The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero exchange system

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Abstract

High intensity, zero exchange shrimp ponds contain a high density of flocculated particles, rich in bacteria and phytoplankton, compared with flow-through systems. The flocculated particles provide a potential food source for shrimp. Short term tank experiments were conducted to determine the retention of nitrogen (N) from natural biota, dominated by flocculated particles, in white shrimp (*Litopenaeus vannamei*) at a high intensity, zero exchange shrimp farm in Central America (Belize Aquaculture Ltd (BAL)). There were two treatments: 'floc' and 'floc + 20%' (3 x 1000 l replicate tanks each) based on two densities of flocculated particles. The floc density in the 'floc' treatment was typical of shrimp growout ponds at BAL, whereas the 'floc + 20%' treatment had a 20% higher density of flocculated particles. Three consecutive experiments were conducted with 1, 5 and 9 g shrimp respectively. At the start of the experiment, ^{15}N-ammonium was added to the tanks and assimilated by the natural biota. Shrimp were maintained in these tanks for 48 h after the ^{15}N-nitrogen enrichment. After this time, shrimp were found to be enriched with ^{15}N-nitrogen. It was calculated that between 1 and 3% of the particulate nitrogen in the tanks, principally from the flocculated particles, was retained by the shrimp. The proportion of estimated daily nitrogen retention of the shrimp contributed by the natural biota was calculated to be 18 to 29% for 1 to 9 g animals in the floc treatment. There was a tendency for greater retention in the floc + 20% treatments, but this trend was not consistent. This study suggests that natural biota, which in this system was largely flocculated particles, can contribute substantially to the nutrition of *L. vannamei*. There are, therefore benefits for shrimp in the promotion of flocculated particles in *L. vannamei* ponds. Whether this translates into improvements in shrimp growth and production efficiency remains to be established.

1. Introduction

Shrimp farming is a major industry in tropical and subtropical areas around the world with current production estimated at 1,090 Mt p.a. (FAO, 2000). However there are concerns about the ecological sustainability of shrimp farming including the discharge of nutrient-rich waters into coastal waters that may result in the deterioration of ecosystem health (Eng et al., 1989; Naylor et al., 1998). The industry is, therefore, under increasing pressure from resource managers and non-government organizations to improve their environmental credentials while still needing to remain viable and profitable businesses.

One approach to improving sustainability has been the development of high-intensity growout systems with no water discharge during the crop cycle. This technology was developed at an experimental scale through the 1980's and 1990's (Aquacop, 1985; Wyban and Sweeney, 1990; Hopkins et al., 1993; Sandifer and Hopkins, 1996). In the mid-90's a commercial shrimp farm, BAL in Belize, Central America, was designed and constructed using this technology (McIntosh et al., 1999). BAL developed an integrated approach to farming shrimp using high health, selectively bred stocks, low-protein (~ 20%) feed, high stocking densities in ponds, no water exchange throughout the growth season and cycling of water through treatment ponds at harvest time. Harvest tonnages have averaged 15 t ha^{-1} $crop^{-1}$ (Browdy et al., 2001).

Litopenaeus vannamei, the species grown in this system, has a protein requirement reported to be between 30 to 44%, depending on the study, and the size of the shrimp

(Guillaume, 1997; Rosas et al., 2001). However, reducing protein levels from 40 to 20% in ponds resulted in acceptable levels of shrimp production in several outdoor trials, possibly because natural foods were also supplying protein (Hopkins et al., 1995; Teichert-Coddington, 1995). At BAL, three feeds are used: two fish meal-based feeds, and a grain feed, the proportion of each being applied to a pond varying with crop age. In theory, the protein shortfall is met by the natural biota in the ponds, particularly the flocculated material which contains high numbers of bacteria and senescent phytoplankton (Burford et al., 2003). Studies using stable isotopes have shown that the natural biota can contribute to shrimp nutrition in less intensive systems (Parker et al., 1989, 1991; Cam et al., 1991; Burford, 2000). This short term feeding study sought to determine the retention of nitrogen from the natural biota, particularly the flocculated particles, by *L. vannamei* in BAL ponds.

