim Report was completed in August 2007. Part A of the Interim Report is a review of literature pertinent to the management of the environmental effects of tropical marine finfish cage culture, with emphasis on studies directly relevant to the Hinchinbrook Channel area. Higher temperatures in the tropics mean biological rate processes are higher than in temperate environments, and many tools developed for managing temperate aquaculture cannot be applied in the northern Australian tropics because of the environmental differences (highly turbid, macrotidal environments) and because of differences in biological communities. Part B of the Interim Report is a desk study of all accessible historical monitoring data required by the licence to operate, placed in the context of previous studies conducted in the Hinchinbrook area. There was insufficient scientific evidence to show that the farm has had a significant impact on the adjacent marine environment since monitoring began in 1998.

For the final report, AIMS was asked to estimate the area of influence of the farm, the carrying capacity of the environment in this area, and the fate of uneaten feed and other aquaculture wastes. AIMS was also asked to synthesise the results of this study to assist predictive modelling of the environmental impacts of sea cage aquaculture in similar areas, and to recommend and design a meaningful continuing monitoring program.

Our results indicate that:

- Water within Conn Creek is well mixed by tidal currents.
- Physical oceanographic models indicate the tidal flushing times in Conn Creek are rapid. Tidal exchange removes 60% of the water within Conn Creek within 12h during spring tides and within 24h on neap tides. This is an effective mechanism for the removal of suspended and dissolved wastes from Conn Creek.
- Seasonal climatic variation is the major factor affecting the water quality of Conn Creek.
- Overall, water quality within Conn Creek, including the farm site, conforms with Queensland Water Quality standards and there is no clear evidence of differences to similar mangrove environments in North Queensland.

- The footprint of the farm on the benthos appears to be restricted to the approval area, based on sediment chemistry and nutrient transformation processes.
- There is no evidence of accumulation of organic waste in the sediments underneath the cages.
- Phytoplankton within the water column of Conn Creek do not have sufficient assimilative capacity to absorb all dissolved wastes from the farm because the volume of the creek is too small.
- Mangroves facing Conn Creek contain N likely to have originated from farm activities, and play a significant role in nitrogen cycling within the ecosystem.
- Tidal flushing of Conn Creek (with Hinchinbrook Channel) is a vital route by which nutrients are exchanged and dissolved oxygen is replenished.
- Our results indicate that there has been no significant impact from the farm's operation on the adjacent marine environment, despite it being in operation for over 20 years.

We estimate that wastes from the Bluewater Barramundi farm add significantly to the N budget status of Conn Creek, and in a minor way to the C budget. In spite of this, water quality standards established by the EPA for partially enclosed waters of the wet tropics region were only marginally exceeded during the wet season, and are still well within the range of values typical of undisturbed mangrove waterways in North Queensland. In the water column there is a slight degree of enrichment of dissolved N in the immediate vicinity of the farm, but tidal mixing and turbulence rapidly dissipate these nutrients. The ratio of dissolved nutrients (N and P), together with the long turnover time of the dissolved N pool in the water column, both point to nutrients not limiting primary production within the system, and therefore that the assimilative capacity of the Conn Creek water column is saturated. The reason for this is that the water volume of Conn Creek is too small to support sufficient primary producers to absorb all aquaculture wastes.

Flushing by tides is a major physical process for dissipation of aquaculture wastes from Conn Creek. Our results suggest a 60% replenishment of the water within the farm area can occur within a single tidal cycle (~12h) during spring tides. During neap tides however, this increases to two tidal cycles (~24h). The water column of Conn Creek was well mixed, both vertically through the water column and horizontally, from the mouth to the headwaters of the creek. There was, however, a trend toward lower dissolved oxygen concentration in the upper reaches of the creek compared to the mouth, as is typical in these estuarine environments. The tides caused diurnal fluctuations in water temperature, salinity, dissolved oxygen concentration and pH. Based on a hydrodynamic transport model, we predict material originating from the farm potentially disperses as much as 2km both upstream and downstream from the farm, based on the movement of passive, non diffusive, virtual particles (i.e. those not dissipated by chemical or biological processes).

During low tides during the wet season we observed dissolved oxygen concentrations falling below 2mg l⁻¹ for up to 3h, leading us to believe that oxygen is likely to be a limiting factor for the carrying capacity of aquaculture within Conn Creek. Accordingly, we applied two carrying capacity models based on dissolved oxygen budgets. The model predictions seem reasonable, and are consistent with the upper limits of historical production at the site, but both models suggest that the farm is currently near the maximum carrying capacity of the site. However:

- Model predictions should be interpreted with caution since these models were primarily developed for grouper aquaculture in SE Asia.

- There are no documented cases of mortality as a direct result of oxygen depletion at the Bluewater Barramundi farm, probably because the site is very well flushed.
- There is no published information on the critical oxygen concentrations for barramundi – making it impossible to fully understand the implications of the periodically low oxygen concentrations in Conn Creek.
- Farm management practices (e.g. stocking density, cage location and design, feeding rate and timing of feeding) will all influence carrying capacity, and have not been taken into consideration in our predictions.

We have identified a range of possible monitoring indicators that may be suitable for future environmental monitoring. The costs and benefits of these monitoring strategies should be discussed at a workshop of stakeholders to develop the design of the monitoring program, since in our opinion conventional monitoring strategies are inappropriate in macrotidal tropical mangrove estuaries such as Conn Creek.

1. Introduction

In July 2007, a research agreement was signed by Queensland Department of Tourism, Regional Development & Industry (DTRDI), Queensland Department of Primary Industries and Fisheries (DPI & F), Lyntune Pty Ltd trading as Bluewater Barramundi and the Australian Institute of Marine Science (AIMS) entitled *Environmental Impacts of Sea Cage Aquaculture in a Queensland Context – Hinchinbrook Channel Case Study (SD576/06)*.

The goals of the project are to:

- ▶ Obtain an independent assessment of the environmental impacts of sea cage aquaculture on water quality and ecosystem function under best management practices likely to be applied to sea cage aquaculture in Queensland.
- ▶ To develop a predictive model suitable for extension to other regions, in order to support better management of coastal zone use.
- ▶ To provide this information to Government to assist in the design and recommendations for a meaningful continuing monitoring program at the Bluewater Barramundi farm site.
- ▶ To provide this information to Government to assist in the development of the State's policy on intensive marine aquaculture.

The first deliverable (Milestone 2) from this contract was the completion of an Interim Report comprising:

- ▶ A literature review pertinent to the management of the environmental effects of tropical marine finfish cage culture, with emphasis on studies directly relevant to the Hinchinbrook Channel area (Part A).
- ▶ A desk study of all accessible monitoring data collected by the farm and by various monitoring agencies with the relevant historical studies conducted in the Hinchinbrook area included (Part B).

This Interim Report was completed in August 2007.

The following report constitutes Milestone 3 – delivery of a draft report on the results of AIMS' fieldwork at Bluewater Barramundi farm, addressing the Project Tasks outlined in the Research and Collaboration Agreement:

- ▶ Estimate of the extent of the “area of influence” of the farm.
- ▶ Estimate of the carrying capacity of the environment in this area.
- ▶ Determination of the fate of uneaten food and other waste materials.
- ▶ A preliminary study of the role of wild fish attracted to sea cages.
- ▶ Synthesis of results to assist predictive modelling of the environmental impacts of sea cage aquaculture in other areas.
- ▶ Design and recommendations for a meaningful continuing monitoring program.

2. Design of the study

2.1 Study site

Bluewater Barramundi farm is located in an extensive mangrove ecosystem within the Great Barrier Reef World Heritage Area (GBRWHA) (Figs. 1 and 2). The farm is comprised of 32 synthetic mesh cages permanently moored in the main channel of Conn Creek, a side branch of Hinchinbrook Channel (18° 23.26' S, 146° 08.07' E). It is licensed to grow a maximum tonnage of 450t of barramundi, *Lates calcarifer*, but usually holds less than 250t. The farm feeds an average of 365t of commercial fish pellets (Ridley Barramundi Aquafeed) annually and achieves a food conversion ratio (FCR) of 1.5–2.0 (SBMP 2003). For most of the year fish are fed daily but during winter feeding is reduced to once every two days.



Figure 1: Location of Conn Creek in the Hinchinbrook Channel system.
Source: Google Earth® image.



Figure 2: Bluewater Barramundi farm, 22 April 2008 (Photo: S. Clarke, AIMS).

2.2 Sampling trips

We conducted five field trips to the Bluewater Barramundi farm (Table 1). The first of these trips was to take measurements of water movement over a tidal cycle using an Acoustic Doppler Current Profiler (ADCP) and was therefore restricted to physical measurements. Our experience from previous research in similar environments is that there are two primary forcing factors on water quality – season and tide. We therefore designed our sampling to concentrate on wet and dry season conditions, and neap and spring tides.

Table 1: Dates and environmental conditions for the AIMS field trips.

Trip	Dates	Season/Tides
1	30 July–2 August 2007	Dry season, springs
2	15–17 October 2007	Dry season, neaps
3	23–25 October 2007	Dry season, springs
4	19–21 February 2008	Wet season, springs
5	26–28 February 2008	Wet season, neaps

We were fortunate that our sampling dates spanned climatic extremes which gave us the opportunity to estimate the widest possible range in water quality due to natural influences: our October 2007 field trips occurred during a period of drought, whereas our February 2008 trips occurred at the tail-end of extreme flood conditions in North Queensland (Fig. 3).

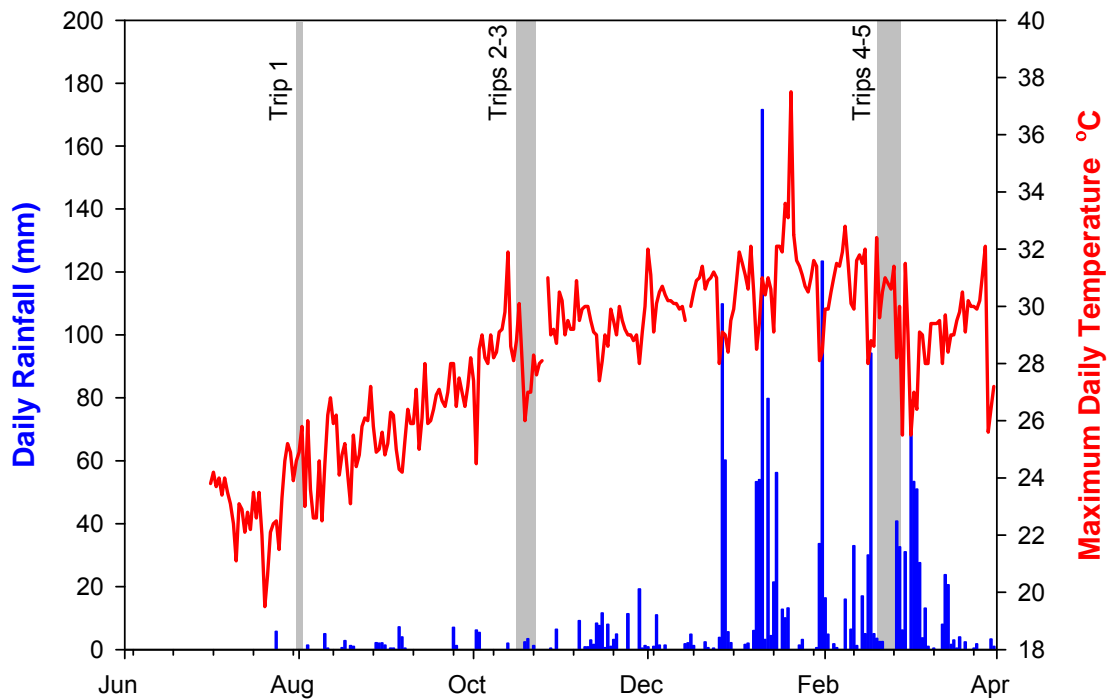


Figure 3: Climate data from Cardwell over the period of the study (July 2007– February 2008). Source: Australian Government Bureau of Meteorology <http://www.bom.gov.au>

In order to best understand currents and exchange rates in Conn Creek, Trip 1 was scheduled to coincide with a period of spring tides (Fig. 4). Transects across Conn Creek were run over a full tidal cycle, with an Acoustic Doppler Current Profiler mounted on the transom of the boat continuously monitoring water movement throughout the water column and at the same time recording bathymetry.

Biological measurements were made on Trips 2–5. Trip 2 was chosen to coincide with dry season neap tides (Fig. 5). We returned the following week to sample on the spring tides (Fig. 6). Similarly, in February 2008 we returned to make parallel sets of measurements on spring tides (Trip 4; Fig. 7) and neap tides (Trip 5; Fig. 8). Technical methods for all measurements are given in Appendix I.

The farm feeds Ridley Barramundi grower diets, which have a C content of 50.8%, an N content of 7.7%, and a P content of 1.96%. During the October 2007 field trips the farm managers estimated the farm was holding 150–180t of barramundi. During the February 2008 field trips the farm was holding ~170t.

In our calculations, we have assumed the area of cages to be 3,200m² (32 cages, 10m by 10m), the area containing these cages to be 40,000m² and the approval area to be 83,200m². We calculate the Conn Creek catchment to be approximately 25km², and average annual rainfall to be 2,119mm (based on Bureau of Meteorology data). The area of water in Conn Creek at mid tide is approximately 1.5km².

CARDWELL

Local Standard
Timezone: -10:00 U.T.

18° 16' S 146° 2' E

PREDICTION DATUM: 1.94 (m)

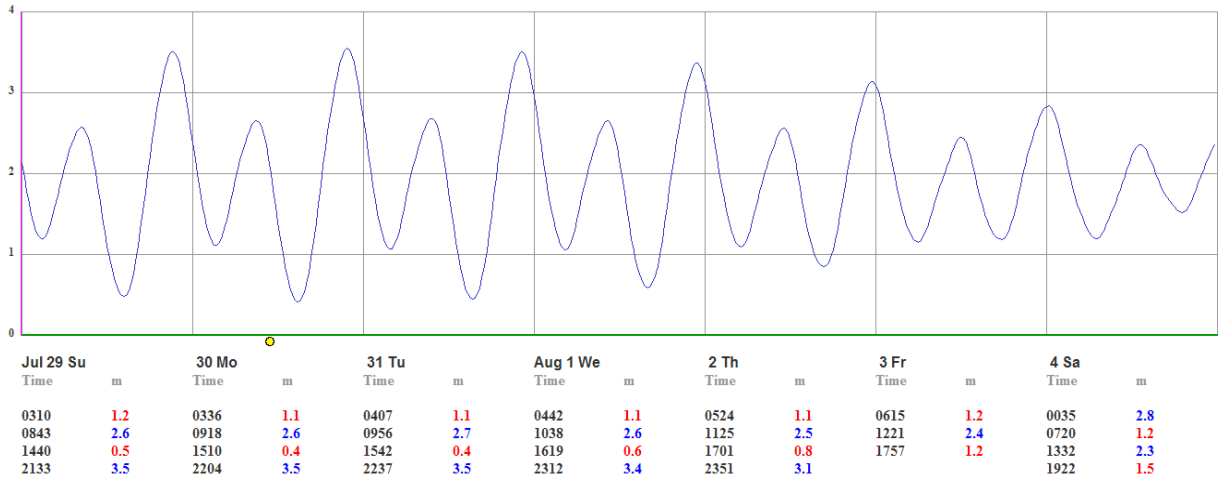


Figure 4: Tides during Trip 1. Source: Seafarer tides 2007.

CARDWELL

Local Standard
Timezone: -10:00 U.T.

18° 16' S 146° 2' E

PREDICTION DATUM: 1.94 (m)

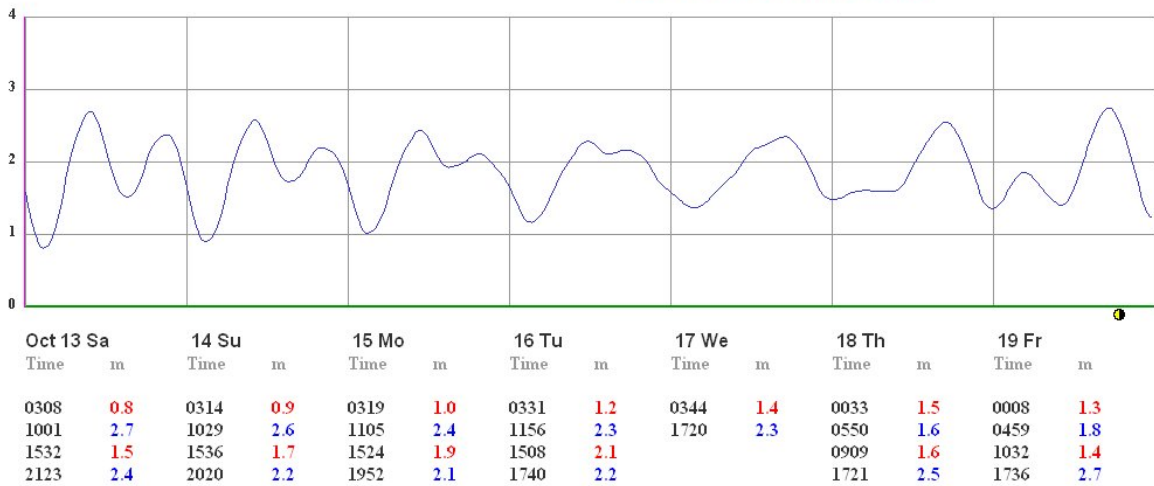


Figure 5: Tides during Trip 2. Source: Seafarer tides 2007.

CARDWELL

**Local Standard
Timezone: -10:00 U.T.**

18° 16' S 146° 2' E

PREDICTION DATUM: 1.94 (m)

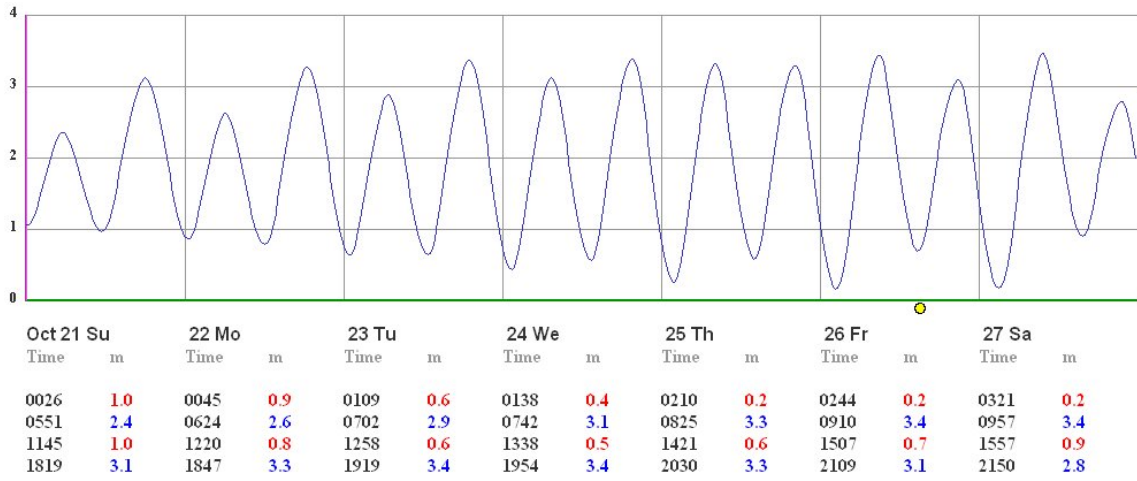


Figure 6: Tides during Trip 3. Source: Seafarer tides 2007.

CARDWELL

**Local Standard
Timezone: -10:00 U.T.**

18° 16' S 146° 2' E

PREDICTION DATUM: 1.94 (m)

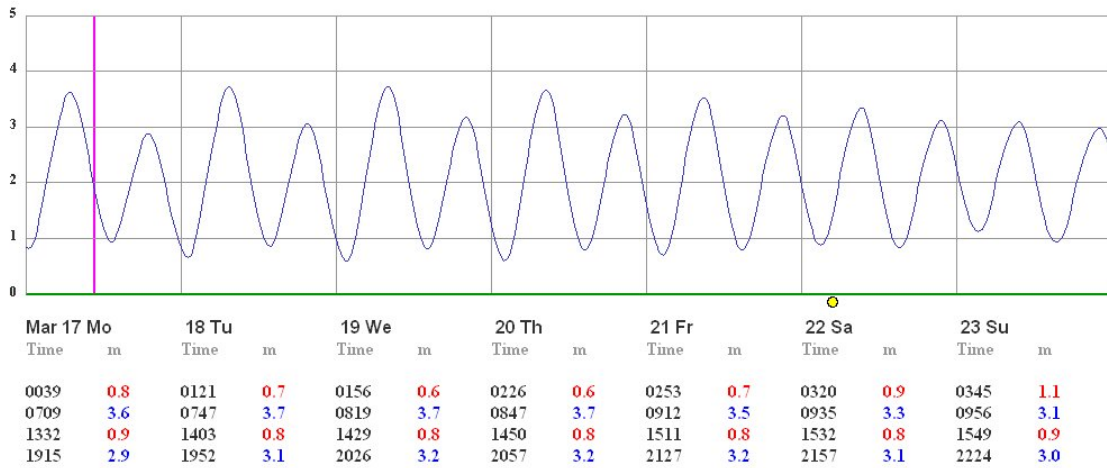


Figure 7: Tides during Trip 4. Source: Seafarer tides 2008.

CARDWELL

**Local Standard
Timezone: -10:00 U.T.**

18° 16' S 146° 2' E

PREDICTION DATUM: 1.94 (m)

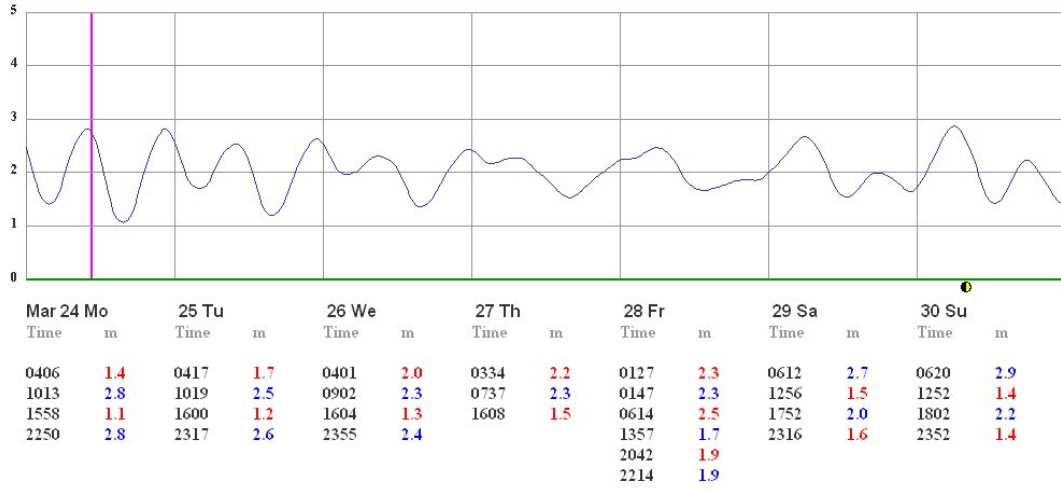


Figure 8: Tides during Trip 5. Source: Seafarer tides 2008.

3. Results

3.1 The physics of the study area

Understanding the movement of water in the vicinity of the farm is crucial to understanding ecosystem processes, as well as to estimate the physical removal of wastes. To understand the physics of Conn Creek, we first described the physical structure of the water and monitored diurnal change in physical variables. We then developed a two-dimensional model of water movement in Conn Creek, and validated this by direct measurement of currents.



Figure 9: Sampling sites used for CTD profiles. The station notation indicates approximate distance (m) north or south of the Cage site. Note that the farm approval area indicated by the black polygon extends north of the “Cage” site by ~300m. The physical location of the “Cage” site differed between trips, as we sampled at the most heavily stocked cage, wherever that was within the farm.

3.1.1 Conductivity, Temperature, Depth (CTD) profiles

Generally, there was little vertical structure in the water column of Conn Creek, because the creek is shallow and well mixed by the tides. The selection of CTD profiles described below corresponds to the locations of the production and respiration experiments (Fig.9).

The mean dry season temperature was 26.8°C and salinity was 35.7.

In the neap tides during the dry season (Fig. 10) the Reference station was the most turbid and there was slight stratification, which was most pronounced with lower oxygen concentration near bottom. The Cage site showed a slight increase in turbidity roughly corresponding to the bottom of the cage structure. The site 100m south of the cages

(S1000) had a turbid patch near-surface. In other respects, all the Conn Creek stations were well mixed and had identical values of salinity, temperature, chlorophyll and oxygen.

In the spring tides during the dry season (Fig. 11) the most pronounced feature was an increase in turbidity at a depth corresponding to the bottom of the cage. Dissolved oxygen concentration was marginally lower at the Cage site than at the concurrent station on the upstream side of the cages (4.9 vs 5.3mg l⁻¹ at high tide, 3.8 vs 4.0mg l⁻¹ at low tide). The mean wet season temperature was 29.1°C and salinity was 23.3.

In the spring tides during the wet season (Fig. 12) the high tide stations were more turbid than the low tide stations, though S1000 was very turbid near bottom. The turbidity anomaly at the Cage site, at a depth corresponding to the bottom of the cage structure, was again apparent at the high tide, but not at low tide. Dissolved oxygen concentrations did not differ between the stations, but were higher on the high tide (4.6–4.8mg l⁻¹ at high tide, 3.4–3.5mg l⁻¹ at low tide).

In the neap tides during the wet season (Fig. 13) there was some stratification in salinity, with lower salinity near the surface. Because the CTD profiler itself is ~1m deep we do not have estimates of surface salinity. There was also some indication of slightly higher chlorophyll near the surface of all stations. The Reference station had a pronounced turbidity layer mid-water. Dissolved oxygen concentrations were similar at all stations (3.3–3.5mg l⁻¹).

The structure of Conn Creek during neap tides in the wet season is best illustrated by a section taken through the creek (Fig. 14), based on CTD profiles throughout the system. Warm, turbid, low salinity water is mixing up the creek from Hinchinbrook Channel. This is seen in the low salinity layer (top panel) in the Hinchinbrook Channel, which probably has its origin from the Herbert River at the south of the channel. It therefore appears that the main source of low salinity water was from the mouth of Conn Creek, rather than from the head of the creek. However, the fresh water layer is mixed through the water column at the head of the creek as a result of tidal mixing.

Dissolved oxygen concentration decreased with distance into Conn Creek. There is a turbidity anomaly just south of the farm, where an adjoining creek enters. In other respects the creek is well mixed i.e. there is little difference in physical structure throughout the creek system.

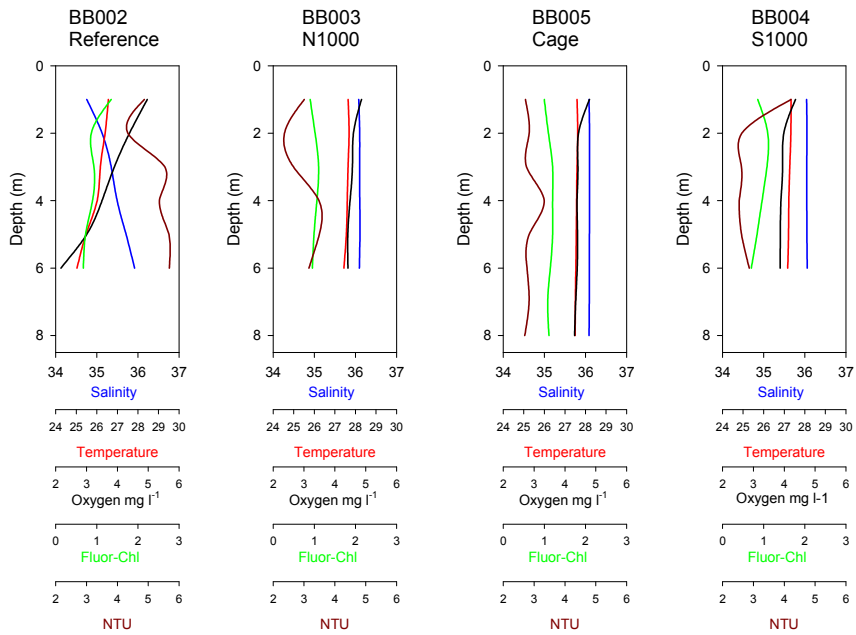


Figure 10: Dry season neap tide (Trip 2) spatial comparison of water column structure.

