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Food sources of the sergestid crustacean, *Acetes sibogae*, in shrimp ponds

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Abstract

A combination of stable isotope measurements and gut contents analysis was used to determine the major food sources of the sergestid crustacean *Acetes sibogae*, in commercial shrimp ponds at two farms in southeast Queensland, Australia. Slight differences were observed between farms but overall the results were consistent. Although gut contents analysis gave a good indication of the range and temporal occurrence of food items consumed by *Acetes*, it was difficult to ascertain the contribution each item made to the diet. This was mainly due to the large proportion of unidentifiable material in the guts. All specimens examined contained unidentifiable material and about half the *Acetes* from both farms contained nothing identifiable. This unidentifiable material may be the result of processing by the *Acetes* gastric mill or the consumption of detritus, sediment or processed material from shrimp pellets. Only resilient items such as crustacean remains, diatoms and tinntinnids were commonly identified from the guts, and although present over the majority of the sampling period, FOCs were never greater than 25%.

Stable isotope signals were measured for *Acetes* and likely food sources including pelleted shrimp feed, zooplankton and macroalgae. The pattern of changes in isotopic signals of *Acetes* across the season showed that zooplankton was a primary food source. Changes in the signals of zooplankton were reflected by changes in *Acetes*, but the changes in *Acetes* signal were less pronounced. At both farms, *Acetes* were more enriched in ^{13}C and ^{15}N (−15‰ to −20‰ and 12‰ to 13.8‰) than the zooplankton (−18.9‰ to −23.7‰ and 5‰ to 13.1‰), during the whole season. The absolute difference between the $\delta^{13}C$ values of *Acetes* and zooplankton were more consistent than for $\delta^{15}N$, but both were greater than might be expected based on fractionation over a single trophic level. Furthermore, laboratory feeding trials showed that fractionation could not explain the greater than expected enrichment of the *Acetes* signal compared to that measured for zooplankton in the ponds. This, together with evidence from gut content analysis, showed that a food source other than zooplankton must also be important to *Acetes*. Macroalgae are the most likely additional source, although some minor contribution of pellets or microalgae cannot be ruled out entirely. There was no evidence from either gut contents or stable isotope signatures of dramatic dietary changes for *Acetes* either through a season or as they grew. It would appear unlikely that *Acetes* would have a great effect on shrimp production in ponds unless they were extremely abundant early in the season when the postlarvae are also feeding on zooplankton.
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Keywords: Stable isotopes; Sergestids; Diet

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1. Introduction

The assemblages of zooplankton and epibenthic fauna in shrimp ponds in southeast Queensland have been well described (Preston et al., 2003; Coman et al., 2003), and factors influencing their dynamics have been investigated. In shrimp farms in this region and elsewhere in the world, shrimp gain nutrition from zooplankton (Chen and Chen, 1992; Martinez-Cordova et al., 1997; Coman et al., 2003), particularly early in the season (soon after stocking). As the grow-out season progresses the amount of nutrition gained from zooplankton decreases so that by the end of the season the shrimp are almost exclusively gaining their nutrition from formulated pellets (Preston, 1998).

Generally it is accepted that the pond zooplankton assemblages, which are usually dominated by copepods and barnacle nauplii, feed primarily on the phytoplankton blooms in the pond (Martinez-Cordova et al., 1997) and are not likely to compete with the stocked shrimp for resources. Due to their larger size, the feeding habits of the epibenthos may have a more direct impact on the stocked shrimp, however their feeding habits within the ponds have not been investigated. The sergestid, *Acetes sibogae*, is the most prominent epibenthic animal occurring in ponds in the study region (Coman et al., 2003). *Acetes* have feeding mechanisms to allow them to effectively prey on zooplankton (McLeay and Alexander, 1998) and will probably feed on the smaller zooplankton in the ponds. Many studies have concluded that *Acetes* species are omnivorous (Xiao and Greenwood, 1993), so it would seem possible that they also feed on pond phytoplankton, other pond fauna and flora, and pellets. The contribution of these different food sources may change across the season and could place sergestids as competitors for feed with the shrimp stocked into the ponds.

Two approaches commonly used in determining the diet of animals are gut contents analyses and stable isotope analyses. Gut content analyses have been more widely used, despite several problems with this technique. Generally this technique can detect only what was eaten very recently by an animal, and may not be very useful for looking at soft bodied prey (Gee, 1989). Also, the technique reveals ingestion but does not give an indication of what is assimilated. Further, for species such as *Acetes*, which have a gastric mill at the anterior end of their alimentary tract, much of the prey is broken down to fragments too small to identify confidently. Stable isotope analysis has advantages in that it indicates what organisms have assimilated and integrates this over time (Peterson and Fry, 1987; Grey et al., 2004). However, there can be problems in interpretation when more than one combination of dietary items can result in a similar isotopic signature for the consumer. This can be overcome to some degree by analysing multiple elements.

The aim of this study was to determine whether the major direct food source of *Acetes* occurring in the shrimp ponds was pellets or zooplankton, using gut contents and ^{13}C and ^{15}N stable isotope signatures. By simultaneously analysing the diet using these two techniques it was hoped this would overcome problems associated with using either technique in isolation.

2. Materials and methods

2.1. Pond sampling

2.1.1. Sampling sites

Samples were collected from a single pond at each of two shrimp farms in southeast Queensland, Australia. Moreton Bay prawn farm (MBPF) produced *Penaeus monodon* at Cleveland (27°30′S, 153°20′E). The grow-out season ran from December 1998 to April 1999. Rocky Point prawn farm (RPPF) reared *Penaeus japonicus* at a site several kilometres to the south of MBPF. The grow-out season at this farm ran from December 1998 to July 1999.