2. Materials and Methods

2.1. Facility

The BAL production facility is located in the Stann Creek District of Belize, in Central America (17°20'N, 88°30'W). The lined 1.8 m deep ponds were stocked with disease resistant white shrimp (*L. vannamei*) at a typical density of 120 animals m^{-2} (Browdy et al., 2001). Ponds were aerated with paddlewheels and propeller-aspirators (50 hp ha^{-1}). Sludge was removed via central drains and siphons. Lime, iron and silicate were added to promote the formation of flocculated particles. No water was exchanged during the growth season.

Three feeds were added to the ponds: a low-protein (18-22%), grain feed made from soybean meal, wheat grain and corn; and two fish meal-based feeds containing

24 and 31% protein, respectively. The 31% protein feed was added early in the growth season and was replaced with 24% feed when the shrimp were 4 g in weight (Jory et al., 2001). The grain feed was fed throughout the season, the proportion, relative to other feeds, decreasing as the shrimp increased in size. Grain constituted 90% of the feed at the start of the season, reduced to 25% by the end of the season. Feeding rates were determined by feed trays. High protein feed was added to the ponds three times a day, grain feed two times a day.

2.2. Feeding experiments

A feeding experiment was conducted to determine the amount of nitrogen derived from natural biota, principally flocculated material, retained by shrimp over 48 h in the presence of formulated feed. This involved the addition of the stable isotope, ^{15}N-nitrogen, to tanks with shrimp pond water and shrimp, which in turn became labelled with the isotope. At the end of the experiment, the amount of ^{15}N-nitrogen accumulated in shrimp in the tanks was determined.

Water from a commercial pond (pond 5), which had been regularly sampled as part of a study of the microbial community and nutrient cycling, was used (Burford et al., 2003). There were two treatments testing two densities of flocculated material, to be known as ‘floc’ and ‘floc + 20%’ treatments. The ‘floc’ treatment had water pumped from pond 5 into 3 x 1000 l square outdoor tanks (0.8 m deep) while the ‘floc + 20%’ treatment tanks (3 x 1000 l square tanks) were filled with water from the same pond used for the ‘floc’ treatment, plus concentrated flocculated material giving the same final volume as the ‘floc’ treatment tanks. The flocculated material was concentrated by passing 250 l of water from pond 5 through a 10 μm mesh size nylon

mesh and collecting the material trapped on the screen. Tanks were vigorously aerated with airstones to ensure that the flocculated material remained in the water column and did not settle on the tank floor.

Water samples were collected from the pond at the start of the study for bacterial, phytoplankton and protozoan counts, and chlorophyll *a* and phaeopigment analysis. For bacterial and phytoplankton counts, a subsample was fixed in 1% borate buffered formaldehyde and stored at 4°C. For chlorophyll *a* and phaeopigment analysis, subsamples were filtered onto GF/F glass fibre filters and frozen. Water samples were also collected from the tanks and fixed in 1% borate buffered formaldehyde for measurements of the density of flocculated particles prior to the addition of shrimp.

Shrimp (approx. 1 g ea.) were collected from commercial growout ponds using cast nets and added to each tank (60 individuals $tank^{-1}$) to give a density equivalent to the stocking density of the ponds, i.e. 120 animals m^{-2}. Each tank was fed the same 31% protein formulated feed as that used in the commercial ponds (feed formulation, Jory et al., 2001) twice a day from stocking of the shrimp and throughout the experiment at a rate based on the percentage of biomass, i.e. 14% of biomass d^{-1} for 1 g animals (Jory et al., 2001). Shrimp were acclimatized to the tanks for approx. 15 h prior to the start of the experiment when the stable isotope tracer, ^{15}N-ammonium was added. A parallel study at BAL showed that the natural biota in the shrimp pond water becomes enriched with ^{15}N-tracer in 1 to 2 h (Burford et al., 2003). ^{15}N-ammonium chloride (120 mg 98% ^{15}N) was dissolved in deionized water and 20 mg was added to each tank to give a theoretical addition amount of 10% of the total ammoniacal nitrogen concentration (TAN) (based on a spot check with the Hach

