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# Bacterial Productivity in Ponds Used for Culture of Penaeid Prawns

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**Abstract.** The quantitative role of bacteria in the carbon cycle of ponds used for culture of penaeid prawns has been studied. Bacterial biomass was measured using epifluorescence microscopy and muramic acid determinations. Bacterial growth rates were estimated from the rate of tritiated thymidine incorporation into DNA. In the water column, bacterial numbers ranged from $8.3 \times 10^9$ $l^{-1}$ to $2.57 \times 10^{10}$ $l^{-1}$ and production ranged from 0.43 to 2.10 mg C $l^{-1}$ $d^{-1}$. In the 0–10 mm zone in sediments, bacterial biomass was 1.4 to 5.8 g C $m^{-2}$ and production was 250 to 500 mg C $m^{-2}$ $d^{-1}$. The results suggested that most organic matter being supplied to the ponds as feed for the prawns was actually being utilized by the bacteria. When the density of meiofauna increased after chicken manure was added, bacterial biomass decreased and growth rates increased.

## Introduction

Detrital food chains, based on the use of manures and compost, have been used in aquaculture for centuries, especially in Southeast Asia [4]. Heterotrophic bacteria convert organic detritus into protein and presumably constitute an important food source for fish in ponds [23]. In concert with protozoa and meiofauna, aerobic bacteria mineralize organic matter, providing inorganic nutrients such as N and P for algal growth [7, 11]. The mineralization process may be detrimental in ponds where primary production is not desired, e.g., in high intensity farming where feed pellets are supplied, because not only will feed pellets be decomposed by the microbes, but oxygen will be depleted in the water column.

The role of bacteria in aquatic ecosystems can now be quantified with modern techniques to measure their numbers, biomass, and production [26]. A particularly valuable approach is the estimation of production rates, using the rate of tritiated thymidine incorporation into DNA, because there is a direct correlation between DNA synthesis and bacterial division rate [9, 10, 15, 18, 19]. This method specifically measures the growth of heterotrophic bacteria; it does not measure the growth of cyanobacteria (blue-green algae), eukaryotic algae, or fungi.

In the work reported in this paper, production of bacteria was determined in aquaculture ponds to which a pelleted food was fed to penaeid prawns. The

influence of chicken manure on bacterial production was also studied. From these results it was possible to estimate how much of the organic matter was decomposed by bacteria. The effect of meiofauna on bacterial biomass and production was also investigated. Only a short time was available for the studies reported here, but it was sufficient to assess the usefulness of the methodology for studies of bacterial production in ponds.

## Materials and Methods

### *Site*

The ponds were constructed at Gelang Patah, near Johore Bahru, Malaysia, on acid-sulfate mangrove soil. Due to problems with alkalinity and pH, about half the water in each pond was exchanged daily on a tidal cycle [25]. Penaeid prawn species were cultured in the ponds. Salinity was between 26 and 28 parts per thousand, and water temperature ranged from 28–32°C.

The ponds had a peripheral canal about 3 m wide and 1.5 m deep, with a layer of soft sediment. The center of the ponds was 1 m deep, with a thin (20–30 mm) layer of soft sediment over a heavy clay substrate. Water depth was lowered to depth of about 50 cm at low tide once per day, and then refilled on the next high tide from a river fringed by extensive mangrove forests.

### *Pond Treatments*

*Ponds Stocked with Prawns.* At the time of this study, pond 11 (0.5 ha) was supplied with pelleted food at a rate of 10 kg dry weight $d^{-1}$ (1 g C $m^{-2}$ $d^{-1}$). Pond 29 (0.25 ha) was supplied with pelleted food at a rate of 16 kg $d^{-1}$ (3.2 g C $m^{-2}$ $d^{-1}$). Pond 23 (0.25 ha) contained a nursery pen (10 m × 20 m) which was supplied with a food mash at a rate of 5 g dry weight $m^{-2}$ $d^{-1}$ initially, and then 8 g $m^{-2}$ $d^{-1}$ a day before the measurements reported here were made. Pond 26 (0.15 ha) was untreated, except for water exchange, and was used as a control.