Farm management practices were similar at the two farms despite the stocking of different shrimp species. Both farms grew shrimp in earthen ponds up to 1 ha in surface area and 1.8 m depth in the centre. The farms were supplied water from nearby tidal creeks that was screened to approx. 1000 μm before entering the ponds. Water quality in the ponds was maintained by exchanging water as necessary. Ponds were filled several weeks before the shrimp postlarvae (PL15) were stocked. Stocking densities across the farms varied between 25 ind. m^{-2} and 50 ind. m^{-2}. The shrimp were fed a fishmeal based commercial pellet diet, usually between two and five times per night. The major difference between the farms was that RPPF used pellets with higher protein levels to grow *P. japonicus* than the pellets required to grow *P. monodon* at MBPF. Paddlewheels were used to circulate pond water and maintain dissolved oxygen levels in the ponds. Lime was added to maintain pH at close to 8 throughout the season. Phytoplankton blooms were maintained by fertilisation of the pond with chicken manure.

2.1.2. Sample collection

Samples were collected monthly from a single pond at each farm. Sergestids were caught using a beam trawl, with mouth 500 mm wide and 300 mm high, 1400 mm in length, constructed of 1 mm mesh. A metal tickler chain (20 mm links) was suspended across the front of the net. Zooplankton was sampled using a conical 140 μm mesh plankton net with a mouth of 350 mm diameter. Both nets were towed over approximately 50 m. Macroalgae was an incidental capture in both nets. Samples from both nets were rinsed into plastic bags and frozen immediately after collection. A sample of the pellets fed to the shrimp at that time was collected on each sampling occasion.

As the sergestids collected from the ponds were to be used for both gut contents and stable isotope analyses, it was necessary to establish the optimal time of day to sample such that there was a high likelihood that a large proportion of the sergestids collected would have food in their guts. To determine this a preliminary trial was conducted at RPPF. Sergestids were collected from three ponds over a 24 h period, two of the ponds were sampled every 3 h, the third every 6 h. Sergestids were immediately placed into an ice slurry, prior to having their foreguts dissected out. Gut fullness was estimated as described below. In all of the ponds sampled the largest average gut fullness was recorded from sergestids collected at either 0900 h or 1200 h (Fig. 1). As a result of this preliminary trial, further sampling was conducted in the later part of the morning at both farms.

2.1.3. Sample processing

Sergestids were dissected so that the cephalothorax was separately preserved in 70% ethanol for gut content analysis and the tail tissue was obtained for stable isotope analysis. Zooplankton samples were rinsed through a 90 μm mesh with distilled water, in preparation for stable isotope analysis.

2.1.4. Gut contents analysis

The carapace length (CL) of each sergestid was measured using callipers before it was removed from the cephalothorax and the foregut was dissected out. Foregut fullness was determined visually, by placing the gut on a Sedgewick Rafter slide under a stereo microscope, and applying pressure to the middle of the foregut until it was flattened without breaking the gut lining. The foregut was then opened and the lining carefully removed. A dilute solution of Rose Bengal was added to the contents to stain the chitin. Food items were highly fragmented due to the action of the gastric mill, and despite several attempts to ascribe body parts to specific taxa, ultimately items had to be grouped into high level taxa. The frequency of occurrence (FOC) for each dietary category was recorded as the percentage of *Acetes* in the sample with at least one of that item in their foregut (Heales et al., 1996). The guts from 24 *Acetes* were examined each month.

2.1.5. Stable isotope analyses

Individual *Acetes* and pooled zooplankton and pelleted feed samples were dried at 60 °C in a drying oven for 24 h, ground with a mortar and pestle, weighed into tin capsules, and oxidized at high temperature with analysis of the resultant CO_2 and N_2 in a continuous flow-isotope ratio mass spectrometer . Ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were expressed as the relative per mil (‰) difference between the

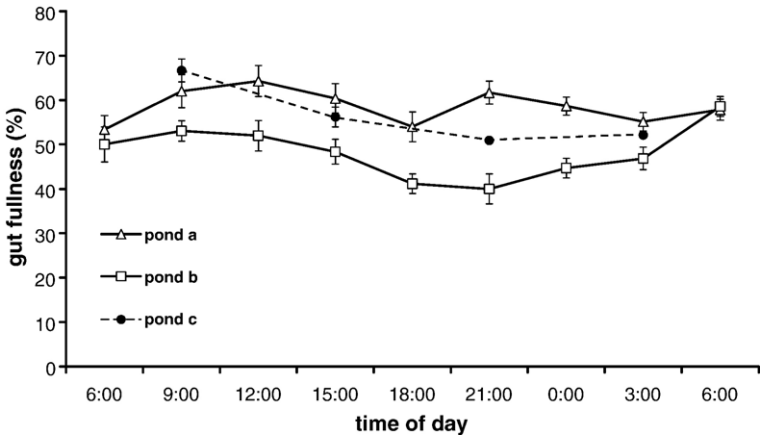

Fig. 1. Mean foregut fullness (%) of *Acetes sibogae* collected at 3 or 6 h intervals from 3 ponds over a 24 h period.

sample and conventional standards (PDB carbonate and N_2 in air) where:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \ (‰)$$

where $X = {}^{13}C$ or ${}^{15}N$ and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

2.2. Validation trial

Acetes collected from a single pond at RPPF and a sample of pellets being fed to that pond at that time were used in a validation trial. The trial was conducted to measure the response of the isotopic signature of *Acetes* when fed one dietary item exclusively. The *Acetes* were transported live to the laboratory, where 5 animals were sacrificed for stable isotope analysis. The remaining sergestids were split into two groups of approximately 30 animals. Each group was placed into cylindrical, polyethylene tanks (100 L) continuously supplied with filtered seawater and aeration via an airstone. The tanks contained no substrate and the outlets were covered by a 140 μm nylon mesh screen. Food was added to each tank twice daily. One tank received pellets from the farm, the other received one day old *Artemia salina* nauplii (as a proxy for pond zooplankton). The pellets were ground prior to feeding as *Acetes* had previously been observed to not readily consume whole pellets. To reduce the chance of cannibalism *Acetes* were stocked at a low rate and fed to excess. Only 2 deaths were recorded and the bodies were removed before they were cannibalised. Each fortnight, 5 *Acetes* were sampled from both tanks, with the final samples collected 56 days after the trial began. Individual *Acetes*, and pooled *Artemia* and pellet samples were processed for stable isotope analysis as described above.

