Tracing the fate of ^{15}N -enriched feed in an intensive shrimp system

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Abstract

The fate of ^{15}N -nitrogen enriched formulated feed fed to shrimp was traced through the food web in shallow, outdoor tank systems (1000 L) stocked with shrimp. Triplicate tanks containing shrimp water with and without sediment were used to identify the role of the natural biota in the water column and sediment in processing dietary nitrogen (N). A preliminary experiment demonstrated that ^{15}N -nitrogen enriched feed products could be detected in the food web. Based on this, a 15 day experiment was conducted. The ammonium (NH_4^+) pool and particulate N fraction in the water column became rapidly enriched (within 1 d) with ^{15}N -nitrogen after shrimp were fed ^{15}N -enriched feed. By day 15, 6% of the added ^{15}N -nitrogen was in this fraction in the 'sediment' tanks compared with 0.4% in the 'no sediment' tanks. The particulate fraction in the water column, principally autotrophic nanoflagellates, accounted for 4 to 5% of the ^{15}N -nitrogen fed to shrimp after 1 d. This increased to 16% in the 'no sediment' treatment, and decreased to 2% in the 'sediment' treatment by day 15. It appears that dietary N was more accessible to the phytoplankton community in the absence of sediment. The difference is possibly because a proportion of the dietary N was buried in the sediment in the 'sediment' treatment, making it unavailable to the phytoplankton. Alternatively the dietary N was retained in the NH_4^+ pool in the water column since phytoplankton growth, and hence N utilization was lower in the 'sediment' treatment. The lower growth of phytoplankton in the 'sediment' treatment appeared to be related to higher turbidity, and hence lower light availability for growth. The percentage ^{15}N -nitrogen detected in the sediment was only 6% despite the high capacity for sedimentation of the large biomass of plankton detritus and shrimp waste. This suggests rapid remineralization of organic waste by the microbial community in the sediment resulting in diffusion of inorganic

1 N sources into the water column. It is likely that most of the dietary N will ultimately
2 be removed from the tank system by water discharges. Our study showed that ^{15}N -
3 nitrogen derived from aquaculture feed can be processed by the microbial community
4 in outdoor aquaculture systems and provides a method for determining the effect of
5 dietary N on ecosystems. However a significant amount of the dietary N was not
6 retained by the natural biota and is likely to be present in the soluble organic fraction.

7

8 Keywords: stable isotopes, nitrogen, formulated feeds

9

10 **1. Introduction**

11 Current production of farmed shrimp, mostly from southeast Asia and Central
12 America, is 810,000 t p.a. (Rosenberry, 1999). Many shrimp farms have intensive
13 earth ponds stocked with high densities of animals (25 to 100 animals m^{-2}) fed high-
14 protein formulated feeds. About 90% of the nitrogen (N) input to a pond comes from
15 the formulated feed; however, much of the N (70 to 80%) is not retained by shrimp
16 (Briggs and Funge-Smith, 1994). Instead it enters the pond ecosystem and acts as an
17 expensive fertilizer, stimulating the growth of bacteria and plankton (Burford and
18 Glibert, 1999). The resulting microbial and plankton biomass exerts considerable
19 oxygen demand. Unless the ponds are flushed with cleaner water, hypoxic conditions
20 which are detrimental to shrimp growth and health can readily develop.

21

22 The discharge of eutrophic water with high loads of N and suspended solids can
23 damage the coastal environment, particularly in areas with high farm densities, by
24 promoting harmful or nuisance algal blooms, increasing turbidity and creating anoxic
25 conditions. This is the focus of increasing criticism of intensive shrimp farming from

1 environmentalists and environmental regulators worldwide (Naylor et al., 1998). The
2 unregulated expansion of the industry, and a lack of understanding of the
3 environmental consequences have also resulted in mass mortalities of shrimp,
4 significantly affecting production in, for example, China and Taiwan (Chen, 1995;
5 Qingyin et al., 1995). Countries with an emerging shrimp farming industry, such as
6 Australia, are keen to avoid these mistakes by licensing farmers for maximum
7 allowable nutrient and solids discharges, and controlling the density and siting of
8 farms (Preston et al., 1995).