*Pen Experiments with Chicken Manure.* Pond 32 (1.0 ha) contained 3 pens, each 3 m in diameter, constructed with plastic mesh (5 mm square holes) in the central area of the pond. Pen A was supplied with chicken manure at a rate of 0.6 g dry weight $m^{-2}$ $d^{-1}$, for 1 week prior to sampling for bacterial biomass, organic C, and N determinations and meiofauna numbers. Pen B was treated with chicken manure for 2 weeks, and pen C was treated with manure for 3 weeks at the same rate as pen A.

A sample of the complete water column was obtained by using a long plastic tube which was pushed down to about 5 cm from the bottom; it was then stoppered and removed. Water was brought back to the laboratory for analysis within 10 min after collection. All analyses were carried out on pond water collected between 8 a.m. and 2 p.m. before the daily tidal exchange. Samples for microscopy were preserved with formaldehyde (3% v/v final volume). Bacteria were counted using epifluorescence microscopy after staining with acridine orange [28, 29] with modifications described by Moriarty [13]. Cell volume was determined from the sizes of bacteria measured on photographs, and biomass was calculated with a conversion factor of 0.22 g C $cm^{-3}$ [2]. For muramic acid measurements, 50 ml of water were filtered through Whatman GF/F filters and then polycarbonate membrane filters (0.2 μm pore size). The filters were combined and dried at 100°C. Muramic acid was extracted and measured on a high pressure liquid chromatograph after precolumn derivation with *o*-phthaldialdehyde [14].

Bacterial production measurements were carried out according to the principles described by Moriarty and Pollard [18, 19]. The following procedure was used for the pond survey. Before collecting the water, 30 μl (30 μCi) [5-methyl-$^3$H]thymidine (51 Ci $mmol^{-1}$) were added to each of a series of polypropylene tubes. The final concentration of thymidine was 60 nM, after pond water (10 ml) was added. Tubes were capped, shaken, and then incubated at *in situ* temperature for 15

min. Each sample was filtered through cellulose acetate filters (0.2 μm, 25 mm diameter) to stop the incubation. Each filter was washed five times with 2 ml of 3% (w/v) ice-cold trichloroacetic acid (TCA). Filters were stored, and later 2 ml of 5% TCA were added, and the filters were heated at 100°C for 5 min. One milliliter was removed for liquid scintillation counting. Zero time controls were used for blanks, i.e., the samples were filtered immediately after adding pond water.

To check whether macromolecules other than DNA were being labeled, two time courses were carried out. One was stopped and treated with TCA as described above; this method does not separate DNA from other macromolecules. In the other experiment the DNA was extracted and separated from RNA and protein. The incubation was stopped with cellulose nitrate filters, and each filter was washed twice with ice-cold tap water and transferred to polypropylene tubes containing 2 ml of 0.3 M NaOH. The DNA was extracted as described by Moriarty and Pollard [19]. Recovery was found to be 100%, using $^{14}$C-DNA as a standard [19]. Isotope dilution experiments were also conducted to show that the concentration of thymidine was high enough to inhibit *de novo* synthesis, i.e., dilution did not occur (see [19, 21]).

### *Sediment Measurements*

Sediment was cored using 50 ml plastic syringes with their bases cut off. The top 10 mm was collected for analysis of organic C and N and muramic acid. Cores from canal and center areas were analyzed separately; four cores were pooled for each zone. Sediments were sun-dried then weighed and stored. Muramic acid was measured as described by Moriarty [14].

For the measurement of thymidine incorporation rates, preliminary experiments were carried out using a sediment slurry to measure isotope dilution and to do a time course on the rate at which tritiated thymidine was incorporated into DNA. The top 10 mm from three cores (50 ml) were combined and a small plastic spoon was used to dispense about 1.0 g into each of a series of polypropylene tubes containing 50 μCi of tritiated thymidine (see [19]). Incubations were carried out at *in situ* temperature for 15 min (or various intervals for the time-course experiment) in a water bath on the pond bank, out of direct sunlight, as soon as the samples were collected. The incubations were stopped by adding 2 ml 0.6 M NaOH. The DNA was extracted by heating the samples in a pressure cooker for 30 min and proceeding as described by Moriarty and Pollard [19].