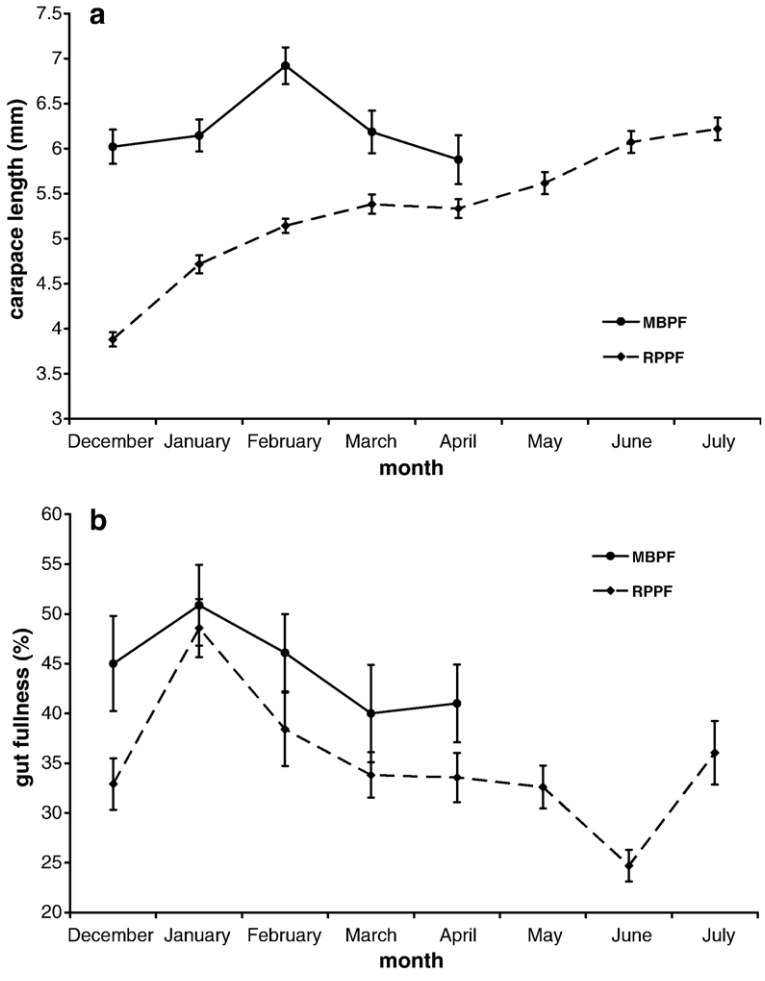

Fig. 2. Characteristics of *Acetes sibogae* over time (a) carapace length (mean±S.E.), (b) foregut fullness (mean±S.E.).

2.2.1. Data analysis

Differences in stable isotope signals of *Acetes* and zooplankton across months and between farms were investigated with a multiple factor ANOVA one each for $\delta^{13}C$ and $\delta^{15}N$, where overall significant differences were detected pairwise comparisons were performed using Tukey tests. Relationships between $\delta^{13}C$ and $\delta^{15}N$ signals of *Acetes* and zooplankton from each farm were investigated with linear regression. Differences between the two treatment groups in the validation trial were examined using the same ANOVA methods as for the pond data.

3. Results

3.1. Gut contents analysis

At MBPF the mean carapace length of *Acetes* varied from 5.9 to 6.9 mm and was greatest in the middle of the season. In contrast, at RPPF the mean carapace length increased almost steadily from 3.9 mm early in the grow-out season samples to 6.2 mm by the end of the grow-out season (Fig. 2a).

Although the average gut fullness of the samples examined from both farms was variable over the season, for the majority of months it was between 30% and 45% (Fig. 2b). The most notable feature of the *Acetes* gut contents was that the majority of the material could not be identified. All specimens contained unidentifiable material (frequency of occurrence [FOC] 100%, Fig. 3a, b), and about half of all *Acetes* from both farms contained nothing identifiable.

A further similarity between the farms was the prevalence of crustacean remains in the guts of *Acetes*. Crustacean remains were categorised as barnacle nauplii, copepods or other unidentifiable crustacean remains. Unidentified crustacean remains were recorded from between 22% and 25% of *Acetes* guts at both