126 salicylate method). At the same time, the tank water was also sampled for TAN
127 analysis using the more precise phenate method. Water was filtered through 0.45 μm
128 membrane filters and frozen for subsequent analysis. A datalogger (Yeokal) was
129 deployed to measure temperature, salinity, pH and oxygen at 0600 and 1400 h each
130 day for the duration of the experiment.
131
132 The experiment was run for 48 h after the addition of ^{15}N-ammonium chloride.
133 Water samples were taken to determine the density of flocculated material after 24
134 and 48 h. At the end of the experiment, water was also filtered onto precombusted
135 glass fibre filters (GF/F) for total particulate nitrogen and $^{15}N/^{14}N$ isotope analyses.
136 Tanks were drained and shrimp were trapped with nets and individually weighed.
137 Twenty shrimp from each tank were placed in seawater for ~2 h to allow gut
138 evacuation. After sacrificing the shrimp, they were dried at 60°C and ground using a
139 mortar and pestle for $^{15}N/^{14}N$ isotope and total nitrogen analyses.
140
141 The experiment was repeated twice within 8 d using the protocol outlined above
142 but with 5 and 9 g animals. Water from the same pond and the same 31% formulated
143 feed was used. The feeding rates were 5% of biomass (5 g animals) and 4% of
144 biomass (9 g animals) d^{-1} as per the commercial ponds (Jory et al., 2001).
145
146 *2.3. Analyses*
147 The density of flocculated particles, phytoplankton and protozoa was determined
148 by counting particles using a Sedgewick Rafter chamber under phase-contrast
149 microscopy (Burford et al., 2003). Flocculated particles were identified as clumps of
150 material with sufficient integrity to withstand moderate agitation. Loose aggregations

of cells were not counted. Total bacterial counts were determined by staining with acridine orange and counting under epifluorescence microscopy (Hobbie et al., 1977). Samples were counted before and after sonication (1 min duration) to determine the number of bacteria associated with flocculated particles (Burford et al., 2003). Chlorophyll *a* and phaeopigments were determined by extraction of filters with sonication (1 min duration) in acetone, then measuring the extract spectrophotometrically before and after acidification with 0.1N hydrochloric acid (Jeffrey and Welshmeyer, 1997).

Filtered water samples were transported to the CSIRO Marine Laboratories, Australia. TAN was analyzed using the phenate method (4500-NH3 G) (American Public Health Association, 1995).

Glass fibre filters for stable isotope analysis were dried at 60°C for 24 h. Shrimp and filters were analyzed for $^{15}N/^{14}N$ isotope ratios using a mass spectrometer and particulate nitrogen using a CHN analyzer.

2.4. Calculations

The total amount of nitrogen in the natural biota in the tanks was determined by multiplying the particulate N concentrations by the tank volume. Particulate nitrogen was assumed to be principally in the flocculated particles since particulate nitrogen was correlated with the density of flocculated particles but not correlated with chlorophyll *a* in the BAL ponds (Burford et al., 2003).

175 The amount of nitrogen derived from natural biota retained by the shrimp (*F*, mg
176 N g shrimp^{-1} d^{-1}) was calculated as:

177 $$F = (e * n)/(f * wt * d) \quad (1)$$

178 where e = ^{15}N-ratio (atom% excess) of the shrimp
179 n = total amount of nitrogen in shrimp (mg)
180 f = ^{15}N-ratio (atom% excess) of the natural biota
181 wt = weight of prawns in tank (g)
182 d = number of days of trial
183
184 The percentage of total particulate nitrogen in the tanks retained by the
185 shrimp (L) over the 48 h period was therefore calculated as follows:
186 $$L = (F * wt * d * 100)/(N) \quad (2)$$
187 where N = total nitrogen content of the tank (mg)
188

189 **3. Results**

190 Water temperature, oxygen and pH in the tanks measured at 0600 and 1400 h were
191 similar in all three experiments (Table 1). Salinity was high (42.5) but constant
192 between experiments. TAN concentrations ranged from 0.01 to 0.14 mg l^{-1} but there
193 was no consistent difference between the 'floc' and 'floc + 20%' treatments or
194 between experiments (Table 2).
195
196 The density of flocculated particles in the water in the 'floc' treatment was similar
197 in experiments 1 and 2 (6300 and 6700 particles ml^{-1}) and approximately 40 to 50%
198 lower in experiment 3 (Table 2). Densities were 25 to 50% higher in the 'floc + 20%'
199 treatment. The mean size of the flocculated particles in all experiments was 270 μm.