Subsequent measurements of thymidine incorporation rates were made using the top 10 mm of sediment taken with small corers (5 ml syringes) and treating each core as an individual sample. This treatment minimized oxygenation of anaerobic sediment. Duplicate samples were analyzed for both center and canal zones in each pond. Because the preliminary experiments showed only a small amount of isotope dilution, 100 μCi (100 μl) of [methyl-$^3$H]thymidine (51 Ci mmol$^{-1}$) were used for later experiments. No significant isotope dilution was observed with this amount of tritiated thymidine (see [21]). Incubations were carried out as described above and were stopped with 2 ml of 0.1 M NaOH. This lower concentration (0.1 M) of NaOH was used to minimize losses of DNA during storage. The tubes were capped and stored for 1 week, and the DNA was extracted after 2 ml of 0.6 M NaOH was added. Recovery of DNA, measured with $^{14}$C-DNA, was 80%. See Moriarty and Pollard [19] for further details of the technique for extracting DNA and determining rates of DNA synthesis.

For enumeration of the meiofauna, three cores 30 mm in diameter and 20 mm deep were taken and combined for each sample. The cores were extruded into beakers containing formaldehyde (3% v/v) and rosebengal. Clay was washed out through a fine sieve (0.5 mm) and 30% colloidal silica (Ludox, Dupont) was used to separate the meiofauna from the remaining sediment and detritus [3]. Two or three treatments with Ludox were used. Very few, or no, animals were found to be left in the sediment when it was checked after the Ludox treatment.

## Results

The rate of incorporation of tritiated thymidine into DNA in sediments was linear for 20 min, and in the water column the rate was linear for 1 hour, but

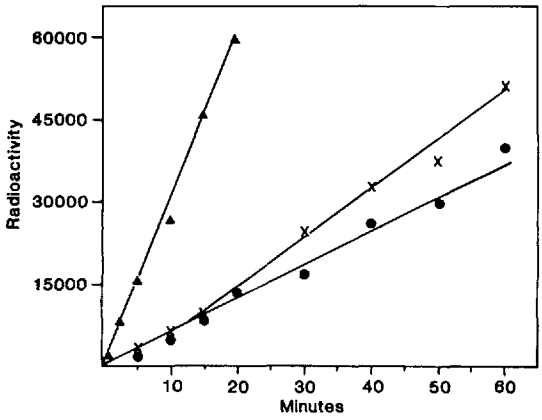


**Fig. 1.** Time course for tritiated thymidine incorporation into DNA. Radioactivity units are disintegrations per min per 10 ml. Symbols: ▲ incorporation into extracted DNA in sediment samples; ● incorporation into extracted DNA in water column samples; × incorporation into macromolecules insoluble in trichloracetic acid in water column.

the rate of incorporation into TCA-insoluble compounds increased after about 20 min (Fig. 1). The time periods chosen for the pond survey experiments were less than these limits for linear rates of DNA synthesis.

Bacterial numbers in the water column were higher in most ponds receiving organic matter than in control ponds (Table 1). The presence of particles with attached bacteria, which could not be counted accurately, contributed to the variability. Most bacteria were between 0.4 and 0.8 $\mu$m in diameter and 0.5 and 1.5 $\mu$m in length. The average cell volume was 0.14 $\mu m^3$, and thus biomass was 30 fg (a total of 546 bacteria was measured). Biomass values were also calculated from the muramic acid content of bacteria in the water. With the factor of 30 fg C $cell^{-1}$, the mean ratio of biomass calculated from both methods for six ponds was 1.1 with a 9% coefficient of variation. Thus, there was a good agreement between the two methods. The same conversion factor was used to calculate bacterial production rates in terms of carbon.

