

Effect of Removing Accumulated Sediments on the Bacteriology of Ponds Used to Culture *Penaeus monodon*

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Abstract

The bacteriology of sediment was studied at two farms and mangrove habitats on the Clarence River, Australia, from 1990 to 1995. *Penaeus monodon* was grown semi-intensively at both farms. Accumulated sediment was removed between crops at one farm while it was spread over pond bottoms at the other farm. Spatial and temporal distributions of vibrios were not significantly different between the farms, with maximum levels occurring in the center of ponds during times of peak temperature and feed rate. Vibrio levels in pond sediment were usually an order of magnitude higher than in mangrove sediment. Vibrios levels in mangrove sediment near effluent canals were also significantly higher than those from mangrove sites 2 km from the farms. Both farms had similar spatial and temporal patterns for sulfate-reducing bacteria. As for heterotrophic bacteria, no significant difference was found for farm type or location on pond bottom. It was concluded that a) drying sediment between crops temporarily reduced vibrios and sulfate-reducing bacteria; b) removing accumulated sediment did not reduce vibrios, sulfate-reducing bacteria or heterotrophic bacteria; and c) levels of vibrios and sulfate-reducing bacteria were better biological indicators of pond health and environmental conditions than heterotrophic bacteria. It is hypothesized that the bacteriology of pond sediments is largely driven by input of pelleted feed, ambient temperature and limiting levels of oxygen.

Introduction

Vibriosis is a form of bacterial septicaemia which is one of the most common and serious diseases in the culture of *Penaeus monodon* and other shrimp (Bell and Lightner 1992; Anon. 1994a). It occurs in all countries which culture shrimp and the most frequent causative agents are *Vibrio parahaemolyticus*, *V. alginolyticus*, *V. harveyi* and *V. vulnificus* (Lightner 1996). *Vibrio* infections are generally thought to occur when either stresses in the environment or primary pathogens, such as viruses, weaken the shrimp's immune system, leading to fatal secondary infection (Lightner 1983; Flegel *et al.* 1992; Fulks and Main 1992; Nash *et al.* 1992; Mohny *et al.* 1994; Lightner 1996). However, some researchers report that some highly virulent vibrios are the primary causative agents in outbreaks of mass mortalities (Muir 1990; Lee *et al.* 1996; Lightner 1996).

Smith (1993) reported that levels of vibrios were 50 to 100 times higher in pond sediment than in pond water at Australian shrimp farms, hence the

level of *Vibrio* spp. in pond sediment is an important parameter to monitor because it is an indicator of bacterial load and stress on the immune system of cultured shrimp.

Dissimilatory sulfate-reducing bacteria in the sediment of earthen ponds may also be a major cause of stress and reduced productivity in shrimp farming. During anaerobic respiration, sulfate-reducing bacteria generate hydrogen sulfide which is either released as a gas or converted to iron monosulfide. Hydrogen sulfide is toxic to shrimp at trace levels (Apud *et al.* 1983) and accumulated sediments contain, on average, 0.12 % S as iron monosulfide (Masters and Smith 1995, 1996). However, there have been few reports on levels of sulfate-reducing bacteria in shrimp pond sediment (Owens 1996; Suplee and Cotner 1996).

Management of pond sediment may be a key factor influencing the bacteriology of earthen shrimp ponds and health of shrimp during growout (Anon. 1994b). In standard management practices, aerators are used to accumulate the organic wastes, detritus and clay particles in the central regions of ponds. Accumulated sediments are usually removed at the end of the season, though Boyd *et al.* (1994) suggested that the accumulated material, which is mostly eroded soil (Smith 1993, 1996a), should be spread over the pond bottom. However there have been few reports on the effect of management practices on the bacteriology of pond sediment in shrimp farms.

This paper reports on a study carried out on the Clarence River, Australia, over five years at two semi-intensive farms which grew *P. monodon* and used different management practices for pond sediment. The levels of bacteria (heterotrophs, *Vibrionaceae* and sulfate-reducing bacteria) in pond sediment were monitored with the aims of determining whether a) removing accumulated sediments could reduce levels of bacteria, b) there was a temporal or spatial pattern in the levels or types of bacteria in pond bottoms, and c) the levels of bacteria in pond sediment were different from those in mangrove sediment. The results of this study could increase our understanding of the bacteriology of pond sediments and indicate appropriate methods for managing accumulated sediments.

Materials and methods

Study sites

The two farms selected for this study were located approximately 1 km apart on a broad, fast-flowing section of the Clarence River, Australia (29.26° S, 153.23° E). There were three other shrimp farms operating on the Clarence River, but they were either not on the same branch of the river, or more than 5 km away. Both farms in this study carried out similar practices of animal husbandry: they were usually stocked early in October at a density of 30-40 m⁻², paddle-wheel aerators were positioned around their periphery at 8 - 10 ha⁻¹, water depths averaged 1.5 m, water exchange rates were usually minimal in the first months of growout but increased to 5% of pond volume per day by week 11 and were maintained at this level until harvest. Harvest

at farm A usually commenced in week 20 and continued until about week 30, while at farm B a partial harvest was often carried out in week 17 to sell to the Christmas market. The same brands of imported commercial pellet feeds were used at both farms and feed trays were monitored daily. Records of feeding rates were only available from farm A (Table 1). Average feed rates reached a maximum of 72 kg ha⁻¹ d⁻¹, though rates for individual ponds reached maximums of up to 105 kg ha⁻¹ d⁻¹ from week 16 to 24. Table 1 shows that by week 27, the feeding rate was only 35 kg ha⁻¹ d⁻¹ because a) food consumption was lower in cooler temperatures and b) prawn biomass had been reduced by previous harvesting. During the study, harvests ranged from 2,500-8,000 kg ha⁻¹ crop⁻¹ at both farms.