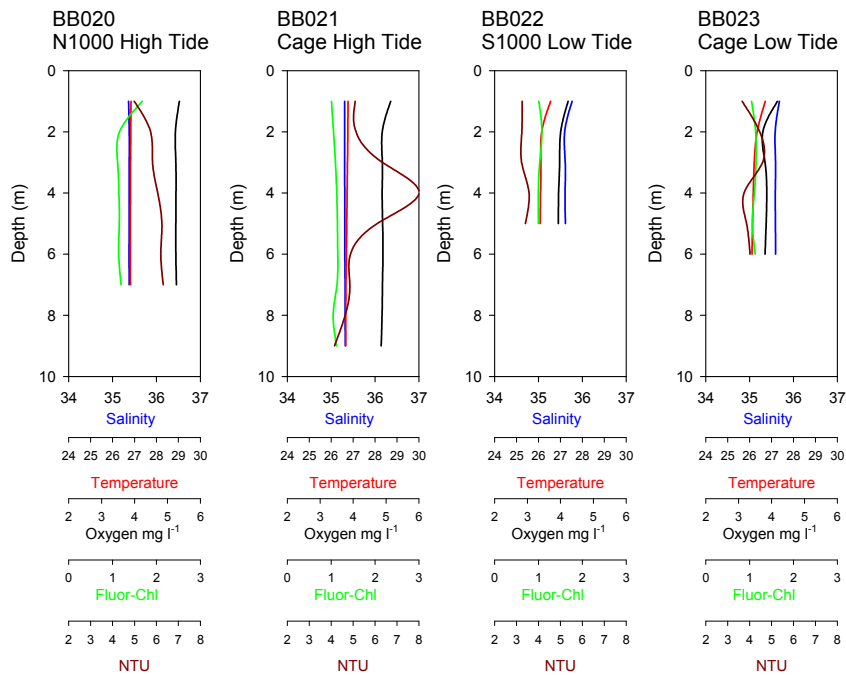


Figure 11: Dry season spring tide (Trip 3) spatial comparison of water column structure.

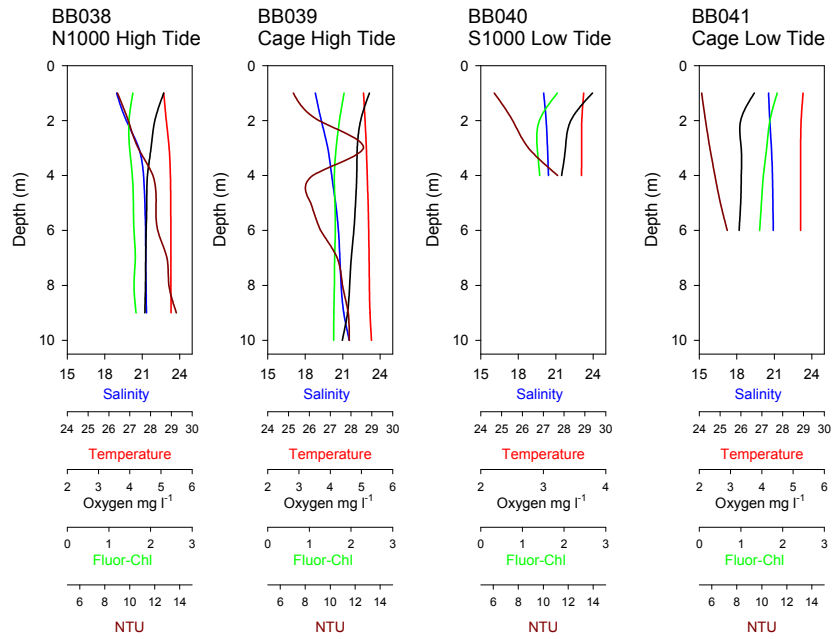


Figure 12: Wet season spring tide (Trip 4) spatial comparison of water column structure.

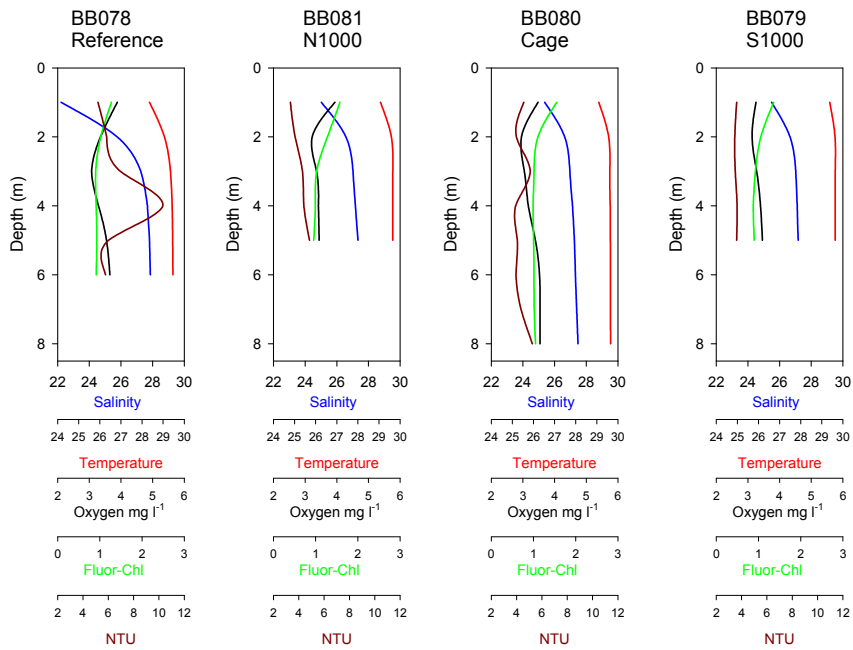


Figure 13: Wet season neap tide (Trip 5) spatial comparison of water column structure.

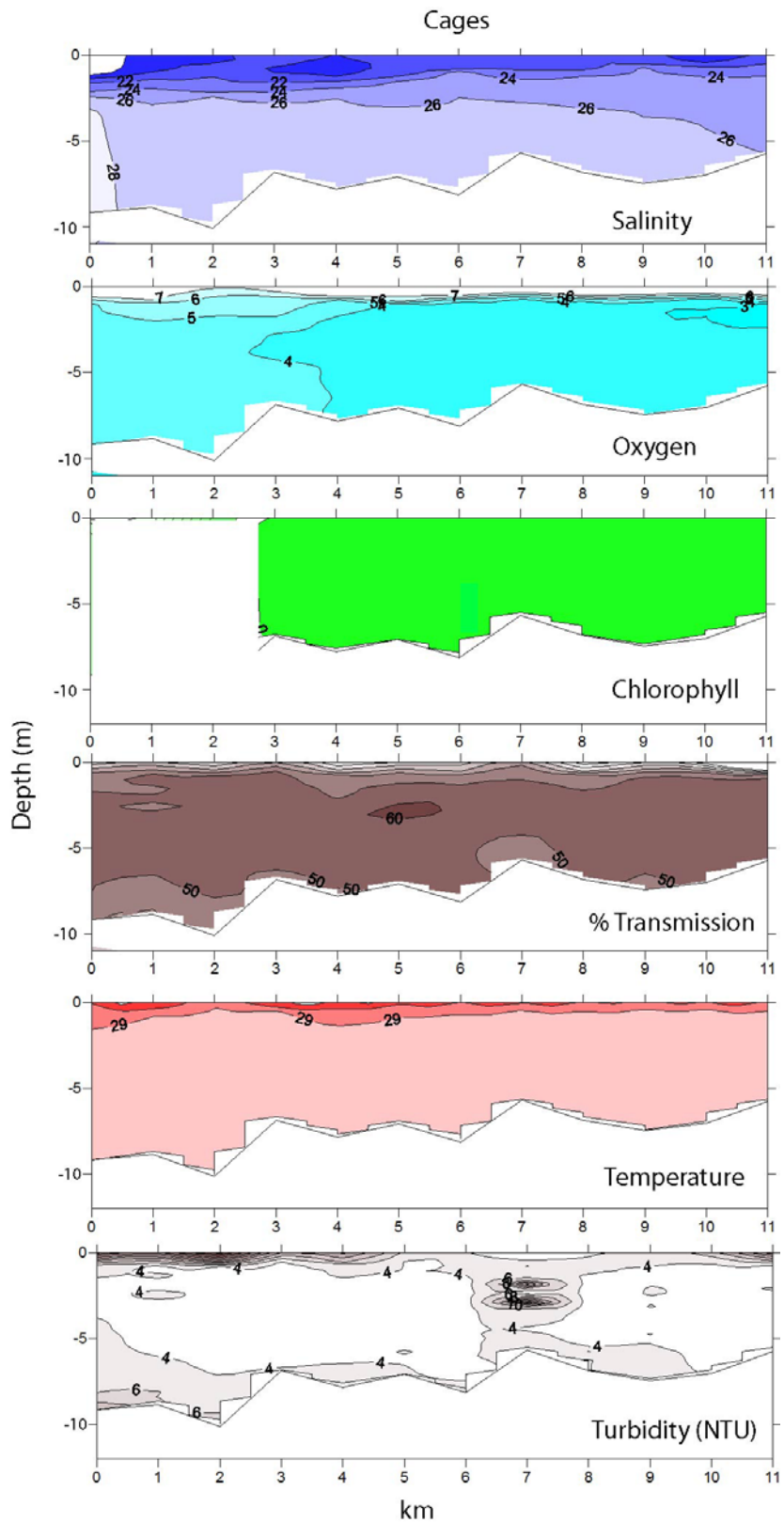


Figure 14: Cross section of Conn Creek in wet season (Hinchinbrook Channel = 0km; Bluewater Barramundi farm cages = 6km; top of creek = 11km).

3.1.2 Diurnal variation in water quality

To estimate diurnal change in water quality, on each trip we deployed Hydrolab DataSonde data loggers at three locations within the farm approval area. These instruments measure and store the following variables over continuous 24h periods:

- Temperature
- Salinity
- Conductivity
- Depth
- Dissolved oxygen
- pH

During the neap tides in the dry season (Fig. 15) we recorded a pattern of slight warming during the day, and cooling at night that coincided with incoming and outgoing tides. Dissolved oxygen concentration showed a slight trend toward higher concentrations in the middle of the day, possibly reflecting phytoplankton production during daylight and respiration overnight. However, these peaks and troughs in dissolved oxygen also coincide with oxygen rich incoming tides and depressed oxygen during outgoing tides. During dry season spring tides (Fig. 16) both oxygen and temperature closely tracked the tidal signal, with peaks in each corresponding to the high tide. Dissolved oxygen concentrations inside the cages also tracked the tidal signal but remained slightly below those observed outside the cages, implying that movement and mixing of water inside the cages was reduced and that fish respiration within the cages was lowering the dissolved oxygen concentration.

During the wet season spring tides, both dissolved oxygen and temperature closely tracked the tidal signal (Fig. 17), as was the case during spring tides in the dry season. There is a marked increase in fluctuation of dissolved oxygen concentration inside the cages. While the maximum dissolved oxygen concentrations reached were similar inside and outside the cages, there is greater draw-down of dissolved oxygen inside the stocked cage during ebb tide slack water. During this period, the dissolved oxygen concentration inside a stocked cage was 1.5mg l⁻¹ lower than outside the cage. These lower concentrations reached ~1.5mg l⁻¹ for the duration of the slack water, followed by a rapid rise to ~4.5mg l⁻¹ during the maximum run-in of the flood tide. Outside the cage during the flood tide slack water, dissolved oxygen concentrations remained higher, within the range 2.75–4.5mg l⁻¹. Over the 2.5 days of deployment there was a gradual lowering of salinity (to below 20), as freshwater was mixed into Conn Creek from the Hinchinbrook Channel (see Section 3.1.1.). Other water quality variables such as pH tracked the salinity signal.

In wet season neap tides (Fig. 18) there was no pattern of diurnal warming or nocturnal cooling. Oxygen consumption in the water column was particularly pronounced, with dissolved oxygen dropping to less than 2mg l⁻¹ at night. As was the case during the spring tides, there was a gradual pattern of lowering salinity during the logger deployment, as freshwater mixed with higher salinity creek water. With the exception of wet season salinity immediately after the January 2008 extreme rainfall event, and dissolved oxygen inside and outside cages at slack water, the other water quality variables measured by the Hydrolabs remained within normal ranges.

Generally, outgoing tides in the dry season lower the dissolved oxygen and temperature and raise the salinity, while incoming tides raise the dissolved oxygen and temperature and lower the salinity. Similarly, in the wet season outgoing tides lower the dissolved oxygen and temperature, and incoming tides raise the dissolved oxygen and temperature. Salinity fluctuations, however, in the wet season vary depending on whether the freshwater is coming from the headwaters of the creek (lower salinity in outgoing tides) or from

surrounding river catchments via Hinchinbrook Channel (lower salinity in incoming tides). During these flood periods, temperature may track the salinity fluctuations more closely. The degree of variation between extreme values of dissolved oxygen, salinity and temperature is usually closely correlated to the height and duration of the tides.

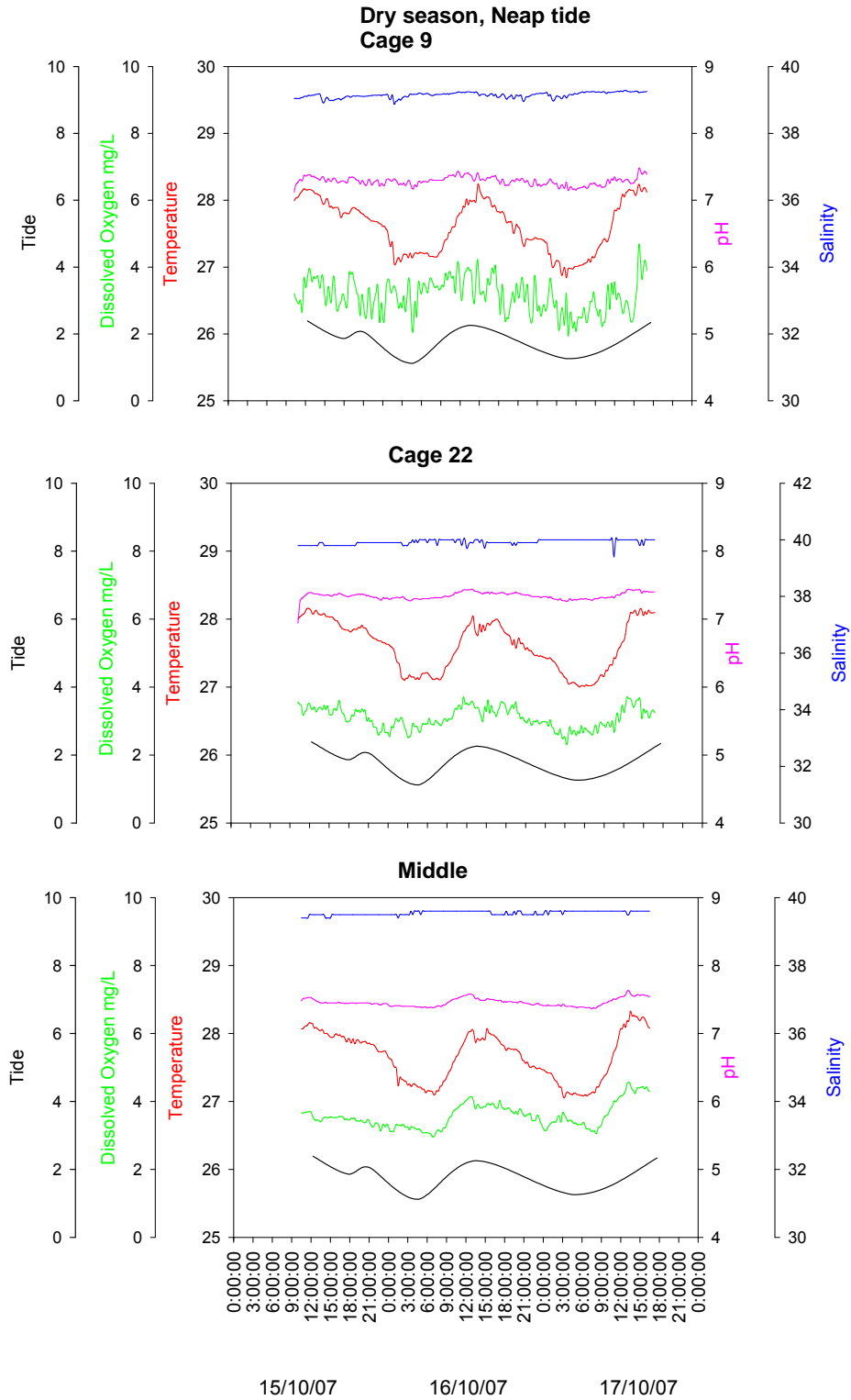


Figure 15: Hydrolab data records from Trip 2; dry season neap tides.

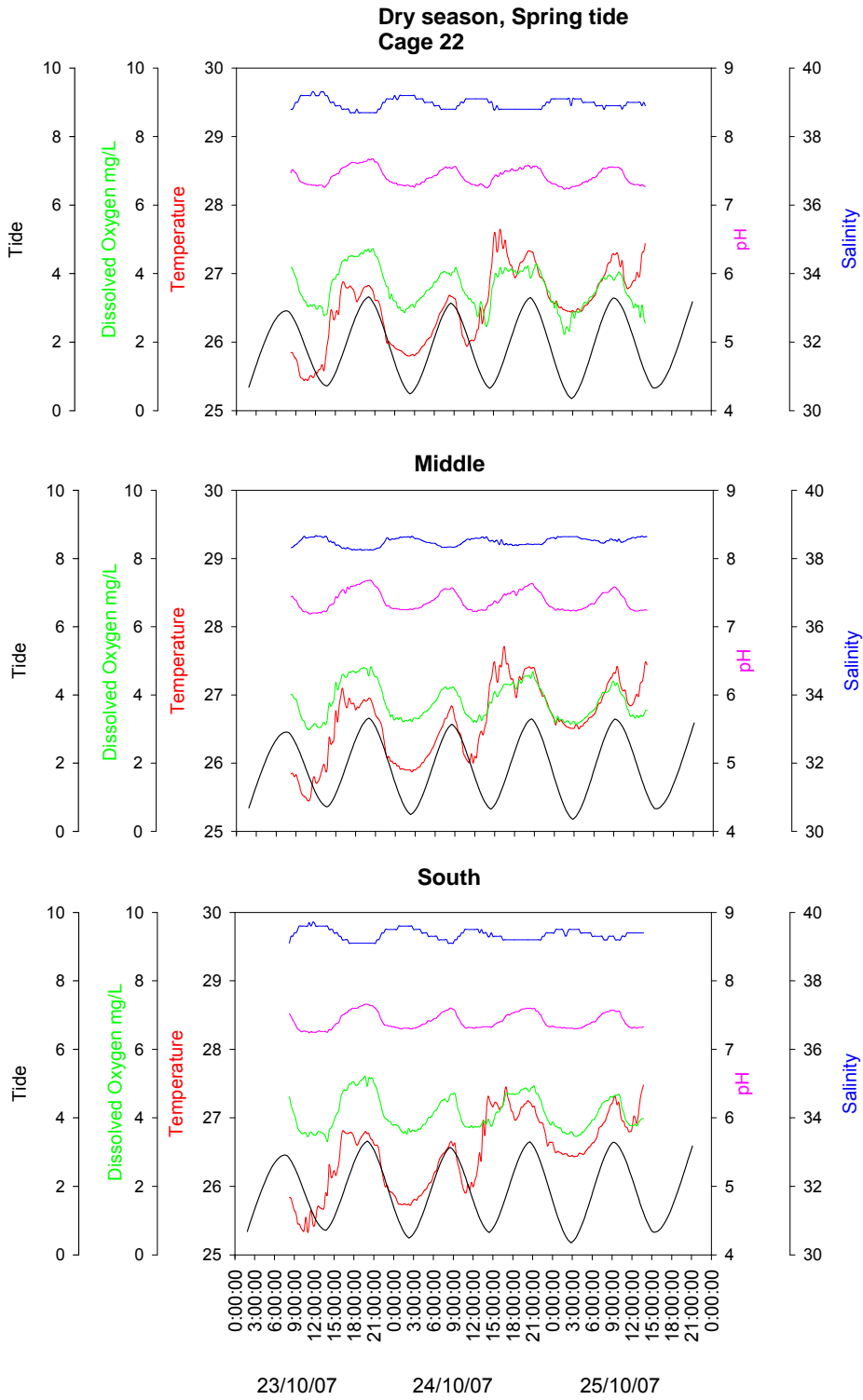


Figure 16: Hydrolab data records from Trip 3; dry season spring tides.

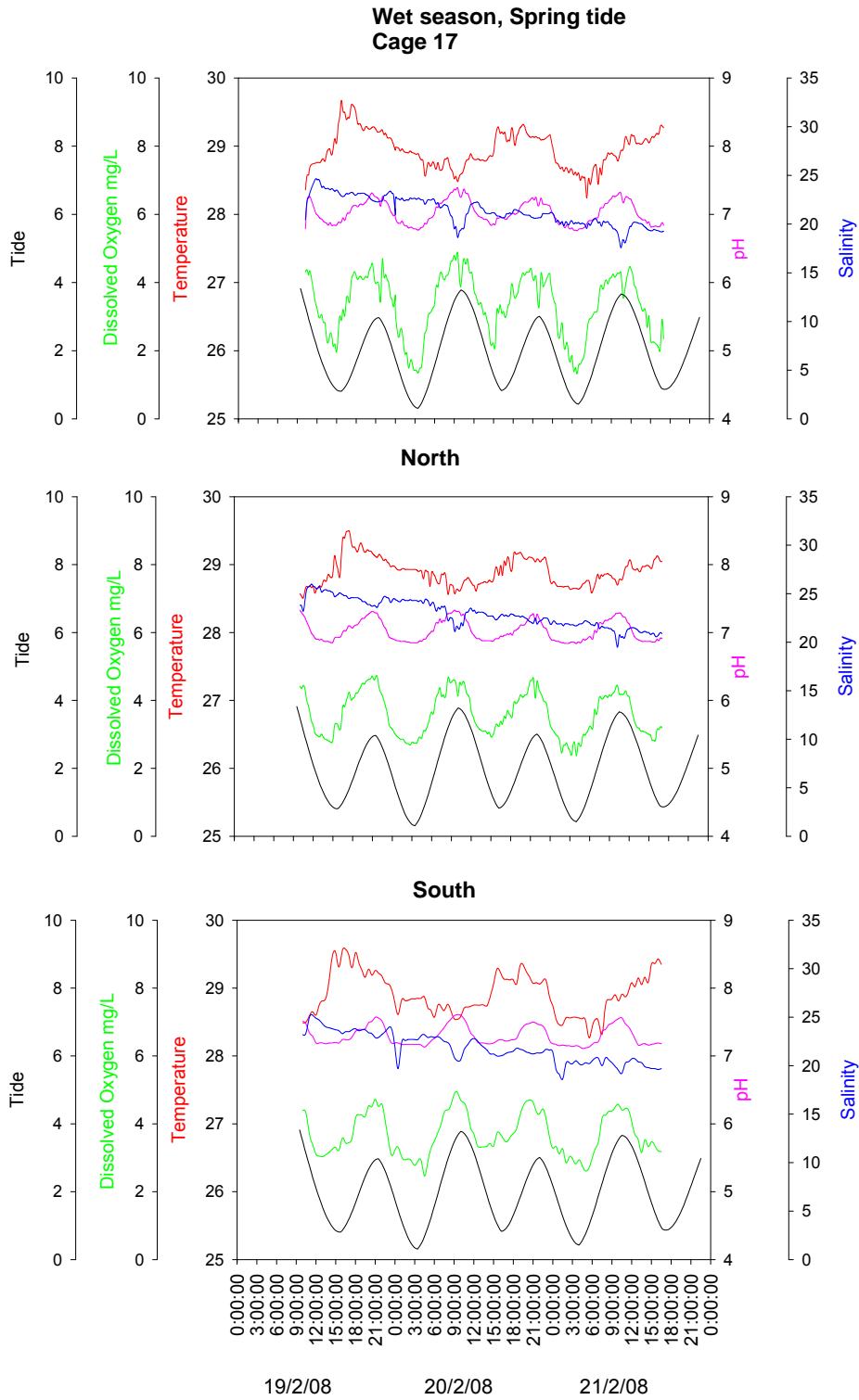


Figure 17: Hydrolab data records from Trip 4; wet season spring tides.

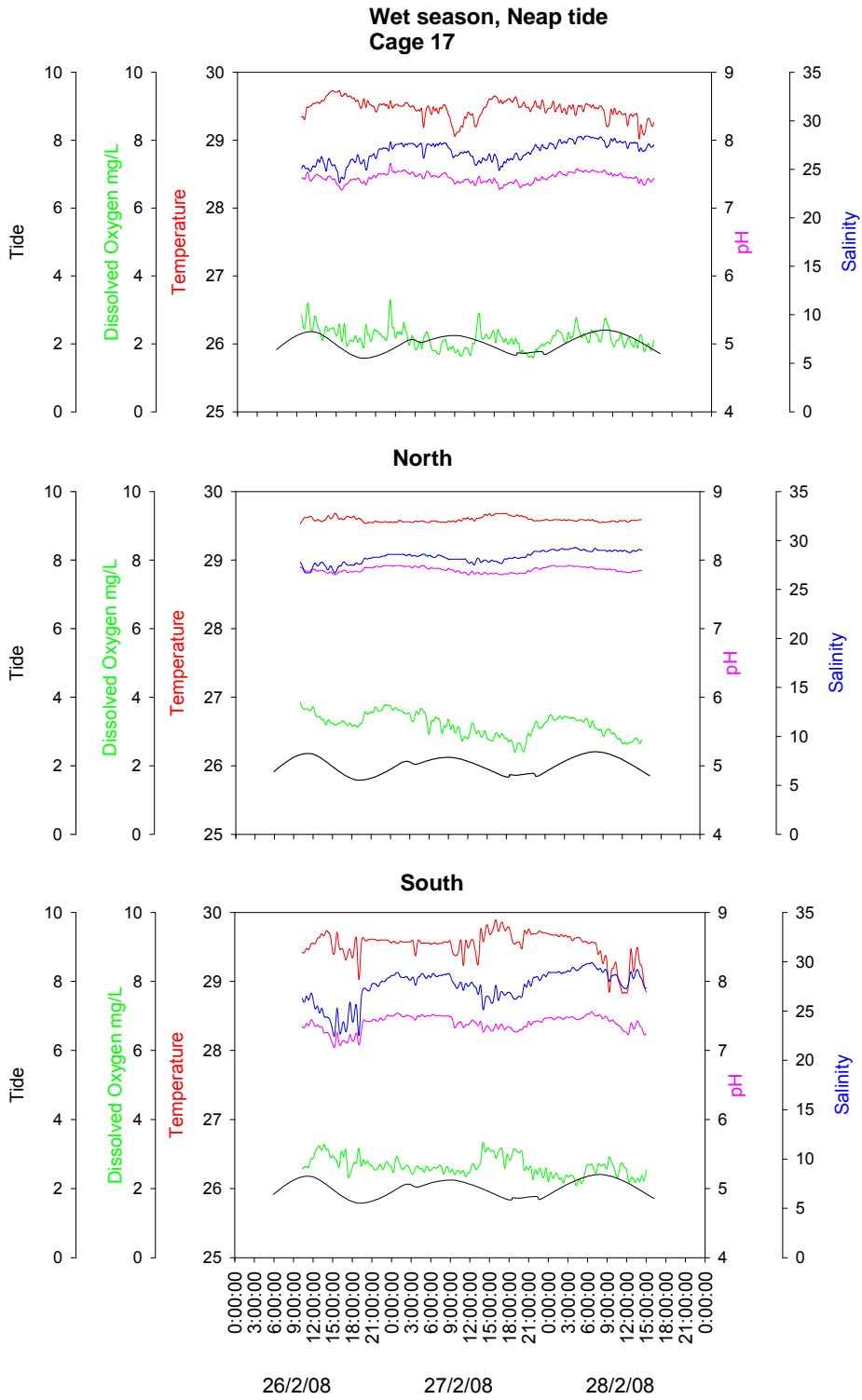


Figure 18: Hydrolab data records from Trip 5; wet season neap tides.

3.1.3 Secchi depth

Water clarity, as measured by Secchi disc, was greater during the dry season (Table 2). In the wet season Conn Creek was more turbid, as a result of heavy rainfall washing in sediment and detritus from the mangroves. The 1% light level is often taken as the depth of the euphotic zone (the lowest limit for photosynthesis). We calculated the value of the 1% light level as 3.5 times the Secchi depth, as is appropriate for waters with Secchi depth less than 5m (Holmes 1970).

Table 2: Mean Secchi depth values (standard deviation) and depth of the 1% light level from each season and tide (N, neap; L, low spring; H, high spring) combination.

Season	Tide	Secchi depth (m)	1% Light level (m)
D	N	3.6 (0.6)	12.6
D	H	1.8 (0.4)	6.3
D	L	2.5 (0.4)	8.7
W	N	1.6 (0.2)	5.6
W	H	0.9 (0.4)	3.1
W	L	1.1 (0.2)	3.8

3.1.4 Physical modelling

In order to better understand the movement of water in the vicinity of the farm, we employed a two dimensional finite element numerical hydrodynamic model (King 2005; King 2006) to simulate the depth-averaged hydrodynamics within Conn Creek. Finite element models allow the model domain to contain elements of non-uniform area and shape (Fig. 19) that enables the model grid to accurately resolve areas of complex topography such as the Conn Creek shoreline. The numerical model computed water surface elevations and horizontal velocity components on a computational domain comprised of 1700 elements, which covered the majority of Conn Creek and surrounding mangrove catchment (Fig. 19). The bathymetric information supporting the model grid was obtained from Australian Hydrographic Charts AUS259 and augmented by bathymetric data collected during Trip 1.