Total bacterial counts in the pond water used in the experiments ranged from 3.64 to 5.06 x 10^7 ml^{-1} and 27 to 51% of the bacteria were associated with flocculated particles. Total phytoplankton counts in the pond water ranged from 12 to 40 x 10^4 ml^{-1} and was comprised principally of chlorophytes, chrysophytes and cryptophytes all less than 20 μm in size. Protozoa counts in the pond water were lower, 0.2 to 0.9 x 10^4 ml^{-1}, and dominated by heterotrophic nanoflagellates, with few ciliates or rotifers.

Chlorophyll *a* and phaeopigment concentrations in the pond water used in the experiment ranged from 164 to 314 μg l^{-1}, and 544 to 791 μg l^{-1} respectively (Table 2). In experiments 1 and 2 (1 and 5 g animals), densities of flocculated particles in both the 'floc' and 'floc + 20%' treatments were relatively constant over the first 24 h then decreased (Figs. 1a, b). In contrast, in experiment 3 (9 g animals), the density of flocculated particles changed little (Fig. 1c).

Particulate nitrogen concentrations ranged from 6.79 to 11.18 mg l^{-1} between the 'floc' treatments at the end of the experiments with ^{15}N-enrichment levels of between 0.157 and 0.267 atom% excess (Table 2). Particulate nitrogen concentrations ranged from 7.63 to 11.00 mg l^{-1} between the 'floc + 20%' treatments with ^{15}N-nitrogen enrichment levels of between 0.134 and 0.294 atom % excess. Particulate nitrogen concentrations were correlated with the density of flocculated particles across treatments (R^2 = 0.85), but not with chlorophyll *a* (R^2 = 0.01). This suggests that most of the particulate nitrogen was present in the flocculated particles rather than free-living phytoplankton. There was no correlation between the level of ^{15}N-nitrogen enrichment and the concentrations of flocculated particles.

Mean shrimp weights were 1.07, 5.03 and 8.85 g in experiments 1, 2 and 3 respectively (Table 2). Weights were similar in the 'floc' and 'floc + 20%' treatments. Shrimp survival ranged from 91 to 99%. Shrimp were enriched with ^{15}N-nitrogen at levels ranging from 0.003 to 0.010 atom% excess (Fig. 2). The ^{15}N-nitrogen enrichment levels were higher in 1 g shrimp than 5 and 9 g shrimp, and statistically higher in the 'floc + 20%' treatment than the 'floc' treatment for the 9 g animals. The reverse was true for the 5 g animals. There were no statistical differences ($P > 0.05$) between treatments for the 1 g animals.

The percentage of the particulate nitrogen in the water column of each tank that was retained by shrimp was calculated to be between 1.4 and 2.6% for the 'floc' treatment, and between 1.6 and 2.0% for the 'floc + 20%' treatment with little variation between replicate tanks (Table 2). Based on these values, the theoretical reduction in the density of flocculated particles at the end of the experiment was compared with the measured densities (Fig. 1a,b,c). In general, the reduction in the density of flocculated particles was greater than that due to shrimp feeding, except in experiment 3.