9
10 While the link between nutrients in feed inputs and waste loads is well established,
11 little is known about the nutrient pathways in ponds and adjacent waterways. This
12 information is an essential first step to research aimed at reducing nutrient waste from
13 shrimp farming. Stable isotope ratios, such as those of $\delta^{13}\text{C}$ -carbon and $\delta^{15}\text{N}$ -nitrogen
14 can be used to determine major nutrient sources in natural food webs in marine and
15 freshwater environments (Shearer and Kohl, 1993; Coffin et al., 1994) and
16 aquaculture ponds (Parker et al., 1989). They have been used in pond systems
17 principally to quantify the relative roles of natural and formulated feeds in shrimp and
18 fish nutrition (Lilyestrom et al., 1987; Parker et al., 1989). Labeling by enrichment of
19 N and carbon in ponds provides a method of tracking the fate of these compounds in
20 the natural biota. The use of ^{15}N -enriched feeds to determine N retention by shrimp
21 has already proved successful (Preston et al., 1996).

22
23 Previous studies of N cycling in aquaculture ponds have focussed principally on
24 whole-pond, whole-season N budgets (Boyd, 1985; Neori and Krom, 1991; Briggs
25 and Funge-Smith, 1994). However by using ^{15}N -nitrogen tracers in formulated feeds,

it is possible to trace the link between aquaculture feeds and their effect on N cycling, and ultimately on the environmental loads resulting from shrimp aquaculture. The aim of this study was to trace ^{15}N -nitrogen derived from formulated feed through the biota in an aquaculture ecosystem.

2. Materials and Methods

Experimental design

Preliminary experiment: A preliminary experiment was conducted to determine how effectively ^{15}N -labelled formulated feed can be traced through the natural biota in tanks containing shrimp pond water, sediment and shrimp. This experiment was conducted in November 1996 outdoors at a commercial shrimp farm near a tidal, mangrove-lined creek in subtropical Queensland, Australia ($27^{\circ}30'\text{S}$, $153^{\circ}20'\text{E}$). Six 1 000 l fibreglass tanks (1.15 m dia x 1 m deep) were used for two treatments: one with sediment on the tank floor and one without (three replicates of each). The sediment, which was a mixture of sand, mud and rubble, came from commercial shrimp ponds, and was spread to a depth of 5 cm. The tanks were filled with water pumped from a shrimp pond to a depth of 0.9 m and aerated with air diffusers to ensure that the tanks were well mixed. The tanks were maintained for 1 week before the experiment to stabilize the sediment and allow the natural biota to establish.

Three days before the experiment, tanks were drained and refilled, then shrimp (*Penaeus monodon* Fabricius, mean weight 1.2 g) were added at a density of 50 animals m^{-2} . The total biomass was 60 g m^{-2} . The animals were fed dry, fishmeal-based, formulated feed (7% N content) three times a day (Table 3). The amounts (between 7.6 and 8.8 g d^{-1} tank $^{-1}$) were determined by checking how much feed was

1 left on mesh trays on the tank bottom after 1 to 2 h. Feeding rates were adjusted to
2 minimize overfeeding. Feed enriched with ^{15}N -nitrogen was fed to the shrimp three
3 times a day for the first 3 d (see section on feed preparation), and unenriched feed was
4 used for the remaining 3 d of the experiment. Water quality and sediment variables
5 were sampled or measured every 24 h, except for dissolved oxygen and temperature
6 (measured every 12 h) (Table 1).

7

8 Water samples were maintained on ice and processed in the laboratory within 3 h
9 of sampling. Macrozooplankton were sampled once a day by towing a 90 μm mesh
10 net up through the water column three times and removing samples after each tow.
11 On days 3 and 5, four shrimp were removed from each tank for ^{15}N -nitrogen analyses
12 (Table 2) and replaced with tagged shrimp to maintain the stocking density. Only
13 untagged shrimp were used for ^{15}N -nitrogen analyses.

14

15 The sediment was sampled on day 6 with a 24 mm diameter corer. Six cores to a
16 depth of 5 cm were bulked and mixed into a slurry. Subsamples were taken for
17 bacterial counts, total N and ^{15}N -nitrogen analyses and stored on ice (Tables 1, 2).

18

19 Experiment – Tracing the fate of ^{15}N -enriched feed: Based on the knowledge
20 gained from the preliminary experiment, the main experiment, was conducted in
21 February 1998. This experiment used the same preconditioning of tanks and
22 experimental design as experiment 1 with a few exceptions. Airlift pumps were used
23 to circulate water in the tanks. Two weeks before the experiment, tanks were stocked
24 with shrimp (mean weight 15.30 g) at a density of 25 animals m^{-2} . The total biomass
25 was 380 g m^{-2} . The tanks were drained and refilled on the morning of the experiment.

1 The ^{15}N -labelled formulated feed was fed to shrimp three times over the first 24 h.
2 For the remaining 14 days of the experiment, shrimp were fed unlabelled feed three
3 times a day. Samples were taken for ^{15}N -nitrogen and nutrient analyses, and
4 quantification of the biota (Tables 1, 2). On day 4, 20% of the water was removed to
5 prevent NH_4^+ reaching toxic levels and replaced with the inlet water for the farm.