Model simulations were performed for a 30 day period (i.e. longer than one complete neap-spring-neap tidal cycle) commencing on 20 July, 2007, during which time the model was forced along the northern boundary of the domain with a synthesised tidal sea surface elevation generated using the tidal constituents observed at Cardwell. The model was validated against current velocities observed during Trip 1. These velocities were observed with cages *in situ*, and the model is therefore based on actual velocities measured in Conn Creek with the present cage arrangement.

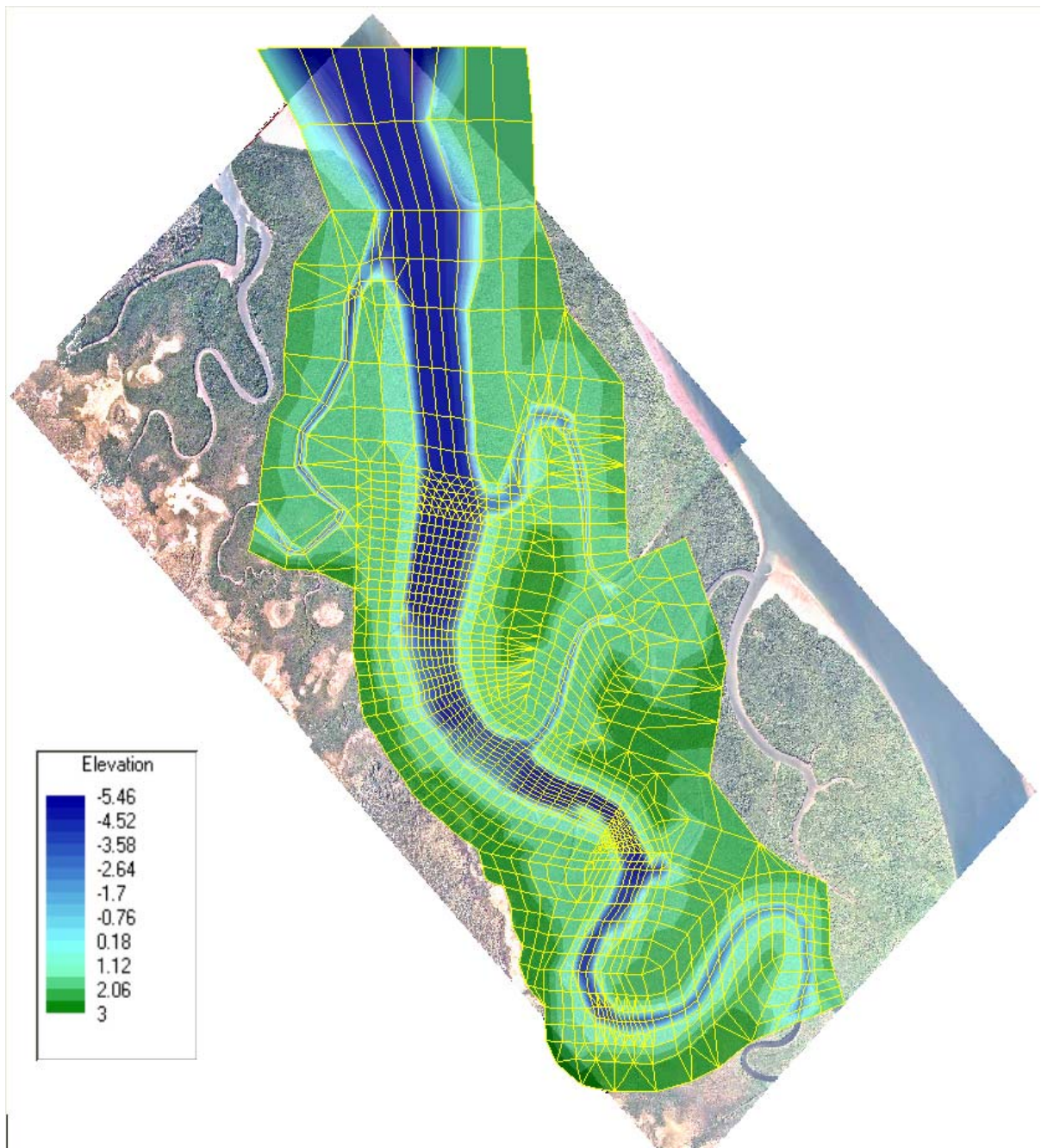


Figure 19: Finite element mesh established for the numerical hydrodynamic model of Conn Creek.

To provide the context in which to interpret water and sediment quality observations, we used model simulations to assess the general hydrodynamics in the vicinity of the farm and estimate material dispersal, flushing rates and water mass exchanges.

Material dispersal from the farm was estimated by tracking passive, non-diffusive virtual particles released from the farm and advected by simulated current fields (Fig. 20). These virtual particles are not intended to resemble real aquaculture waste products such as fish faeces or pellets of food. They are best regarded as indicators of an individual “packet” of water, and the concentration of the particles indicate how quickly that “packet” is diluted and dispersed by physical processes alone. Since the model is two-dimensional, there is no buoyancy term. During spring tides, the model predicts that passive waterborne material

released from a location within the farm will traverse a maximum area extending approximately 1500m to the north and 1300m to the south of the farm. During neap tides the area traversed contracts to 800m to the north and 600m to the south of the farm.

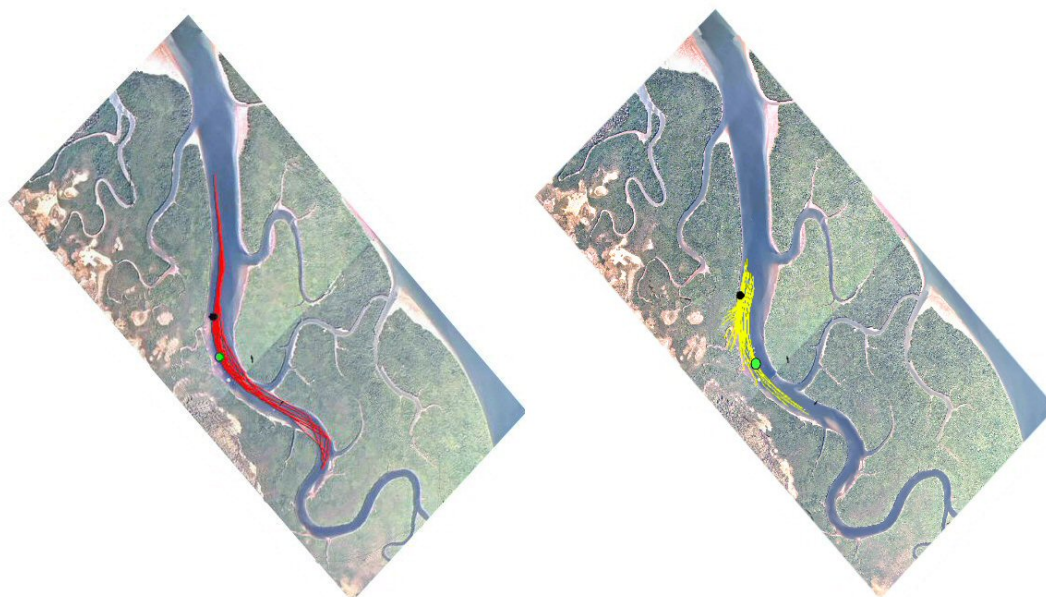


Figure 20: Trajectories of simulated passive particles released from a site within the fish cages under spring (left) and neap (right) tides. The green circle indicates the location of particle release. Black circle shows location of particle after 10 days.

A commonly used measure of the flushing of tidal estuaries and water bodies is the *e-folding* time, which is encountered when the concentration of a conservative, non-decaying tracer within a particular region of the estuary is reduced to $1/e$ (~38%) of its initial value. We simulated the flushing characteristics of Conn Creek by releasing a virtual passive tracer into the model from within a sub-region of Conn Creek that included the area of the farm (Figs. 21 and 22). The tracer was initialised within the sub-region with a concentration of 100mg l^{-1} and zero elsewhere. Tidal forcing was applied to the domain and the advection/diffusion of the tracer was simulated using the computed hydrodynamics during both spring and neap tide. A time series of the normalised mean concentration within the sub-region (Fig. 23) shows that while concentrations oscillate at the tidal frequency as the tracer is mixed with water brought into the domain on the flood and removed on the ebb, the general flushing times are very rapid. The flushing times are greater during neap tides (18–24 hours) compared to less than 12 hours during spring tides.

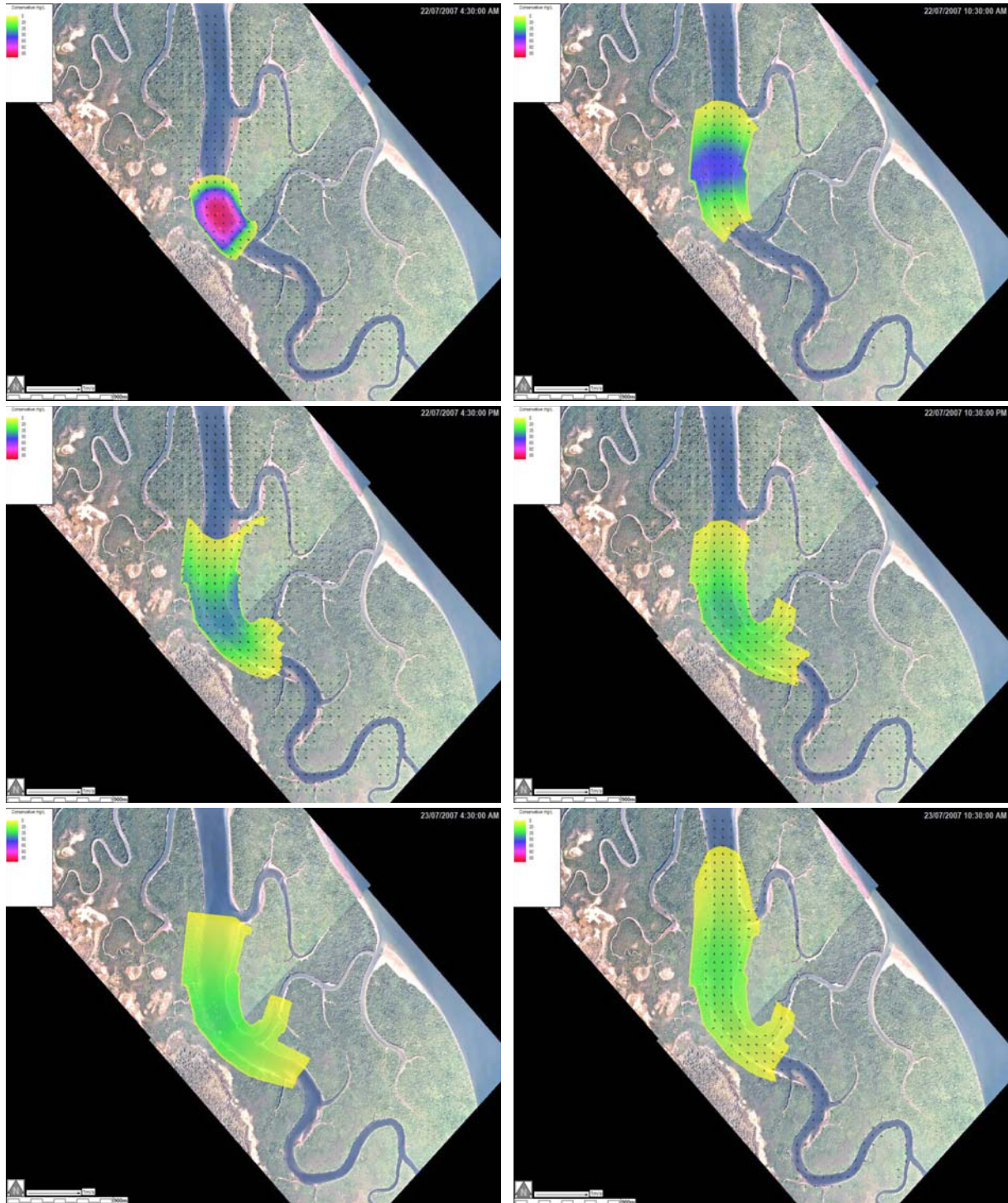


Figure 21: Synoptic views of predicted concentration of a virtual passive tracer released from sub-region of Conn Creek (top left frame) under neap tides. Snap shots are at 6 hourly intervals. Times are shown at top-right corner of each panel. Animations are available at: <http://www.aims.gov.au/docs/research/sustainable-use/tropical-aquaculture/sea-cage-aquaculture.html>

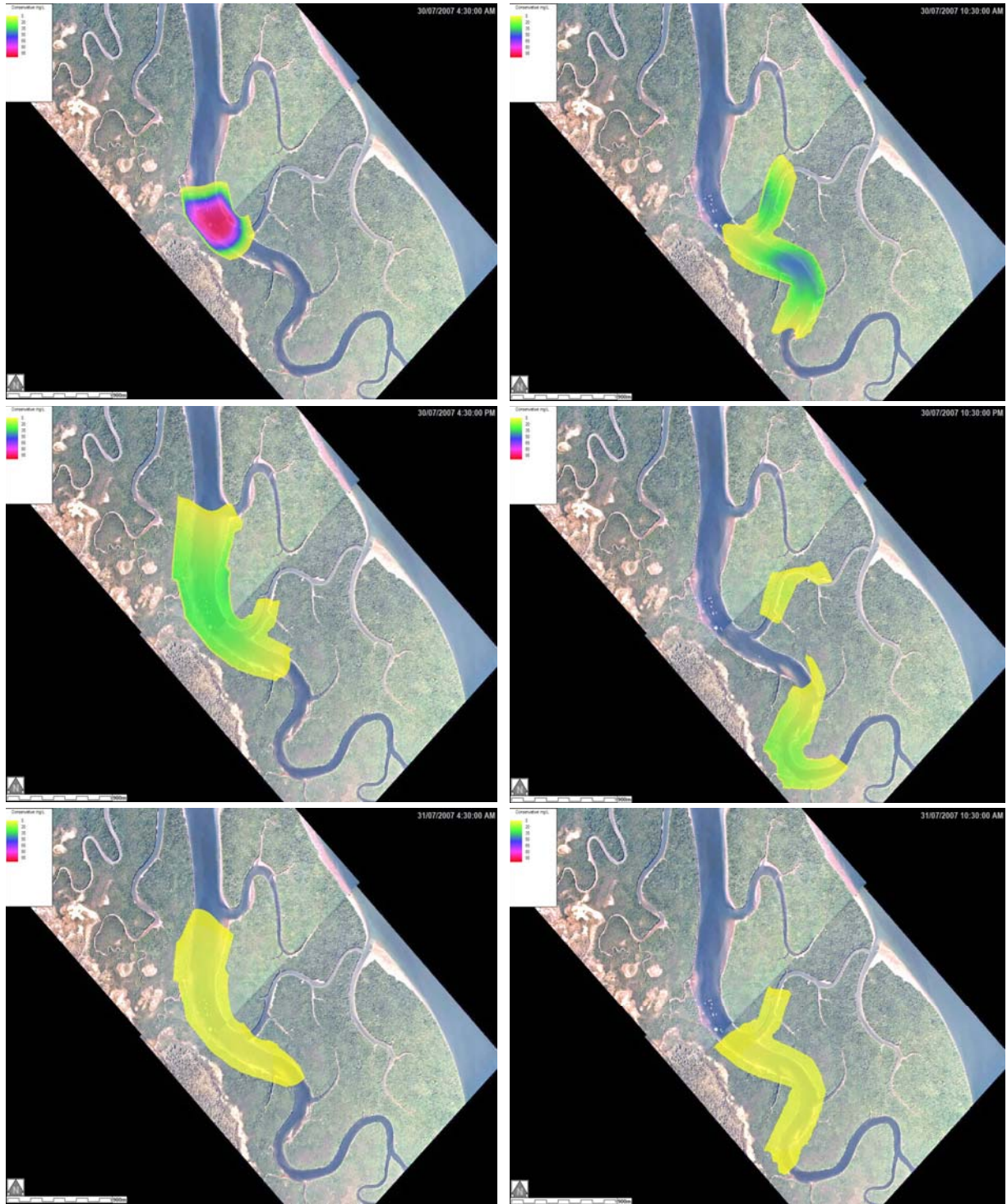


Figure 22: Synoptic views of predicted concentration of a virtual passive tracer released from sub-region of Conn Creek (top left frame) under spring tides. Snapshots are at 6 hourly intervals. Times are shown at top-right corner of each panel.

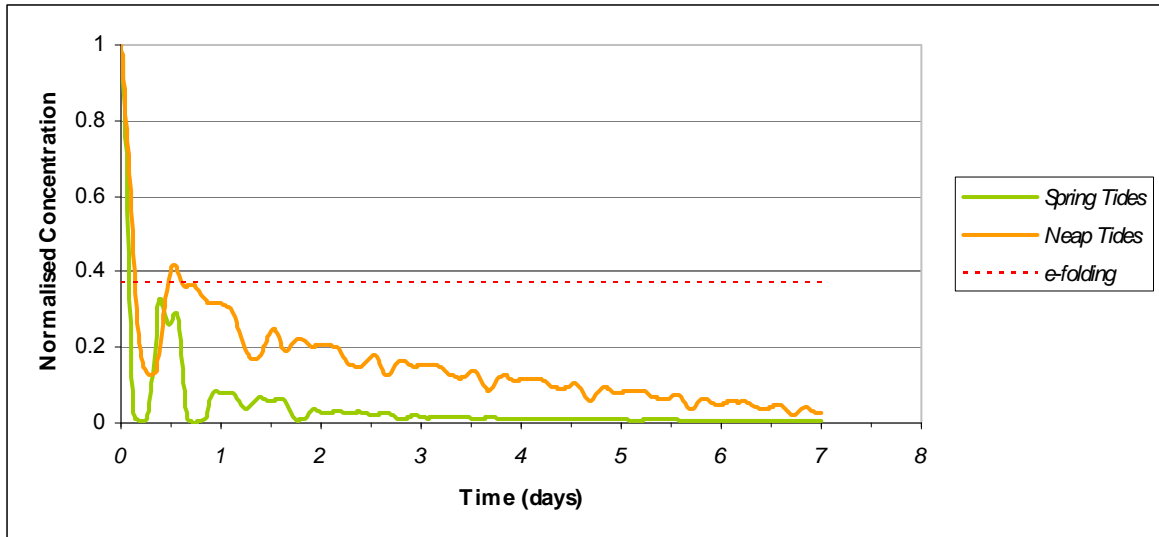


Figure 23: Normalised mean concentration of passive tracer within the sub-region of Conn Creek (see Fig. 20).

Daily water volume exchanges within the farm area under neap and spring tidal regimes (Table 3) were estimated from model output time series of current velocity and water level at sites within the farm area.

Table 3: Total water volume exchange passing under a cage/day estimated for spring and neap tides. Also given are characteristic values of non-directional current speed.

	Spring (Range 3.1m)	Neap (Range 0.9m)
Total volume passing under cage/day (m^3)	5,400,00	1,100,000
Speed ($m s^{-1}$)		
Mean	0.13	0.03
Median	0.12	0.03
Max	0.33	0.06
Std Dev	0.09	0.02

Commentary: the physics of the study area

Observation of physical properties of the water column and modelling of tidally driven circulation indicate that Conn Creek is in general vertically well mixed with significant tidal exchange, the magnitude of which is modulated by the spring-neap cycle.

Modelled flushing times in the vicinity of the farm were estimated at less than 12 hours during spring tides, suggesting complete replenishment of farm water within a tidal cycle. During neap tides however, the flushing times increase to around one day. This is confirmed and best illustrated by instrumental measurements (Hydrolab DataSonde dataloggers) of dissolved oxygen (see Figs. 15–18). During spring tides dissolved oxygen concentration oscillates at a tidal frequency, however during neap tides, dissolved oxygen concentration may remain low for up to 24 hours. The exception to this is during the wet season when fresh water flows may dominate the replenishment of the water within the farmed area of the creek, particularly during neap tides.

Passive non-diffusive waterborne material originating from the farm dispersed approximately 1500m to the north and 1300m to the south of the farm. This is a conservative estimate based only on advection during the dry season, and will change in response to other processes that influence the hydrodynamics of the creek, such as fresh water inflow and sub-tidal period changes in sea level.

3.2 Area of influence of the Bluewater Barramundi farm

To identify the area of influence of the farm, we measured nutrient concentrations in the water column on transects through the farm, as well as elemental composition of creek sediments. In addition, we traced the area of influence of the farm by looking at stable isotope signatures of mangrove leaves, which can vary depending on the degree of exposure to sources with elevated stable isotope signatures (e.g. breakdown products of aquaculture feed).

3.2.1 Water quality transects

To better understand the effect of the farm on water quality, on each trip we sampled along a transect running through the farm (Fig. 24). This was conducted only once on neap tides, but at both the low and the high tide on spring tides. In the wet season trips the sampling was initially conducted at only one depth (for comparative purposes) and was then repeated, taking samples at top and bottom, since there was salinity stratification as a result of heavy rain.

The stations are coded with nominal distance N or S of the farm, taking the cage to the south of the farm at which we commenced our studies as the zero point. Accordingly, the names and actual distances from this point are: Cage = 0; N 1000 = 1000m N, N 2000 = 2200m N; S 250 = 250m S; S 500 = 575m S; S 1000 = 1100m S. The Reference station was chosen to represent a similar but independent location accessible to the farm, but hydrologically isolated from it.



Figure 24: Sampling sites used for water quality and primary production measurements. The station notation indicates approximate distance (m) north or south of the Cage site. Note that the farm itself extends north of the “Cage” site by ~300m. The physical location of the “Cage” site differed between trips, as we sampled at the most heavily stocked cage, wherever that was within the farm.

The transects comprised single water samples taken subsurface at each station throughout Conn Creek, with the purpose of establishing a longitudinal pattern in nutrients throughout the creek to see if there was any repeatable evidence of enrichment adjacent to the cages. We did not replicate samples at each station other than to collect analytical duplicates from each water sample because of the difficulty in getting a true replicate, especially in spring tides, and because of the additional cost both in time and for analysis.

For all variables measured there was no indication of any difference in concentration along the transect and no evidence of enrichment within the farm approval area (dissolved nitrogen species Fig. 25, dissolved phosphorus species Fig. 26, particulate nutrients Fig. 27 and suspended solids and dissolved silica Fig. 28). There was no consistent difference between samples taken at spring and neap tides. There are, however, strong indications of differences in concentrations between seasons (higher in the wet season) that will be explored statistically in the next section. Silica in particular had the largest seasonal difference (Fig. 28).

As was the case for the nutrients, there was no indication of elevated chlorophyll *a* nor of bacterial abundance at the Cage site (Fig. 29), but both were higher in the wet season. The chl:bacteria ratio has been used elsewhere as an indicator of organic pollution (Harrison et al. 2005). In Conn Creek the chl:bacteria ratio is $5.5 * 10^{10}$, which is significantly below the global average of $7 * 10^{12}$ (Li et al. 2004). This indicates that it is unlikely that the farm is adding sufficient organic material to the water column of Conn Creek to trigger this indicator of pollution.

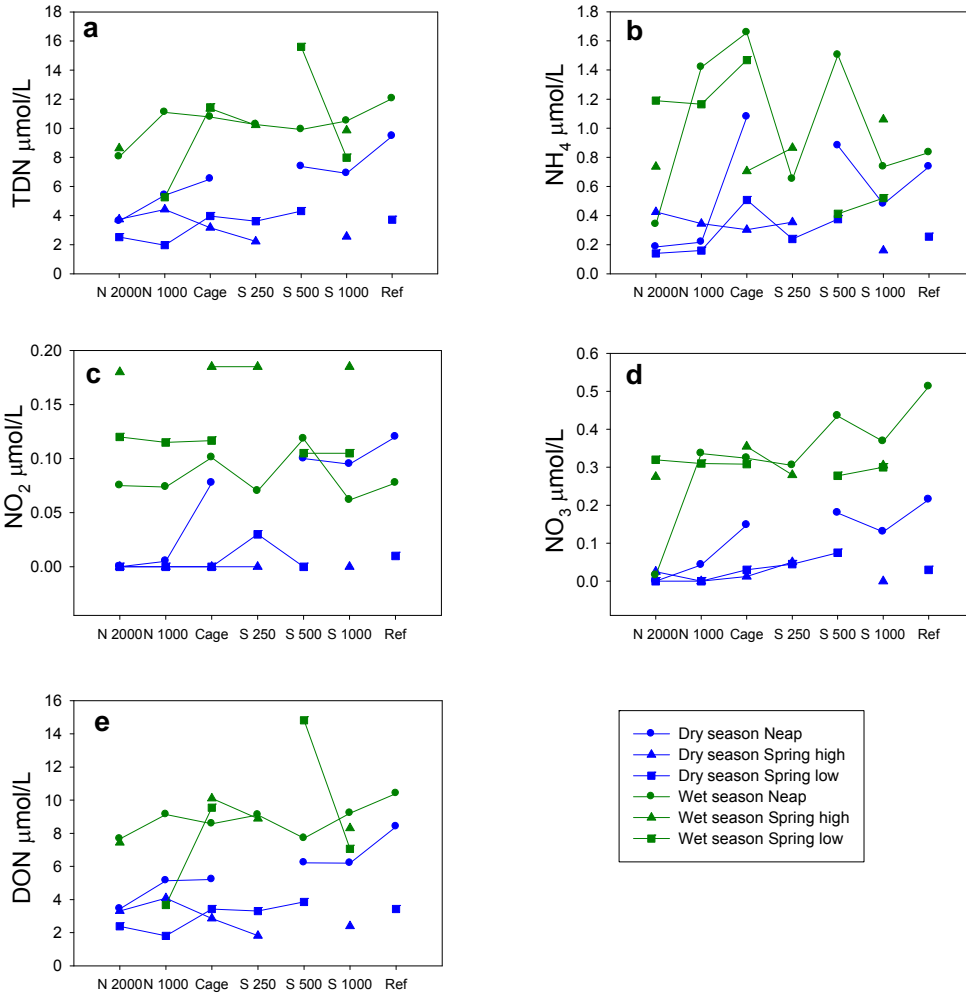


Figure 25: Variation in dissolved nitrogen species along the transects; (a) total dissolved N (TDN); (b) ammonium (NH_4); (c) nitrite (NO_2); (d) nitrate (NO_3); (e) dissolved organic nitrogen (DON).

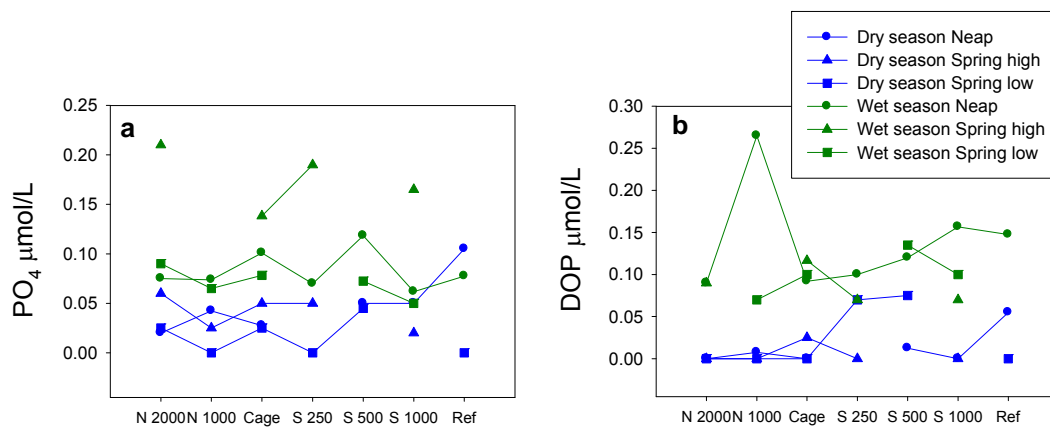


Figure 26: Variation in dissolved phosphorus species along the transects; (a) phosphate (PO_4); (b) dissolved organic phosphorus (DOP).