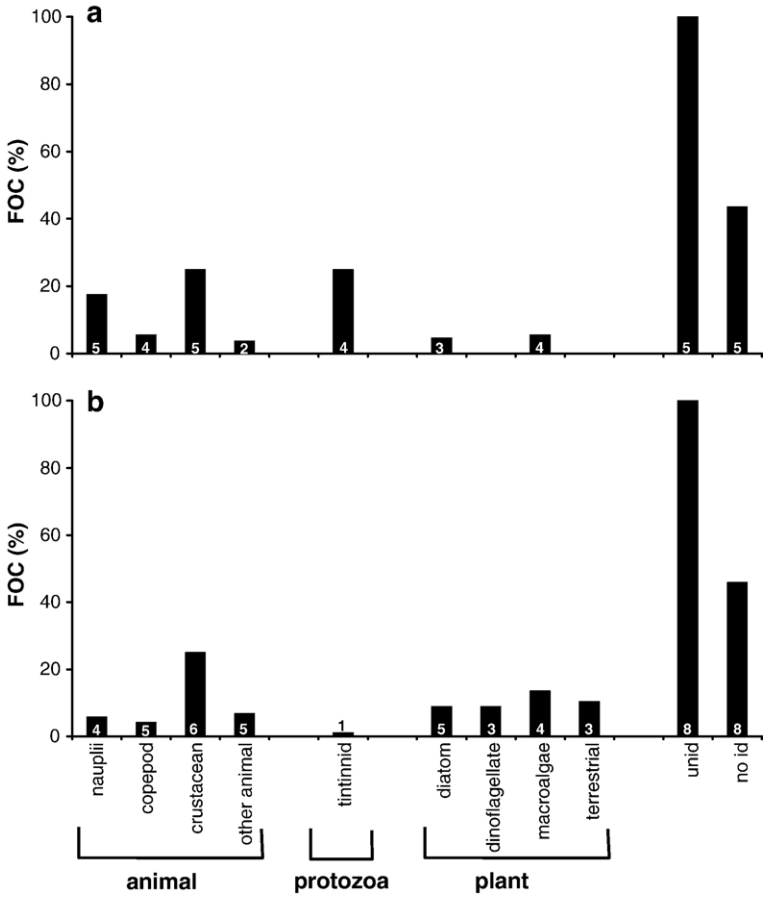

Fig. 3. Frequency of occurrence (FOC) of dietary categories in the foregut of *Acetes sibogae* collected over the whole season. Numbers at the bottom of each bar represent the number of months in which the taxon was identified (a) MBPF, 5 month season (b) RPPF, 8 month season. unid=unidentifiable material (gut may also contain material which was identified); no id=no identifiable material in gut.

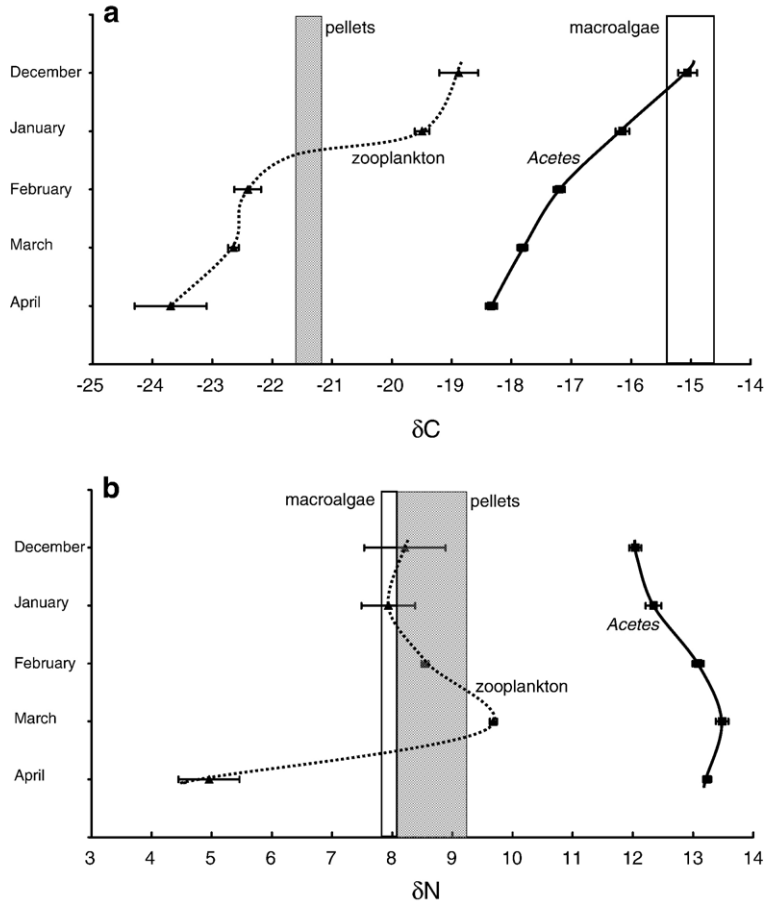

Fig. 4. Monthly stable isotope values; (a) δ^{13}C (mean±S.E.) and (b) δ^{15}N (mean±S.E.) for *Acetes sibogae* and potential food sources (widths of columns show S.E.) sampled from MBPF over the grow-out season.

farms (Fig. 3a,b). Although whole barnacle nauplii were occasionally observed in the guts the majority of the identifications were made from fragments. Barnacle nauplii were more numerous and encountered more frequently in guts of *Acetes* from MBPF (FOC 15.3%). Copepods were also mostly identified from fragments. Copepods were recorded in a low proportion of *Acetes* guts (<5%), but consistently over the season at both farms (Fig. 3a,b). Non-crustacean animal remains were encountered at RPPF at about twice the frequency recorded at MBPF. These remains were unable to be accurately identified but appeared to be of arthropod origin (possibly insects).

Tintinnids, diatoms and dinoflagellates were most often encountered as whole specimens. Tintinnids were present in 22.5% of guts examined from MBPF but were rare at RPPF (Fig. 3a,b). Diatoms were recorded over several months at both farms, and were encountered about twice as frequently in samples from RPPF. Dinoflagellates were recorded from nearly 9% of *Acetes* collected at RPPF but were not recorded at MBPF.

Macroalgae fragments were recorded at both farms, but were much more common at RPPF (13.5%) than at MBPF (4.8%). The macroalgae fragments appeared to be the same as the algae collected in the trawl samples, which resembled the green filamentous algae *Enteromorpha*. Terrestrial plant fragments were found in *Acetes* from RPPF but not from MBPF. The size of these fragments was large compared to other plant and animal fragments observed in the guts. Grasses grow in close proximity to pond edges at RPPF, but not at MBPF.