6

7 Formulated feed preparation

8 Soybean plants were grown in pots in a glasshouse for 4 months until beans had
9 begun developing on the plants. Then ^{15}N -potassium nitrate (98 atom%) was then
10 added as fertilizer. At maturity, beans were harvested, thrashed to remove husks and
11 ground in a blade mill to a grain size of less than 0.7 mm. The ground beans were
12 then extracted three times in hexane at 45°C (first extraction 70 min, second and third
13 extraction 40 min) to remove lipids. After each extraction, the hexane was drained
14 and the beans left to dry in a fume hood at room temperature. They were then spread
15 out on a tray, covered in foil and autoclaved at 121°C, 100 kPa for 15 min.

16

17 The feed formulation is outlined in Table 3. Soybean meal, either unenriched or
18 enriched with ^{15}N -nitrogen was used. Fishmeal was screened through a 0.7 mm metal
19 sieve before adding to the rest of the ingredients. The ingredients were mixed
20 together with water, extruded through a mincer, steamed for 5 min and dried at room
21 temperature for 24 h. The ^{15}N -enrichment level of the feed used in experiment 1 was
22 1.43 atom% excess (ie. isotopic abundance which is greater than the ^{15}N -nitrogen
23 natural abundance value) while in experiment 2 it was 1.95 atom% excess.

24

Sample analyses

Nutrient samples collected from the tanks in both experiments were filtered through 0.45 μm membrane filters and frozen immediately. For chlorophyll a samples, water was filtered through glass fibre filters (GF/F) which were stored frozen. The thawed water and filters were analyzed for nutrients and chlorophyll a (see Table 1). In order to characterize the DN more fully, samples were also processed for molecular weight differentiation. This involved centrifuging filtered water samples for 90 min at 4°C in 10 kDa and 3 kDa centrifugal ultrafiltration devices (Amicon) to give three molecular size fractions, i.e. > 10, 3 to 10 and < 3 kDa. Samples were then analyzed for DN. DON was calculated as the difference between the DN and NH_4^+ plus nitrate plus nitrite concentrations. This method could not be used for many samples in the second experiment because NH_4^+ concentrations were high relative to the DN concentrations, resulting in erroneous and artificially low DON values. The indication of this was higher NH_4^+ concentrations than DN concentrations. The persulfate method used in this study is suited to the range of concentrations of DON and NH_4^+ found in seawater and it not appropriate for significantly higher concentrations. It was, therefore, decided not to calculate DON values when NH_4^+ concentrations reached 200 μM .

Porewater nutrient concentrations were also measured in the main experiment (Table 1). Bulk sediment cores were homogenized to a slurry and centrifuged at 10,000 rpm for 10 min. The supernatant was decanted off, filtered through a 0.45 μm membrane filter, and frozen for nutrient analyses as described above.

Samples for bacteria, phytoplankton and protozoa counts were fixed in 1% glutaraldehyde. The macrozooplankton were frozen in the laboratory. Bacteria and nanoflagellates were counted using epifluorescence microscopy and, in the case of nanoflagellates, quantified in major taxonomic groups. Larger phytoplankton, protozoa and zooplankton were counted using an inverted microscope and quantified in major taxonomic groups (see references in Table 1).

Water samples for ^{15}N analysis were filtered onto pre-combusted GF/F glass fibre filters. Filters, sediment, shrimp and feed samples were dried at 60°C for 24 h and analyzed by mass spectroscopy (Glibert et al., 1991). The filtrate from filtered water samples were also prepared for ^{15}N - NH_4^+ analysis (Main expt. only) by adding saturated magnesium oxide solution and distilling samples into 0.0025 N hydrochloric acid (Glibert and Capone, 1993). The atom% enrichment, ie. the proportion of the total NH_4^+ in the water column which is labelled with ^{15}N -nitrogen, was calculated from the equations of Glibert and Capone (1993). Samples of known volume were also filtered onto pre-combusted glass fibre filters for elemental analysis of particulate N and carbon. The recovery of ^{15}N -nitrogen added to the tanks was determined for the water column, sediment and shrimp samples.

Benthic flux measurements

Two perspex benthic chambers that enclosed ~ 7 l of water (Nicholson et al. 1999) were placed on the sediment in one of the sediment treatment tanks at the end of the main experiment. At 0.5, 1, 2 and 3 h, samples were taken for nutrient analyses. They were filtered through $0.45\ \mu\text{m}$ membrane filters and frozen until analyzed.