4. Discussion

The presence of enriched ^{15}N-nitrogen in the shrimp 48 h after the addition of isotope to tanks in our study demonstrated that nitrogen derived from the natural biota was retained by *L. vannamei*. Much of the biomass of natural biota in the water column of the pond water used in the tank experiments was flocculated particles, which contain bacteria and dead phytoplankton. The densities of flocculated particles in these

249 experiments were equivalent to the highest densities measured in BAL ponds in a
250 parallel study (Burford et al., 2003).
251
252 The growth of the natural biota in BAL ponds was promoted by the routine addition
253 of grain feed (18-22% protein) and molasses as carbon sources, and fish meal-based
254 feeds and ammonia from shrimp excretion as nitrogen sources. This resulted in
255 densities of bacteria and phytoplankton higher than those in conventional shrimp
256 ponds (Moriarty, 1986; Burford, 1997, 2000). A 3 week study of BAL ponds showed
257 that these biota bloom and crash resulting in fluctuating net autotrophic
258 (phytoplankton dominated)/heterotrophic (bacterial dominated) system (Burford et al.,
259 2003). The flocculated particles are promoted by the addition of silicate and lime
260 (Browdy et al., 2001), and are composed of a mixture of bacteria, senescent
261 phytoplankton, protozoa and inorganic particles (Burford et al., 2003). A previous
262 study showed that flocculated particles from BAL ponds had a high protein content
263 (43%) and contained levels of some essential amino acids similar to those found in
264 whole shrimp homogenates, ie. arginine (2%), methionine (0.5%) and lysine (2.1%)
265 (Jory et al., 2001).
266
267 The contribution of natural biota to the nitrogen retention by shrimp over 48 h was
268 estimated by determining the ^{15}N-labelled nitrogen in the shrimp as a proportion of
269 the theoretical total daily protein retention. Firstly, protein retention (*P*, mg protein g
270 shrimp^{-1} d^{-1}) was calculated as:
271 $$P = i * p * r \quad (3)$$
272 *i* = daily food intake, for 1 g shrimp the daily food intake was assumed to be 14%
273 d^{-1} (Jory, 1995)

274 p = protein level in the formulated feed (31%)

275 r = protein retention efficiency, assumed to be 40% (Mendoza et al., 2001).

276 This equates to 17 mg protein retained g shrimp^{-1} d^{-1} for 1 g shrimp.

277

278 Secondly, the amount of protein derived from natural biota retained by the shrimp

279 (S, mg protein g shrimp^{-1} d^{-1}) was calculated from formula (1) in the Results section

280 as follows:

281 $$S = F * 6.25 \quad (4)$$

282 6.25 is the value for conversion from nitrogen to protein. This equates to 3.7 and 4.7

283 mg protein shrimp^{-1} d^{-1} for the 'floc' and 'floc + 20%' treatments respectively. It was

284 assumed that protein was the main form of nitrogen assimilated. Additionally, it was

285 assumed that protein turnover was low so that little of the nitrogen was likely to be

286 metabolised over 48 h (Smith and Dall, 1991; Hewitt, 1992).

287

288 Given these assumptions, the percentage of daily protein retention contributed by the

289 natural biota (R) was calculated as follows:

290 $$R = (S * 100) / P \quad (5)$$

291 This equates to 22 and 28% of daily protein retention for the 'floc' and 'floc + 20%'

292 treatments, respectively.

293

294 Using the same formula for 5 g shrimp but assuming a daily feed intake of 5% of

295 body weight, 29 and 26% of daily retention for the 'floc' and 'floc + 20%' treatments

296 respectively was attributable to the natural biota. For 9 g shrimp, assuming a daily

297 intake of 4%, 18 and 22% of daily retention for the 'floc' and 'floc + 20%' treatments

298 was attributable to natural biota. Based on these estimates, natural biota, which was

299 dominated by flocculated particles, do contribute substantially to nitrogen retention
300 irrespective of the shrimp size. While the data supports this statement, it is not
301 possible to compare the nitrogen retention rates relative to the size of the animals
302 since the experiments with different size animals were not conducted simultaneously,
303 but over an 8 d period, and the nutritional quality of the pond water and flocculated
304 particles may have changed over time.
305
306 Protein levels in *L. vannamei* diets have been reduced from the reported protein
307 requirements of 30 to 44% (Guillaume, 1997) to 22% in high intensity, zero-exchange
308 ponds, including BAL ponds, based on the theory that the flocculated particles in
309 these systems contribute substantially to shrimp nutrition (Hopkins et al., 1995;
310 McIntosh and Avnimelech, 2001). Our study supports the theory that natural biota
311 can provide a nitrogen source for shrimp, and that flocculated particles are likely to be
312 a significant proportion of this. However, despite the grazing by shrimp, the density
313 of flocculated particles was not substantially reduced. Less than 3% of the flocculated
314 particles in the tanks were consumed by shrimp irrespective of the treatment.
315 Phytoplankton and protozoa in the water column may also provide a food source for
316 shrimp. However, given the relatively small size of the phytoplankton (< 20 μm) and
317 low numbers of protozoa, it is unlikely that 1 to 9 g shrimp were targeting and
318 consuming significant numbers.
319
320 Previous studies have shown that growth rates of *L. vannamei* grown in pond water
321 were higher than in water with a low load of particulate organic matter (Leber and
322 Pruder, 1988; Moss and Pruder, 1995; Otoshi et al., 2001). The presence of structures
323 with epiphytic growth also increased *L. vannamei* production (Bratvold and Browdy,