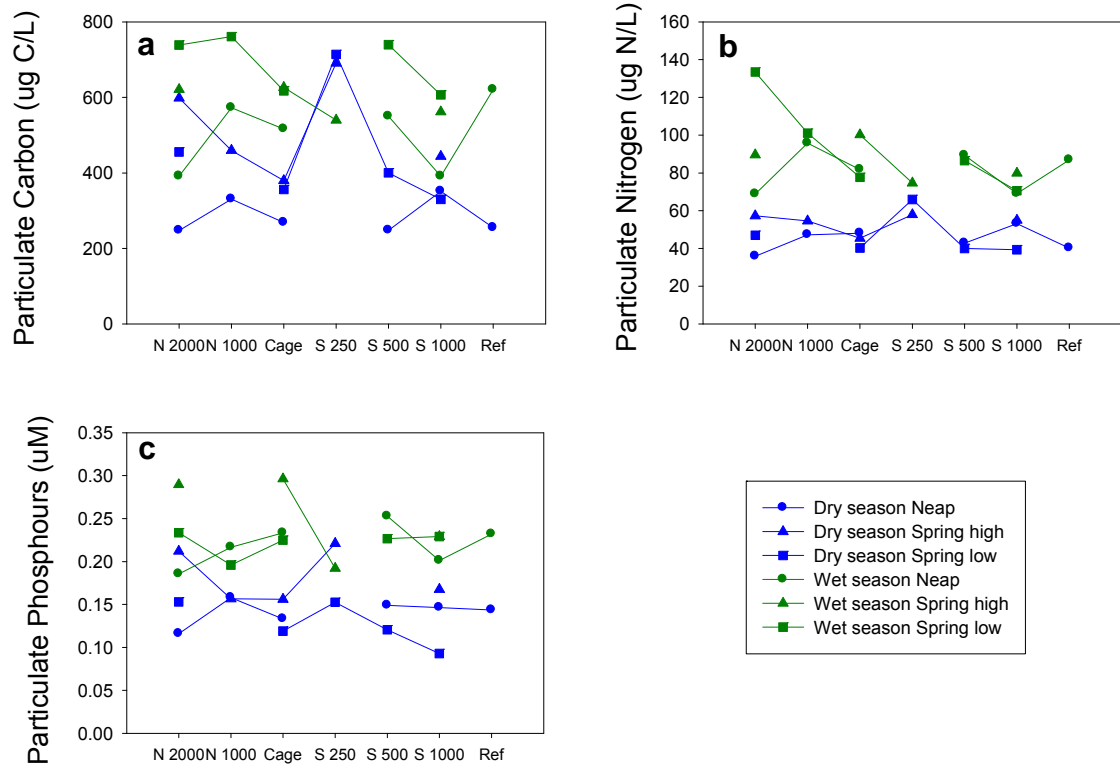


Figure 27: Variation in particulate nutrients along the transects; (a) carbon (PC); (b) nitrogen (PN); (c) phosphorus (PP).

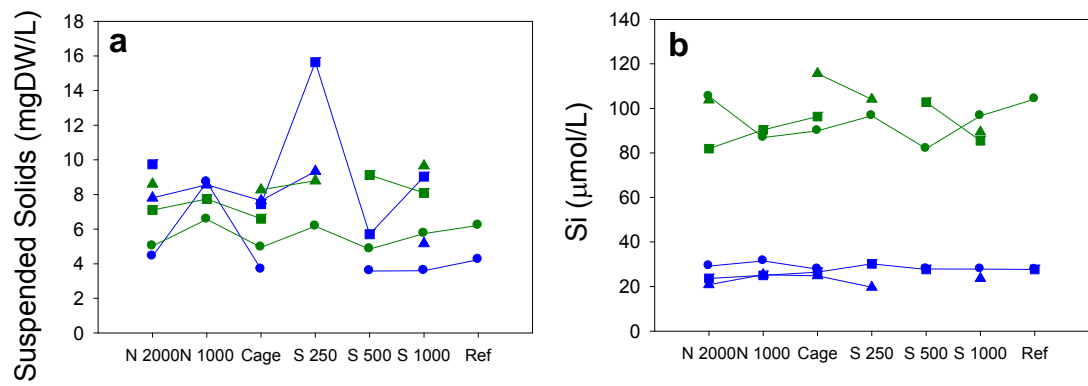


Figure 28: Variation in (a) suspended solids (SS); (b) dissolved silica (Si) along the transects.

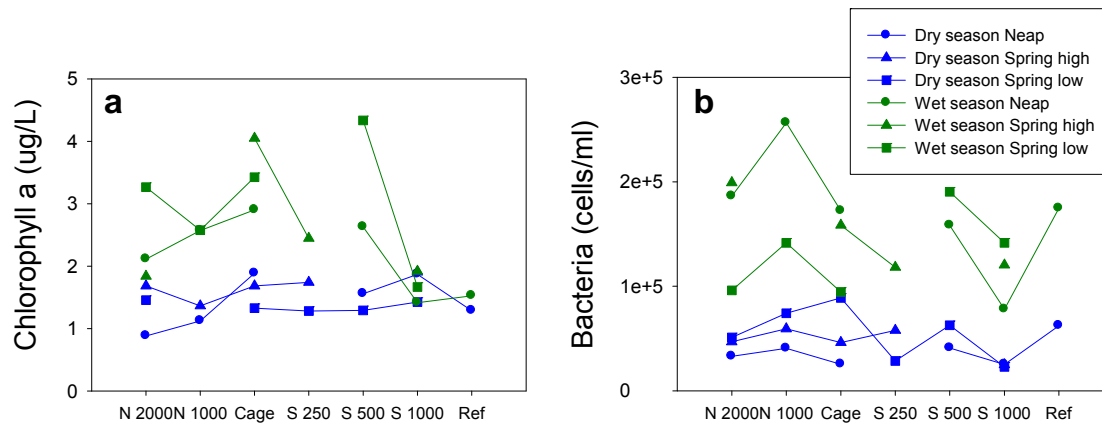


Figure 29: Variation in (a) chlorophyll *a*, (b) heterotrophic bacteria along the transects.

3.2.2 Water quality

To facilitate comparison of water quality at different locations within Conn Creek, we pooled all measurements by location and season to summarise the influence of these main stressors. Despite applying square root transformations to all the data and, where necessary, log transformation to normalise the data, it remained heteroscedastic. Results of statistical analyses should therefore be interpreted conservatively. The Reference site was not included in analyses of variance because of the small number of samples collected. Sample sizes for all comparisons are indicated in Fig. 30.

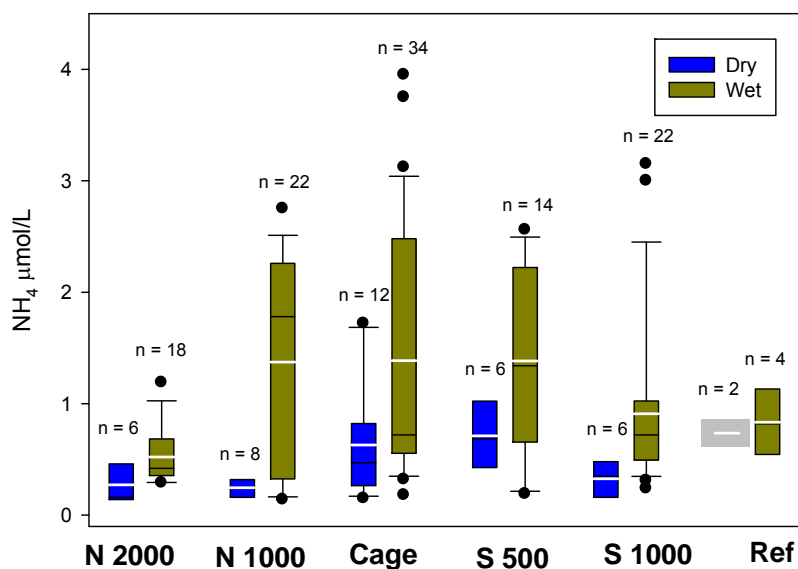


Figure 30: Box plot of ammonium vs location in both wet and dry seasons. The boxes represent the 25th and 75th percentile, the whiskers represent the 10th and 90th percentiles. The black bar represents the median, the white bar the mean. Outliers are indicated by ●. There was a statistically significant difference between seasons and stations (ANOVA, Station: $F_{(5)} = 3.68$, $p = 0.004$; Season: $F_{(1)} = 48.25$, $p < 0.001$).

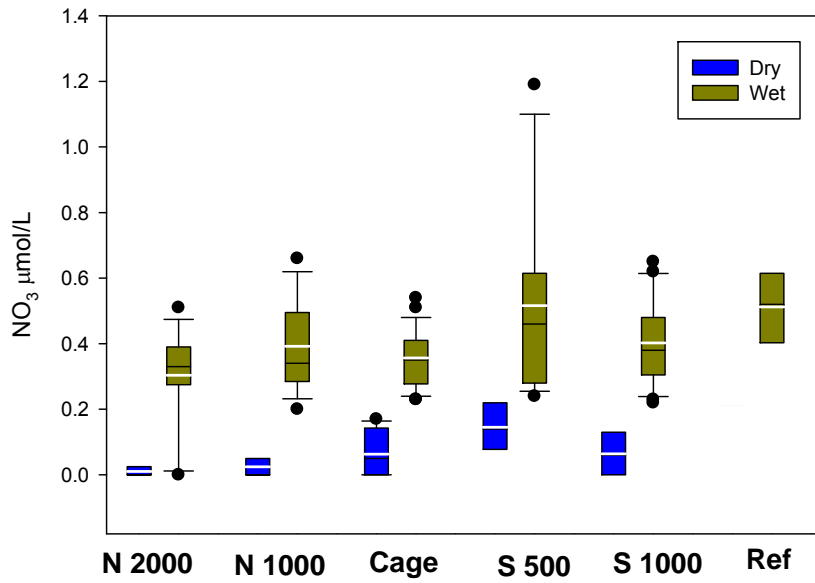


Figure 31: Box plot of nitrate vs location in both wet and dry seasons, as for Fig. 30. There was a statistically significant difference between seasons and stations (ANOVA, Station: $F_{(5)} = 2.49$, $p = 0.033$; Season: $F_{(1)} = 344.89$, $p < 0.001$).

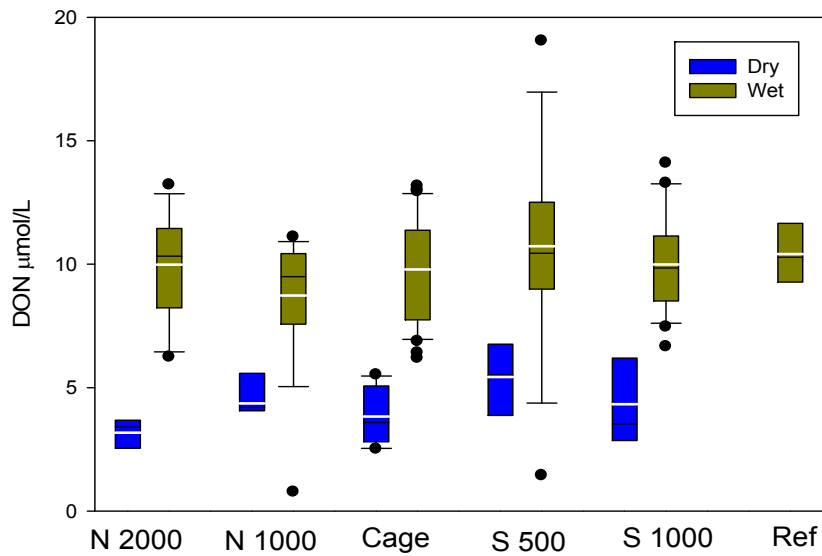


Figure 32: Box plot of dissolved organic nitrogen vs location in both wet and dry seasons, as for Fig. 30. There was a statistically significant difference between seasons as well as the interaction between seasons and stations (ANOVA, Season: $F_{(1)} = 207.78$, $p < 0.001$; Station*Season: $F_{(5)} = 2.53$, $p = 0.03$).

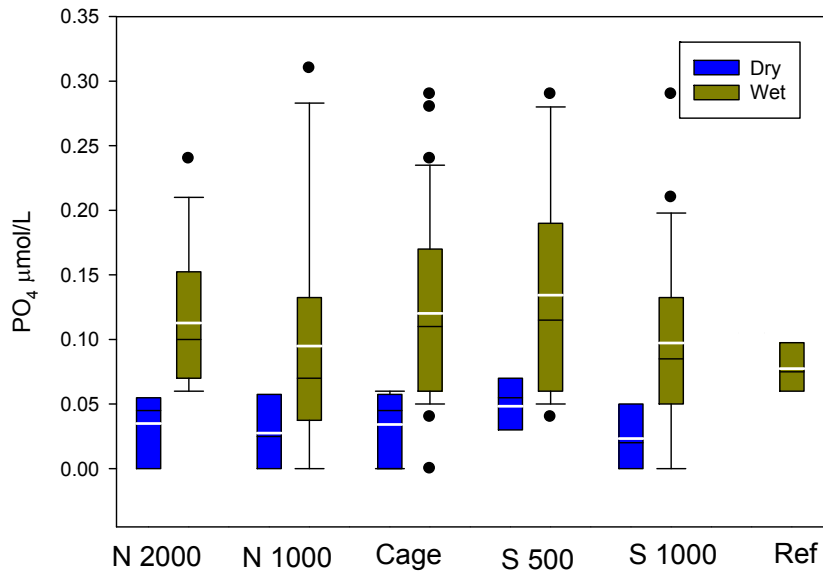


Figure 33: Box plot of dissolved phosphate vs location in both wet and dry seasons, as for Fig 30. There was a statistically significant difference between seasons, but not stations (ANOVA, $F_{(1)} = 28.59$, $p < 0.001$).

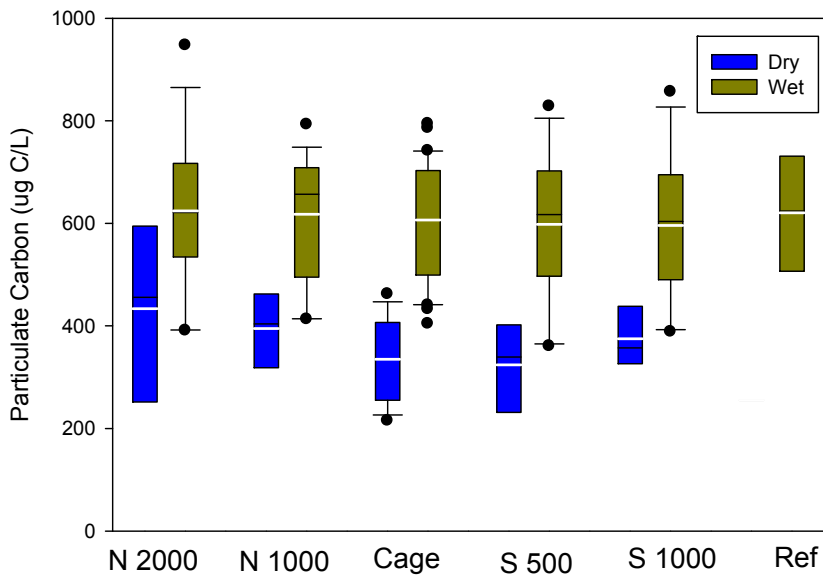


Figure 34: Box plot of particulate carbon vs location in both wet and dry seasons, as for Fig 30. There was a statistically significant difference between seasons and stations as well as the interaction between seasons and stations (ANOVA, Station: $F_{(5)} = 4.55$, $p < 0.001$; Season: $F_{(1)} = 60.43$, $p < 0.001$; Station*Season: $F_{(5)} = 4.78$, $p < 0.001$).

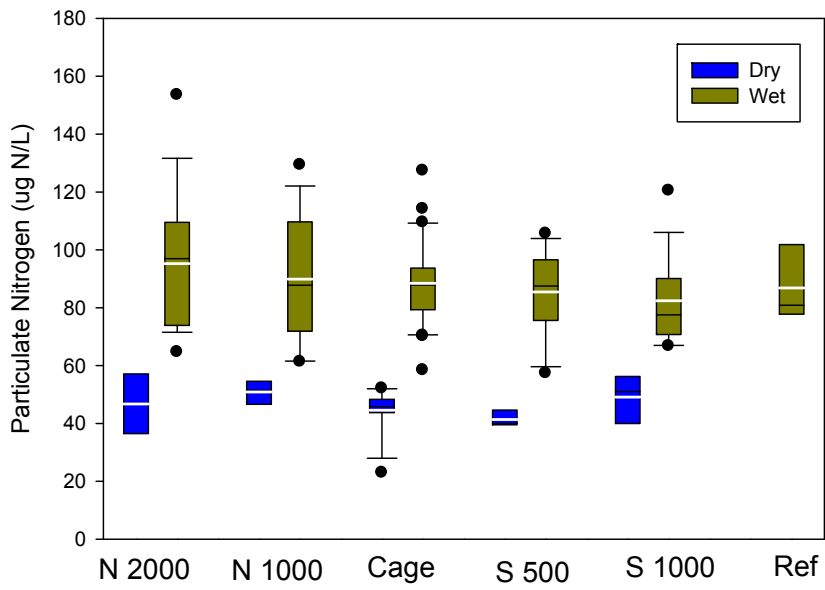


Figure 35: Box plot of particulate nitrogen vs location in both wet and dry seasons, as for Fig 30. There was a statistically significant difference between seasons, but not stations (ANOVA, $F_{(1)} = 173.1$, $p < 0.001$).

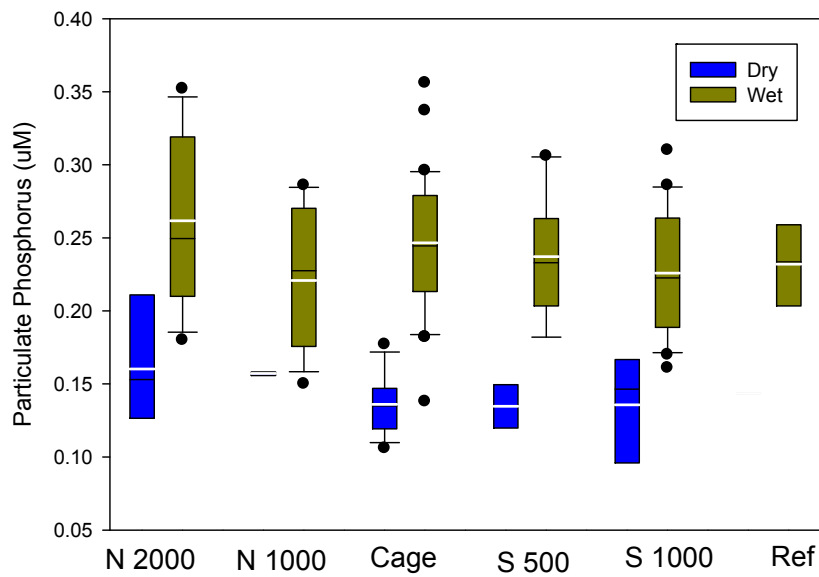


Figure 36: Box plot of particulate phosphorus vs location in both wet and dry seasons, as for Fig 30. There was a statistically significant difference between seasons as well as the interaction between seasons and stations (ANOVA, Season: $F_{(1)} = 94.48$, $p < 0.001$; Station*Season: $F_{(5)} = 2.443$, $p = 0.037$).

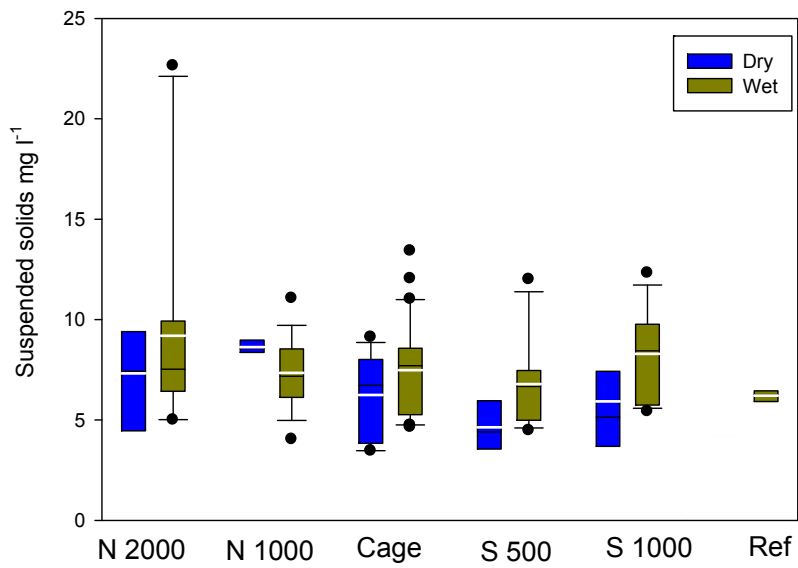


Figure 37: Box plot of suspended solids vs location in both wet and dry seasons, as for Fig 30. There was a statistically significant difference between stations as well as the interaction between seasons and stations (ANOVA, Station: $F_{(5)} = 4.755$, $p < 0.001$; Station*Season: $F_{(5)} = 2.535$, $p = 0.032$. Data was square root transformed.

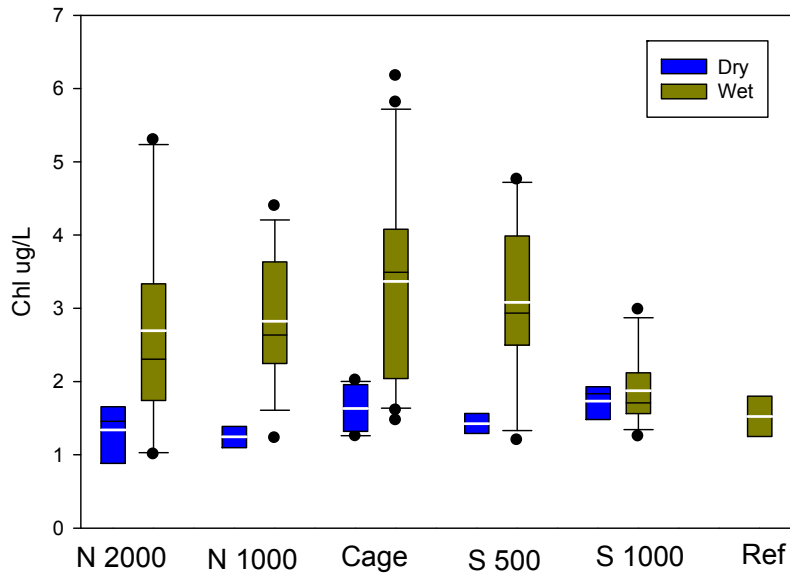


Figure 38: Box plot of chlorophyll *a* vs location in both wet and dry seasons, as for Fig 30. There was a statistically significant difference between seasons as well as the interaction between seasons and stations (ANOVA, Season: $F_{(1)} = 64.49$, $p < 0.001$; Station*Season: $F_{(5)} = 2.53$, $p = 0.03$).

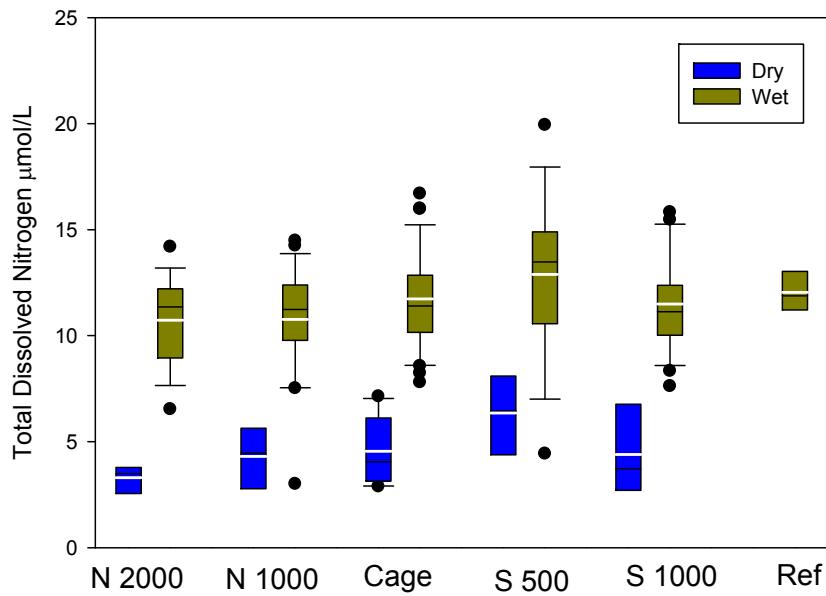


Figure 39: Box plot of total dissolved nitrogen vs location in both wet and dry seasons, as for Fig. 30. There was a statistically significant difference between seasons and stations ANOVA, Station: $F_{(5)} = 3.47$, $p = 0.005$; Season: $F_{(1)} = 370.423$, $p < 0.001$).

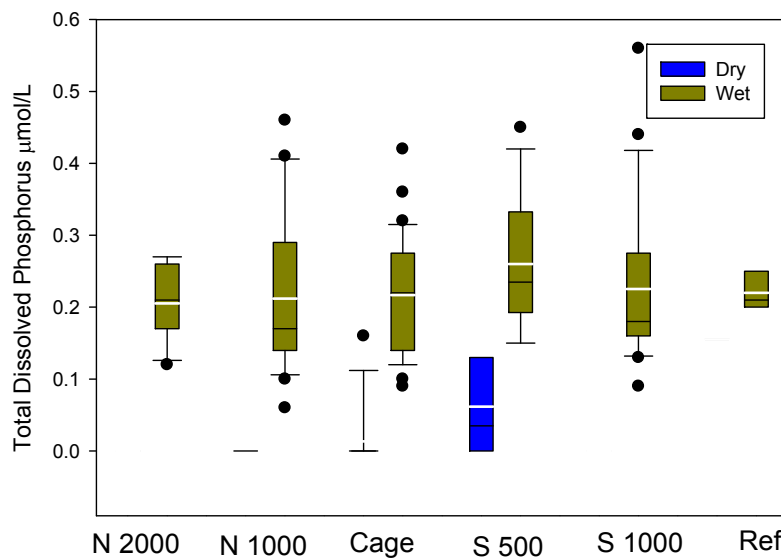


Figure 40: Box plot of total dissolved phosphorus vs location in both wet and dry seasons, as for Fig. 30. There was a statistically significant difference between seasons, but not stations (ANOVA, $F_{(1)} = 102.091$, $p < 0.001$).

During the wet season, the water column was stratified with respect to salinity (see Fig. 14). Differences in the concentration of key nutrients between samples taken near surface and near bottom were minor (Table 4).

Table 4: Top and bottom nutrient concentrations (μM) averaged over all stations for each wet season trip (\pm standard deviation). S = spring tide; N = neap tide; B = bottom; T = top.

Trip	Tide	Depth	NH_4^+	NO_3^-	PO_4^{3-}	PC	PN	PP	
4	S	H	B	0.53 (0.14)	0.44 (0.04)	0.12 (0.03)	100.05 (13.67)	4.62 (0.38)	0.24 (0.05)
4	S	H	T	0.38 (0.11)	0.38 (0.08)	0.13 (0.02)	103.97 (13.32)	5.40 (1.09)	0.25 (0.07)
4	S	L	B	0.46 (0.21)	0.35 (0.06)	0.06 (0.02)	120.81 (16.04)	4.97 (0.76)	0.23 (0.02)
4	S	L	T	0.89 (1.05)	0.32 (0.05)	0.06 (0.04)	127.22 (14.61)	5.47 (0.52)	0.30 (0.03)
5	N		B	2.49 (0.43)	0.63 (0.18)	0.27 (0.03)	76.76 (17.67)	4.54 (1.14)	0.19 (0.02)
5	N		T	1.69 (0.36)	0.59 (0.14)	0.13 (0.06)	104.94 (6.67)	6.06 (0.85)	0.24 (0.05)

Commentary: water quality

The water column nutrient results demonstrate that the major factor determining the concentration of water quality variables within Conn Creek is the season – all 11 water quality variables tested had significantly higher values during the wet season. Only five water quality variables showed differences between locations. Of these, three are dissolved species of nitrogen (NH_4 , NO_3 and Total Dissolved Nitrogen - TDN), and indicate local enrichment around the farm approval area. Two other variables showed spatial differences (Particulate Carbon - PC and Suspended Solids - SS), both with a significant interaction term. During the dry season, these variables were higher at the northern end of the transect, probably reflecting the import of particulate material from Hinchinbrook Channel independent of the operation of the Bluewater Barramundi farm.

The mean values we obtained during this study are slightly lower than the same variables measured in the previous monitoring survey (as reported in section B2 of the Interim Report - note that values reported therein were in mg l^{-1}) (Table 5).

Table 5: Mean (\pm standard deviation) of selected water quality variables for comparison with earlier Bluewater Barramundi monitoring. TDN, Total Dissolved Nitrogen; TDP, Total Dissolved Phosphorus.

Variable	Earlier Monitoring	This study (07/08) Dry Season	This study (07/08) Wet Season
TDN $\mu\text{g l}^{-1}$	70 – 500	69.06 (30.91)	157.83 (32.05)
TDP $\mu\text{g l}^{-1}$	10 – 80	0.89 (1.45)	7.39 (2.81)
Chlorophyll <i>a</i> $\mu\text{g l}^{-1}$	2 – 5	1.52 (0.31)	2.84 (0.97)
Suspended solids mg l^{-1}	5 - 69	7.00 (3.20)	7.85 (2.84)

In general, water quality at the cage sites (taking what might be expected to be the most impacted area) are within the guideline values for Wet Tropics waters given in the Queensland Water Quality Guidelines (EPA 2006).