NH_4^+ , nitrate/nitrite and phosphate were analyzed colorimetrically as described in the previous section.

Statistical analyses

Water quality data were tested for normality and log transformations were done as required. First-order autoregressive, repeated measures, analysis of variance was performed (SAS software).

3. Results

Preliminary experiment

In the preliminary experiment, the mean water temperature was 26°C and the salinity was 39 (Table 4). Salinity, turbidity, DON and zooplankton numbers were statistically higher in the ‘sediment’ treatment than the ‘no sediment’ treatment.

The particulate fraction in the water column of all tanks became enriched with ^{15}N -nitrogen over the first three days, coinciding with the three days when shrimp were fed ^{15}N -labelled formulated feed (Fig. 1). After this time, the enrichment levels plateaued. There was little variation in the level of enrichment between tanks or treatments.

A mass balance was constructed to account for the pools of ^{15}N -nitrogen at the end of the experiment. In the ‘sediment’ treatment, most of the ^{15}N -nitrogen was in the water column particulates ($35 \pm 1.7\%$), while shrimp contained $21 \pm 3.1\%$ and the sediment contained $13 \pm 2.5\%$ (Table 5). In the ‘no sediment’ treatment, more of the ^{15}N -nitrogen was in the water column particulates ($57 \pm 9.1\%$).

Experiment – tracing the fate of ^{15}N -enriched feed

The results from the preliminary experiment were used to design a second experiment which involved sampling more intensively, and for a longer period. In addition, greater resolution was gained in characterizing the phytoplankton, protozoan and bacterial communities, and the role of these organisms in processing dietary N.

The mean water temperature in this experiment was 29°C while salinity was 32 (Table 6). Turbidity levels were significantly higher by two to three times ($P < 0.005$) in the ‘sediment’ treatment than the ‘no sediment’ treatment, but there was a significant interaction between treatment and time. Oxygen, pH and particulate N values were significantly higher in the ‘no sediment’ treatment than the ‘sediment’ treatment.

NH_4^+ concentrations were significantly higher ($P < 0.005$) in the ‘sediment’ treatment than the ‘no sediment’ treatment but increased at a higher rate in the ‘sediment’ treatment resulting in an interaction between treatment and time (Fig. 2). Nitrate + nitrite concentrations were much lower than NH_4^+ concentrations.

Mean DON concentrations were similar in the ‘sediment’ and the ‘no sediment’ treatments at the start of the experiment (Fig. 3). DON could not be measured for the entire experiment as the high NH_4^+ concentrations resulted in inaccurate DON concentrations. Molecular weight fractionation of DON at the end of the experiment showed that 40% was in the greater than 10 kDa size range, 34% was between 3 and 10 kDa and 26% was less than 3 kDa.

1
2 Urea was a small proportion of the DON concentrations (10%) (Fig. 3). Dissolved
3 primary amines were less than 10% of the DON and mean concentrations were
4 significantly higher ($P < 0.005$) in the 'sediment' treatment than the 'no sediment'
5 treatment. However there was a significant interaction between treatment and time.
6

7 Chlorophyll a concentrations increased dramatically in the 'no sediment' treatment
8 but remained similar in the 'sediment' treatment (Fig. 4). Levels of chlorophyll a
9 were highly correlated with the concentration of particulate N (PN) in the water
10 column ($R^2 = 0.9$, Fig. 5). Approximately 54 μM was non-phytoplankton PN
11 calculated from the extrapolation to a chlorophyll a concentration of zero. When this
12 was compared with the total PN value, approximately 80 and 70% of the PN in the
13 'sediment' and 'no sediment' treatments respectively was phytoplankton.
14

15 Chlorophyll was correlated with turbidity in the 'no sediment' treatments ($R^2 =$
16 0.49). However this was not the case in the 'sediment' treatment ($R^2 = 0.25$). The
17 phytoplankton community was dominated by small cyanobacteria and eukaryotic
18 plankton (defined as picoplankton $< 2 \mu\text{m}$) (Table 6). Small diatom species (> 10
19 μm) and cryptophytes were also common. Numbers of all the dominant
20 phytoplankton groups were higher in the 'no sediment' treatment than the 'sediment'
21 treatment. Bacterial numbers were significantly lower in the 'no sediment' treatment
22 than the 'sediment' treatment. Carbon:nitrogen (C:N) ratios of the particulate matter
23 in the water column were close to the Redfield (1958) ratio of 6.6.
24

1 The nutrient concentrations, bacterial counts and nutrient fluxes were quantified in
2 the sediment of the ‘sediment’ tanks (Table 7). Carbon:nitrogen ratios in the
3 sediment were higher than in the water column. Ammonium was released from the
4 sediment at a rate of $5.38 \text{ mmol m}^{-2} \text{ d}^{-1}$ which was substantially higher than the rate of
5 nitrate/nitrite flux.