2001). In semi-intensive *L. vannamei* and *Penaeus setiferus* ponds, 52% of the retained nitrogen was supplied by the pond biota (Parker et al., 1989, 1991). The consumption of detrital matter has previously been shown for other shrimp species (Moriarty and Barclay 1981; Focken et al., 1998). However, less is known of the role of the natural biota in supplying the other nutrients required by shrimp, such as lipid, minerals, and vitamins.

There have been few previous studies tracing ^{15}N-enriched dietary sources into cultured shrimp species. ^{15}N nitrogen labelling of natural food has been used to determine the contribution of this source to the nutrition of *Penaeus monodon* and *Litopenaeus vannamei* in tank systems (Burford, 2000; Epp et al., 2002). In the case of *P. monodon*, between 4 and 10% of the ^{15}N-enriched natural biota was retained by shrimp after 48 h. Using a similar technique with ^{13}C-carbon enrichment, carbon from the natural biota has been shown to contribute up to 30% of the feed intake for *Marsupenaeus japonicus* (Cam et al., 1991). The main differences in these studies compared with our study were the lower densities of flocculated material, lower stocking densities, and the presence of earthen floors. Tracer studies have also been done to determine the short- and long-term retention of ^{15}N-enriched formulated feeds by *P. monodon* (Preston et al., 1996; Burford and Williams, 2001; Burford et al., 2002).

In conclusion, our study suggests that *L. vannamei* are capable of ingesting and retaining nitrogen derived from natural biota. A significant proportion of the natural biota in our study was flocculated particles, and it is likely that they were a dominant contributor. However, further research is needed to determine the optimal ways to

produce natural biota, principally flocculated particles, and optimize the nutritional composition. It would also be beneficial to determine the role of natural biota in supplying the other nutritional requirements of the shrimp, and ultimately to determine the effect of the natural biota on shrimp growth.

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485

486 **Figure Legend**

487

488 Figure 1: Mean (± SD) density of flocculated particles for the ‘floc’ and ‘floc + 20%’
489 treatments prior to and during the 48 h feeding experiments with (a) 1, (b) 5
490 and (c) 9 g shrimp. *Solid triangles and squares show theoretical densities
491 of flocculated particles based on calculated consumption of flocculated
492 material by shrimp.

493

494 Figure 2: ^{15}N-nitrogen enrichment (atom% excess) in shrimp after 48 h in the ‘floc’
495 and ‘floc + 20%’ treatments in three experiments with shrimp weights of 1,
496 5 and 9 g.

Table 1: Physical parameters (mean) at 0600 and 1400 h in the tanks containing *L. vannamei* during the three feeding experiments at BAL.

Parameter		Experiment 1	2	3
Water temperature (^oC)	0600 h	27.3	27.1	27.5
	1400 h	28.9	28.7	30.2
Oxygen concentration ($mg\ L^{-1}$)	0600 h	5.6	5.5	5.6
	1400 h	5.4	6.1	5.7
pH	0600 h	7.4	7.4	7.3
	1400 h	7.9	7.8	8.1
Salinity		42.5	42.5	42.5

Table 2: Biological parameters (mean ± SD, n = 3) in pond water used to fill tanks, and at the start and end of each experiment for the 'floc' and 'floc + 20%' treatments. TAN = total ammoniacal nitrogen, W/C = water column.