During the wet season, chlorophyll *a*, organic nitrogen and total nitrogen concentrations exceeded the guideline values (bold numbers in Table 6). In the case of chlorophyll *a*, the mean dry season value is still well within the range of values for mangrove systems in Australia (0.7-15.7 $\mu\text{g l}^{-1}$; see Table B3 of Interim Report). Oxygen saturation was half that recommended as a trigger value in both seasons, but is nonetheless typical of the values recorded in undisturbed northern Australian mangrove waterways (Boto and Bunt 1981).

Table 6: Water quality variables from the cage sites compared to trigger values for enclosed coastal waters given in Table 2.5.3.1 of the Queensland Water Quality Guidelines (EPA 2006). Data are mean \pm standard deviation. Bold numbers indicate data outside the guideline values.

Indicator	QWQG trigger	Dry season	Wet season
Ammonia N $\mu\text{g l}^{-1}$	15	4.48 (3.64)	9.73 (7.84)
Oxidised N* $\mu\text{g l}^{-1}$	10	1.25 (1.45)	7.75 (2.50)
Organic N** $\mu\text{g l}^{-1}$	135	53.6 (15.48)	137.00 (28.83)
Total Nitrogen [^] $\mu\text{g l}^{-1}$	160	108.3 (24.49)	250.97 (32.11)
Filterable Reactive P+ $\mu\text{g l}^{-1}$	5	1.06 (0.81)	3.73 (2.21)
Total P++ $\mu\text{g l}^{-1}$	20	4.63 (1.42)	14.50 (2.53)
Chlorophyll a $\mu\text{g l}^{-1}$	2.0	1.63 (0.30)	3.36 (1.34)
Dissolved Oxygen % saturation	85-100	41.22 (12.82)	50.20 (6.71)
Turb NTU	10	4.22 (1.64)	6.63 (2.86)
Secchi m	1.0	2.68 (0.87)	1.3 (0.36)
Suspended solids mg l^{-1}	nd	6.25 (2.08)	7.48 (2.28)
pH	7.5-8.4	7.38 (0.10)	7.24 (0.25)

* Oxidised N = $\text{NO}_2 + \text{NO}_3$ ($\mu\text{g L}^{-1}$)

** Organic N = Total Organic Nitrogen

[^] Total Nitrogen = Total Dissolved Nitrogen + Particulate Nitrogen ($\mu\text{g L}^{-1}$)

+ Filterable Reactive P = PO_4^{3-} ($\mu\text{g L}^{-1}$)

++ Total P = Total Dissolved Phosphorus + Particulate Phosphorus ($\mu\text{g L}^{-1}$)

Using the sum of the inorganic nitrogen species to calculate nitrogen availability (i.e. assuming that most Dissolved Organic Nitrogen (DON) is not available to primary producers), our data indicates that the dissolved N:P ratio is 17.6 (= $0.635\mu\text{M} / 0.036\mu\text{M}$) during the dry season and 15.9 (= $1.688\mu\text{M} / 0.106\mu\text{M}$) during the wet season. These ratios approximate the Redfield N:P ratio of 16:1 (see Appendix II), indicating that phytoplankton within the Conn Creek system are probably not limited by N or P.

3.2.3 Sediment nutrient fluxes

Solid wastes from sea cage farming in the form of uneaten food and faeces can accumulate in the sediments underneath and adjacent to cages. This solid material usually has higher concentrations of N, P, OC, trace elements (e.g. Zn) and other components included in feeds for nutritional advantage. Deep water and strong currents help to disperse these wastes and minimise localised effects of accumulation, however, there may be detectable effects on sediment nutrient processes or concentrations of elements if site selection is poor or excess wastes are generated. We studied variables that could detect changes in the sediment due to sea cage farming activities and compared these to reference sites where no such effect would be expected. Initially sites were selected at increasing distance north and south of the cages, however, due to a lack of statistical difference between adjacent sites, in the final analysis individual sites were grouped into "Locations" as to whether they were within 1km north of the cages (North), within 1km south of the cages (South), within the

immediate area of the cages (Cage), or at the reference sites (Reference). See Figure 41 for the locations of the samples sites. We chose sediment oxygen consumption rates (respiration) as an indicator of OC (organic Carbon) input, and ammonium (NH_4^+), oxidised nitrogen ($\text{NO}_2^- + \text{NO}_3^-$) and phosphate (PO_4^{3-}) flux rates as indicators of organic N and P input and breakdown (mineralisation). Sediments were also analysed for grain size, OC, TC (Total Carbon), TN (Total Nitrogen), TP (Total Phosphorus), Zn, Li, Redox and pH in order to determine the “footprint” of the cages.

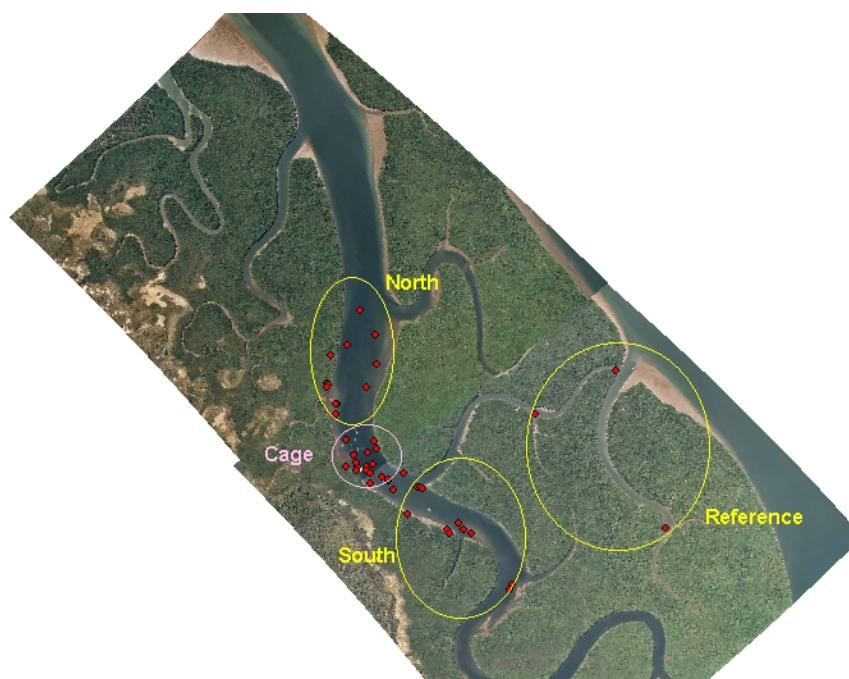


Figure 41: Sediment sample sites, indicated by red dots, and locations used in analyses, indicated with ellipses.

3.2.3.1 Ammonium (NH_4^+) flux

Ammonium (NH_4^+) flux in to the water column was significantly greater ($F=9.2905$, $p=0.000024$) under the cages than other locations in the dry season only ($11,926 \pm 970$ (SD) $\mu\text{mol m}^{-2} \text{day}^{-1}$) (Fig. 42). The mean NH_4^+ flux under the cages in the wet season (neap tide = $2,315 \pm 1,492$ (SD) $\mu\text{mol m}^{-2} \text{day}^{-1}$; spring tide = $2,077 \pm 2,276$ (SD) $\mu\text{mol m}^{-2} \text{day}^{-1}$) was not significantly different to the Reference, North or South locations. Except for under the cages during the dry season, there was no significant difference between neap and spring tide mean NH_4^+ flux rates at any other sites. There was a significant positive correlation between NH_4^+ and PO_4^{3-} flux rates ($r^2=0.654$, $p < 0.05$). The N:P molar ratio of fluxes was approximately 14:1 at the Cage location, compared to an average of 32:1 from the pooled data at non-cage locations (Redfield ratio C:N: = 6.6:1), indicating a greater flux of N from the sediments under the cages. The large dry season spike in NH_4^+ flux at the Cage location was most probably due to a temporary accumulation of farm waste as there was no significant difference between any location in the following wet season.

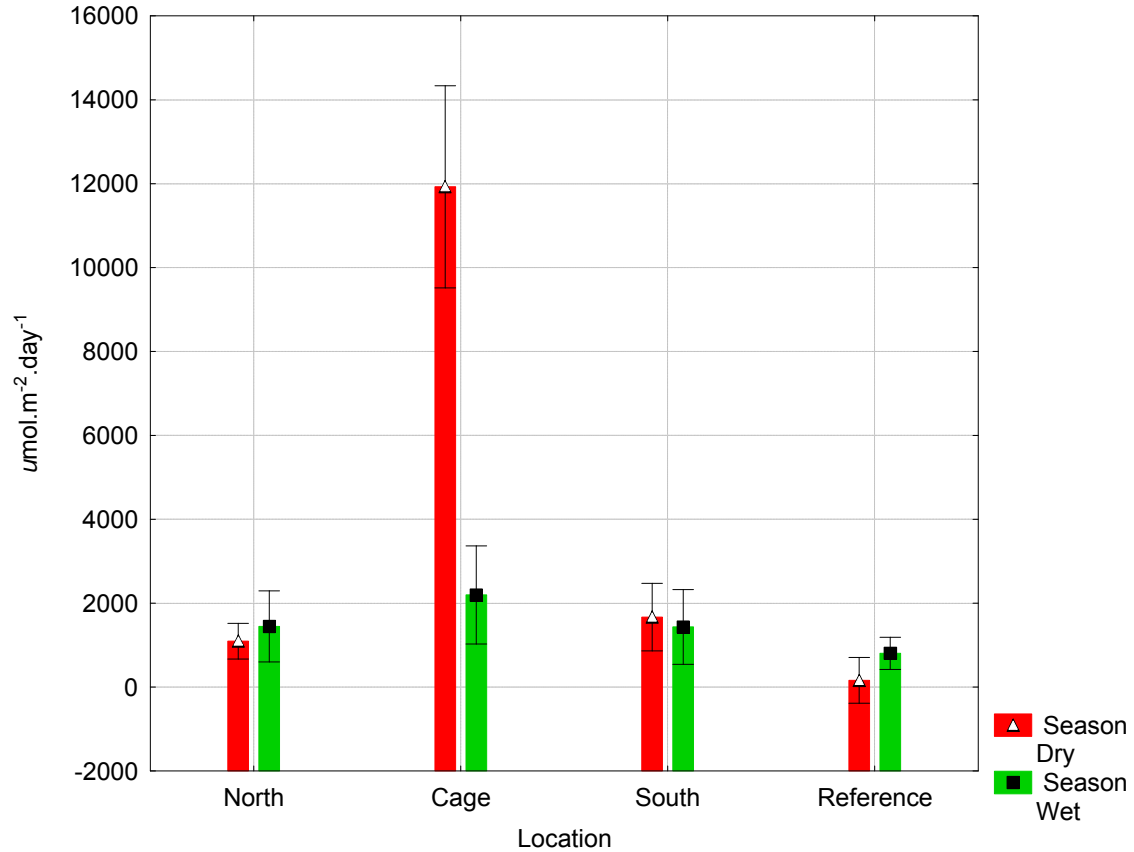


Figure 42: Mean sediment ammonium (NH_4^+) fluxes (and 95% confidence interval) in dry and wet seasons, as a function of location.

3.2.3.2 Phosphate (PO_4^{3-}) flux

Phosphate (PO_4^{3-}) flux rates into the water column (Fig. 43) were also significantly greater ($F=5.8631$, $p=0.001$) under the cages than at the North, South and Reference locations during the dry season only (dry season mean = 51.3 ± 13.4 (SE) $\mu\text{mol m}^{-2} \text{day}^{-1}$; wet season mean = -19.4 ± 13.2 (SE) $\mu\text{mol m}^{-2} \text{day}^{-1}$). Similar to the NH_4^+ fluxes, there was no significant difference between cage and non-cage areas in the wet season.

3.2.3.3 Oxidised Nitrogen ($\text{NO}_2^- + \text{NO}_3^-$) flux

There was no significant difference in mean oxidised nitrogen ($\text{NO}_2^- + \text{NO}_3^-$ [NO_x]) flux rate between season or tide (Fig. 43). Data for pooled (all seasons, all tides) oxidised nitrogen fluxes showed there was a significantly greater negative flux (in to the sediments) under the cages ($F=5.8631$, $p=0.001$). Rates fluctuated between + and - $100 \mu\text{mol m}^{-2} \text{day}^{-1}$ (dry season mean = 18.4 ± 26.1 (SE) $\mu\text{mol m}^{-2} \text{day}^{-1}$; wet season mean = 0.28 ± 25.7 (SE) $\mu\text{mol m}^{-2} \text{day}^{-1}$).

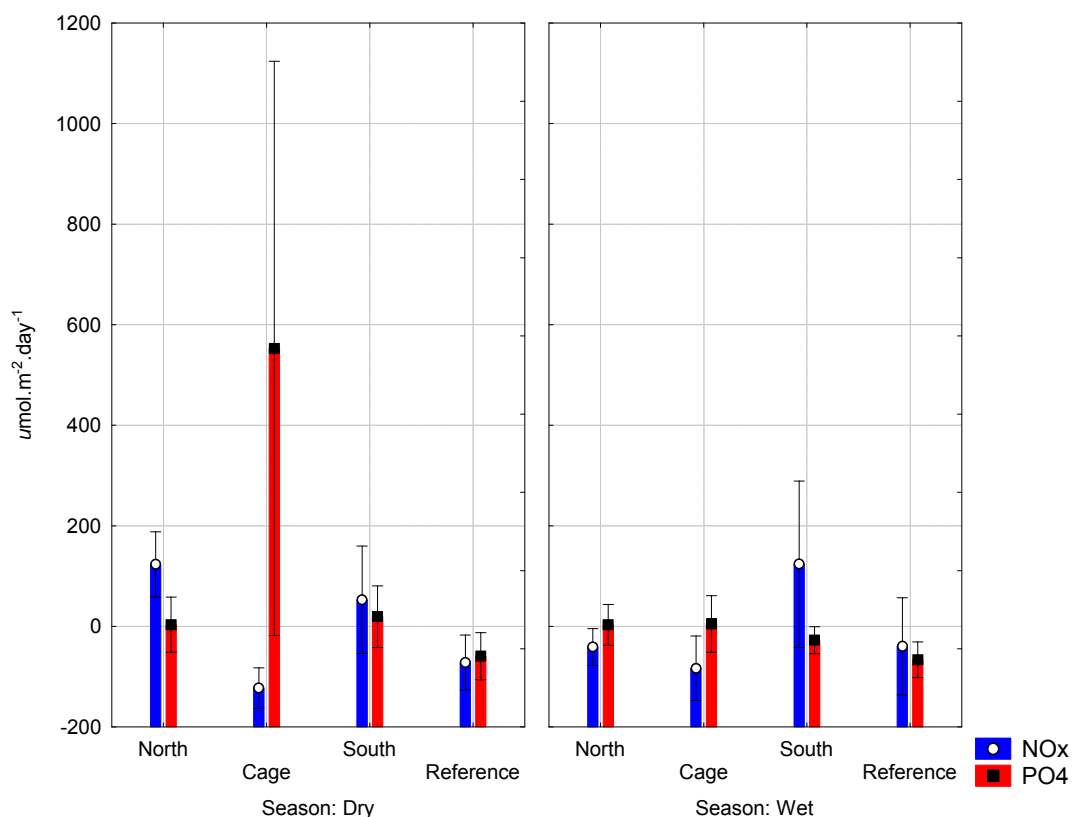


Figure 43: Mean sediment oxidised nitrogen (NO_x) and phosphate (PO_4^{3-}) fluxes in dry and wet seasons (mean and 95% confidence interval).

3.2.3.4 Sediment grain size

Grain size analysis showed that the sediments were predominantly well sorted fine silt (4–31 μm) and clay (0.06–3.9 μm) with a patchy distribution of quartz sand (62.5–500 μm) and coarse material (leaves, wood, rocks and shell) (Folk 1974). There was no major difference in average sediment grain size between the sites, except for the numerous mangrove litter components in some samples at the reference site.

3.2.3.5 Sediment C, N and Total Organic C (TOC)

Total Organic Carbon (%TOC), inorganic %C and %N content were within normal ranges for similar mangrove environments. The highest %TOC and C:N ratios were recorded from the Reference location where there was a large accumulation of C rich mangrove litter: a feature not observed at Conn Creek locations (Table 7). The lowest C:N ratio was at the Cage location, indicating the higher N content compared to C content of the location, possibly due to aquaculture wastes rich in N. For statistical analysis, a single anomalous data point from the Reference location was eliminated. This resulted in there being no significant difference between the C:N ratio, %TOC or %N under the cages compared to adjacent and remote sites, indicating that there was not an accumulation of organic material under the cages.

Table 7: Mean sediment characteristics pooled from all samples from all trips (+/- SD).

Location	%TOC (SD)	%N (SD)	C:N (SD)	Respiration (SD)
Cage	1.22 (0.54)	0.10 (0.05)	13.73 (1.79)	39.05 (14.80)
North	4.13 (4.71)	0.13 (0.09)	26.38 (11.00)	31.26 (14.94)
Reference	9.89 (11.05)	0.21 (0.12)	37.54 (20.03)	32.54 (16.19)
South	5.64 (1.81)	0.20 (0.07)	31.18 (13.04)	34.63 (17.93)

3.2.3.6 Zn:Li and P:Li Ratios in sediments

Yeats et al. (2005) used the ratio of Zn:Li in sediments to trace the footprint of aquaculture activities, as this ratio can be used as a signature of aquaculture feed. We also included P:Li as an additional indicator due to the reported low levels of natural P in sediments from these mangrove locations (Boto and Wellington, 1988) and the fact that P:Li provides a higher signal-to-noise ratio than the Zn:Li ratio (Hargrave 2005). Feed pellets from Bluewater Barramundi farm had a Zn:Li ratio of 44.7 and P:Li ratio of 12,637. The Zn levels recorded in Conn Creek sediments (range 5–65ppm) were within the normal ranges of naturally occurring Zn, where the ranges expected are:

- in mangrove soils (10–400ppm),
- in mangrove trees (2–20ppm), and
- in fish feed (~150ppm).

Using pooled data from all seasons and sampling trips, ratios of Zn:Li and P:Li in sediments from directly under the cages were significantly higher than at other locations (Zn:Li = 0.27 +/- 0.12 (SD), P:Li = 65.83 +/- 30.79 (SD), $p < 0.05$) (Fig. 44). There were no significant differences between the adjacent non-cage locations (North and South) and the Reference location. There was a significant correlation between the Zn:Li and P:Li ratios ($r^2 = 0.86$, $p < 0.05$) indicating that either of these ratios might prove useful as indicators where there is a single source of Zn and P input. Figure 45 shows a contour map displaying where the Zn:Li ratios within Conn Creek are clustered near the farm.

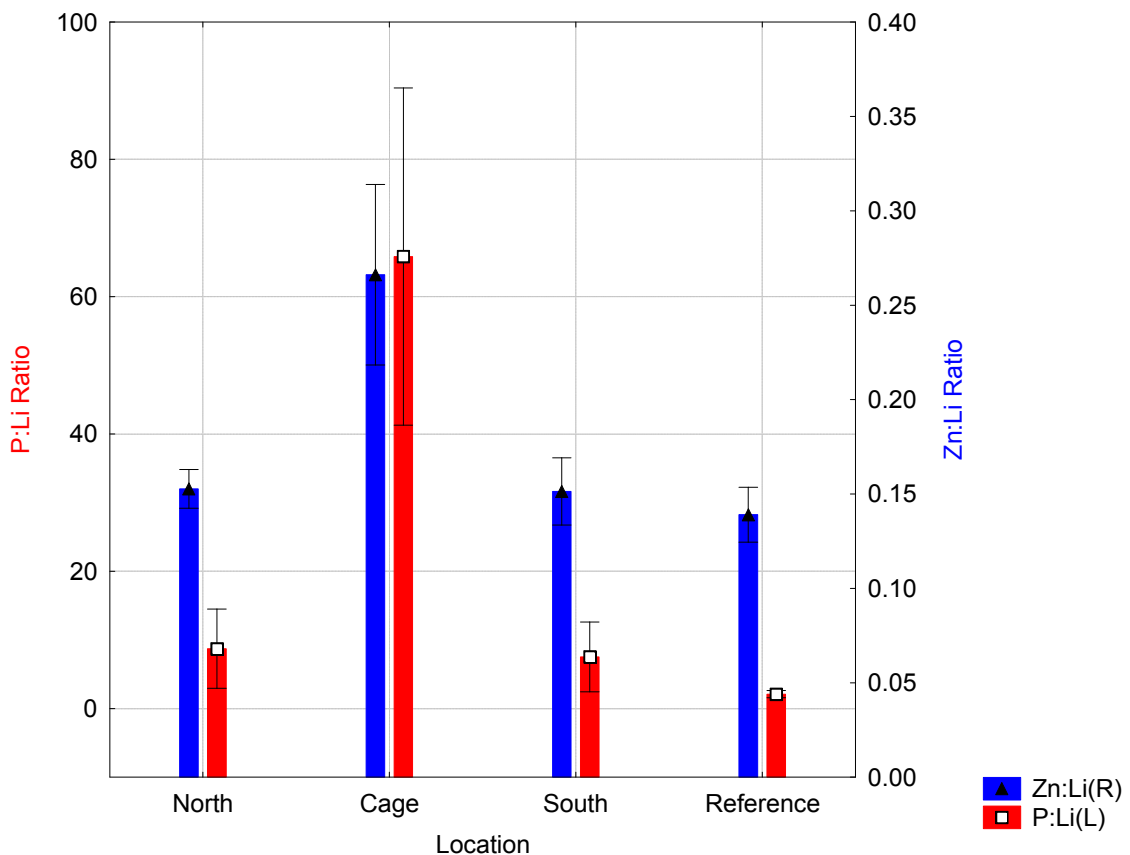


Figure 44: Mean Zn:Li and P:Li ratios in sediments as a function of location using pooled data from all samples (mean and 95% confidence intervals).

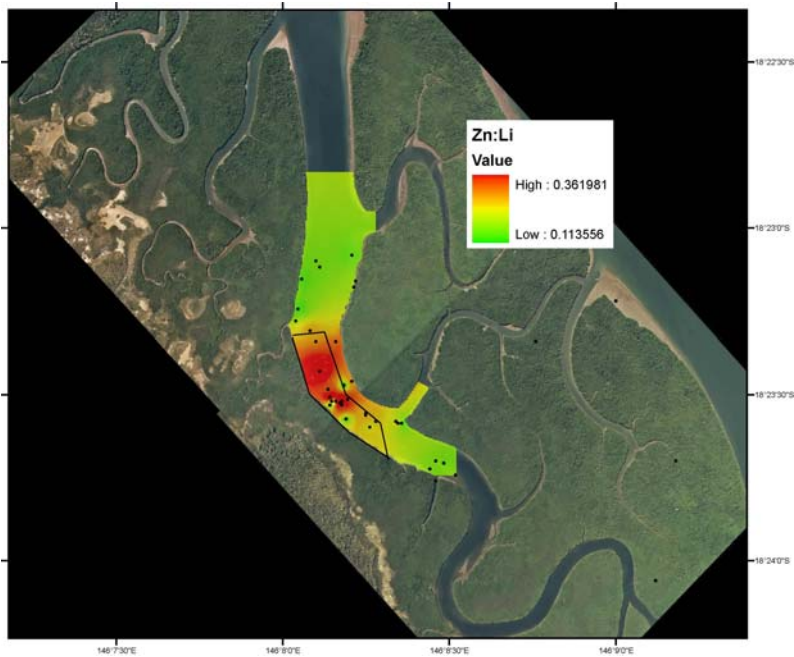


Figure 45: Contour plot of Zn:Li ratio in Conn Creek sediments. Black dots indicate where samples were taken; lined region is the approval area of the farm.

3.2.3.7 Redox potential (Eh) and pH status of sediments

We sampled at the Reference locations as well as along a transect through the cages and sites at adjacent mudbanks for sediment Redox potential (Eh), pH, temperature, and surface dissolved oxygen, temperature and salinity. Sites adjacent to stocked cages and within the farm approval area showed Redox values within the range -200mV to -250mV, and were similar to values obtained at mudbanks adjacent to the farm and to the reference sites. There was some indication that Eh was slightly lower under the cages, but more data are required to confirm this. Dissolved oxygen was depressed immediately downstream and within the cage area (approx 3.5mg l⁻¹) compared to upstream and outside the cage area (approx 5.2mg l⁻¹). The pH of sediments within the cage area was in the range 7.0–7.3, which was higher than some adjacent sites and reference sites.

Commentary: sediments

The highest positive NH₄⁺ and PO₄³⁻ flux rates (i.e. from the sediments to the water column) were observed under the cages in the dry season. These NH₄⁺ flux rates were significantly higher than those observed elsewhere in Hinchinbrook Channel, while the PO₄³⁻ flux rates were at the higher end of the observed range (Alongi et al. 1992). Nutrient flux rates from other mangrove studies in this region were frequently low and variable except where excess organic material was available for mineralisation (Alongi et al, 1992). Significantly elevated positive flux rates of NH₄⁺ and PO₄³⁻ under the cages compared to all other locations in the dry season indicate that excess organic material was being mineralised by bacteria under the cages. These rates were not significantly elevated compared to other locations in the wet season. The farm was holding a similar fish biomass at both the wet and dry season sampling times (~150–180t). The high rates observed under the cages in the dry season were presumably due to farm wastes (excess feed and/or faeces) containing N and P rich material and may have accumulated during the low currents of the neap tide period and were dispersed during the stronger flows of the spring tides. High positive NH₄⁺ and PO₄³⁻ flux rates were not evident during the following wet season sampling trip.

Elevated NH_4^+ and PO_4^{3-} flux rates and consumption of NO_x indicate a disturbed metabolic process within the mud (Holmer et al. 2005) where excess organic matter is being mineralised and denitrification is consuming NO_3^- . This effect was limited to the area underneath or immediately adjacent to the cages.

Zn:Li and P:Li ratios were both significantly elevated under the cages and may be useful to indicate the extent of the footprint of the farm.

Redox and pH results from sediments around the cages and in reference sites indicate variable reducing environments in all locations. Redox values in sediments from under the cages and at reference sites and intertidal mudbanks were similar in range, but more are required to clarify this.

3.2.3.8 ^{15}N in mangrove leaves

Many chemical elements have non radioactive forms, or isotopes, which do not decay over time (hence the term “stable” isotope). These isotopes are distinguishable from other isotopes of the same element using sophisticated and sensitive equipment such as stable isotope mass spectrometers. Stable isotopes are frequently used as tracers in biological systems, and their ability to track changes and processes over time has made them increasingly important to ecological research. For ecologists, stable isotopes provide a natural and safe way to directly trace details of element cycling in the environment and can help identify the food source or contaminant source in biological systems. The ratio of one isotope to another (e.g. the heavier ^{15}N to the lighter, more prevalent ^{14}N) is called the delta value (in this case $\delta^{15}\text{N}$). Variations in the $\delta^{15}\text{N}$ content within the tissues of bacteria, plants and animals can indicate the source and relative uptake of N from the environment. For example, variations in the $\delta^{15}\text{N}$ in coastal flora or fauna can indicate a source of anthropogenic input if the source of excess N in the system is sewage and/or fertilisers that have elevated $\delta^{15}\text{N}$ values. Mangroves take up essential elements via their roots from the environment (e.g. N, P, Fe) to support growth of leaves, propagules and flowers, and also for ongoing metabolism. Mangrove leaves and tissues will eventually reflect the $\delta^{15}\text{N}$ values of the environmentally available N. Aquaculture feeds have elevated $\delta^{15}\text{N}$ and lower $\delta^{13}\text{C}$ values. This elevation in the $\delta^{15}\text{N}$ ratio has previously been traced from a prawn farm to mangroves in a creek system within Hinchinbrook Channel (Costanzo et al. 2004). We analysed leaves from mangroves along the entire length of Conn Creek and at the Reference location, in order to detect if N from aquaculture feeds was being incorporated by the trees, and was detectable as altered $\delta^{15}\text{N}$ values. $\delta^{13}\text{C}$ values were also obtained from the same mangrove leaf analyses and this helps to further discriminate locations and sources of these elements.

Leaves from mature *Rhizophora stylosa* trees in Conn Creek showed only a small increase in the $\delta^{15}\text{N}$ values (+3.2‰) indicating only minor uptake of N with an elevated $\delta^{15}\text{N}$ ratio. Ridley Aquaculture feed pellets from the Bluewater Barramundi farm were found to have the following ratios: $\delta^{15}\text{N} = +9\text{‰}$, $\delta^{13}\text{C} = -21.7\text{‰}$. There was no significant difference between mangrove leaf concentrations of %C, %N, or $\delta^{13}\text{C}$ from any sample site. %N content of leaves from all sites was consistently within the range 0.9–1.0%, indicating that there was no difference in the absolute amounts of leaf N. Reference location leaves had significantly lower ($F=4.738$, $p=0.016$) mean $\delta^{15}\text{N}$ (mean = +1.38‰ +/- 0.29 SE) than the Cage (mean = +2.65‰ +/- 0.17 SE) location (Table 8). A significant difference ($F=5.847$, $df=5$, $p=0.0048$) was observed between the $\delta^{13}\text{C} : \delta^{15}\text{N}$ ratios from Reference and pooled data from Conn Creek Locations (Fig. 46). Zones of elevated $\delta^{15}\text{N}$ extend throughout the length of Conn Creek for several km upstream and downstream of the farm (Figs. 47 and 48).