6
7 The particulate fractions (< 2 , $2\text{-}10$, $> 10 \mu\text{m}$) in the water column were all
8 enriched within 24 h (Fig. 1). Shrimp were only fed ^{15}N -enriched feed for 24 h, but
9 enrichment levels in the particulate fractions generally increased until day 4, although
10 values were higher in the ‘sediment’ treatment.

11
12 Only 52 to 57% of the ^{15}N -nitrogen added as feed was accounted for in the water
13 column, sediment and shrimp (Table 8). Considerably more ^{15}N -nitrogen was present
14 in the water column particulates in the ‘no sediment’ treatment than the ‘sediment’
15 treatment, and this increased in the ‘no sediment’ treatment until day 9. The
16 proportion of ^{15}N -nitrogen in the shrimp was lower in the ‘no sediment’ treatment
17 than the ‘sediment’ treatment.

18
19 The results from the experiment were used to construct a schematic N budget 14
20 days after feeding ^{15}N -enriched feed for both ‘sediment’ and ‘no sediment’ tanks (Fig.
21 6). This budget incorporates both data from this study and literature for NH_3
22 volatilization (Gross, et al., 1999). It was assumed that ^{15}N was lost from the system
23 via denitrification, NH_3 volatilization and water exchange.

24

4. Discussion

Our experiments showed that ^{15}N -enriched feed fed to shrimp can be detected in the food web. Between 67 and 81% of the N was not retained by the shrimp but entered the food web. It could do so via a number of pathways: direct leaching of N from shrimp faeces; microbial breakdown of feed and shrimp faeces in the sediment; and by shrimp excretion through the gills, mainly in the form of ammonia (Regnault 1987; Burford and Williams, 2001).

The ^{15}N -enriched NH_4^+ was detected in the water column within one day of feeding ^{15}N -enriched feed to shrimp, presumably by shrimp gill excretion. The rapid ^{15}N -nitrogen enrichment of all size fractions of plankton in the water column in our experiments is consistent with preferential assimilation of this NH_4^+ source by phytoplankton (McCarthy, 1977; Glibert et al., 1982; Dortch, 1990). Previous studies have shown that NH_4^+ is rapidly assimilated by phytoplankton in shrimp aquaculture systems (Burford and Glibert, 1999). In addition, previous studies have shown that ^{15}N leached from enriched feed and shrimp faeces is rapidly assimilated by the plankton and/or bacterial communities (Burford and Williams, 2001). In our experiment, the proportion of ^{15}N -nitrogen assimilated in the water column by the fraction less than $2\text{ }\mu\text{m}$ was relatively high, compared with the larger size fractions. This fraction contained picoplankton as well as bacteria, and either or both may have assimilated N.

There were distinct differences in the processing of dietary N in the 'sediment' and the 'no sediment' tanks. One obvious difference was the combination of higher ammonium and turbidity levels, and lower chlorophyll and particulate N

1 concentrations in the 'sediment' treatment than the 'no sediment' treatment. It
2 appeared that the high turbidity levels in the 'sediment' treatment reduced light
3 availability thus limiting the growth of the phytoplankton, and hence their uptake of
4 ammonium relative to the 'no sediment' treatment. The high turbidity in the
5 'sediment' treatments may have been caused by shrimp bioturbating the sediment
6 (Ritvo et al., 1997).

7

8 More ^{15}N -nitrogen was accounted for in the particulate fraction, which was mostly
9 phytoplankton, in the 'no sediment' treatment than the 'sediment' treatment. This
10 was due to both a higher biomass of phytoplankton in the 'no sediment' treatment but
11 also a higher level of ^{15}N -enrichment. It appears that more dietary N was cycled
12 through the phytoplankton community in the absence of sediment. The difference is
13 possibly because a proportion of the ^{15}N -nitrogen was buried in the sediment in the
14 'sediment' treatment, making it unavailable to the phytoplankton. Alternatively it
15 may have been retained in the NH_4^+ pool in the water column since phytoplankton
16 growth, and hence N utilization was lower in the 'sediment' treatment.