Bacterial production was higher in the water column of all ponds receiving organic matter than in the control ponds (Table 1). Bacterial production was significantly lower in the water of the control ponds than in the river water ($P < 5\%$). Production in the three ponds receiving pellets was significantly higher than in the control ponds ($P < 1\%$). Doubling times were fastest in pond 29, which was the only pond that was being aerated. Bacterial biomass and production were generally higher in the sediments of ponds with an organic matter input, but the effects of organic matter on either biomass or production were not as marked as in the water column (Table 2). Where large amounts of food were being added (ponds 29 and 23) there was a significantly higher rate of bacterial production than in control pond sediments ($P = 1\%$). Production rates were similar in the mangrove sediment and the control pond, but the doubling time was slower in the mangrove sediments (Table 2).

Chicken manure had a marked effect on bacteria in pond sediment. One week after adding manure, bacterial biomass had increased, and then over the next 2 weeks it fell 5-fold (Table 3). Bacterial growth rates (average for the whole community) increased over the same period. The number of animals in the meiofauna increased markedly after manure was added (Table 4). As it was

**Table 1.** Effect of pelleted food on bacterial numbers and production in the water column

| Pond | Treatment | Food input g C $m^{-2}$ $d^{-1}$ | Number of bacteria $10^9$ $l^{-1}$ | Production* g C $m^{-2}$ $d^{-1}$ | Doubling time (h) |
|---|---|---|---|---|---|
| River: | | 0 | 8.8 ± 0.8 | 0.76 ± 0.2[a] | 6 |
| 26 | Control | 0 | 8.3 ± 0.6 | 0.43 ± 0.04[b] | 10 |
| 32 | Control | 0 | 8.0 ± 1.0 | 0.45 ± 0.01[b] | 9 |
| 11 | Pellets | 1.0 | 8.8 ± 0.7 | 1.07 ± 0.09[c] | 4 |
| 29 | Pellets | 3.2 | 11.3 ± 1.4 | 1.32 ± 0.11[c] | 4 |
| 23 | Pellets | 4.0 | 25.7 ± 4.3 | 2.10 ± 0.19[c] | 8 |

* Analysis of variance: [a] > [b] significant at 5% level; [b] < [c] significant at 1% level The water depth was 1 m, thus 1 mg C $l^{-1}$ = 1 g C $m^{-2}$. Values shown are mean ± SE with n = 9 for number and n = 5 for production

not possible to count the meiofauna before manure was added, an example of normal density is taken from data for another pond [16]. Growth rates ranged from 0.03–0.17 for nematodes; 0.03–0.15 for copepods, and 0.11–0.3 for polychaetes.

## Discussion

### *Bacterial Contribution to Pond Carbon Budgets*

Bacterial production in the water column was due partly to input from the river, because half the water in the ponds was exchanged daily. The river water drained a large area of mangroves, and so the value of 0.76 mg C $l^{-1}$ $d^{-1}$ (Table 1), although large, is not surprising. In pond 26, which received no organic matter except that in the river water, the bacterial productivity in the water column was about half that in river water, as expected when half the water was exchanged daily.

Much of the added organic matter in feed pellets was supporting bacterial growth, particularly in the water column. Productivity of bacteria was closely correlated with input of organic matter. Approximate estimates of the amounts of organic matter needed to support both bacterial production and respiration would be twice the production values in water and at least 3 times the production values in sediment. These estimates are based on average conversion efficiencies of 50% in the water column and 30% in sediments for the utilization of organic matter by bacteria. Aerobic bacteria in culture are often more than 50% efficient [20]. Anaerobic bacteria, such as sulfate reducers, are much less efficient (around 12–15%) [12, 27]. As bacteria in the pondwater were utilizing pellets comprised of protein and readily digestible carbohydrate, a conversion efficiency of 50% is not unreasonable. In fact, it fits well with the data, because with this factor, utilization of carbon by bacteria balances the input. If the conversion efficiency were much lower, bacterial production and respiration would be in excess of input. It is possible, however, that the average carbon