The environmental conditions at the two farms were comparable. Soil types were the same at both farms (Smith 1996a). Salinities in the ponds were 25-30 ppt during the first 12 weeks of growout and 15 - 25 ppt for the remainder. Average water temperature for ponds were not significantly different ($P < 0.01$) at the two farms, but were 1-2°C warmer than the river (Table 1).

Farm A had 14 ponds with a total pond area of 26 ha, with pond sizes ranging from 1.0-2.7 ha. At farm B, there were 5 ponds with a total pond area of 2 ha, with ponds ranging from 0.25-1.0 ha. During winter, farm A usually dried the pond bottoms for at least 4-6 weeks then removed most of the accumulated sediment from the gutters and central regions, whereas farm B usually dried the pond bottoms for 3-4 weeks and spread the accumulated sediments over the bottoms and walls.

Sampling techniques

Field trips to the farms occurred on three to five occasions each year; usually in October, November, December, January and April. Hierarchical sampling was employed for the farms and the river samples were taken from a few representative sites using replicates and repeats over a number of seasons. Sediment samples were routinely collected in duplicate from at least five ponds at each farm at three locations: 5m from the wall, 20m from the wall and in the centre of ponds. Six mangrove habitats were at least 2 km from prawn farms and six sites were within 100 m of the outlets of the effluent canals (Smith 1996a). For the sites near the effluent canals, one site was 100 m upstream, while the others were downstream by 10 m, 20 m, 30 m, 50 m and 100 m.

Table 1. Average water temperature and feed rates for farms on the Clarence River in 1990-95.

Week	River temperature (°C)	Pond temperature (°C)	Average feed rate (kg · ha ⁻¹ d ⁻¹)
1	21.5 ± 0.5 (35)	22.0 ± 0.5 (55)	2 ± 1 (16)
5	23.0 ± 0.4 (23)	24.5 ± 0.7 (48)	13 ± 2 (24)
11	26.1 ± 0.6 (35)	28.3 ± 0.4 (58)	38 ± 4 (25)
16	26.5 ± 0.4 (28)	29.1 ± 0.7 (36)	72 ± 5 (15)
27	22.4 ± 0.6 (31)	23.2 ± 0.5 (42)	35 ± 4 (23)

Sediment samples were collected in 100-ml sterile plastic containers with sufficient water to keep the sediment moist. Samples were processed within 3 hrs of collection by mixing with a clean glass rod, withdrawing 0.5 ml of moist sediment with a sterile pipette, homogenizing in 4.5 ml of sterile seawater and transferring into tenfold serial dilutions.

Determining abundance and speciation of bacteria

Levels of viable heterotrophic bacteria and presumptive *Vibrio spp.* were determined by counting the colonies which grew on plates of Marine Agar (Difco Laboratories) and TCBS (Oxoid), respectively, inoculated with diluted sediment samples (Cameron *et al.* 1988). Also, isolates of vibrios were randomly selected and subcultured from plates with less than ten colonies (i.e. highest dilutions). *Vibrio* isolates were classified according to key diagnostic traits (Baumann and Schubert 1984). Comparisons were made between the vibrio counts for duplicate sets of TCBS plates grown aerobically and anaerobically (in hydrogen and carbon dioxide gas).

Levels of dissimilatory sulfate-reducing bacteria were determined by counting the colonies that grew on plates of selective agar as well as by estimating the most probable number (MPN) from broths with poised redox potential and 2.5% NaCl. The agar and broth were both based on Medium E (Postgate 1984) and the method described by Pankhurst (1971). Plates and broths were inoculated with duplicate diluted samples and incubated at 30°C in anaerobic jars (in hydrogen and carbon dioxide gas) for at least 30 days. Because counts from agar plates were between 3 and 20 times higher than those for broths, the results presented here were calculated from plates only.

Levels of bacteria are quoted in colony forming units per ml of sediment (cfu ml⁻¹). The means and standard error of the means are presented in accompanying tables and figures. Student's t-test was used to detect differences.

Results

Vibrionaceae

Fig. 1 shows that levels of vibrios in prawn pond sediment increased significantly during the growout season, with the highest values measured in week 16 ($2.6 \times 10^6 \pm 0.4 \times 10^6$ cfu ml⁻¹, n=95). *Vibrio* levels in the sediment increased slightly during the same period to $2.4 \times 10^5 \pm 0.2 \times 10^5$ cfu ml⁻¹ (n=60) in mangrove habitats Fig. 2 shows that average levels of vibrios in pond sediment have a similar seasonal pattern at farm A and farm B.