Table 8: %C, %N, $\delta^{13}\text{C}\text{‰}$, $\delta^{15}\text{N}\text{‰}$ and $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio in mangrove leaves (Mean and (Standard Deviation)) from all locations.

Location	%C	%N	$\delta^{13}\text{C}\text{‰}$	$\delta^{15}\text{N}\text{‰}$	$\delta^{13}\text{C}/\delta^{15}\text{N}$	N
Cage	44.62 (1.03)	1.03 (0.08)	-29.07 (0.56)	2.65 (0.49)	-11.30 (2.10)	5
North	43.08 (2.27)	1.01 (0.09)	-29.00 (0.42)	2.49 (0.18)	-11.70 (0.73)	6
Reference	45.69 (0.98)	1.01 (0.02)	-28.19 (0.80)	1.38 (0.44)	-21.37 (6.25)	2
South	44.74 (1.14)	0.99 (0.04)	-28.57 (0.66)	2.35 (0.47)	-12.51 (2.20)	6

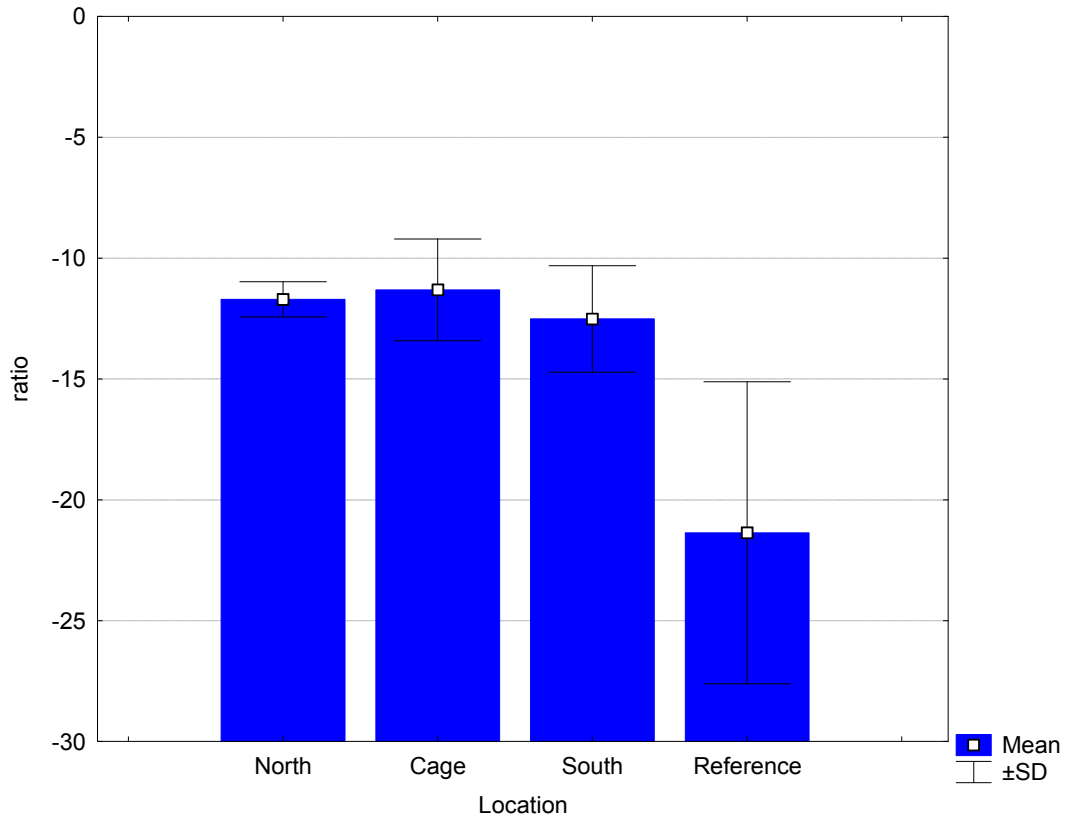


Figure 46: $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio in *Rhizophora stylosa* leaves from all locations (mean \pm SD).

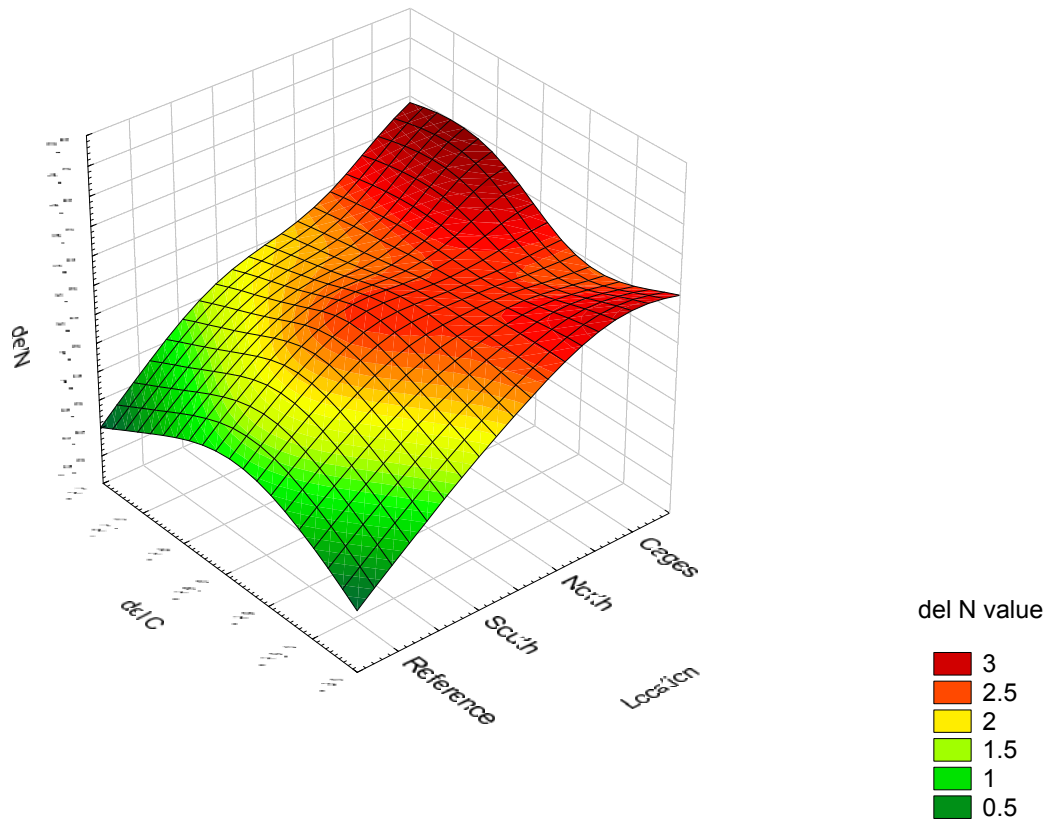


Figure 47: $\delta^{15}\text{N}$ vs $\delta^{13}\text{C}$ values in *Rhizophora stylosa* leaves from all locations.

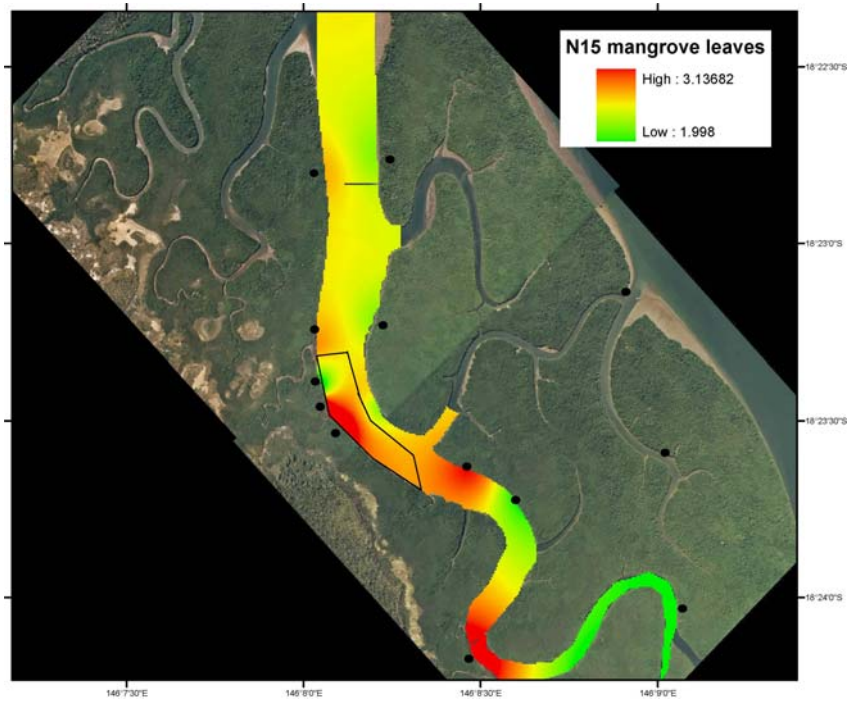


Figure 48: Contour plot of $\delta^{15}\text{N}$ in *Rhizophora stylosa* leaves in Conn Creek. Black dots indicate where sample were taken; lined region is the approval area of the farm.

Commentary: stable isotope signatures in mangrove leaves

The characteristic ^{15}N signature of waste from aquaculture feed ($\delta^{15}\text{N} = +9\text{‰}$, $\delta^{13}\text{C} = -21.7\text{‰}$) can be detected in mangroves on the creek bank along the entire length of Conn Creek (Fig. 48). Given the long period of time that a farm has been established in Conn Creek, this result is quite plausible. We did not analyse leaves from trees located further inland than the intertidal creek bank, but in future it would be useful to obtain $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratios from trees that are close to Highest Astronomical Tide (HAT) in order to clarify the source of the altered ratios.

The low $\delta^{15}\text{N}$ values observed here ($+3.2\text{‰}$) are within the values observed in mangrove leaves from a similar nearby tidal creek ($\delta^{15}\text{N}$ range = $+2.2$ to $+7.3\text{‰}$, Costanzo et al. 2004) receiving prawn farm effluent with a $\delta^{15}\text{N}$ content of $+6\text{‰}$ (Preston et al. 2000). This might indicate that there is less input of high $\delta^{15}\text{N}$ material from the Conn Creek fish cages and/or that mixing and dilution of tidal water was greater in Conn Creek. We consider that high $\delta^{15}\text{N}$ in the feed pellets added to the fish cages is the most likely source for these slightly elevated values in Conn Creek, as there are no other known or potential sources. There are, however, several other unquantified factors reported from the literature that could be contributing to the small difference in $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratios observed here. Small differences in $\delta^{15}\text{N}$ (-5‰ to $+2\text{‰}$) can be attributed to different soil conditions, growth and proximity to ocean input (Fry et al. 2000), groundwater nitrogen input (Page, 1995), bacterial partitioning of $\delta^{15}\text{N}$ by nitrification and denitrification (Nadelhoffer and Fry, 1994) and preferential N uptake by trees (Fry et al. 2000). Differences in the supply of direct groundwater input between the Conn Creek catchment and the Reference location may also be a factor in the observed difference. The $\delta^{15}\text{N}$ data should be interpreted with caution and a wider distribution of sites should be included if this indicator is to be used to detect the footprint of the farm. To further confirm that the farm is the source of the elevated $\delta^{15}\text{N}$, it may be useful to employ a $\delta^{15}\text{N}$ indicator that responds in a shorter time period (e.g. 3–7 days) and can be located directly adjacent to the cages, such as the caged algae used by Costanzo et al. (2004). This would help to indicate a more precise source of the high $\delta^{15}\text{N}$, and could be used in conjunction with the mangrove data that reflects integration of N uptake over a time span of years.

In summary, the sediment nutrient fluxes, sediment chemistry and stable isotope studies have allowed us to estimate the footprint of the farm in several ways.

1. Sediment chemistry – the ratios of Zn, a micronutrient added to aquaculture feeds, to Li, an inert element, indicates that the footprint of the farm is confined to the approval area.
2. Leaves of the dominant mangrove tree (*Rhizophora stylosa*) along Conn Creek have a higher concentration of ^{15}N that is enhanced in aquaculture feeds, indicating that N is being dispersed by tidal mixing throughout Conn Creek and is being assimilated by the mangrove forests.
3. Nutrient fluxes from the sediment (ammonium and dissolved phosphate), were greater directly under the cages during the dry season, but not during the wet season. Uptake of oxidised nitrogen by the sediments was greater under the cages in the dry season, but not during the wet season.
4. Sediment respiration (oxygen consumption) in benthic samples from underneath stocked cages was not significantly higher than adjacent sites or Reference locations.
5. Other commonly used indices of the footprint of sea cage farms, such as sediment Redox values, sediment grain size and organic content, have not proven useful due to the large natural variation observed in mangrove waterways.

3.3 Carrying capacity

To estimate carrying capacity, we conducted experiments to measure primary production by phytoplankton and we calculated mangrove primary production on the basis of forest area and literature values of litter production and growth rate. Using these results we then estimated the nutrient demand necessary to sustain the estimated level of primary production within Conn Creek. From our calculations of nutrient losses from the farm, we can then place farm effluents in overall ecosystem perspective.

We have also applied two carrying capacity models (for grouper aquaculture in SE Asia) incorporated in CADS_TOOL, a decision support system developed under our parallel ACIAR project, and available at: <http://www.aims.gov.au/docs/research/sustainable-use/tropical-aquaculture/cads-tool.html>

3.3.1 Water column primary production and respiration

We undertook spatial sampling on the neap tides, since water movement would have least influence on the spatial distribution of water column variables. Production: Respiration (P:R) experiments were conducted at the Reference Site, at S1000, at the cage identified by the farm manager as being the most heavily stocked and at N1000 (Fig. 49).

On the spring tides we sampled according to tidal state, taking measurements at the most heavily stocked cage at both high and low tides and at a station upstream of the farm; the physical location of which was south of the farm on the ebb tide and north of the farm on the flood tide. In this way, we had pairs of stations under assumed maximum influence of the farm and little influenced by the farm. The upstream station might conceivably have some influence of the farm but only from the last tidal cycle. Given the degree of mixing within Conn Creek, it is unlikely to have been significantly affected. The water column structure at the P:R experiment stations is discussed in Section 3.1.1.



Figure 49: Locations of P:R experiments.

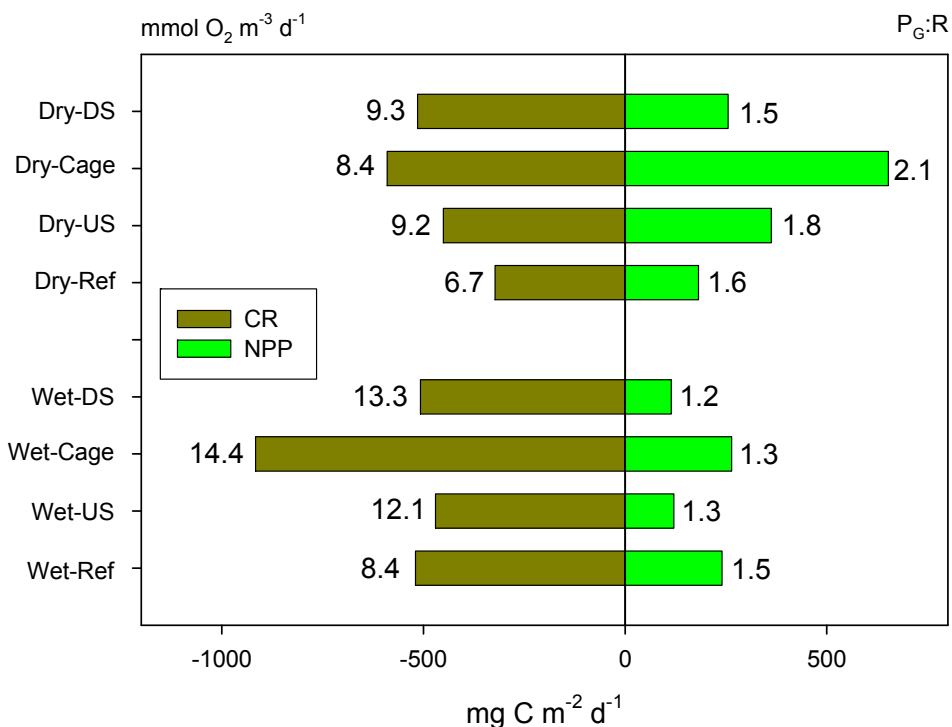


Figure 50: Spatial comparison of water column production and respiration (neap tides). Bars to the left of the origin indicate rates of Community Respiration (CR), bars to the right the rates of Net Primary Production (NPP), expressed in units of carbon per square metre of water area ($\text{mg C m}^{-2} \text{d}^{-1}$). Gross Primary Production (P_G) is the sum of CR and NPP. The ratio of $P_G:R$ is referred to as the P:R ratio and is indicated by the number to the right of the bars. The mean water column respiration rate per unit volume is indicated by the number to the left of the bar ($\text{mmol O}_2 \text{m}^{-3} \text{d}^{-1}$). DS = downstream of cages; US = upstream of cages.

Net Primary Production (NPP), as shown in Fig. 50, represents the net amount of carbon fixed by phytoplankton in a column of water with a surface area of 1m^2 , after respiration by all micro-organisms in the water, including the phytoplankton themselves, is subtracted. However, phytoplankton production only occurs where there is light, and the waters of Conn Creek are very turbid. In our experiments the compensation point (the depth at which production and respiration is equal) was always less than 1.5m , and less than the depth of the euphotic zone predicted from transparency measurements (Table 2). Community Respiration (CR) represents the respiration rate of all microorganisms in the water, as measured in zero light (i.e. in the absence of photosynthesis). Simply put, respiration is the combustion of organic matter to form CO_2 ; production is the assimilation of CO_2 and nutrients to form organic matter. The ratio of $P_G:R$ is referred to as the P:R ratio (see legend to Fig. 50) – when positive the system is autotrophic, when negative the system is heterotrophic. Autotrophic systems assimilate nutrients; heterotrophic systems generate nutrients.

Overall, during neap tides the waters of Conn Creek were moderately productive, fixing an average of 274 ± 173 (SD) $\text{mg C m}^{-2} \text{d}^{-1}$. Net primary production was significantly higher in the dry season (t test, $p < 0.001$) and community respiration was significantly higher in the wet season (t test, $p = 0.022$, Fig. 50). During the dry season there was no difference in production at different stages of the tide. However, in the wet season the combination of spring tides, turbid water and overcast conditions resulted in the waters in Conn Creek becoming heterotrophic at low tide (Fig. 51). Since nutrients are apparently not limiting

(Section 3.2.2), and since the productive layer of water is often less than 1m in depth, light must limit phytoplankton production in Conn Creek. Overall, respiration rates per unit volume were significantly higher (t test, $p < 0.001$) in the wet season than in the dry season, probably because of the combination of higher temperatures and bacterial decomposition of allochthonous material (e.g. organic matter in land based runoff) in the water column as a result of high rainfall. There was no indication of enhanced phytoplankton growth at the cage site, since the rates of oxygen production at the surface of cage sites were not significantly higher than at other sites (t test).

Assuming an overall mean production rate of $21 \text{ mmol C m}^{-2} \text{ d}^{-1}$, and a creek area of 1.56 km^2 , the annual N demand by phytoplankton is 25.4 t N . The farm feeds 365 t of pellets per year (SBMP 2003), equivalent to 28 t N . The estimates of total C and N input to Conn Creek are discussed in Section 3.5.

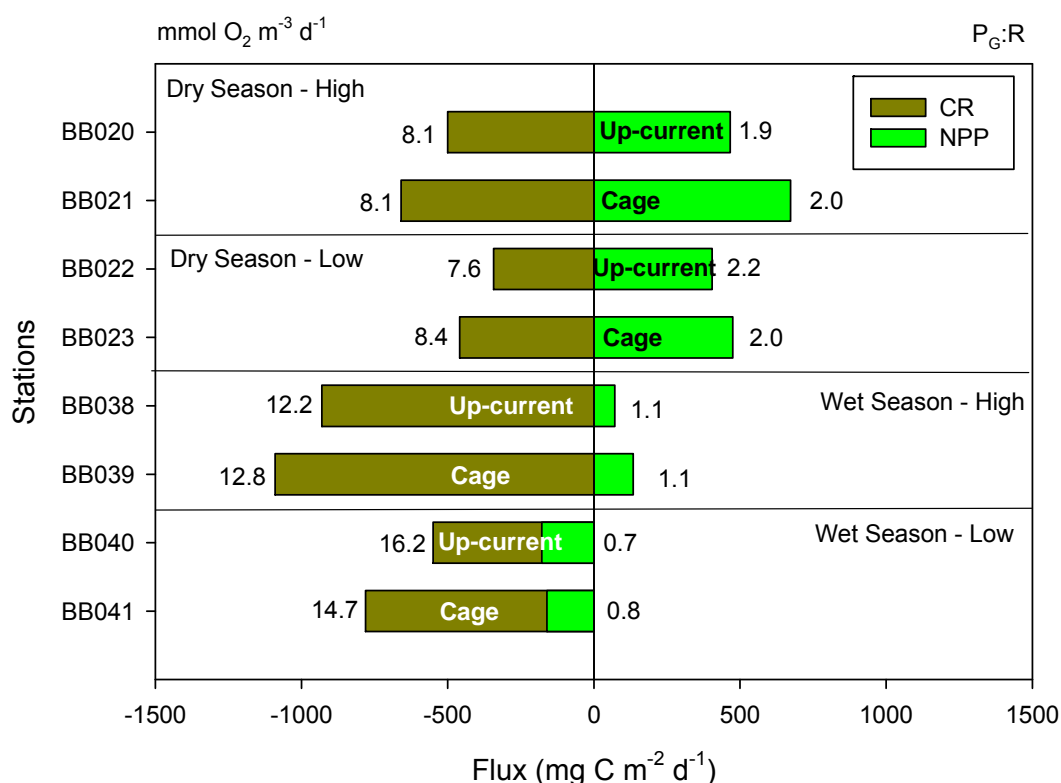


Figure 51: Seasonal and tidal comparison of water column production and respiration (spring tides), as for Figure 50.

3.3.2 Mangrove primary production

We calculated an approximate area of 542 ha of mangrove forest in the Conn Creek catchment. Assuming that most of the forest is mixed *Rhizophora* spp and similar in age to that along Coral Creek, Hinchinbrook Island, the mangrove net primary production would average $14,840 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ for a total of $8,043 \text{ t C yr}^{-1}$. This equates to about 43 t N yr^{-1} and about 6 t P yr^{-1} (using primary production data in Robertson and Alongi 1992 and the C:N:P ratio of whole *Rhizophora* forests in Alongi et al. 2003a). We estimated average litter flow into the creek at approximately $1,800 \text{ t C yr}^{-1}$, 34.6 t N yr^{-1} and 4.3 t P yr^{-1} (C:N:P in litter from Alongi et al. 1992 and litter data from Robertson and Alongi 1992). See Section 3.5 for a more detailed explanation of the total C and N inputs.

3.3.3 Carrying capacity on the basis of an oxygen budget

We applied two models incorporated into the CADS_TOOL decision support system developed under our ACIAR work in Indonesia, and available at the AIMS web site. Our measurements on the farm with both the CTD and the Hydrolabs indicate dissolved oxygen concentrations often fall to $\sim 2\text{mg l}^{-1}$. Consequently, we consider dissolved oxygen is likely to be the main limiting factor for the carrying capacity of the site, especially if concentrations were to fall lower than $\sim 1\text{mg l}^{-1}$ (Rimmer and Russell 1998).

The application of the model of Tookwinas et al. (2004; Fig. 52) predicts a carrying capacity for the site of 280t, using the mean dry season high tide values of oxygen concentration from the CTD records detailed in Section 3.1.1. However, this represents a best case calculation; using the low tide values from the same measurement series results in a carrying capacity of only 135t. We have also applied the maximum current speed observed during spring tides in Trip 1 i.e. 30cm s^{-1} . During the wet season there was no difference in oxygen concentration throughout the creek area, making this model inapplicable.

Application of the model of Hanafi et al. (2006; Fig. 53) predicts somewhat less, 235t, using an overall mean oxygen concentration from CTD records taken at the cage sites (Section 3.1.1). Again, this represents a best case situation. Applying the minimum oxygen concentration observed in the CTD data at the cage site (2.68mg l^{-1}) results in a carrying capacity of only 46t.

The screenshot shows the CADS_TOOL software interface. The 'Holding Capacity' tab is active, and the 'Tookwinas et al' model is selected. The interface displays the following input parameters and their values:

Parameter	Value
Oxygen Inflow [mg/l]	5.3
Oxygen Outflow [mg/l]	4.9
Oxygen Consumption Water Column [mg O ₂ /m ² /h]	2.7
Oxygen Consumption Sediment [mg O ₂ /m ² /h]	52
Fish Oxygen Consumption Rate [g O ₂ /kg fish/h]	0.081
Water Flow [m/s]	0.03
Area [m ²]	40000
Cage Depth [m]	7.5

The resulting **Carrying Capacity** is **279.8963 Tonnes**.

Reference: Tookwinas, S., Songsangjinda, P., Kajonwattanakul, S., Singharachai, C. 2004. Carrying capacity estimation of marine finfish cage culture at Pathew Bay, Chumpon Province Southern Thailand. Southeast Asian Fisheries Development Centre. TD/RES/91 LBCFM-PD No. 34.

Figure 52: CADS_TOOL calculation of carrying capacity based on the model of Tookwinas et al. (2004).

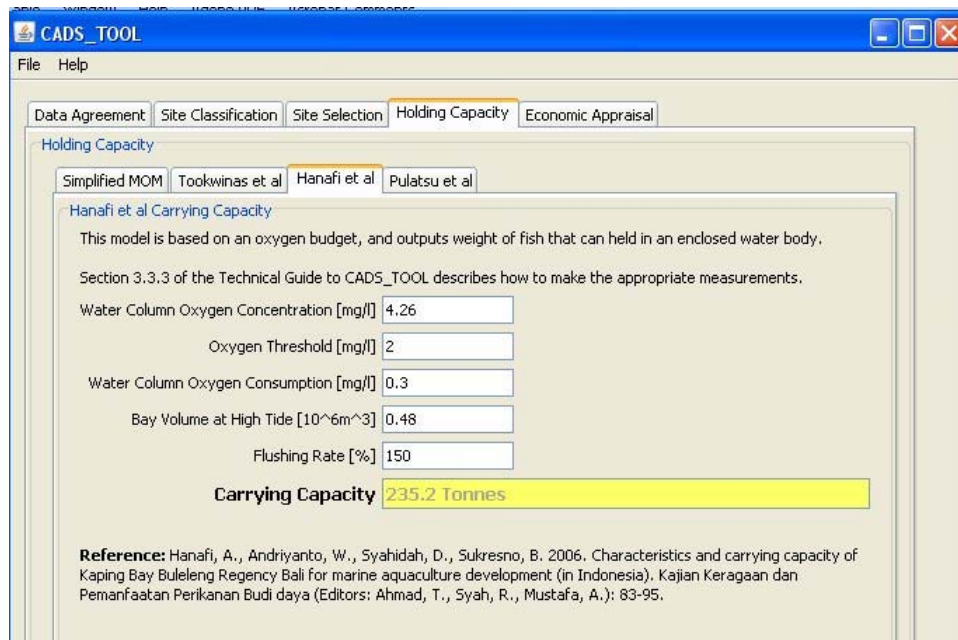


Figure 53: CADs_TOOL calculation of carrying capacity based on the model of Hanafi et al. (2006).

Commentary: carrying capacity

On the basis of our calculations, waste N from the Bluewater Barramundi farm adds significantly to the nutrient status of the system because of the small size of Conn Creek. Our data suggest that phytoplankton are light-limited rather than nutrient-limited. The main demand for N by primary producers within the creek system is by the mangroves (43t) and phytoplankton (25t), compared to the feed input from the farm alone of 28t N. For a more detailed description of the C and N budget within Conn Creek, refer to the Nutrient Budget (Section 3.5 below).

Results from the carrying capacity models that we have applied should be interpreted with caution, since they were primarily developed for grouper culture in SE Asia. There are no documented cases of mortality caused by oxygen depletion at the Bluewater Barramundi farm, probably because the site is very well flushed (see Section 3.1.4). Nevertheless, the estimates of 235–280t seem reasonable, and are consistent with the upper limits of historical production at the site. Note that our estimates of carrying capacity are the amount of fish held at any one time, not the annual production, and that we have used the high tide oxygen data because of the short residence time of Conn Creek and the resilience of barramundi to short periods of low oxygen. There is no published data available on barramundi respiration rates at various temperatures and salinities, or during feeding activity. Recommended minimum dissolved oxygen concentrations from the literature are: $>2 \text{mg l}^{-1}$ for larval rearing, and $>1 \text{mg l}^{-1}$ for grow out (Rimmer and Russell 1998).