**Table 2.** Effect of pelleted food on bacterial biomass and production in sediment

| Pond | Treatment | Food input g C $m^{-2}$ $d^{-1}$ | Biomass g C $m^{-2}$ | Production* g C $m^{-2}$ $d^{-1}$ | Doubling time (d) |
|---|---|---|---|---|---|
| Mangrove | | 0 | 3.6 (3.1–4.2) | 0.25 (0.20–0.27) | 10 |
| 26 | Control | 0 | 1.5 (1.4–1.7) | 0.26 (0.20–0.40)$^a$ | 4 |
| 11 | Pellets | 1.0 | 2.7 (2.1–3.4) | 0.24 (0.11–0.35)$^a$ | 9 |
| 29 | Pellets | 3.2 | 2.6 (2.3–3.0) | 0.41 (0.36–0.47)$^b$ | 4 |
| 23 | Pellets | 4.0 | 5.8 (5.2–6.4) | 0.50 (0.47–0.52)$^b$ | 8 |

* Analysis of variance: $^a < ^b$ significant at 1% level
Values for a mangrove sediment on the river bank away from the ponds are also given. Mean and range are given for 4 determinations of production and for duplicates of biomass

**Table 3.** Effect of chicken manure on bacterial biomass, growth rates, and meiofauna in sediment

| | | Bacteria | | | |
|---|---|---|---|---|---|
| Pen | Treatment | Biomass g C $m^{-2}$ | Production mg C $m^{-2}$ $d^{-1}$ | Specific growth rates* $d^{-1}$ | Meiofauna number per 10 $cm^2$ |
| | Open pond | 3.4 ± 0.00 | 440 (340–520) | 0.13 ± 0.03 | 144 |
| A | Manure, 1 week | 4.3 ± 0.20 | 350 (280–420) | 0.08 ± 0.01$^a$ | 300 |
| B | Manure, 2 weeks | 1.9 ± 0.20 | 180 (140–220) | 0.10 ± 0.03$^b$ | 1,500 |
| C | Manure, 3 weeks | 0.8 ± 0.07 | 230 (170–290) | 0.25 ± 0.04$^c$ | 1,500 |

* Analysis of variance: $^a < ^c$ and $^b < ^c$ significant at 5% level
The experiment was carried out in 3 pens as described in Materials and Methods. Values are mean ± range; n = 2 for biomass and number. See Table 4 for details of meiofauna numbers

content of the bacteria was lower than the value used here (see [2]), and thus a lower growth efficiency could apply.

To obtain an estimate of organic matter from added food pellets utilized by bacteria in the water, an average value of 0.44 mg C $m^{-2}$ $d^{-1}$ for the control ponds was deducted from the production values for ponds 11, 29, and 23, and the result was doubled to allow for respiration. For ponds 11, 29, and 23, these estimates are 1.26, 1.76, and 3.32 respectively. In pond 11, the estimate is close to the amount of added food pellets, and indicates that bacteria were utilizing most of the added food. In pond 29, the estimate is only about half the value for added food, but values for bacterial production in sediment are significantly higher in pond 29 than 11. In the nursery area of pond 23, the very high level of bacterial production (Table 1) required most of the added food mash supplied to the young prawns to sustain it. Thus, the food material was dissolving or remaining as small suspended particles in the water column

**Table 4.** Effect of manure treatment on numbers of meiofauna

| Pen | Sample | Nematodes | Harpacticoid copepods | Polychaetes | Others | Total |
|---|---|---|---|---|---|---|
| Open pond[a] | | 132 | 8 | 0 | 4 | 144 |
| A | 1 | 104 | 40 | 112 | 136 | 392 |
| | 2 | 96 | 24 | 16 | 64 | 200 |
| B | 1 | 200 | 480 | 256 | 320 | 1,256 |
| | 2 | 328 | 560 | 520 | 464 | 1,872 |
| C | 1 | 160 | 640 | 440 | 208 | 1,448 |
| | 2 | 368 | 652 | 304 | 200 | 1,524 |