At the start of the 1992-93 season, the vibrio levels were measured in ponds that had been dried for 12 weeks between crops. Levels of vibrios in pond sediment were below detectable limits of 100 cfu/ml (n=6) at both farms. However, in ponds which had been dried for a similar period but filled with river water to a depth of 400 mm for 48 hr, levels of vibrios in pond sediment averaged $1.0 \times 10^6 \pm 0.3 \times 10^6$ cfu ml⁻¹ (n=9) at Farm A, and 8.0×10^5 (n=3) at farm B.

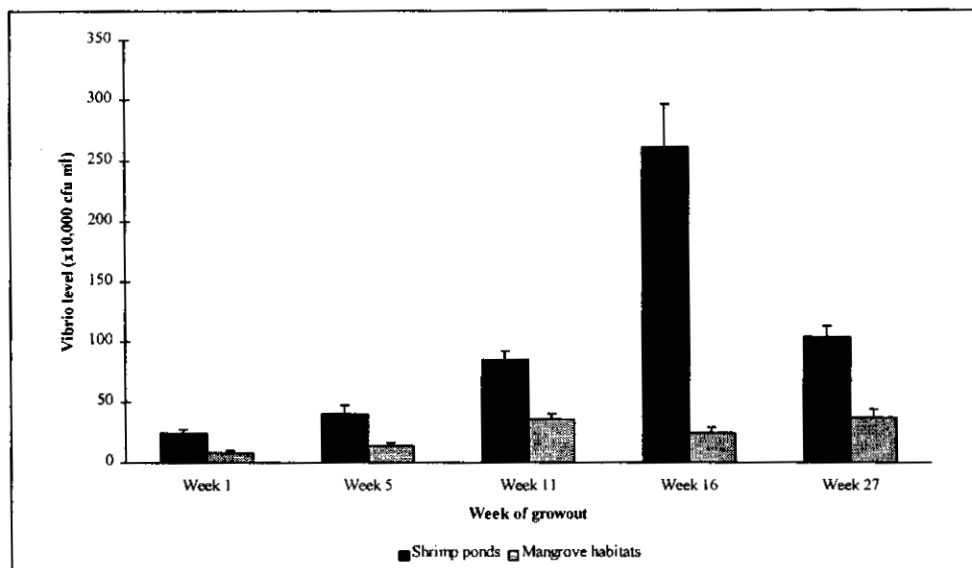


Fig. 1. Average levels of vibrios in sediment from farms and mangroves. Samples were from shrimp ponds and mangrove habitats during the growout seasons 1990-1995. October was the first month of growout.

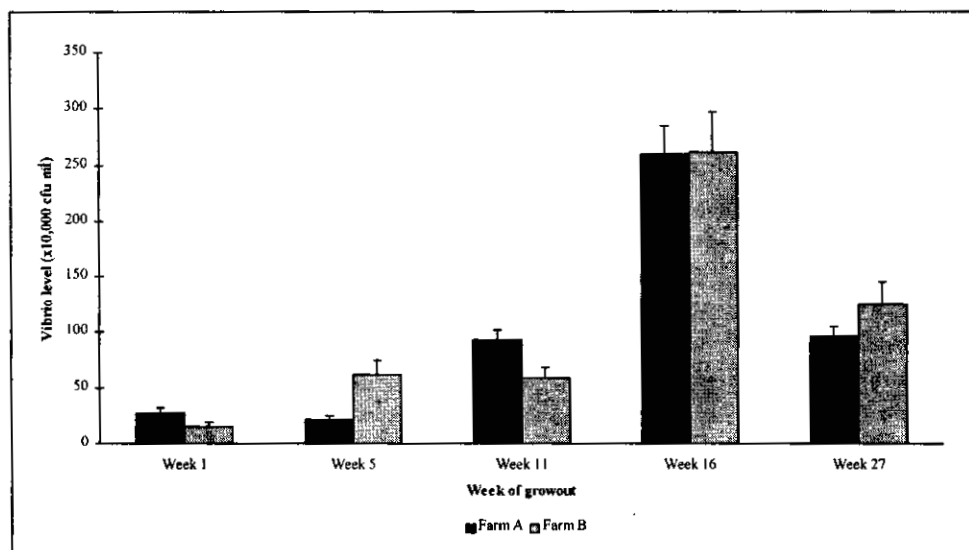


Fig. 2. Average levels of vibrios in pond sediment. Samples were from farm A and farm B during the growout seasons 1990-1995.

At mangrove habitats near the farms, the vibrio levels in the sediment increase throughout the season, reaching a maximum average of $8.3 \times 10^5 \pm 0.2 \times 10^5$ cfu ml⁻¹ (n=30) in April, i.e. week 27 of growout (Fig. 3). In comparison, at mangrove sites away from the farms, the vibrio levels reached a peak in week 16 of growout, the warmest period, at $1.9 \times 10^5 \pm 0.5 \times 10^5$ cfu ml⁻¹ (n=28). The differences were significant ($P < 0.05$) for all periods of measurements.