3.2. Pond stable isotope values

The δ^{13}C and δ^{15}N signatures of both *Acetes* and zooplankton varied significantly across the season at

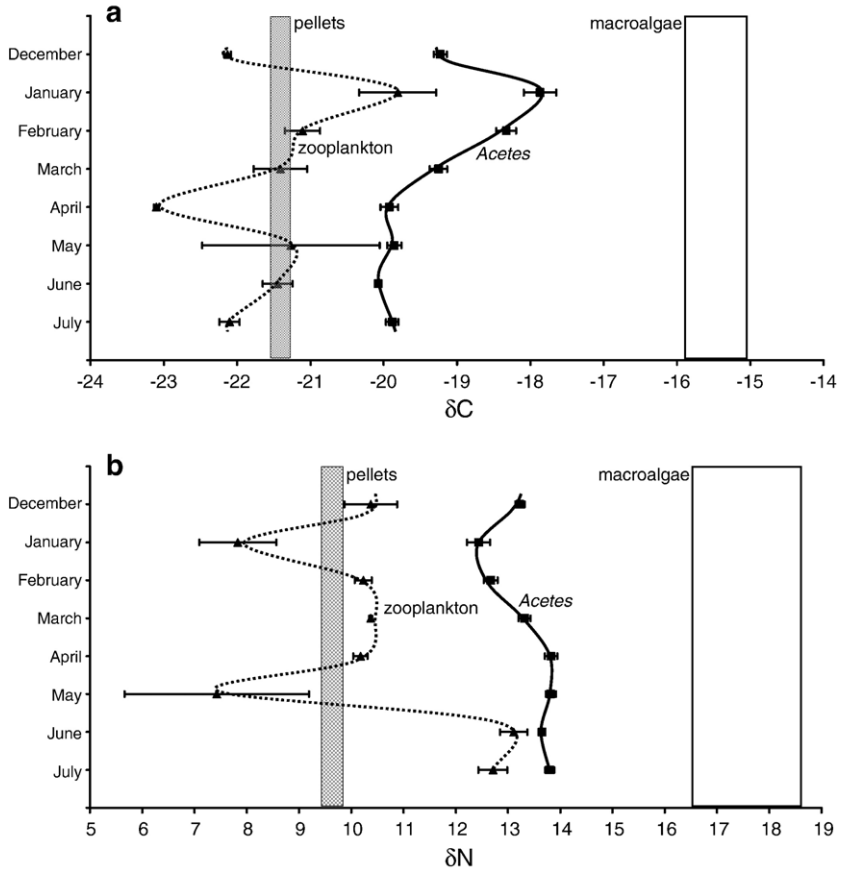

Fig. 5. Monthly stable isotope values; (a) δ^{13}C (mean±S.E.) and (b) δ^{15}N (mean±S.E.) for *Acetes sibogae* and potential food sources (widths of columns show S.E.) sampled from RPPF over the grow-out season.

both MBPF and RPPF (Figs. 4 and 5; Table 1 [farm × organism] and Table 2). Significant differences were found between the δ^{13}C and δ^{15}N signatures of *Acetes* and zooplankton from MBPF compared to those from RPPF (Figs. 4 and 5; Table 1 [organism × month]) except for δ^{13}C zooplankton samples in January and April; δ^{15}N zooplankton samples collected in January and δ^{15}N *Acetes* samples collected from January, February and March.

Table 1
ANOVA results for pond stable isotope analysis of δ^{13}C and δ^{15}N

Source	*F* value	*df*	*P* value
$\delta^{13}C$			
Organism	284.51	1, 114	<0.0001
Farm	26.54	1, 114	<0.0001
Month	8.52	7, 114	<0.0001
Organism × farm	15.94	1, 114	<0.0001
Organism × month	7.06	7, 114	0.0017
Farm × month	7.32	4, 114	<0.0001
$\delta^{15}N$			
Organism	503.87	1, 114	<0.0001
Farm	57.24	1, 114	<0.0001
Month	36.37	7, 114	<0.0001
Organism × farm	54.44	1, 114	0.0001
Organism × month	3.61	7, 114	<0.0001
Farm × month	32.26	4, 114	<0.0001

Table 2
Tukey's pairwise comparisons of δ^{13}C and δ^{15}N for months, grouped by farm and organism

Farm × organism comparisons [a]	δ^{13}C	δ^{15}N
MBPF *Acetes*	12^{A} 1^{B} 2^{C} 3^{D} 4^{E}	3^{A} 4^{A} 2^{A} 1^{B} 12^{B}
RPPF *Acetes*	1^{A} 2^{A} 12^{B} 3^{B} 5^{C} 7^{C} 4^{C} 6^{C}	5^{A} 4^{A} 7^{A} 6^{A} 3^{AB} 12^{AB} 2^{BC} 1^{C}
MBPF zooplankton	12^{A} 1^{A} 2^{B} 3^{B} 4^{B}	12^{A} 1^{A} 2^{AB} 3^{B} 4^{C}
RPPF zooplankton	1^{A} 2^{AB} 5^{AB} 3^{AB} 6^{B} 7^{B} 4^{B} 12^{B}	6^{A} 7^{A} 12^{AB} 2^{AB} 3^{AB} 4^{AB} 1^{B} 5^{B}

Months with the same superscripts, within each row, are not significantly different.
[a] 1=January, 2=February, 3=March, 4=April, 5=May, 6=June, 7=July, 12=December.

The mean $\delta^{13}C$ signature of zooplankton from MBPF became steadily more depleted from −18.9‰ to −23.7‰ across the season (Fig. 4a). The *Acetes* had more enriched signatures than the zooplankton, varying from −15‰ to −18.3‰, but followed a similar pattern of depletion over time to the zooplankton. The mean $\delta^{13}C$ of the pelleted feed averaged over the whole season was −21.4±0.2‰. Macroalgae, resembling the fragments found in the guts, were only collected from the pond in December, and had a mean $\delta^{13}C$ of −15± 0.4‰, which is very similar to the most enriched *Acetes* samples collected in December.