In our opinion, the farm is currently operating at or near the maximum carrying capacity for the site based on the assimilative capacity for nutrient uptake within the Conn Creek ecosystem (see Section 3.5 below) and on oxygen budgets. To our knowledge, the only published information on barramundi respiration is that of Glencross and Felsing (2006), which is based on experiments on fish in fresh water saturated with oxygen. Based on their relationship of gross oxygen consumption by barramundi, we calculate a 1kg fish consumes $74 \text{mg O}_2 \text{ h}^{-1}$. Extrapolating this to farm scale, we calculate that 200t of fish consume $14.8 \text{kg O}_2 \text{ h}^{-1}$. Assuming the approval area is $40,000 \text{m}^2$ (Section 2.2) and an average depth within the approval area of 5m (this is conservative – it is probably less), we calculate that the volume

of water within the approval area contains 400kg O₂ when oxygen levels fall to 2 mg l⁻¹, as we have observed to be the case for periods up to 3h (Section 3.1.2). This stock of oxygen would be turned over in only 27h on the basis of fish respiration alone, and less if the water column was heterotrophic as was the case during wet season low tides (Fig. 51). Though this calculation makes no allowance for water renewal as a result of tidal flushing, for lowering of respiration rate in suboptimal conditions, for differences in respiration rate of different sized fish, or for differences in barramundi respiration in salt water, it does lend credence to our assertion that oxygen is a limiting factor for the carrying capacity of Conn Creek. Glencross and Felsing (2006) also note that feeding fish when oxygen is low may force them into oxygen debt, and that this may have long-term effects on fish performance. However, we note the absence of published information on critical oxygen concentrations for barramundi, without which it is impossible to fully understand the implications of the periodically low oxygen concentrations observed in Conn Creek.

3.4 The fate of uneaten food and other wastes

To trace the fate of dissolved wastes such as ammonia, an important excretory product of fish, we conducted experiments to measure the uptake rate of dissolved forms of nitrogen by phytoplankton. Particulate wastes (such as uneaten food pellets and fish faeces) that reach the sediment underneath seacages are rich in organic carbon. To determine the rates of microbial degradation of these materials, we measured respiration rates in the sediment under the farm and compared these to locations nearby. In sea cages elsewhere, wild fish consume significant quantities of waste feeds. Consequently, we made some preliminary observations to find out if this was the case at Bluewater Barramundi farm.

3.4.1 ¹⁵N uptake experiments

To estimate the time it takes for biota to absorb dissolved nutrients such as ammonia, which may be higher around fish farms because of fish excretion, we conducted experiments in which a small amount of ammonia or nitrate labelled with a stable isotope of nitrogen was incubated with natural plankton communities. At the conclusion of the experiment the sample was filtered and the amount of ¹⁵N incorporated into particulate material (phytoplankton) measured with a mass spectrometer.

For these experiments, ¹⁵N-ammonium chloride and potassium nitrate were added to triplicate bottles that were incubated for 1–1.5h at different light levels – 100% natural light, within bags that admitted only 30% of natural light, and in the dark. The goal was to have addition rates in the range of 10% of background nitrate (NO₃⁻) and ammonium (NH₄⁺) (see Appendix I for more detailed description of methods used).

Ammonium (NH₄⁺) was the preferred nutrient in all experiments, as indicated by the lower amounts of nitrate (NO₃⁻) assimilated per unit time (Table 9). This is the expected result, since it is more energetically efficient for phytoplankton to assimilate NH₄⁺ than NO₃⁻, and the ambient concentrations of NH₄⁺ are fairly high. The unexpected result from these experiments was that uptake rates were greater in the shaded and dark experiments than in the full sunlight, which may indicate that phytoplankton were photo-inhibited in the surface waters. In our experimental design, we did not acclimate phytoplankton to the shaded treatments, in which case they would still have sufficient energy reserves from previous exposure to sunlight to uptake nutrients. In our calculations, we have used the mean uptake rate over all three light treatments, figuring that this best represents the overall light climate to which algal cells are exposed as they are moved through the water column by turbulent water motion. To calculate the time in which the entire nutrient pool is turned over, we took the ambient concentration of each nutrient and divided it by the mean uptake rate, to

result in turnover times for NH_4^+ of 10–86h (mean 44hrs), and NO_3^- of 13–343hrs (mean 157hrs).

Table 9: Results of $^{15}\text{NH}_4$ and $^{15}\text{NO}_3$ uptake rates by water column micro-organisms, sampled at the farm pontoon. The data are in units of $\mu\text{M m}^{-3} \text{h}^{-1}$. Station BB007 was on Trip 2, BB014 and BB028 on Trip 3, BB033 on Trip 4 and BB082 on Trip 5. BB082 was on a very low tide and had high turbidity.

Station	Nutrient	100% Light		30% Light		Dark		Ambient Conc. μM	Turn-over hours
		mean	stdev	mean	stdev	mean	stdev		
BB007	NH_4^+	0.0049	0.0011	0.0218	0.0011	0.0255	0.0025	1.49	86
	NO_3^-	0.0006	0.0002	0.0012	0.0007	0.0012	0.0010	0.21	214
BB014	NH_4^+	0.0079	0.0018	0.0234	0.0030	0.0209	0.0070	0.40	23
	NO_3^-	0.0007	0.0004	0.0032	0.0001	0.0035	0.0008	0.04	16
BB028	NH_4^+	0.0415	0.0086	0.0557	0.0115	0.0481	0.0149	0.48	10
	NO_3^-	0.0025	0.0086	0.0078	0.0004	0.0089	0.0007	0.08	13
BB033	NH_4^+	0.0173	0.0012	0.0315	0.0021	0.0434	0.0078	2.03	66
	NO_3^-	0.0011	0.0003	0.0012	0.0006	0.0020	0.0002	0.29	201
BB082	NH_4^+	0.0212	0.0044	0.0362	0.0059	0.0536	0.0007	1.22	33
	NO_3^-	0.0009	0.0001	0.0011	0.0005	0.0023	0.0004	0.50	343

3.4.2 Sediment respiration rates

Sediment respiration refers to the combined oxygen consumption of the benthos and all organisms within, including microorganisms, plants and animals. Organic C is the main fuel for the consumption of oxygen, although some sediment can have substantial chemical oxidation due to high levels of reduced Fe, S and Mn. Where there is excess organic C input, sediment can become anaerobic favouring the production of reduced S (e.g. toxic H_2S), release of reduced N (NH_4^+ flux), low Redox and pH, fluxes of solubilised heavy metals and P. We have used sediment respiration rates as an indicator of organic C input.

There was no significant difference in mean respiration rates between the wet and dry season, neap and spring tides, or between locations (season x tide x location) (Table 10). However, at the Reference location, the mean wet season respiration rates (43.06 ± 4.50 (SE) $\text{mmol m}^{-2} \text{d}^{-1}$, $p < 0.05$) were significantly higher than in the dry season (18.5 ± 2.4 (SE) $\text{mmol m}^{-2} \text{d}^{-1}$, $p < 0.05$). No difference between the wet and dry season rates were observed at the other locations (Fig. 54). Higher respiration rates in the wet season might be expected due to higher input of organic C in the form of litter from the surrounding mangrove forests and higher temperature induced metabolic rates.

Table 10: Sediment respiration rates ($\text{mmol m}^{-2} \text{d}^{-1}$) for all locations, seasons and tides.

Treatment	Factor	N	Mean	St Dev	SE
		79	34.38	16.29	1.83
Dry		36	31.28	13.20	2.20
Wet		43	36.98	18.24	2.78
Neap		39	36.06	17.76	2.84
Spring		40	32.75	14.76	2.33
Cage		20	39.05	15.05	3.37
South		19	34.63	18.33	4.20
Reference		21	32.54	16.51	3.60
North		19	31.26	15.27	3.50
Dry	Neap	18	32.80	15.48	3.65
Dry	Spring	18	29.75	10.68	2.52
Wet	Neap	21	38.85	19.43	4.24
Wet	Spring	22	35.19	17.28	3.68
Dry	Cage	9	40.19	13.04	4.35
Dry	South	9	33.04	11.88	3.96
Dry	Reference	9	18.50	7.16	2.39
Dry	North	9	33.38	10.86	3.62
Wet	Cage	11	38.13	17.10	5.16
Wet	South	10	36.06	23.27	7.36
Wet	Reference	12	43.06	13.29	3.84
Wet	North	10	29.35	18.79	5.94

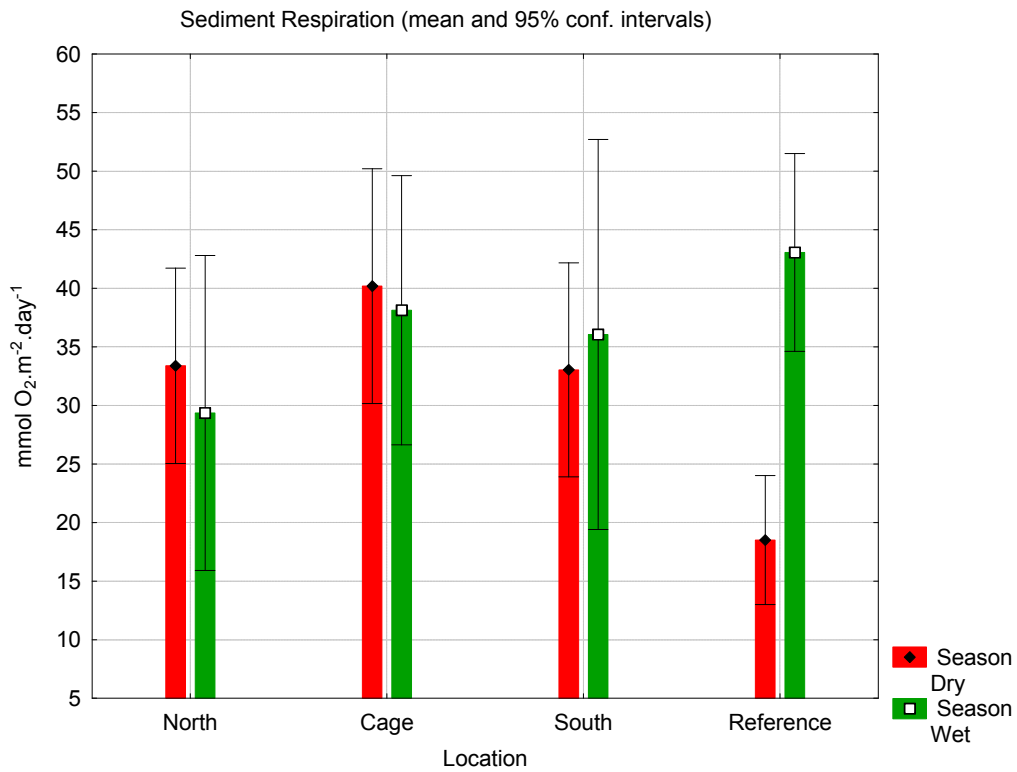


Figure 54: Sediment respiration rates in dry and wet seasons, as a function of location.

3.4.3 Role of wild fish

Aggregations of wild fishes are a ubiquitous feature of sea cage farms and were first documented by Carss (1990) around salmonid cages in Scotland. Dempster (2005) suggested these aggregations were caused by habitat complexity provided by the sea cage structure itself and the attraction of wild fishes to waste foods. Tuya et al. (2006) were able to separate these effects in a study of a decommissioned farm site and found that the daily feeding and presence of caged fishes outweighed the attractive features of the structure. The role of wild fishes around tropical sea cage farms is not well known (Sudirman et al. in press), but could represent a significant loss term for wastes. Accordingly, we attempted to determine whether this was the case at Bluewater Barramundi farm.

On Trip 2 we hired a DIDSON (Dual frequency Identification Sonar; Sound Metrics Corp., Washington U.S.A.) from NSW Department of Primary Industries and Fisheries. The DIDSON was deployed around cages during periods of feeding and non-feeding periods. Using this instrumentation we were able to image wild fish communities in the immediate vicinity of the cages irrespective of the turbidity of the water.

We deployed the DIDSON around cages during non-feeding periods, and prior to and during feeding. Though there was evidence of aggregations of small fishes around the cages, we did not witness any convincing difference in fish abundance or behaviour between feeding and non-feeding periods. Most of the fish observed around the cages were schools of sardines which were highly mobile (Fig. 55), making quantitative analysis difficult.

An interesting observation by the farm managers, and confirmed by DIDSON imagery, is the occurrence of a resident community of siganids living in between the predator nets, where these are fitted, and the main net structure (Fig. 56). Siganids are primarily herbivores and are likely to have been feeding on the organisms fouling the net structure, and at the same time protecting themselves from predators outside the net.

Fishes are attracted to sea cages for three main reasons: (i) the cage structure provides a refuge; (ii) the fish feed on fouling organisms on the cage structure, and (iii) waste feed and faeces coming from the cage provide a food source. Within the time available and with the current level of resourcing, we were unable to separate these effects. Our suspicion based on the absence of any perceivable change in behaviour between feeding and non-feeding periods is that waste feeds from the cage are not the primary attractant at this farm.

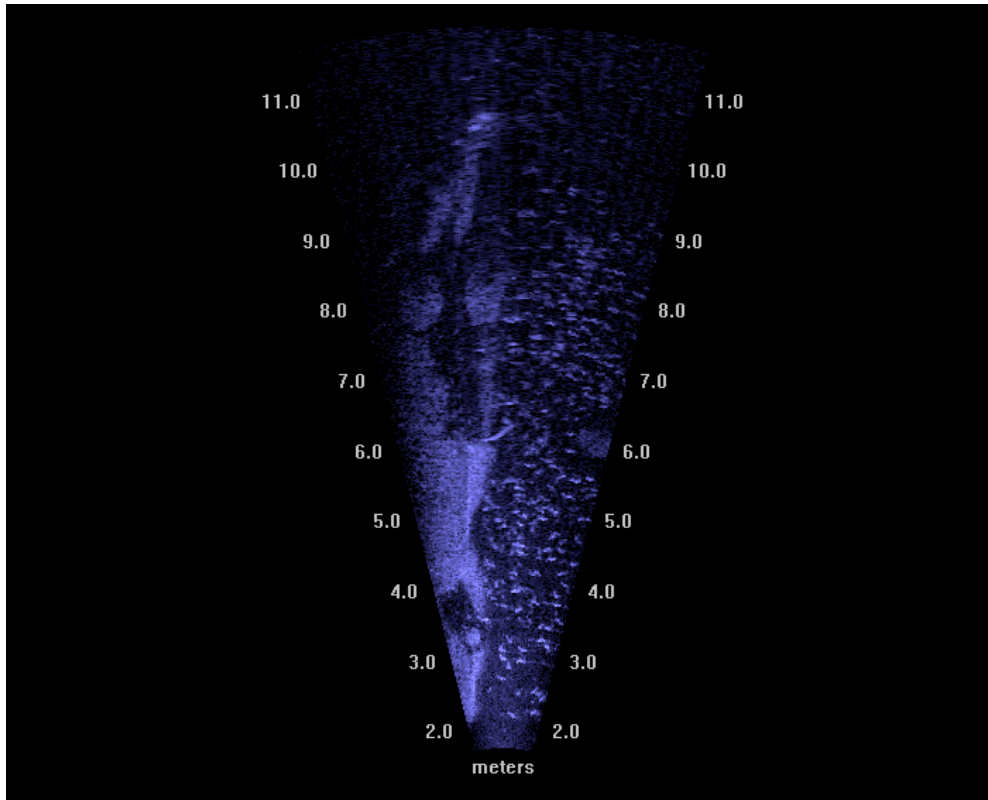


Figure 55: Small fishes, probably sardines, aggregating around Cage 9 during a feeding period on 17 October 2007. This cage was stocked with 14t barramundi. The cage netting is indicated by the opaque areas on the left of the image. Movies are available at : <http://www.aims.gov.au/docs/research/sustainable-use/tropical-aquaculture/sea-cage-aquaculture.html>

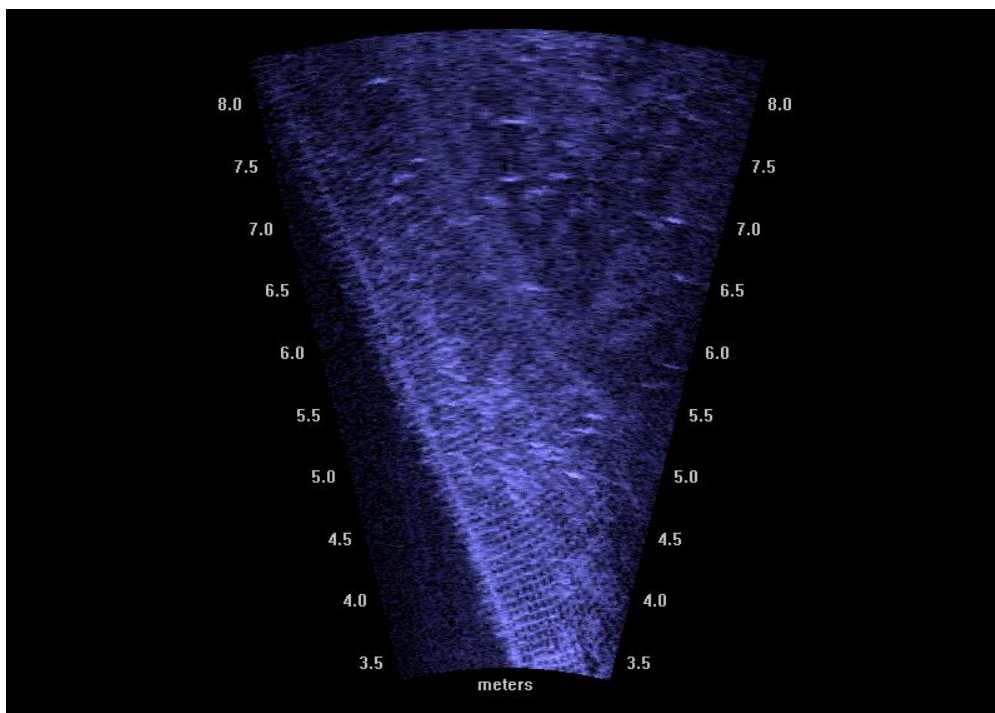


Figure 56: Fish aggregation around the predator net of Cage 13, 15 October 2007. The view is upward between the predator net (left) and the main cage net. Cultured barramundi are visible as dark shadows in the upper right quadrant. A school of sardines is visible to the left at 5.0m distance, and larger fishes (probably siganids) between 5 and 8m distance, more evenly dispersed.

Commentary: fate of wastes

The results from the ^{15}N experiments indicate that the pool of dissolved nitrogen within Conn Creek is turning over quite slowly: 33h in the case of $^{15}\text{NH}_4$, and 157h for $^{15}\text{NO}_3$. In North Queensland mangrove creeks receiving prawn farm effluent, $^{15}\text{NH}_4$ turnover times were similar (~30h; Burford et al. 2003). By comparison, in Darwin Harbour, a larger mangrove estuary with equally turbid water, $^{15}\text{NH}_4$ turned over every 5h and $^{15}\text{NO}_3$ every 21h (Burford et al. 2008). McKinnon et al. (2006) concluded that the water column of Darwin Harbour had an unsaturated assimilative capacity for absorbing nitrogenous wastes. Placing the results of these three studies in perspective, the long turnover times of both these forms of dissolved N suggest that the growth rate of phytoplankton within Conn Creek is not limited by the supply of dissolved forms of nitrogen i.e. that the assimilative capacity of the water column for dissolved nitrogen is saturated. It appears from the data of Burford et al. (2003) that this is also the case in other creeks in the area receiving aquaculture wastes, but since there is no comparative data from undisturbed North Queensland mangrove creek systems it is not possible to conclude that these observations are necessarily different from the natural situation.

The significant difference in sediment respiration rates at the Reference location between wet and dry season reflects the patchy nature of mangrove forest litter accumulation and dispersal. Large amounts of refractory mangrove material (intact leaves, wood and bark) were found in the grab samples from the reference sites in the dry season that were not found in the wet season, or in Conn Creek sites. This difference showed up in the unusually high TOC values from this Reference location (Table 7). It is interesting to note that there was no significant difference in sediment respiration rates from any Conn Creek location (Cage, North, South), nor between the wet and dry season, nor between neap and spring tides. This indicates that the wastes from the cage are not accumulating directly underneath the cages in sufficient quantities to affect this variable, and that these wastes are not affecting sediment respiration at adjacent locations to any significant degree.

At Bluewater Barramundi, wild fish do not appear important in assimilating waste feeds.

3.5 Nutrient budgets

To place the activities of the farm in an overall ecosystem perspective (i.e. taking account of other sources and sinks of key elements in Conn Creek), we collated our own directly measured data with published data and estimated the major standing stocks of both carbon and nitrogen within the Conn Creek ecosystem, and the major fluxes of these elements.

Nutrient budgets were estimated based on data from the current study and from published literature values. We used this compilation of data to calculate the major sources and losses from the system for C (Fig. 57) and N (Fig. 58). The data, calculations and assumptions used to construct these budgets are outlined in Table 11.

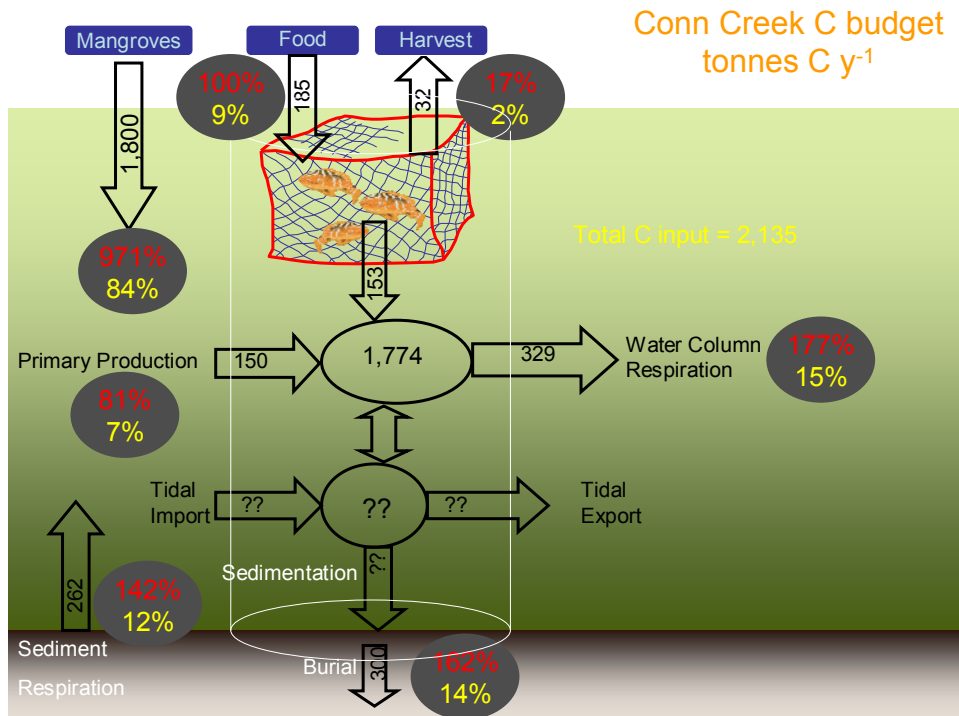


Figure 57: Estimates of C input, uptake and loss at Bluewater Barramundi farm (tonnes C year⁻¹). Red % figures denote the proportion of each process relative to the farm input, while yellow % figures denote the proportion of each process relative to the total nutrient input.

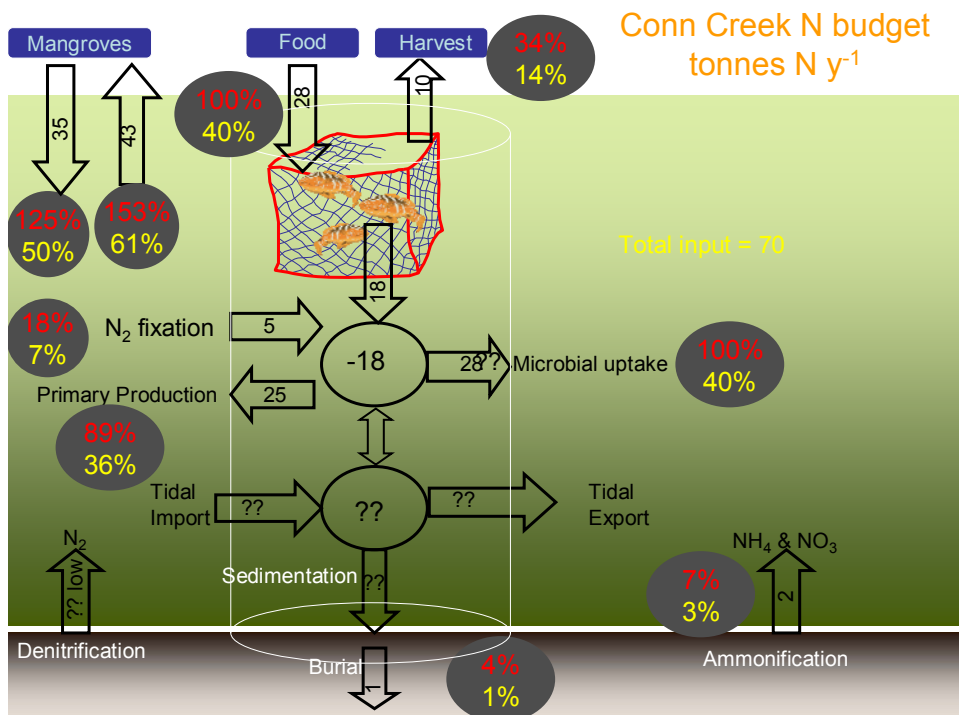


Figure 58: Estimates of N input, uptake and loss at Bluewater Barramundi farm (tonnes N year⁻¹). Red % figures denote the proportion of each process relative to the farm input, while yellow % figures denote the proportion of each process relative to the total nutrient input.

Table 11: Inputs and outputs of C and N to the Conn Creek system based on data from this study and published literature, excluding tidal fluxes between Conn Creek and Hinchinbrook Channel. Assumptions used in constructing the nutrient budget are included as footnotes.

Estimated C and N input output to/from Conn Creek (t yr ⁻¹)	C t yr ⁻¹ (% of total input)	% of farm C input	N t yr ⁻¹ (% of total input)	% of farm N input
Inputs				
¹ Aquaculture input (365 t yr ⁻¹)	185 (9%)	100%	28 (40%)	100%
² Mangrove litter	1,800 (84%)	97%	35 (50%)	125%
³ Phytoplankton production	150 (7%)	81%		
⁴ N ₂ fixation			5 (7%)	18%
⁵ Benthic DIN release			2 (3%)	7%
Total system input	2,135 (100%)		70 (100%)	
Outputs				
⁶ Fish harvest (240 t yr ⁻¹)	32 (2%)	17%	10 (14%)	34%
⁷ Sediment burial	300 (14%)	162%	1 (1%)	4%
⁸ Water column respiration	329 (15%)	177%		
⁹ Sediment respiration	263 (12%)	142%		
¹⁰ Mangrove growth			43 (61%)	153%
¹¹ Phytoplankton growth			25 (36%)	89%
¹² Microbial growth			28 (40%)	100%
Total system output	-924		-107	
Difference (input-output)	+1,212		-37	

¹Aquaculture input = 365t feed at 50.8% C, 7.7% N, 1.96% P (Ridley pellets). Feed input = 185t C and 28t N.

²Mangrove litter from 542ha mixed *Rhizophora* forests (Robertson and Alongi 1992), C:N:P ratio of whole *Rhizophora* forests (Alongi, et al. 2003a).

³Phytoplankton production from Conn Creek, this study. NPP = 274mg C m⁻² d⁻¹

⁴N fixation in mangrove sediments and intertidal areas (median = 2.5µg N m⁻² d⁻¹) from Alongi et al. (1997).

⁵Sediment nutrient fluxes in Conn Creek, this study. The minimum total N mineralisation is 1.5t N yr⁻¹ based on the DIN flux data. It is a minimum as it doesn't include denitrification.

⁶Estimated annual production at Bluewater Barramundi farm, fish nutrient content (dry weight) = 40% C, 12% N, 2.4% P. Dry weight fish = 33%; Wet weight of fish at harvest: 240t ww fish = 79t dw fish = 31.7t C and 9.5t N. (Alongi et al. 2003a). 25% of feed C, N input is removed as harvested fish (Shimoda et al. 2007).

⁷Estimated from flux and sediment TOC, C, N in Conn Creek, this study, and based on the mineralisation: sedimentation rate relationship in Henrichs (1993).