Figs. 4 and 5 compare the levels of vibrios in pond sediment at three locations in farms A and B, respectively. In the first week of growout at both

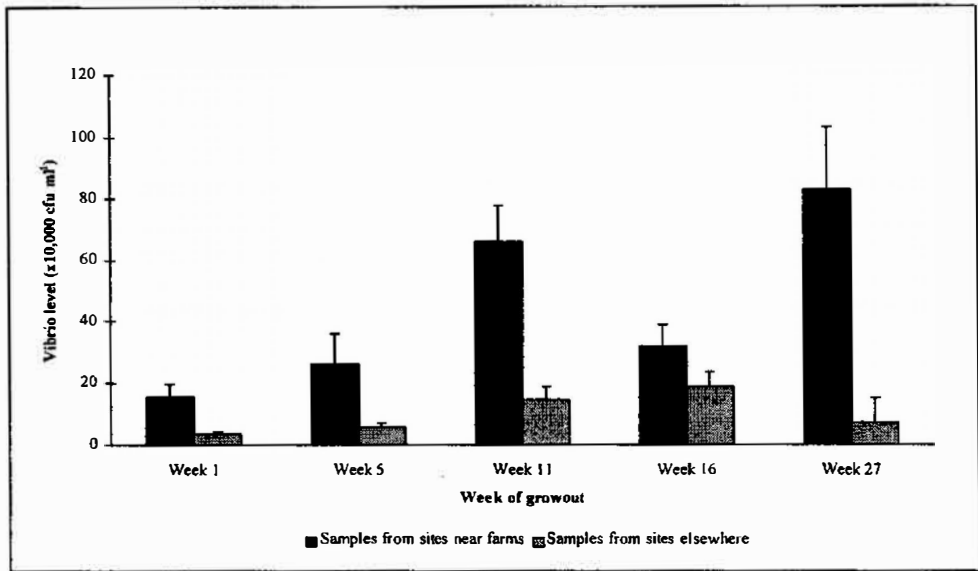


Fig. 3. Average levels of vibrios in sediment from mangrove habitats. Samples were from sites near farms and far from farms during the growout seasons 1990-1995.

farms, the levels of vibrios were usually not significantly different ($P>0.05$). In week 16 at farm A, the levels of vibrios were significantly higher ($P<0.05$) in the centre of ponds than in other areas (Fig. 4). At farm B, the levels 20 m from the wall were higher than other areas, but not significantly higher than in the centre (Fig. 5). By week 27, levels of vibrios at both farms were highest 20 m from the wall.

In the 1995-96 season, farm A applied a commercially available brand of beneficial bacteria at a rate recommended by the supplier. Vibrio levels were $55 \times 10^5 \pm 22 \times 10^5$ cfu ml⁻¹ ($n=6$) in ponds which had been treated throughout the season, while vibrio levels averaged $70 \times 10^5 \pm 21 \times 10^5$ cfu ml⁻¹ ($n=12$) in the untreated ponds ($P>0.05$). Also, the farmer reported that at the end of the season, there had been no obvious difference in growth rates or survivals between treated and untreated ponds.

Vibrio vulnificus was the most common species found in prawn pond sediment, accounting for 30% of all isolates ($n=139$). In total, the group of *V. vulnificus*, *V. alginolyticus*, *V. harveyi* and *V. parahaemolyticus*, accounted for 63% of isolates. The dominance by that group was more pronounced in farm A (79%, $n=74$) than in farm B (45%, $n=65$). This group also dominated at all three sampling points on the pond bottom, with 63% of isolates ($n=43$) at sites 5m from the wall, 64% ($n=49$) at sites 20 m from the wall and 62% ($n=47$) in the center of ponds.

Sulfate-reducing bacteria

Average levels of dissimilatory sulfate-reducing bacteria in pond sediment increased dramatically from week 1 to 5 at both farms, with the highest levels being recorded in week 11 (Table 2). Highest levels of sulfate-reducing