Zooplankton $\delta^{15}N$ values at MBPF ranged between 5.0‰ and 9.7‰ (Fig. 4b). The *Acetes* $\delta^{15}N$ values ranged between 12‰ and 13.5‰. The mean $\delta^{15}N$ of *Acetes* at MBPF was generally 3.8‰ to 4.5‰ more enriched than the zooplankton collected at the same time. The mean $\delta^{15}N$ of the pelleted feed was 8.6± 0.6‰, which is close to the values of zooplankton in all months except April. Macroalgae $\delta^{15}N$ values of 8.0 ±0.1‰ were slightly more depleted than the pellets.

The mean $\delta^{13}C$ of the zooplankton samples from RPPF ranged between −19.8‰ and −23.0‰ (Fig. 5a). The *Acetes* collected from RPPF were more depleted in ^{13}C than those from MBPF, with values ranging between −17.9‰ and −20.0‰. The $\delta^{13}C$ signals of macroalgae collected at RPPF were −15.5±0.4‰, similar to those recorded from MBPF. The $\delta^{13}C$ signal of the pelleted feed from RPPF was −21.4±0.1‰ which was also similar to MBPF.

The $\delta^{15}N$ of zooplankton collected from RPPF ranged between 7.4‰ and 13.1‰ across the season (Fig. 5b). The $\delta^{15}N$ of *Acetes* from RPPF varied much less than the zooplankton, ranging from 12.4‰ to 13.8‰. The mean $\delta^{15}N$ of the pelleted feed was 9.7±0.3‰, which was slightly more enriched than at MBPF. Macroalgae samples collected from RPPF had noticeably enriched $\delta^{15}N$ values of 17.6±1.0‰ (Fig. 5b).

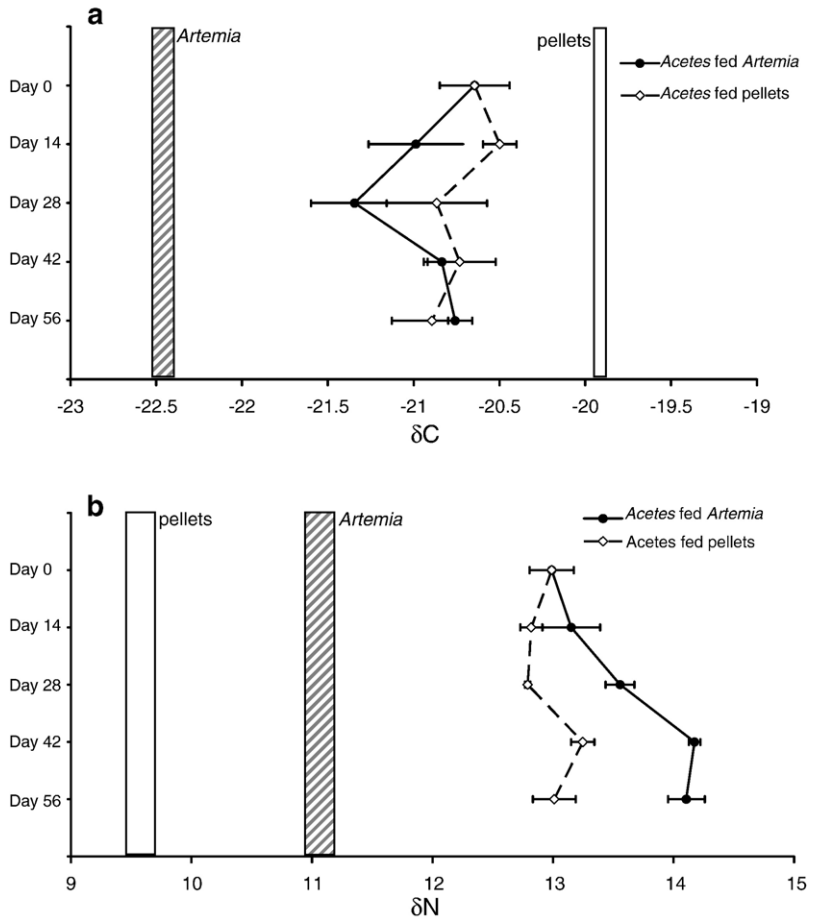

Fig. 6. Fortnightly stable isotope values; (a) $\delta^{13}C$ (mean±S.E.) and (b) $\delta^{15}N$ (mean±S.E.) for *Acetes sibogae* and food sources (widths of columns show S.E.) from the laboratory validation trial.

There were significant differences between the zooplankton and *Acetes* signatures, for both isotopes across the whole season from MBPF. Although the differences between zooplankton and *Acetes* from RPPF were usually less, they were also statistically significant (Table 1 [farm × month]) apart from $\delta^{13}C$ in May and $\delta^{15}N$ in June. While the $\delta^{13}C$ of zooplankton and *Acetes* appeared to be correlated at both MBPF ($r^2=0.972$, $P<0.01$) and RPPF ($r^2=0.724$, $P<0.05$), there does not appear to be a significant correlations between their $\delta^{15}N$ signals at either farm. However at both farms and for both $\delta^{13}C$ and $\delta^{15}N$ *Acetes* signatures followed a pattern of becoming more enriched when the zooplankton became more enriched, or becoming depleted when the zooplankton became depleted, although the magnitude of the change in the *Acetes* signal was much less than for zooplankton. This is particularly apparent in January and May $\delta^{15}N$ samples from RPPF where very large changes in the magnitude of zooplankton samples are reflected by much smaller changes in *Acetes* signals, which are however, in the same direction (Fig. 5b).