Parameter		Experiment 1	2	3
Pond 5 water				
Total bacterial count (x 10^7 ml^{-1})		5.06	3.64	4.36
% total W/C bacteria assoc. floc.		51	27	35
Total phytoplankton count (x 10^4 ml^{-1})		16	12	40
Total protozoa count (x 10^4 ml^{-1})		0.9	0.2	0.2
Chlorophyll *a* conc. (μg l^{-1})		164	314	207
Phaeopigment conc. ((μg l^{-1})		544	539	791
Start of experiment				
TAN (mg l^{-1})	Floc	0.01 (0.007)	0.14 (0.006)	0.08 (0.013)
	Floc + 20%	0.04 (0.007)	0.12 (0.008)	0.04 (0.025)
Floc. particle count (ml^{-1})	Floc	6300 (1600)	6700 (1500)	3800 (200)
	Floc + 20%	9100 (700)	8400 (1600)	5600 (1200)
End of experiment				
Water				
W/C particulate N (mg l^{-1})	Floc	6.79 (0.39)	9.77 (0.48)	11.18 (0.46)
	Floc + 20%	7.63 (0.71)	9.97 (0.65)	11.00 (0.10)
W/C particulate ^{15}N (atom% excess)	Floc	0.157 (0.010)	0.180 (0.006)	0.267 (0.017)
	Floc + 20%	0.134 (0.014)	0.168 (0.008)	0.294 (0.024)
Shrimp				
Shrimp weight (g)	Floc	1.10 (0.24)	5.41 (0.58)	8.84 (0.53)
	Floc + 20%	1.05 (0.13)	4.66 (0.54)	8.85 (0.45)
Shrimp survival (%)	Floc	98	92	91
	Floc + 20%	99	92	99
Floc retained (mg N g^{-1} 48 h^{-1})	Floc	1.2 (0.29)	0.6 (0.13)	0.2 (0.04)
	Floc + 20%	1.5 (0.23)	0.6 (0.07)	0.3 (0.06)
% total floc N load retained by shrimp	Floc	1.4 (0.24)	2.6 (0.36)	1.4 (0.10)
	Floc + 20%	1.6 (0.15)	2.0 (0.22)	1.7 (0.23)