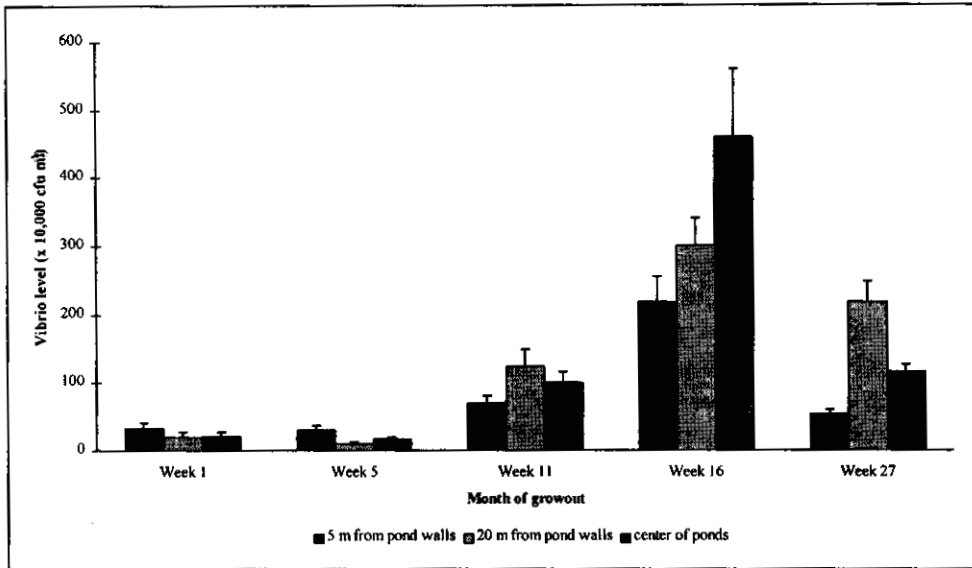


Fig. 4. Average vibrio levels in sediment from ponds at farm A. Samples were from three locations: 5 m from pond walls, 20 m from walls and in the center of ponds.

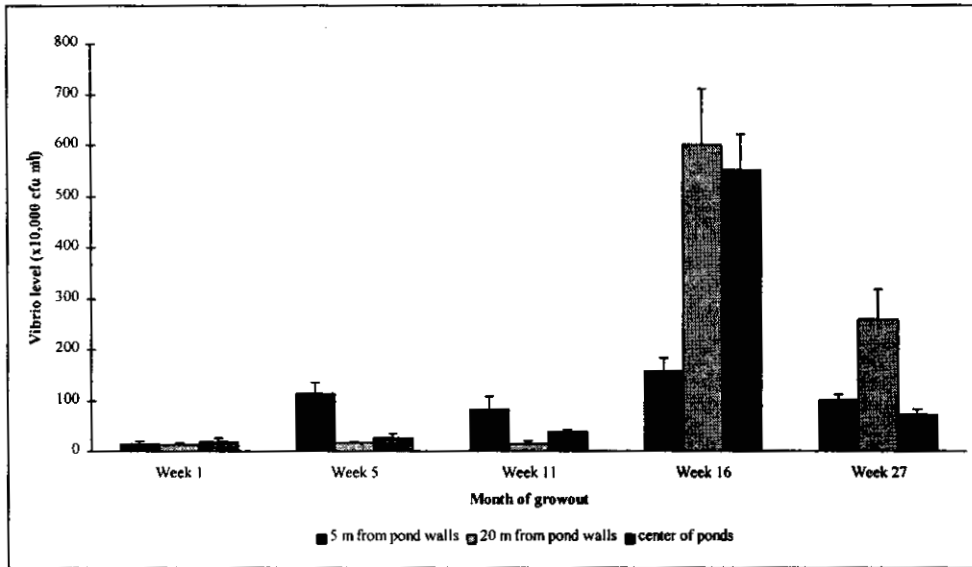


Fig. 5. Average vibrio levels in sediments from ponds at farm B. Samples were from three locations: 5 m from pond walls, 20 m from walls and in the center of ponds.

bacteria were found in the center of ponds at both farms, but the differences in levels between the three locations were not significant. There was one exception which occurred at farm B, where high levels were measured in the center of ponds in week 1 (Table 2). This farm usually had a shorter drying period than farm A between crops, so the center of the ponds had remained slightly moist and the sediment was still black beneath the surface.

Levels of sulfate-reducing bacteria in mangrove sediment did not vary significantly during the monitoring period. Average levels of sulfate-reducing

bacteria were $1.1 \times 10^4 \pm 0.4 \times 10^4$ cfu ml⁻¹ (n=16) in mangrove sediment near the farms and $1.4 \times 10^4 \pm 0.9 \times 10^4$ cfu ml⁻¹ (n=15) away from the farm. The difference was not significant ($P > 0.05$).

The occurrence of black colonies on TCBS agar (i.e. sulfate-reducing facultative anaerobes) was a common occurrence in all years and these levels were monitored during the 1993-94 growout. Table 3 shows that there was no spatial pattern to the distribution or abundance of sulfate-reducers at either farm, but there did appear to be a decrease in abundance at most locations towards the end of the growout. Also, the percentage of black colonies on TCBS agar plates was not significantly different ($P > 0.05$) for anaerobic and aerobic incubation conditions (Table 3). The average results for samples taken in week 5 and 11 show that, at farm A, $16 \pm 2\%$ (n=46) of presumptive vibrios were black, which was significantly lower ($P < 0.05$) than at farm B, where $23 \pm 3\%$ (n=25) were black.

Table 2. Average levels of dissimilatory sulfate-reducing bacteria in pond sediment.

Location	Farm	Dissimilatory sulfate-reducing bacterial (cfu ml ⁻¹)			
		Week 1	Week 5	Week 11	Week 16
5 m	A	160 _a	104 _c	2x10 ⁴ _c	9x10 ⁵ _{cd}
	B	100 _a	3x10 ⁴ _c	4x10 ⁵ _c	8x10 ⁵ _{cd}
20 m	A	100 _a	5x10 ³ _{bc}	3x10 ⁴ _c	3x10 ⁶ _{cd}
	B	500 _a	3x10 ³ _{bc}	3x10 ⁴ _c	6x10 ⁶ _d
centre	A	100 _a	104 _c	2x10 ⁴ _c	106 _{cd}
	B	103 _b	5x10 ⁵ _c	3x10 ⁵ _c	7x10 ⁶ _{cd}

¹Means sharing the same subscript were not significantly different ($P < 0.05$).