17

18 Total N and organic carbon concentrations, and bacterial numbers in the sediment
19 of our tanks were comparable with those in mangrove creeks (Alongi, 1988; 1990).
20 Previous studies suggest that bacterial productivity, rather than biomass, increases
21 with higher N loadings (Moriarty, 1986; Burford et al., 1998). Other studies have
22 shown that N derived from detritus is rapidly remineralized by sediment bacteria in
23 shrimp ponds releasing NH_4^+ into the water column (Burford and Longmore, in
24 press). Additionally N may have been denitrified to produce N_2 gas or volatilized as
25 NH_3 . However low efficiencies of nitrogen removal, via denitrification and NH_3

1 volatilization, have been measured in shrimp ponds (Burford and Longmore, in press;
2 A. Gross, unpubl. data). This compares with a mean of 3.75% of total nitrogen
3 removed via NH_3 volatilization in catfish ponds (Gross, et al., 1999). Further
4 confirmation of these observations is that only a small percentage of the ^{15}N -nitrogen
5 added to tanks was present in the sediment after 2 weeks. This is despite the fact that
6 shrimp feeding products and other pond detritus are likely to accumulate on the
7 sediment floor.

8
9 Intensive pond aquaculture is facing increasing pressure to reduce nutrient loads in
10 coastal waterways (Naylor et al., 1998). An understanding of the role of the natural
11 biota in processing dietary N is an important step in improving feeds and feeding
12 strategies to improve water quality and reduce N discharges. Our study has shown
13 that much of the dietary N, in the form of formulated feed, consumed by shrimp was
14 ultimately not assimilated. Additionally, relatively little of the N was retained in the
15 sediment. Therefore much of the dietary N must inevitably be discharged from ponds.
16 Attempts to reduce N discharges from intensive shrimp ponds must therefore focus on
17 reducing N levels in the water column by promoting removal processes such as
18 nitrification/denitrification, as well as assimilation by autotrophs and heterotrophs,
19 and removal of phytoplankton via biofiltration and/or settling ponds.

20
21 The routine water discharges practiced by farmers is, in part, a response to high
22 ammonium, and hence toxic ammonia levels, in ponds. Our study has shown that the
23 high turbidity caused by the presence of sediment reduces the effectiveness of
24 phytoplankton uptake of ammonium. This is coupled with evidence that sediment
25 remineralization of wastes, particularly from the sludge, produces significant amounts

1 of ammonium (Burford and Longmore, in press). Therefore the use of lined ponds, or
2 improved bank stabilization and sludge removal are likely to be highly effective at
3 reducing ammonium concentrations.

4
5 In summary, our experiments have demonstrated that isotope tracing coupled with
6 quantifying the natural biota, is a powerful technique for determining the role of the
7 natural biota in processing dietary N in aquaculture systems. The phytoplankton and
8 sediment microbial community appeared to play the most important roles in N
9 cycling. The inter-relationship of these roles had a major effect on the fate of dietary
10 N.

11
12 There are still gaps in our knowledge, particularly in relation to quantifying dietary
13 N in the DN fractions. Our results demonstrate that current shrimp farming systems
14 are inefficient at converting dietary N, in the form of soybean, into shrimp biomass.
15 However, the enrichment of other ingredients in the formulated feed, such as the more
16 digestible fishmeal, might have reduced the ^{15}N -dietary N waste. This study has
17 made the first step towards monitoring nutrient processes accurately, with the aim of
18 improving feeding efficiency and minimizing adverse environmental impacts from
19 feed waste.

1

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Table 1: Parameters measured in the water column and sediment of tanks in

experiments 1 and 2. * analyzed only in second experiment. NH_4^+ = ammonium, NO_2^- = nitrite, NO_3^- = nitrate, DPA = dissolved primary amines, DN = dissolved nitrogen.

Variable	Frequency	Technique	Reference
<u>Water column</u>			
NH_4^+	every 24 h	Phenol/hypochlorite	APHA (1989)
$\text{NO}_2^-/\text{NO}_3^-$	every 24 h	Cadmium reduction	APHA (1989)
Urea	every 24 h	Diacetyl monoxime	Rahmatullah and Boyde (1980)
DPA*	every 24 h	OPA/spectrofluorometer	Parsons et al. (1984)
DN	every 24 h	Persulfate oxidation/ Cadmium reduction	Parsons et al. (1984)
Phosphate	every 24 h	Ascorbic acid/antimony	APHA (1989)
Chlorophyll a	every 24 h	Acetone extraction	Jeffrey and Welshmeyer (1997)
Bacterial No.	every 24 h	AODC	Hobbie et al. (1977)
Phytoplankton & protozoa No.	every 24 h	Epifluorescence & Light microscopy	MacIsaac and Stockner (1993)
Zooplankton	every 48 h	Microscopy	
pH	every 24 h	Logger (TPS)	
Salinity	every 24 h	Logger (TPS)	
Turbidity	every 24 h	Logger (TPS)	
Oxygen	every 12 h	Logger (TPS)	
Temperature	every 12 h	Logger (TPS)	
<u>Sediment</u>			
Bacterial No.	end	AODC	Hobbie et al. (1977)
Total N, C	end	CHN analyzer	
Porewater*			
($\text{NH}_4^+/\text{NO}_3^- + \text{NO}_2^-$)	end	as per water samples	APHA (1989)

Table 2: ^{15}N -nitrogen sampling times (days) in experiments 1 and 2.