3.3. Validation trial

The $\delta^{13}C$ signal of the *Artemia* nauplii used in the feeding trial was −22.5±0.05‰, compared with the pellets which had a $\delta^{13}C$ of −19.9±0.03‰ (Fig. 6a). At the commencement of the trial the $\delta^{13}C$ of *Acetes* collected from the pond was −20.6±0.2‰, which was between the values of the two alternative food sources. There were no significant differences between the $\delta^{13}C$ signals of the two treatment groups throughout the trial (Table 3). Over the course of the trial *Acetes* fed only pellets had a $\delta^{13}C$ signature more depleted than the pellets. The $\delta^{13}C$ signal of *Acetes* fed only *Artemia* moved closer to the signal of the *Artemia* up to day 28 of the trial. But the difference between the $\delta^{13}C$ of *Acetes* and *Artemia* increased again by days 42 and 56 due to *Acetes* becoming more enriched in ^{13}C. However, even when this occurred *Acetes* remained more enriched than the *Artemia* and the difference between them remained <2‰.

Table 3
ANOVA results for validation trial stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$

Source	*F* value	*df*	*P* value
$\delta^{13}C$			
Treatment	2.27	232	0.1270
Week	26.54	532	0.7445
Treatment × week	8.52	332	0.8087
$\delta^{15}N$			
Treatment	19.90	232	<0.0001
Week	2.61	532	0.0536
Treatment × week	2.00	332	0.1439

The *Acetes* collected from the pond at the start of the trial were more enriched in ^{15}N than either of the food sources used in the trial (Fig. 6b). The mean $\delta^{15}N$ of *Acetes* was 13.0±0.2‰, which was 2 units more enriched than the *Artemia* ($\delta^{15}N=11.0\pm0.1$) which in turn was another 1.4 units more enriched than the pellets ($\delta^{15}N=9.6\pm0.1$‰). There were significant differences between the signals of the two treatment groups (Table 3), which increased as the trial progressed. By day 56 the $\delta^{15}N$ of *Acetes* being fed *Artemia* or pellets had increased to 14.1±0.2‰ and 13.0±0.2‰ respectively. The difference between the *Acetes* and the *Artemia* at this point of the trial was approximately 3‰, while the difference between the *Acetes* and the pellets was slightly greater at 3.4‰.

4. Discussion

The variety of items ingested by *Acetes* in the ponds indicates that they are omnivorous as has been found in natural ecosystems (Xiao and Greenwood, 1993). However, much of the material in the guts was unidentifiable. While this was in part due to the effect of the gastric mill, which is known to macerate all but the smallest, most resilient items consumed (Donaldson, 1975; McLeay and Alexander, 1998) such as tinntinnids, diatoms and dinoflagellates, it could also represent the consumption of detritus or pellet fragments. Apart from the action of the gastric mill, the fine grinding of ingredients used to produce the formulated pellets would also make them difficult to identify in the guts of *Acetes*. Numerous studies on the diets of sergestids have found a large proportion of unidentified material which makes determination of the diet from gut content analyses difficult (Donaldson, 1975; Flock and Hopkins, 1992; Xiao and Greenwood, 1993). It has also been noted that *Acetes* can expel indigestible material before taking it into the gut (McLeay and Alexander, 1998), so that only the digestible (and unidentifiable) portions of some food items might actually enter the gut.

While FOC is a common estimation technique used in the dietary analysis of crustaceans (Marte, 1980; Wassenberg and Hill, 1993), to be able to determine the relative importance of dietary items from gut contents it is desirable to know not only the FOC, but also the number (or volume) of the each item ingested. This has been achieved in dietary studies of shrimp (O'Brien, 1994), but the very small volume of identifiable material

in the guts of *Acetes* from the ponds does not allow estimation of the relative importance of the ingested items using this method alone.

The analysis of stable isotopes within the ponds provides an indication of the relative importance of different food sources for *Acetes*. At both MBPF and RPPF there was evidence that the changes in both $\delta^{13}C$ and $\delta^{15}N$ values for *Acetes* reflected the changes observed for zooplankton, although the magnitude of the changes was not as great. This suggests zooplankton were the most important part of the diet assimilated by *Acetes* in the ponds. The difference in the magnitude of the change in signals between zooplankton and *Acetes* possibly represents a higher turnover in the tissues of the zooplankton compared to *Acetes* over the month between each sampling occasion. It may also represent a faster response by zooplankton to changes in food. However, evidence from both gut contents and stable isotopes indicates that zooplankton was not the only food source of importance to *Acetes*. These other food sources, such as pellets or macroalgae, may also explain smaller changes in *Acetes* isotopic signatures compared to changes in zooplankton.

Furthermore, the absolute difference between the $\delta^{13}C$ value of *Acetes* and zooplankton was greater than would be expected for fractionation shift over a single trophic level. It is estimated that ^{13}C enrichment between trophic levels is approximately 1‰ (Fry and Sherr, 1984). In trials with *Penaeus vannamei*, animals became enriched up to 2‰ above their food source (Anderson et al., 1987; Parker et al., 1989; Dittel et al., 1997). *Acetes* at MBPF were 3.4‰ to 5.4‰ more enriched in ^{13}C than the zooplankton, and at RPPF the difference was about 1.4‰ and 3.2‰. In contrast, the $\delta^{13}C$ signature of *Acetes* in the validation trial was between 1‰ and 2‰ more enriched than the *Artemia* they were fed.

The expected enrichment of ^{15}N between trophic levels is about 3‰ to 4‰ which is greater than for ^{13}C (Fry and Sherr, 1984). Parker et al. (1989) and Dittel et al. (1997) found the shift in $\delta^{15}N$ between *P. vannamei* and their diets was between 2.4‰ and 2.7‰, while Fry (1988) measured a shift of between 3.4‰ and 3.8‰ between fish and their feed. The differences between the $\delta^{15}N$ signals of *Acetes* and zooplankton at both farms were much more varied than the differences observed for $\delta^{13}C$, and while they were often within this estimated range, they were greater than the differences observed between *Acetes* and the *Artemia* in the validation trial. The *Acetes* from the validation trial were approximately 3‰ more enriched in ^{15}N than the *Artemia* fed to them. A food source within the pond which was significantly more enriched in ^{13}C and ^{15}N may help explain these differences. The shrimp-pelleted feed added to the ponds was always more depleted than the *Acetes*, and never greatly enriched compared to the zooplankton and would not appear to resolve the increased differences observed.