Note: Levels of dissimilatory sulfate-reducing bacteria were monitored at farms A and B at three locations: 5 m from the walls, 20 m from the walls, and in the center of ponds. Five ponds were monitored at each farm during two growouts (1993-95).

Table 3. Spatial and seasonal effects on the percentage of black colonies on TCBS agar for samples collected from pond bottoms in 1993-94. The percentage of black colonies (sulfate reducers) relative to total colonies which grew on plates of TCBS agar at three locations at farms A and B: 5 m from the walls, 20 m from the walls and from the center of ponds. Duplicate sets of TCBS plates were incubated under aerobic and anaerobic conditions.

Location	Farm	Incubation of TCBS plates	Week 5 Sulphate-reducers (%)	Week 11 Sulphate-reducers (%)	Week 27 Sulphate-reducers (%)
5 m	A	aerobic	18 ± 2 (5)	10 ± 4 (10)	3 ± 1 (5)
5 m	A	anaerobic	24 ± 3 (5)	14 ± 3 (10)	n.a.
5 m	B	aerobic	24 ± 3 (4)	6 ± 2 (4)	24 ± 9 (3)
5 m	B	anaerobic	19 ± 6 (4)	n.a.	n.a.
20 m	A	aerobic	38 ± 7 (6)	11 ± 6 (10)	1.0 ± 0.4 (5)
20 m	A	anaerobic	40 ± 10 (3)	24 ± 7 (10)	n.a.
20 m	B	aerobic	26 ± 6 (4)	25 ± 12 (4)	19 ± 6 (3)
20 m	B	anaerobic	44 ± 12 (4)	n.a.	n.a.
center	A	aerobic	15 ± 1 (5)	14 ± 6 (10)	1.8 ± 0.9 (3)
center	A	anaerobic	10 ± 4 (3)	17 ± 4 (10)	n.a.
center	B	aerobic	31 ± 11 (5)	26 ± 19 (4)	4 ± 1 (3)
center	B	anaerobic	9 ± 2 (3)	n.a.	n.a.

¹n.a. = not available

Of 30 black colonies randomly selected from TCBS plates during the season, 20% were identified as vibrios (*V. alginolyticus*, *V. campbelli*, *V. splendidus II*, *V. harveyi*, *V. nigripulchritudo*, *V. Fischeri*), 40% were tentatively identified as *Flavobacterium* spp., 16% were non-vibrio types and 14% did not grow when subcultured. Only 12% of the 30 isolates retained the black color when re-cultured on TCBS agar. Black colonies were also frequently isolated on TCBS agar from mangrove sediment ($14 \pm 4\%$ of colonies, $n=35$), from surface scrapings of seagrass leaves fouled with epiphytes and silt as well as from the hepatopancreas of shrimp.

Heterotrophic bacteria

The average level of heterotrophic bacteria at farm A was $10.9 \times 10^6 \pm 0.5 \times 10^6$ cfu ml⁻¹ ($n=95$), which was significantly higher ($P<0.05$) than the average level of $8.2 \times 10^6 \pm 0.6 \times 10^6$ cfu ml⁻¹ ($n=49$) at farm B. Fig. 6 shows the seasonal changes in levels in heterotrophs in pond sediment at the two farms.

Prior to the 1992-93 season at farm A, the levels of heterotrophic bacteria in pond sediment were measured in ponds that have been dry for 12 weeks. Levels of heterotrophic bacteria were $2.4 \times 10^6 \pm 0.5 \times 10^6$ cfu ml⁻¹ ($n=6$). However, in ponds that have been dry for a similar period but have been filled with river water to a depth of 400 mm in the previous 48 hr, levels averaged $16.0 \times 10^6 \pm 2.6 \times 10^6$ cfu ml⁻¹ ($n=9$), which was significantly higher ($P<0.01$) than those in the dry ponds but comparable to levels found during growout (Fig. 6).

Levels of heterotrophic bacteria in mangrove sediment were usually lower than levels in shrimp ponds (Fig. 6). In mangroves near the farms, levels of heterotrophs in sediment averaged $3.2 \times 10^6 \pm 0.4 \times 10^6$ cfu ml⁻¹ ($n=46$) while, in mangroves far from the farms, levels were $3.7 \times 10^6 \pm 0.3 \times 10^6$ cfu ml⁻¹ ($n=42$).

Discussion

Limitations of the study

The study focused on the ponds at two shrimp farms and the mangrove habitats on the Clarence River. To minimize variations between seasons and temporary local aberrations, measurements were made over five growout periods using replicates and a range of representative sites. While attempts were made to ensure that the farms which were selected were similar in most respects (apart from the practice of removing sediments between crops), the results could have been affected by other less obvious factors, such as daily differences in feeding rates, fertilizing and water exchange rates. Also, although the mangrove sites were chosen so that they were similar in most respects (except for their distance from the farms), it is possible that factors unrelated to shrimp farming, were impacting on the sites. Therefore, results from comparable studies by other researchers are needed before the findings described here can be generalized.