Variable	Expt 1	Expt 2
Water column particulates (total)	2,3,4,6	
> 10 μm water fraction		2,5,9,15
< 10 μm water fraction		2,5,9,15
< 2 μm water fraction		2,5,9,15
NH_4^+		2,5,9,15
Sediment	4,6	15
Sediment porewater NH_4^+		15
Sedimented material		15
Shrimp	4,6	15
Formulated feed	1	1

Table 3: Formulation of feed. *When ^{15}N -enriched soybean was used it replaces the whole portion.

Ingredient	% dry wt
Soybean*	45
Wheat	22
Fishmeal (Peru)	15
Gluten	7
Squid meal	5.7
Squid oil	3.7
Lecithin	1.2
Cholesterol	0.2
Vitamin premix	0.2

Table 4: Mean water quality and sediment parameters ($\pm$ s.e.) in the ‘sediment’ and ‘no sediment’ treatments in the preliminary experiment. Significantly higher values when an asterisk is present. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, ⁺interaction between treatment and time. nd = not detectable.

Parameter	‘Sediment’	‘No sediment’
<u>Water column</u>		
<u>Physical & Chemical</u>		
Temperature (°C)	26.0 (0.58)	25.9 (0.59)
pH	8.0 (0.01)	8.0 (0.01)
Salinity	38.6 (0.08)***	38.9 (0.09)
Turbidity (ntu)	36 (3.9)*** ⁺	11 (0.2)
Oxygen (mg l ⁻¹)	5.7 (0.06)	5.6 (0.07)
NH ₄ ⁺	1.0 (0.11)	1.1 (0.09)
NO ₂ ⁻ /NO ₃ ⁻	nd	nd
DON	72.3 (4.47)***	60.3 (3.55)
Urea	0.6 (0.05)	0.5 (0.05)
Phosphate (μM)	< 0.3	< 0.3
Particulate N (μM)	164.1 (2.97)	156.7 (4.47)
Particulate molar C:N ratio	6.4 (0.1)	6.2 (0.1)
<u>Biological</u>		
Chlorophyll <u>a</u>	75.7 (9.29)	121.5 (8.79)
Bacteria (x 10 ⁷ ml ⁻¹)	2.6 (0.18)	2.4 (0.18)
Ciliates (l ⁻¹)	990 (280)	1170 (260)
Zooplankton (animals l ⁻¹)	23 (3)*** ⁺	5 (1)
Shrimp (animals m ⁻²)	50	50
Shrimp wt (g animal ⁻¹)	1.2 (0.03)	1.2 (0.06)
Feed input (g N m ⁻² d ⁻¹)	0.2	0.2
<u>Sediment</u>		
TN (mmol m ⁻²)	3870 (1030)	
Organic C (mmol m ⁻²)	46440 (1002)	
C:N ratio	12.1	
Bacteria (x 10 ⁷ m ⁻²)	7.5 (0.76)	

Table 5: Mean percentage ($\pm$ s.e.) of added ^{15}N -nitrogen (as formulated feed) present in tank system on day 6 in the preliminary experiment. Other = ^{15}N -nitrogen unaccounted for. W/C = water column.

Day	W/C Particulates	Shrimp	Sediment	Other
‘Sediment’ treatment				
6	35 (1.7)	21 (3.1)	13 (2.5)	31
‘No sediment’ treatment				
6	57 (9.1)	21 (7.3)	-	12

Table 6: Mean water quality parameters ($\pm$ s.e.) in main experiment. Significantly higher values when an asterix is present. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

⁺interactions between treatment and time.