[a] An example of meiofauna density in a stocked pond taken from Moriarty et al. [16]
Pen A: manure treatment for 1 week prior to Feb. 6; Pen B: manure treatment for 2 weeks prior to Feb. 6; Pen C: manure treatment for 3 weeks prior to Feb. 6. Prawns were stocked on Feb. 6
Values are number 10 $cm^{-2}$, 2 cm depth

and providing a substrate for bacterial growth. The very high productivity of bacteria in the sediment would account for the remainder of added mash in the nursery area. The greater bacterial activity in the sediment of pond 29 than in pond 11 is indicated by the faster doubling times (Table 2). It is clear, therefore, that most of the added feed material was not being used by the prawns, but rather was supporting bacterial growth. Net primary production did not contribute significantly to bacterial production in these ponds [16].

Because the time available for this work was limited, it was not possible to study these problems in more detail. The work does demonstrate, however, that aquaculture ponds are useful as experimental sites in microbial ecology because inputs and outputs to the system can be measured. It is possible, for example, to study problems such as the growth efficiency of bacteria in the aquatic environment with different types of carbon source. More research is needed on this topic, in order to improve estimates of bacterial production and carbon cycling.

### *Effect of Bacteria on Oxygen Concentration*

Values for bacterial production in the water column may be used to estimate consumption of oxygen by the bacteria in the planktonic community. Taking pond 23 as an example, at 50% efficiency for utilization of organic C, the bacteria in the water column of the nursery would consume about 5.6 mg $O_2$ $l^{-1}$ $d^{-1}$. More oxygen would be used by aerobic bacteria at the sediment surface. Deoxygenation would occur if aeration was not provided, and, in fact, aeration was needed in this pond. Pond 29 was being aerated and the fast doubling times for the bacterial populations in this pond compared to other ponds are probably a result of the aeration. Bacteria in the water and sediment required about 4.0 mg $O_2$ $l^{-1}$ $d^{-1}$ in pond 29, which is quite high and shows that aeration was desirable. Oxygen supply by exchange from the atmosphere, primary produc-

**Table 5.** Comparison of bacterial numbers and production in the ponds with some other environments

| Site | Number No. $l^{-1}$, or No. $m^{-2a}$ | Production[a] $\mu g\ C\ l^{-1}\ h^{-1}$ or $mg\ C\ m^{-2}\ h^{-1a}$ | Reference |
|---|---|---|---|
| Water | | | |
| Pellet-fed ponds | $8.8 \times 10^9$–$2.6 \times 10^{10}$ | 39–87 | This study |
| Manured ponds | $1.2 \times 10^{10}$–$1.3 \times 10^{10}$ | 37 | This study |
| Seagrass beds | $2.8 \times 10^9$–$6.8 \times 10^9$ | 0.1–0.3 | [19] |
| York River estuary, USA | $1 \times 10^9$–$8 \times 10^9$ | 0.3–3 | [5] |
| Eutrophic lake | $4 \times 10^8$–$2.3 \times 10^9$ | 0.2–7.1 | [1] |
| Open ocean | $5 \times 10^8$–$2.5 \times 10^9$ | 0.08–0.8 | [10] |
| Sediment | | | |
| Pellet-fed ponds | $7 \times 10^{13}$–$2.1 \times 10^{14}$ | 6–21 | This study |
| Manured ponds | $5.3 \times 10^{13}$–$8.3 \times 10^{13}$ | 12–17 | This study |
| Seagrass beds | $4.3 \times 10^{13}$–$1.7 \times 10^{14}$ | 2–7 | [13, 19] |
| Coastal sediment | $9.7 \times 10^{12}$–$4.1 \times 10^{13}$ | 0.7–1.7 | [6] |

[a] Units: for water $l^{-1}$; for sediment $m^{-2}$ and 10 mm depth

tion, and flushing with river water are unlikely to be sufficient for maintaining a level high enough to avoid stress for animals when bacterial production is so high.