Another limitation was the reliance of the study on traditional plate and broth techniques. Unfortunately, viable counts from culture techniques

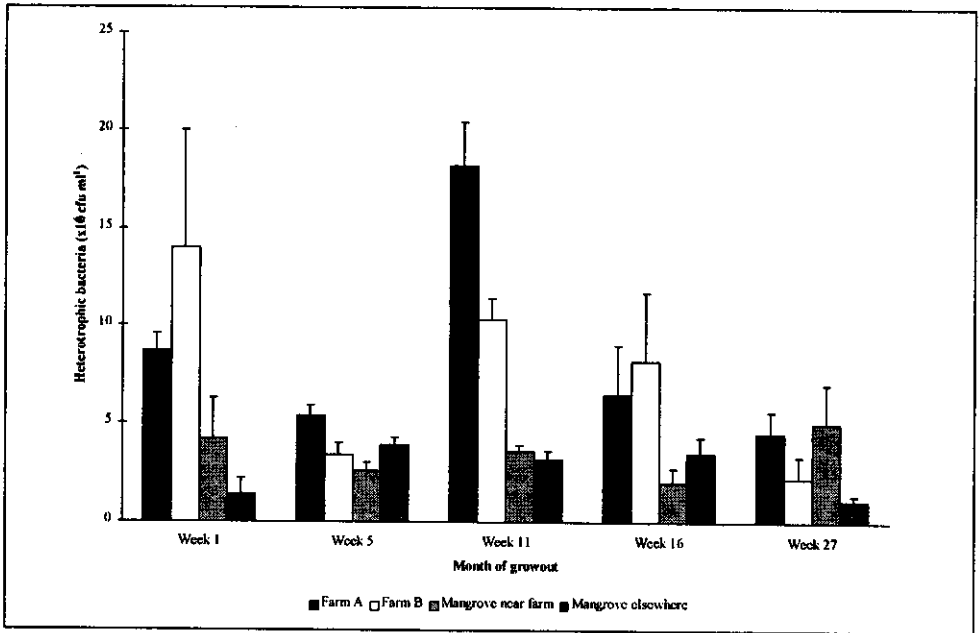


Fig. 6. Average levels of heterotrophic bacteria in pond and mangrove sediment. Samples were collected 5 m from walls at farms A and B and mangrove habitats near from farms and far from farms.

constitute between 0.0001% and 10% of counts obtained by microscopy (Hart *et al* 1996), so the methods used here were unable to provide a complete picture of the bacteriology of the sediment. Hence, further studies which use radioactive tracers (Suplee and Cotner 1996) or alternative techniques for detecting bacteria or their processes are necessary

Nevertheless, the findings have important implications on the industry since shrimp diseases are often a symptom of deteriorating pond conditions and bacteriology may provide bioindicators that can be used to assess pond health.

Levels of bacteria in pond sediment

Bacteria are responsible for many of the dominant chemical processes that occur in earthen shrimp ponds, and they are either the primary or secondary pathogens in many infectious diseases. This case study confirmed that shrimp pond sediment is rich in bacteria associated with both of these activities.

The types of vibrios which were isolated from shrimp pond sediment included a high proportion of strains which are known to be pathogenic to shrimp (Lightner 1996). Also, levels of vibrios in pond sediment increased during the growout (Figs. 1, 2) as temperature and input of feed increased (Table 1). Drying sediments between crops reduced vibrios to minimal levels but levels increased rapidly at both farms when the ponds were refilled.

The practice of using aerators to collect detritus in central regions of the ponds may be expected to produce lower levels of vibrios in the periphery of ponds. However, vibrio levels were generally independent of location on pond bottoms at both farms (Figs. 4, 5). In the first weeks of growout, vibrio levels were slightly higher in the periphery of ponds. Then in week 16, when feeding

rates were high, vibrio levels were lowest around the periphery of the ponds at both farms. However, there was less than an order of magnitude difference between rates at the three locations at any time (Figs. 4, 5). The enrichment of the sediment by the input of feed and decaying algal blooms are likely to be the main cause for these general increases.

Because of the pivotal role that vibrios play in shrimp health and breakdown of organic matter as well as the relative ease with which TCBS counts of pond sediment can be made, it is suggested that the level of vibrios in sediment is a useful biological indicator of pond health. In comparison, the level of heterotrophic bacteria was not a reliable indicator of pond conditions. A relatively narrow range was measured for the level of heterotrophic bacteria during the growout (Fig. 6) and it was not possible to accurately identify effects caused by temperature or feed input.

Sulfate-reducing bacteria in marine sediments have been reported to metabolize 25-50% of the carbon (Hart *et al* 1996) and the present study showed that levels of sulfate-reducing bacteria increased significantly during growout. Levels reported here were more than an order of magnitude higher than those reported by Suplee and Cotner (1996), suggesting that Suplee and Cotner underestimated their results, which were obtained by MPN methods. From flux measurements, they estimated peak levels that were more likely to be 10^6 cells ml^{-1} , which is comparable to the levels obtained here. Suplee and Cotner (1996) also found that new ponds initially had lower levels of sulfate-reducing bacteria than old ponds, but the difference was lost by the 17th week of growout. There was a comparable result in the present study. Table 2 shows that when sediments were dried between crops in the first week of growout, levels of sulfate-reducing bacteria were 100 cfu ml^{-1} while, in sediment which had not been dried sufficiently, levels of sulfate-reducers were significantly higher. However, by week 11, all ponds had similar levels.