Parameter	‘Sediment’	‘No sediment’
<u>Physical & Chemical</u>		
Temperature (°C)	29.2 (0.31)	29.2 (0.28)
pH	7.5 (0.04)	8.0 (0.04)*** ⁺
Salinity	31.9 (0.15)	31.9 (0.14)
Turbidity (ntu)	137 (5.8)***	64 (4.2)
Oxygen (mg l ⁻¹)	4.5 (0.10)	5.0 (0.09)*
Phosphate (μM)	0.3 (0.08)	0.4 (0.10)
Particulate N (μM)	169 (11)	314 (37)*** ⁺
Particulate molar C:N ratio	6.9 (0.1)	6.5 (0.1)
<u>Biological</u>		
Bacteria (x 10 ⁷ ml ⁻¹)	2.3 (0.37)*** ⁺	1.4 (0.24)
Cyanobacteria (x 10 ⁵ ml ⁻¹)	1.3 (0.60)	10.9 (1.31)***
Diatoms (x 10 ⁵ ml ⁻¹)	0.2 (0.12)	0.2 (0.05)
Cryptophytes (x 10 ⁵ ml ⁻¹)	0.1 (0.07)	0.6 (0.21)***
Eukaryotic picoplankton (x 10 ⁵ ml ⁻¹)	0.5 (0.10)	8.2 (1.80)*** ⁺
Ciliates (ml ⁻¹)	160 (60)	730 (180)* ⁺
Dinoflagellates (ml ⁻¹)	130 (60)	1690 (590)
Euglenoids (ml ⁻¹)	1930 (780)	810 (540)
Zooplankton (animals l ⁻¹)	5 (2)	8 (3)
Shrimp (animals m ⁻²)	25	25
Shrimp wt (g animal ⁻¹)	17.2 (0.72)	16.6 (0.61)
Feed input (g N m ⁻² d ⁻¹)	1.3	1.3

Table 7: Sediment characteristics (mean $\pm$ s.e.) and benthic fluxes of ‘sediment’ tanks in the main experiment.

Parameter	
TN (mmol m^{-2})	4400 (310)
Organic C (mmol m^{-2})	49720 (2810)
C:N ratio	11.3
Porewater NH_4^+ (μM)	3180 (320)
Porewater NO_{2+3}^- (μM)	4.0 (3.18)
Bacteria ($\times 10^7 \text{ m}^{-2}$)	3.1 (0.44)
NH_4^+ flux ($\text{mmol m}^{-2} \text{ d}^{-1}$)	5.38
NO_{2+3}^- flux ($\text{mmol m}^{-2} \text{ d}^{-1}$)	-1.06
Denitrification efficiency (%)	53

Day	W/C Particulates	Shrimp		Sediment	Settled Matter	NH ₄ ⁺	Other
‘Sediment’ treatment							
2	4 (0.6)	nd	nd	-		1 (0.6)	
5	4 (0.5)	nd	nd	-		5 (4.7)	
9	2 (0.5)	nd	nd	-		2 (0.4)	
15	2 (0.7)	33 (2.6)		6 (0.6)	-	6 (4.2)	53
‘No sediment’ treatment							
2	5 (0.7)	nd	-	nd		1 (0.0)	
5	12 (1.3)	nd	-	nd		4 (1.9)	
9	19 (5.4)	nd	-	nd		1 (0.3)	
15	16 (4.9)	24 (3.9)	-	-	2 (0.2)	0.4 (0.4)	57.6

Figure Caption:

Fig. 1. Mean ($\pm$ s.e.) ^{15}N -nitrogen enrichment (atom% excess) of particulates in the water column (W/C particulates) in tanks $\pm$ sediment in the preliminary experiment and the main experiment. Arrows show the number of days during which shrimp were fed. In the second experiment, the particulates were size fractionated (> 10 , $2-10$, < 2 μm).

Fig. 2. Mean ($\pm$ s.e.) NH_4^+ and nitrate/nitrite concentrations (μM) in tanks with and without sediment over the course of the main experiment. Statistical differences between treatments noted on graphs. $^+$ denotes interaction of treatment and time.

Fig. 3. Mean ($\pm$ s.e.) dissolved organic nitrogen (DON), urea and DPA concentrations (μM) in tanks with and without sediment in the main experiment. Statistical differences between treatments noted on graphs. $^+$ denotes interaction of treatment and time.

Fig. 4. Mean ($\pm$ s.e.) chlorophyll a concentrations ($\mu\text{g l}^{-1}$) in tanks with and without sediment over the course of the main experiment. Statistical differences between treatments noted on graphs. $^+$ denotes interaction of treatment and time.

Fig. 5. Linear regression of chlorophyll a ($\mu\text{g l}^{-1}$) and particulate N (PN, μM) concentrations in the water column in the main experiment.

Fig. 6. N budgets for the sediment and water column (% ^{15}N inputs) in tanks (a) with sediment and (b) without sediment in the main experiment.