⁸Pelagic respiration in water column = 600mg C.m⁻².d in Conn Creek, this study.

⁹Sediment respiration in Conn Creek, this study.

¹⁰C:N:P ratio of whole *Rhizophora* forests from Alongi et al. 2003a.

¹¹Phytoplankton production N demand from Conn Creek, this study.

¹²Pelagic microbial N demand in Conn Creek, this study.

Commentary: Estimated Nutrient Budget

The construction of nutrient budgets allows us to assess the inputs from the farm relative to the processing inputs and outputs of nutrients from the broader ecosystem, and from individual activities (e.g. feed input from cages). Our estimates for this budget do not include the import and export of material through tidal exchange, and this will certainly account for the unaccounted material in our estimates.

Our estimates indicate that the farm input represents 40% and mangrove litter 50% of the total N input into Conn Creek (Table 11). Mangrove growth (61%), phytoplankton growth (36%), and microbial growth (40%) represent the major losses of N from the system, while fish harvesting removes 14% of the total N input budget. Percentage N losses attributed to each of these processes total more than 100% because the data is expressed as % of the

total N input. This budget exercise indicates that the Conn Creek ecosystem must import around 37t N yr⁻¹ to support its N demand (predominantly by the mangrove forests), and the most likely mechanism for this is via tidal exchange with Hinchinbrook Channel.

Mangrove litter represents the major C input (84%), while the farm (9%) and phytoplankton production (7%) are relatively minor contributors to the total C input. Fish harvesting removes only 2% of the total input, while sediment burial (14%), water column respiration (15%), and sediment respiration (12%) are the other estimated losses from the system.

These calculations indicate that 1,212t C yr⁻¹ is exported from the system, and the most likely mechanism for this is via tidal exchange with Hinchinbrook Channel. Our experience with similar environments (Ayukai et al. 1998) demonstrates that tidal exchange is an important source of nitrogen and carbon exchange within the mangrove lined estuaries in Hinchinbrook Channel that tend to conserve (import) nitrogen and phosphorus (Boto and Wellington 1988) and export organic carbon (Alongi et al. 1998). The estimation of tidal exchange of carbon and nitrogen has been carried out in similar mangrove creeks receiving aquaculture discharge (Wolanski et al. 2000, Trott et al. 2004, Paez-Osuna et al. 1999) but requires a very substantial field and analytical campaign over several complete tidal cycles in order to achieve meaningful results. Wolanski et al. (2000) showed that the concentrations of several water quality variables (dissolved and particulate C,N,P, TSS, chl *a*) were strongly influenced by tidal mixing in a narrower and poorly flushed tidal creek with longer flushing times (4 days during spring tides, 10–15 days during neap tides). They also showed that this system exported some of these components and imported others, depending on the volume of discharge from a prawn farm, the season and the state of the tide. Trott et al. (2004) estimated the removal of prawn farm discharge nutrients by sediment and water column nutrient processes. They also showed that, using an annual budgeting calculation, tidal creeks can export significant amounts of carbon and nitrogen. McKinnon et al. (2002) measured the rates and steps of natural trophic processes involved in the assimilation and dispersion of these nutrients within, and beyond tidal ecosystems receiving aquaculture discharge.

In order to put the relative input of farm carbon and nitrogen in perspective, we estimated the various inputs and outputs as a % of the farm feed input (red % numbers in Figs. 57 and 58). Mangrove litter input is almost ten times (971%) the C input and 1.25 times (125%) the N input from the farm. Primary production by phytoplankton in Conn Creek contributes similar amounts of C (81%) as the feed input, but also removes similar amounts of N (89%) relative to the feed input. Microbial growth can consume the same amount of N (100%) as the N input from the farm. Harvesting of fish accounts for 17% of the C and 34% of the N from the feed input. Sediment burial (162%), water column respiration (177%), and sediment respiration (142%) are each around 1.5 times the C input from the farm. N demand for mangrove growth is 1.5 times (153%) the N contained in the feed from the farm. The largest contribution to this system is the C and N in mangrove litter, and the largest output is the N demand of the mangrove forest. The largest loss of C from the system is most likely to be particulate organic C (in the form of leaves, twigs, flowers and trunks), via tidal flushing. The budgeted export of C and import of N agrees with previous work, mentioned above, in this and adjacent Hinchinbrook Channel creeks.

4. Synthesis of results to assist predictive modelling of the environmental impacts of sea cage aquaculture in other areas

Our results indicate that:

- Water within Conn Creek is well mixed by tidal currents.
- Seasonal climatic variation is the major factor affecting the water quality of Conn Creek.
- Overall, water quality within Conn Creek, including the farm site, conforms with Queensland Water Quality standards, and there is no clear evidence of differences to similar mangrove environments in North Queensland.
- The footprint of the farm on the benthos, on the basis of sediment chemistry, appears to be restricted to the approval area.
- Phytoplankton within the water column of Conn Creek do not have sufficient assimilative capacity to absorb all wastes from the farm.
- Mangroves facing Conn Creek contain N likely to have originated from farm activities, and play a significant role in nitrogen cycling within the ecosystem.
- Tidal flushing of Conn Creek (Hinchinbrook Channel) is a vital route by which excess nutrients are removed from the system and dissolved oxygen is replenished.

Our research at other Australian and overseas locations (e.g. Alongi et al. 2003) illustrates that the environmental impact of a sea cage farm depends on the habitat in which it is placed. Because of this, we believe that appropriate site selection is critical to minimising environmental impacts. A comparison of the advantages and disadvantages of the Bluewater Barramundi farm location is outlined below.

Advantages of the Conn Creek site:

- Shelter from waves and wind for farm infrastructure.
- Limited visual impact due to reduced public accessibility.
- Reasonable access to land based infrastructure/markets.
- Good tidal flushing and current velocities.
- Mature mangrove forest, “pristine” (= undeveloped) environment.
- Mesotrophic waters.

Disadvantages

- Limited assimilative capacity within the water column to absorb all wastes.
- Shallow, prone to high turbidity events leading to low light conditions and limited plankton production (limited nutrient uptake capacity).
- Periods of low dissolved oxygen concentration.
- The water column is intermittently heterotrophic.
- Sudden salinity fluctuations during flood events can stress fish.

Site selection criteria should include optimising sites for water quality and shelter. Water quality requirements will vary according to the species that is to be produced. For instance, mangrove systems offer advantages in terms of placement and assimilative capacity, but the water quality is unsuitable for many cultured species. Our study has demonstrated that the oxygen concentration episodically falls below 2.0mg l⁻¹ in this area, which would be lethal to many fish species if they were exposed for several hours. Tides in North Queensland are semi-diurnal, with a range of ~4m, and represent a good compromise between flushing rate and undue stress on cage moorings caused by stronger currents in macro-tidal settings.

In terms of predictive modelling, our study demonstrates that the extent of the impact of aquaculture development depends upon:

1. The physical exchange rate i.e. residence time or exchange. This is greatly influenced by the tides experienced at the site.
2. The assimilative capacity of the site. For nutrients (primarily ammonium) this depends upon the primary production of phytoplankton, seagrasses, macroalgae and mangroves exposed to water containing nutrients from the facility.

We have found the nutrient-budget approach instructive for estimating the degree of impact. As a first estimation, a preliminary budget was constructed based on an estimate of the area of various habitats (i.e. area of mangroves, area and volume of the receiving water), combined with literature values for rates of key processes taken from similar habitats (see Section 3.5). In other locations used for aquaculture, or for proposed new developments, similar budgets could be prepared based on literature values, and would allow farm activities to be put in the context of the assimilative capacity of the environment.

5. Recommendations for future monitoring

Monitoring design, selection of indicators and recommendations

Aquaculture is a large and expanding industry, which is being fuelled by an increasing international demand for seafood that is predicted to reach an extra 37 million tonnes within the next two decades (Thyer 2008). Cage aquaculture of finfish plays a significant role in meeting this demand. In response to the expanding sea cage aquaculture industry, there have been numerous studies carried out to investigate the environmental effects of certain aspects such as: sea cages and infrastructure effects, impacts of high densities of fish on sediments and water column, distant effects of nutrient inputs from feed and fish wastes, and the effects of escaped fish on local species. Most of these studies have been carried out in clear water temperate regions (ECASA 2008; Hargrave 2005; Beveridge 2004). However, the correlation between farming intensity and environmental impacts indicated in these northern hemisphere studies may not be applicable to temperate Australian waters (Macleod and Forbes 2004). Tropical mangrove environments and waterways that are typified by intertidal forests, muddy water and macrotidal environments with extremes in nutrient and oxygen concentrations and where microbial communities dominate the benthos (Alongi et al. 1998) require a different suite of environmental indicators. Some recent studies have begun to focus on tropical sea cage aquaculture in these coastal mangrove environments (PHILMINAQ 2008). We have recommended a suite of indicators for use at the Bluewater Barramundi farm that were developed during our previous research on sea cage impacts in tropical environments, and we have also reviewed the published literature for any suitable inclusions to this group.

One of our goals was to identify a suite of indicators that were appropriate for mangrove waterway environments and the sea cage aquaculture activity causing the observed disturbance. At the same time it was important to remain within the bounds of the time and technical expertise required to acquire the data, the affordability of collecting and analysing data, the interpretive capacity of the users, and the sensitivity of the indicators to identify anthropogenic rather than natural variation. For example, Scheltinga et al. (2004) suggested several criteria for consideration in the selection of appropriate indicators:

- Is the indicator appropriate to the stressor?
- How easy is it to distinguish between anthropogenic impacts and natural variation?
- Complexity – is it easily measured (not highly technical)?
- Complexity – is it easily interpreted (data analysis)?
- Cost per measurement (sampling/analytical cost).
- Capital costs for indicator measuring equipment.
- Overall rating of usefulness/practicality of indicator.
- Is there a manual or protocol on how to measure the indicator?
- Do any other organisations use this protocol?
- Do guidelines/reference points exist to determine if the indicator measurement result is good or bad?

Scheltinga et al. (2004) provided a matrix of environmental indicators and broad descriptions of the stressors that could have an impact on them (See Table I: Appendix III). The activities of Bluewater Barramundi farm produce the following stressors as defined in that matrix – aquatic sediments, bacteria, nutrients, organic matter, pH. We have suggested indicators that consider the suggested framework and address the criteria for consideration, as noted above.

Recommendations for indicators with reference to the appropriate stressors and selection criteria:

- Continuous logging of dissolved oxygen within the heaviest stocked region of the farm. This could be within a cage, or from a surface buoy mooring supporting a sensor near the surface and another at the same depth as the base of the stocked cage, or this sensor could be near sediment under the cage. This data would be available for inclusion in, and refinement of, stocking capacity models (e.g. CADS_Tool) and serve as a vital management tool to indicate day-to-day control of stocking densities, feeding regimes and (if other variables are included such as temperature and salinity) may provide important strategic information on factors affecting growth, survival and disease, and therefore possibly assist in reducing farm wastes (through better FCR, closer management of low feeding periods, higher survival and improved growth).
- Use of a carrying capacity model (e.g. CADS_Tool) based on hydrodynamic information specific for the site, species cultured and feeding regime. This should be based on sound data on the physiological responses of the fish for the ranges of environmental variables likely to be encountered (e.g. dissolved oxygen, temperature, salinity). This could be used as a predictive tool for farm derived wastes and could incorporate data from monitoring programs to improve accuracy. These predictive tools can contribute to better regional resource planning and management, as well as less cost/time to the farm by reducing the requirement for costly on-site monitoring of several parameters.
- ¹⁵N stable isotope analysis of mangroves on an annual basis could indicate if the farm wastes were extending beyond the current footprint. Our study suggests that mangroves along the length of Conn Creek have taken up farm based N, but we did not sample further afield than the mouth of this creek. Uptake of available N by mangroves from the environment is a normal process, and our study did not identify abnormal amounts of N in leaves at any site. Analytical costs are moderate, while sampling and interpretation is relatively straight forward.
- Annual survey of Zn:Li and P:Li ratios of surface sediments under and near cages, as well as from similar locations not subject to anthropogenic inputs (e.g. sewage outfalls, river input, marina operations). These ratios appear to be useful (simple to collect, store, analyse and are cost effective) in identifying the benthic footprint of the farm, however, they do not indicate any altered processes or adverse impact.
- Nutrient budgeting of the sea cage aquaculture activity in order to estimate annual inputs and nutrient flows in terms of regional coastal nutrient inputs. The nutrient input by the farm via feed can be considered relative to other nutrient inputs, and annual feed amounts easily calculated. Losses from the farm can be calculated using published data on FCRs for the species under specified environmental conditions.
- Chlorophyll *a* monitoring may be useful in more transparent coastal waters (e.g. near shallow sand bars of Hinchinbrook Channel) in order to determine the impact of nutrient input where light is not a limiting factor to primary production or where there are benthic primary producers of interest (e.g. seagrass, inshore corals, macro-algae). However, in turbid, nutrient saturated, light limited waters, this variable has not proven to be a sensitive measure of nutrient input. Continuous logging of chlorophyll *a* is now possible by data loggers, and this could be carried out economically (e.g. WetLabs or Hydrolab dataloggers) for comparison between specified time periods (e.g. full tidal cycles of neap and spring tides, wet season and dry season, full stocking periods or during fallow periods).

Other possible indicators considered, but not recommended at this stage because they do not fully satisfy the selection criteria above, are:

- C:N:P ratios in water column to detect enhanced or altered nutrient ratios and concentrations, organic and total C, N, S and P content, pore water nutrient status, Redox potential, benthic macrofauna, meiofauna in sediments at cage and reference sites. Most of these variables require the measurement of several other variables for interpretation as they are co-variant with these factors (e.g. grain size, Eh, salinity). In Mediterranean studies (ECASA 2008) several of these indicators performed well as indicators of fish cage activities (Porello et al. 2005), however due to the expertise required (e.g. invertebrate taxonomy), costs of analyses (e.g. sediment and water column nutrients) and naturally high background levels with significant variability (e.g. Redox potential) we do not recommend these approaches.
- Lipid profiles and altered sediment bacterial metabolism, diversity and biomass may be specific for estimating the range of the farm's footprint. Some lipids are specific to the aquaculture feed or the fish, while sediment bacterial diversity and counts are potentially useful to indicate altered nutrient availability and processes specifically caused by aquaculture activities. Analytical costs, sampling procedures and expertise in the interpretation of results is not readily available amongst farmers or resource managers, which prevents these indicators from being recommended.
- Diver operated samplers and video/still images of benthic sites have been recommended for clear water environments (Macleod et al. 2004), however, diving in the waters of Hinchinbrook Channel is not recommended for safety reasons (e.g. crocodiles, stingers), and low visibility and strong currents precludes the use of visual assessments. Epibenthic macrofauna do not establish extensive or stable communities in these tidal creeks, and previous work indicates the populations can be highly seasonal with high spatial variability related to sediment type (Alongi and Sasekumar 1992).

We conclude that a meaningful monitoring program in mangrove environments such as Conn Creek would be different from those in operation elsewhere. In view of this, the concerns of the regulating agencies and the need for any monitoring program to be cost-effective, we recommend that a workshop of all parties be convened to develop a satisfactory monitoring design.

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Appendix I. Technical Methods

Field sampling

Vertical Profiling

Vertical profiles of temperature (T°C), salinity (S), chlorophyll fluorescence (Wetlabs Wetstar), optical backscatter (D & A Instruments OBS-3) and oxygen (O₂; Seabird SBE43) was determined at all sampling stations with a Seabird SBE19+CTD profiler. The SBE19+ samples at 4 Hz. During profiles, the instrument package is lowered/raised at ca. 1 m sec⁻¹.

Discrete Water Samples

Discrete water samples were collected by hydrocasts using Niskin bottles closed at depth. Sub-samples of water were drawn from the Niskin bottles in order of priority: dissolved oxygen (when run), dissolved nutrients, chlorophyll, total suspended solids (TSS), particulate nutrients (PN, PP, PC) and bacteria.

Water Clarity (Secchi Disk)

At stations occupied between approximately 0900 and 1500, estimates of water clarity were determined using a Secchi disk (Preisendorfer 1986). The Secchi disk is a 30 cm weighted white disk with a line marked at 1 m intervals. The Secchi disk is lowered through the water until just disappears from view and the depth is recorded. This measure can be used to calculate the depth of the euphotic zone (ca. 1% of surface irradiance – I₀).

Water-column ¹⁵N turnover

Water samples collected with Niskin bottles were used to fill acid-washed 500ml polycarbonate bottles for ¹⁵N-nitrogen uptake. ¹⁵N-ammonium chloride and potassium nitrate were added to triplicate bottles incubated *in situ* for 1–1.5h, at 100% natural light, within bags that admitted only 30% of natural light, and in the dark. Our goal was to have addition rates in the range of 10% of background NO₃⁻ and NH₄⁺ concentrations. Following incubation, known volumes from each bottle were filtered using 25mm pre-combusted GF/F filters (Whatman) and the filters were then frozen. In the laboratory, filters were dried and the ¹⁵N/¹⁴N isotopic ratio was determined using a mass spectrometer (GV Isoprime, Manchester UK). Uptake rates were calculated using the equations of Glibert et al. (1991), using the ambient nutrient concentrations obtained from the methods described in Section 2.3.

Plankton photosynthesis and respiration

Water samples were taken with Niskin bottles at 2 depths corresponding to pre-determined light levels and sets of calibrated acid-washed iodine flasks (nominal volume of 125ml) were filled immediately upon retrieval. Nine flasks were filled from each depth at every station; three were fixed for Winkler titrations immediately (zero-time samples), three were placed in a lightproof bag (dark respiration) and three were placed on a transparent rack. Incubations were conducted *in situ* at the depths of collection for 24h, after which the incubated flasks were fixed. The entire set of flasks from each experiment was titrated as a single batch as soon as possible after completion of the experiment.

Dissolved oxygen concentration was determined with an automated precision Winkler titration system (Oceanographic Data Facility, Scripps Institution of Oceanography, La Jolla, CA, USA) which uses the absorption of 365 nm UV light for endpoint detection. Net

community production (NCP) and community respiration (CR) were estimated as the change in oxygen concentration during a 24hr period in iodine flasks incubated in the light and dark respectively. Oxygen flux data was converted to carbon units assuming a P_Q of 1.2 and a R_Q of 0.8 (Laws 1991). Gross primary production (GPP) was calculated as the sum of NCP and CR, and the P:R ratio calculated as the ratio GPP:CR. We computed area-specific community rates by trapezoidal integration of volumetric data to the sea bottom, in the case of respiration, or to either the bottom or the 1% isolume in the case of production.

Sediment sampling and elemental analysis

Sediment samples for each analysis were taken at the sites indicated in Figure 41 using a stainless steel van Veen grab. Grab samples at each site were composited for sediment respiration and nutrient flux estimates. Redox potential (Eh) and pH were measured at approx 2cm depth in undisturbed surface sediments using calibrated Model PBFC pH and calomel Eh electrodes connected to a TPS LC 80 meter. Analysis of sediment grain size and sorting were performed on a Malvern Mastersizer 2000. Sediments were classified based on the definitions in Folk (1974). Subsamples for Zn/Li ratio, TOC, C, and N were stored frozen at -20°C . On return to the laboratory, samples were freeze-dried, and ground to fine powder for analysis. Grain size analysis was performed on frozen, un-ground samples. Total C and total N was determined on a Perkin-Elmer 2400 CHNS/O Series II Analyzer and total organic carbon (TOC) was determined on a Shimadzu TOC Analyzer with solid sampler. For TOC, a small volume (usually 100 μl) of 2 M HCl was added to the sediment, and the sample was then evaporated to dryness and combusted at 950°C . Total inorganic carbon was assumed to be incorporated into CaCO_3 , as determined by the difference between the total C and TOC concentrations. Zn, Li and P were determined after strong acid digestion on a Varian Liberty inductively coupled atomic emission spectrometer following the procedure of Loring and Rantala (1992).

Sediment respiration and solute fluxes

Solute fluxes across the sediment–water interface and oxygen consumption within the chamber were measured in three opaque chambers (volume: 1litre; area: 64 cm^2) from which O_2 and dissolved inorganic nutrient (NH_4^+ , $\text{NO}_2^- + \text{NO}_3^-$, PO_4^{3-}) samples were taken at one hourly intervals for three to four hours. Each chamber had a propeller-electric motor unit and two sampling ports on opposite sides of the chamber (Alongi et al. 2006). Dissolved oxygen was measured in dark chambers using an O_2 probe (TPS Model WP-82 DO meters) placed into one sampling port; the other port was fitted with plastic tubing to draw off 10ml samples for solutes. The samples for dissolved inorganic nutrients were filtered (0.45 μm Minisart filters) and kept cool and dark (6h) until being frozen at -20°C until analysis. Concentrations of NH_4^+ , $\text{NO}_2^- + \text{NO}_3^-$ and PO_4^{3-} were determined using automated techniques (Ryle et al. 1981, Ryle and Wellington 1982).

^{15}N in mangrove leaves

Rhizophora stylosa trees on the bank of Conn Creek were sampled for ^{15}N 13C, %C, %N content following the method of Costanzo et al. (2004). The second youngest set of leaves (2–4 leaves) from a rosette from each of five individual mature plants were collected, rinsed in Super Q and stored in the dark at 20°C until being freeze-dried and ground to a fine powder for analysis. A sample of feed pellets was also freeze-dried and ground for comparison of %C and %N content and stable isotope concentrations.

Hydrolab Loggers

Hydrolab DataSonde loggers were deployed for 24hr recording inside and outside fish cages in order to detect changes induced by the fish biomass within the cages, and the effect of the cages on water column mixing and stratification. Loggers were fitted with sensors for:

- depth
- dissolved oxygen
- pH
- temperature
- salinity
- time.

Calibrations were carried out according to the manufacturer's specifications.

Analytical procedures (laboratory)

Chlorophyll (Fluorometry)

Replicate subsamples of water from Niskin bottles or surface buckets were filtered through Whatman GF/F (nominal pore size – 0.7µm: total community). After filtration under subdued light, the samples were folded, stored in pre-combusted foil packet envelopes and frozen (<-10°C) until analysis.

In the laboratory, the filtered samples were ground in a tissue grinder with a 90% acetone:water mixture and extracted in the dark for ca. 1hr. After centrifugation to remove suspended matter, chlorophyll fluorescence in the supernatant extract was determined using a Turner Designs Model 10 fluorometer with a red-sensitive photomultiplier. Following the initial reading, the sample was acidified with 1 drop of 10% HCl and the fluorescence re-measured.

Chlorophyll and phaeophytin concentrations were calculated according to Parsons et al. (1984).

Dissolved inorganic nutrients (NO₂⁻, NO₃⁻, PO₄³⁻, Si(OH)₄)

Duplicate water sub-samples (10ml) were syringe filtered (0.45 µm) into acid-washed screw-capped plastic test tubes (12ml) and stored frozen (ca. -20°C) until analysis.

Dissolved inorganic nutrient concentrations in the filtered samples were determined by standard colorimetric methods (Parsons et al. 1984) implemented on a Braune and Lubbe segmented flow analyser (SFA: Ryle et al. 1981).

Dissolved organic nutrients (DON, DOP)

Dissolved organic nutrient concentrations were estimated as the difference between measured total dissolved nutrient (TDN, TDP) concentrations in oxidised water samples and summed inorganic nitrogen (DIN = NH₄⁺ + NO₂⁻ + NO₃⁻) and phosphorus (PO₄³⁻) concentrations in parallel sets of un-oxidised samples.

The organic matter in 10ml filtered water samples were oxidised by alkaline persulfate digestion under high temperature (110°C) and pressure in an autoclave. After oxidation, the total inorganic nutrient (TDN, TDP) concentrations were determined by segmented flow analysis as above.

Particulate carbon (PC) and Particulate nitrogen (PN)

The particulate carbon (PC) and nitrogen (PN) content of material collected on filters was determined by high temperature combustion (HTC) using a Shimadzu TC-5000 carbon analyser fitted with a solid sample inlet.

Filters containing sampled material were placed in pre-combusted (450°C) ceramic sample boats. After the sample inlet was purged of atmospheric CO₂, inorganic C on the filters (e.g. CaCO₃) was removed by addition of concentrated phosphoric acid and quantified by non-dispersive infra-red gas analysis (IRGA). After this quantification was completed, the filter was introduced into the sample oven (1000°C) where the remaining organic carbon is combusted in an oxygen stream and again quantified by IRGA. The analyses were standardised using certified reference materials (e.g. MESS-1).

Particulate phosphorus (PP)

Particulate P was determined by refluxing the pre-ashed glass fibre filters (through which the seawater had been filtered) and their associated organic matter to dryness with acid persulfate (5%), redissolving the digest in deionised water and colorimetrically determining the PO₄³⁻ content of the supernatant (Parsons et al. 1984). The analysis was standardised with potassium phosphate and an organic sugar phosphate (e.g. fructose-6-phosphate).

Physical and Chemical Analysis of sediments

At each study site sedimentary grain size (ϕ) and total organic carbon (%) were measured. To investigate sediment granulometry replicate samples from each site were sieved to 1.8 mm and subjected to ultrasonication for 1 min to break up aggregate particles. Laser diffraction grain size analysis was subsequently used to detect particles between 0.02–2000 μ m. Graphic mean grain size (M_z) and inclusive graphic standard deviation (σ_1) were determined from percentile values.

For chemical analysis sediment samples were collected and stored frozen until processed. Samples were dried (60°C until constant weight was obtained) and milled. Total organic carbon was analysed using a SHIMADZU solid sample module (SSM-5000A) linked to a SHIMADZU total organic carbon analyser (TOC-V_{CSH}). Inorganic carbon was released by acidification (hydrochloric acid 3%) and remaining non-purgeable CO₂ gas was oxidised and measured.

Bacterial counts (Flow cytometry)

Water samples (in 2ml cryovials) were collected for the analysis of picoplankton populations and are preserved with glutaraldehyde (0.1% final concentration) and stored in liquid nitrogen (Vaulot 1989). Analysis of these samples were performed using a Becton Dickinson FACScan flow cytometer. To enumerate heterotrophic bacteria water samples were tagged with a DNA stain (SYBR Green I), a blue-light-excited nucleic acid dye according to the methods of Marie et al. 1997. After counting on the flow cytometer, Cytowin® software was used to determine the concentrations (cells ml⁻¹) of heterotrophic bacteria in the samples (Vaulot 1989).

Appendix II. Redfield ratios

(From Furnas 2003)

The average chemical composition of marine phytoplankton falls within predictable ranges. The relative abundances of carbon (C), nitrogen (N) and phosphorus (P) in plankton is known as the Redfield ratio after the American oceanographer who discovered this characteristic. To produce biomass containing a given amount of organic C, predictable amounts of bio-available N and P are required as nutrients. Conversely, if a given amount of biomass is broken down and mineralised through respiration, predictable amounts of N and P will be given off as ammonium and phosphate. For marine phytoplankton, the Redfield ratio (as atoms) is C:N:P = 106:16:1.

Appendix III. Extract from the Users Guide

Table 1: Indicators for the Matter for Target "Estuarine, Coastal and Marine Habitat Integrity", aligned to stressors. Extracted from the Users Guide to Estuarine, Coastal and Marine Indicators for Regional NRM Monitoring (Coastal CRC, 2004).

INDICATOR	STRESSORS														
	Aquatic Sediments	Bacteria/pathogens	Biota removal/disturbance	Excess freshwater	Excess salt (hypersaline)	Freshwater flow regime	Habitat removal/disturbance	Hydrodynamics	Litter	Nutrients	Organic matter	Pest (plant, animal) species	pH	Toxicants	Water Temperature
Physical-chemical condition															
Dissolved oxygen										✓					
Estuary mouth opening/closing						✓		✓							
pH													✓		
Presence / extent of litter								✓							
Salinity			✓	✓	✓			✓							
Sedimentation/erosion rates	✓														
Total nutrients in the sediments WITH dissolved nutrients in the sediments										✓					
Total nutrients in the water column WITH dissolved nutrients in the water column										✓					
Toxicants in biota														✓	
Toxicants in the sediment														✓	
Turbidity / water clarity	✓														
Water current patterns								✓							
Water soluble toxicants in the water column														✓	
Water temperature															✓
Biological condition															
Algal blooms								✓		✓					
Animal disease / lesions														✓	
Animal kills											✓		✓	✓	
Animal or plant species abundance	✓		✓				✓								
Animals killed or injured by litter								✓							
Benthic microalgae biomass								✓		✓					
Biomass, or number per unit area, of macroalgae								✓		✓					
Biomass, or number per unit area, of epiphytes								✓		✓					
Chlorophyll a								✓		✓					
Coral bleaching															✓
Death of marine mammals, endangered sharks and reptiles caused by boat strike, shark nets or drum lines			✓												
Occurrence of imposex														✓	
Pest species (number, density, distribution)												✓			
Targeted pathogen counts		✓													
Habitat extent															
Extent/distribution of key habitat types	✓						✓								
Extent/distribution of subtidal macroalgae										✓					
Seagrass depth range	✓														