*Methodology*

It is unlikely that the productivity in sediments has been overestimated due to disturbance or aeration during experimental treatment. DNA synthesis is closely regulated in bacteria, and changes in external conditions do not immediately affect the rate of thymidine incorporation into DNA [15]. The only macromolecule that was labeled significantly during the first 15 min was DNA (Fig. 1). Other compounds, possibly proteins, were labeled after 15 min, so all incubations were kept shorter than this to minimize errors.

The production values have been expressed in terms of 1 day, although measurements were made during only part of the day. Major diel cycles in bacterial production are unlikely to have occurred here as there was not a close link with excretion of organic matter during photosynthesis by a macrophyte such as seagrass [19]. There may have been small variations linked to water exchange or daily food pellet addition. Riemann and Sondergaard [22] found that diel variations in bacterial growth rates were minor in seawater and lakes. As the bacterial production in these ponds was dependent mainly on organic matter already present in the sediment, or from an excess supply of feed pellets, diel variations are likely to have been small and thus any errors in calculating daily carbon budgets would be small. Further work is needed to check this assumption.

### *Role of Meiofauna*

Grazing by meiofauna, and perhaps protozoa, is apparently an important factor limiting bacterial densities and production in the pond sediments. The inverse correlation between bacterial density changes and meiofaunal density in the pen experiment supports the concept that meiofauna can limit bacterial biomass [11] (Table 3). Stimulation of bacterial growth rates by the grazing pressure also occurred. Total bacterial productivity fell because a large proportion of the bacteria were removed. The effect of protozoans was not measured, but presumably was also substantial. Protozoa are probably the main grazers on bacteria in sediments [8]. Further work is needed to quantify the food chain dynamics at trophic levels occupied by protozoa and meiofauna in ponds such as these where the higher consumers are predominantly carnivores. Bacteria were not significant components in the prawns' diet in these ponds [16], so growth efficiencies at the lower trophic levels could control the productivity of these ponds.

### *Comparison with Natural Environments*

These aquaculture ponds support greater numbers and productivity of bacteria than do the ocean or lakes (Table 5). The productivity of bacteria in sediments associated with seagrasses is similar to that in pond sediment. Bacterial biomass and productivity in mangrove sediments was also similar to that in the ponds (Table 2), and indicates that bacterial activities are important in mangrove ecosystems.

Schroeder [23] reported values for bacterial numbers that are 2–3 orders lower than those found for this study in the water column and up to 7 orders of magnitude lower in the sediments. The difference is due to technique. Schroeder [23] counted colonies of bacteria on agar plates, but plate counts considerably underestimate true numbers [26]. Direct counts using epifluorescence microscopy are simple to make in the water column, but more difficult in sediments where many bacteria cannot easily be separated from particles. Muramic acid determinations are, therefore, more useful in sediments and give reasonably accurate results [13, 17]. As shown in the results here, there was a good agreement between direct counts and muramic acid values in the water column.

## Conclusions

The work reported here was a preliminary survey of bacterial productivity in the prawn ponds; there was insufficient time for detailed analyses with many replicates. The work does show, however, that bacterial activities accounted for most of the organic carbon added to the ponds. The prawns needed only a small amount of the pelleted food [16]. Schroeder [24], using ratios of stable carbon isotopes, reached similar conclusions concerning ponds used for culture of common carp and the prawn, *Macrobrachium rosenbergii*. These animals were found to have fed more on natural foods rather than pelleted food.

The techniques for bacterial production, when combined with analysis of nutrient turnover and primary production, can be used to study quantitatively the carbon cycle in ponds, and show what happens to organic matter inputs. If protozoa and meiofauna are studied as well as the animals of commercial interest, a detailed analysis can be made of food chain dynamics. From these types of studies, predictions can be made about the effect of supplemental feeding or how much pelleted food can be substituted by manuring. Such studies would be particularly valuable in ponds where microphagous fish such as mullet or tilapia are cultured.

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