Fig. 1

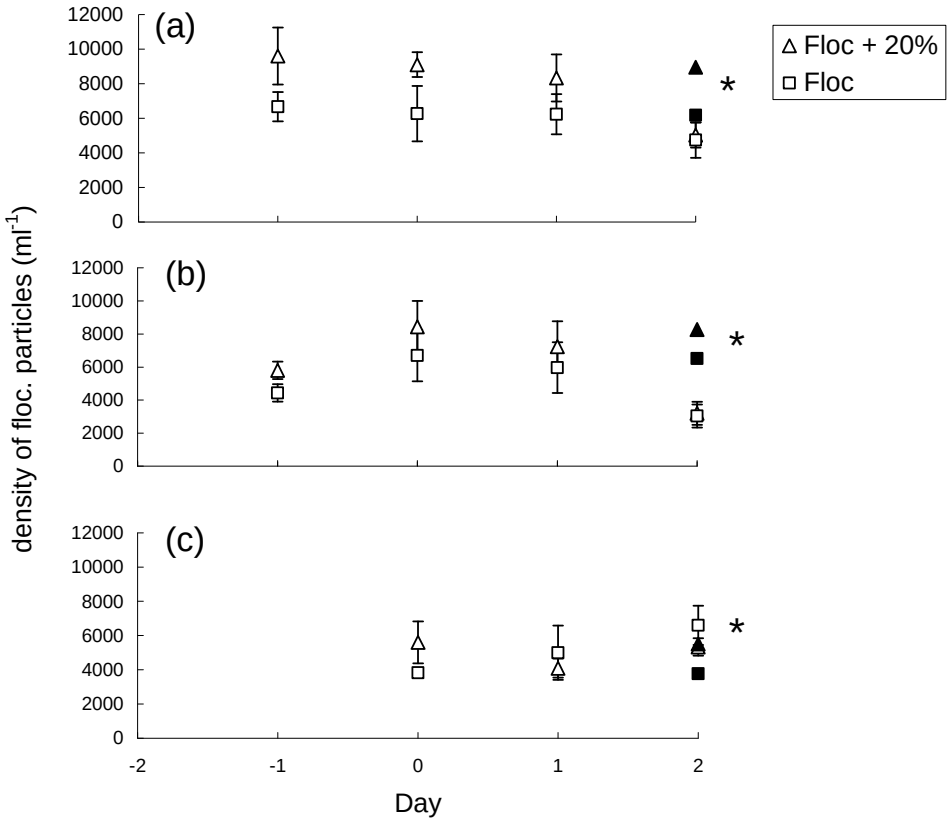
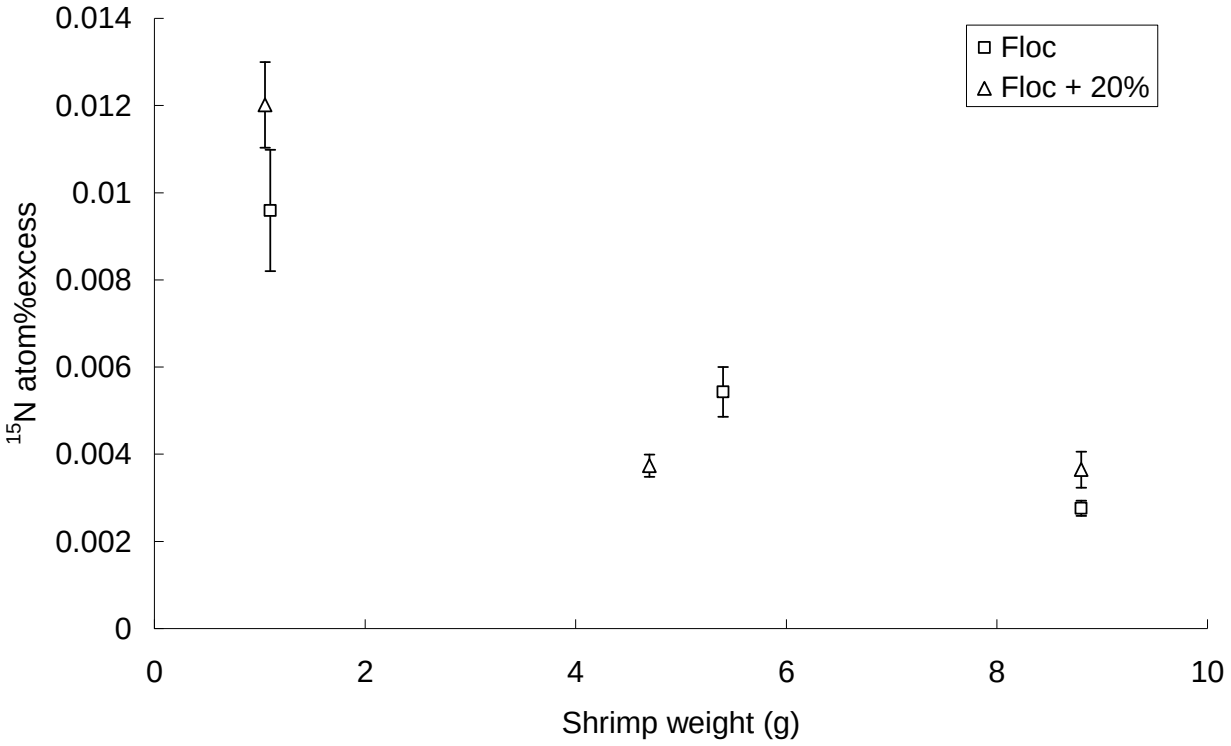

(a)
(b)
(c)
△ Floc + 20%
□ Floc
*
*
*
density of floc. particles (ml-1)
Day
12000
10000
8000
6000
4000
2000
0
-2
-1
0
1
2

Fig. 2


0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
^{15}N atom%excess
0
2
4
6
8
10
Shrimp weight (g)
Floc
Floc + 20%