Schroeder (1983) found that it is possible to selectively assimilate enriched components from a pellet diet resulting in a more enriched $\delta^{13}C$ signature than would have been expected from the average $\delta^{13}C$ signature of the whole pellet. However, the validation trial found that *Acetes* were depleted in ^{13}C compared to the pellet diet they were fed. This suggests that *Acetes* may have selectively assimilated one or more components of the diet with an average value depleted rather than enriched in ^{13}C compared to the whole pellet. These components must have had $\delta^{15}N$ signatures similar to the $\delta^{15}N$ of the whole pellet to result in the ^{15}N enrichment observed between the pellets and the *Acetes*. Consuming pellets in the pond would therefore result in a reduced difference between the $\delta^{13}C$ signatures of *Acetes* and zooplankton, rather than the increased enrichment observed, but would not greatly influence the differences in $\delta^{15}N$ signatures. We can conclude, therefore, that the pelleted feed added to the ponds is not playing any major role in the nutrition of *Acetes*.

The gut content analyses revealed that apart from zooplankton *Acetes* were also consuming phytoplankton and macroalgae. Previous sampling of ponds from this region (Preston, unpublished data) has indicated the $\delta^{13}C$ of phytoplankton (−20‰) would fall between values recorded for pellets and *Acetes* at both farms. The $\delta^{15}N$ of phytoplankton is 8‰ (Preston, unpublished data) and would be slightly depleted when compared to the values obtained for pellets at both farms. These values are not enriched enough to explain the greater than expected differences in $\delta^{13}C$ values observed between *Acetes* and zooplankton in the pond. Macroalgae from the ponds at both MBPF and RPPF were enriched in ^{13}C. It was observed in the gut content analysis that *Acetes* did feed on macroalgae. It appears likely the greater than expected difference between the $\delta^{13}C$ signature of *Acetes* and the $\delta^{13}C$ signature of zooplankton is due to *Acetes* assimilating carbon from macroalgae.

The difference between the $\delta^{13}C$ signals of *Acetes* and zooplankton at RPPF was smaller than at MBPF possibly suggesting they were consuming (and assimilating) less of the more ^{13}C enriched sources than at MBPF. The $\delta^{15}N$ of macroalgae at MBPF was quite depleted, whereas at RPPF it was highly enriched. From

this we may expect the differences between the $\delta^{15}N$ signals of *Acetes* and zooplankton to be slightly reduced at MBPF and slightly increased at RPPF, but this is not the case. The $\delta^{15}N$ of marine algae can be variable, even within a location (Fry et al., 1983), depending on nutrient sources. Macroalgae samples were only collected in a single month at MBPF, so it is possible that $\delta^{15}N$ may not have been representative of the whole season. The contribution of ^{13}C from dietary sources, other than zooplankton, would have to be greater than the contribution of ^{15}N required from other sources, to explain the isotopic enrichment observed in the pond *Acetes*, possibly suggesting that the macroalgae may contribute less to *Acetes* in terms of nitrogen than carbon.

The average size of *Acetes* was not consistent across the season at either farm, but at any one time the size range in the ponds was not greater than 1 mm CL. The average size of *Acetes* at RPPF did increase throughout the season, but there was no indication of a consistent change in gut contents or stable isotope signature across the season to correspond with the increase in size. The small range in the size of *Acetes* at any particular time did not allow for differences between size classes to be investigated, but the evidence from the change in size across the season at RPPF suggests there are not likely to be dramatic changes in the diet as *Acetes* grow.

Direct nutrition from pellets in the ponds is unlikely, and evidence from stable isotope analysis, supported by gut contents, indicates that *Acetes* are probably relying primarily on zooplankton as their direct food source. *Acetes* have been found to have well-developed mechanisms for handling zooplankton (McLeay and Alexander, 1998), therefore it would be expected that zooplankton would be an important part of their diet. However, *Acetes* are known to be omnivorous (Xiao and Greenwood, 1993) and there are indications that they obtain some nutrition from other sources within the ponds. Amongst the potential food sources that were sampled from the ponds macroalgae appear to be one but possibly not the only other food source *Acetes* utilise and assimilate. Other food sources which were not sampled in this study, such as microzooplankton and their bacterial prey or sources derived from pond sediment, may also contribute to the diet of *Acetes*.

Studies using enriched isotope tracers can be very useful in tracing the fate of feeds and the role of the natural biota as a food source where natural abundance studies cannot distinguish among a number of possible scenarios (Burford, 2000; Epp et al., 2002; Burford et al., 2002). However the expense of conducting grow-out pond trials using enriched stable isotope tracers has restricted trials to mesocosms representing ponds. To date these mesocosm experiments have not included epibenthos, but this would be worthwhile if further trials are conducted.

The knowledge of feeding preferences of *Acetes* in shrimp ponds could be useful to pond managers, particularly early in the season. If *Acetes* were very abundant in the ponds at the beginning of the season they may compete with the postlarvae for zooplankton, and it might be worth eliminating them or reducing their numbers to ensure there is adequate zooplankton available for the postlarvae. In this study the maximum abundance of *Acetes* in the ponds was 8 m^{-2}, which is not likely to be high enough to cause concern. However, towards the end of a season *Acetes* may play a useful role for the pond manager, by keeping zooplankton numbers lower, in turn allowing phytoplankton blooms to remain more stable. Stable phytoplankton blooms are considered to play an important role in maintaining shrimp productivity in ponds. Overall it would appear that the presence of *Acetes* in shrimp ponds is likely to have little influence on the production of shrimp providing they are not extremely abundant when postlarvae are first stocked.

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