Sulfate-reducing bacteria are also a useful biological indicator of pond health because of the strong links between them and decreases in available oxygen in the sediment (Suplee and Cotner 1996), input of feed and deterioration of pond sediment through an increase in level of sulfides (Masters and Smith 1995, 1996).

The regular growth of black colonies on TCBS agar from samples taken from pond sediments, mangrove sediments and other sources is of particular interest. Black colonies on TCBS agar plates have been presumed to be sulfate-reducing vibrios (Cropp and Garland 1988) but this study found numerous strains of facultive anaerobes which were capable of reducing sulfate. Table 3 showed that the sulfate-reducing facultive anaerobes occurred throughout the growout at significant levels in all locations in pond sediment. The lowest levels were measured towards the end of the growout, but the number of samples taken in this period was small, so little significance could be attached to this result.

Significantly, the ponds used in the present study have been used for shrimp farming for more than two years before the start of the study, hence the ponds could be categorized as aged. In this case, when the accumulated sediment is removed (as in farm A), the sediment which remained would

contain nutrients and organic matter from previous seasons. This factor could have contributed to the similarity in the levels of the three types of bacteria which were investigated between the farms (Tables 2, 3 and Figs. 2, 4, 5, 6).

Bacterial processes in pond sediments

The bacterial processes in the sediment and benthic layer can be categorized into photosynthesis, breakdown of organic wastes and nutrient recycling. As for photosynthesis, benthic cyanobacteria can be the dominant photosynthetic organisms in shrimp ponds (New and Rabanal 1985), though it has been argued that this is undesirable because some types of cyanobacteria are capable of producing toxins which are harmful to shrimp (Lightner 1978; Smith 1996b).

The processes of degrading organic matter and recycling nutrients are carried out under a combination of anaerobic and reduced conditions. Suplee and Cotner (1996) reported that the oxygen demand rate for shrimp pond sediment was considerably higher than for natural systems and the rate was determined by input of feed. Smith (1996a) reported redox potentials of -160 mV in the sediment in the periphery of shrimp ponds and -368 mV in the central regions. These conditions, which are generated by bacteria in the sediment, also select for the type of bacteria which can inhabit the sediment and the outcomes of bacterial processes. For example, the breakdown of organic matter by dissimilatory sulfate-reducing bacteria requires anaerobic conditions and redox potentials < -150 mV (Postgate 1984) and produces toxic forms of sulfur (H_2S). In ammonification, bacteria decompose nitrogen containing wastes and release ammonia which is also toxic to shrimp. Furthermore, the solubility, reactivity and oxidation state of metals and nutrients in reduced sediments would be significantly different from those for oxidized sediments.

In a related part of this study, the chemical and physical characteristics of sediment in shrimp ponds at farms A and B were also investigated (Smith 1996a). That study found that the accumulating sediment is a complex mixture of quartz, feldspar and clay minerals (70-80%); amorphous oxides of silicon, iron and aluminium (5-10%); organic matter and volatile compounds (5-10%); and elevated trace metals and nutrients. Although input of pelleted feed, feces and decaying plankton enriched the sediment, it was found that the main source of the sediment was soil which was eroded from the walls and periphery of ponds.

Because that study also found that there was no significant difference in the organic content and the nutrient levels of sediments between farms A and B (Smith 1996a), it was hypothesized that microbial processes stabilized these levels in both farms. Hence, Smith (1996a) concluded that the practice of removing accumulated sediments had no significant effect on the chemical and physical characteristics of the sediment. The finding of the present study that removing sediments did not reduce levels of vibrios or sulfate-reducing bacteria provides further evidence to support the hypothesis that bacterial processes in the sediment drive conditions in pond sediments at semi-intensive shrimp

farms to a similar status, regardless of the management practice of removing sediments between crops. The three dominant factors that influence the bacteriology appear to be input of pelleted feed, ambient temperature and limiting levels of oxygen.

The final management decision, as to whether accumulated sediments should be removed, needs to be based on a number of considerations. This study of the bacteriology and a previous study of the nutrients (Smith 1996a) suggest that it is an unnecessary practice. However, Masters and Smith (1995, 1996) showed that undesirable reduced sulfur compounds, such as pyrite and jarosite, are at higher concentrations in the accumulated sediments than in other locations on the pond bottom. That study would suggest removing those sediments as a useful practice.

Effect of farm discharge on the bacteriology of mangrove habitats

It has often been claimed that shrimp diseases and losses in productivity are caused by environmental degradation and self-pollution (Anon. 1990; Revord and Weidner 1992; Rosenberry 1993) However, there have been few environmental studies which have measured environmental impacts. This study has shown that vibrio levels in sediment were 10 times higher in ponds than those in mangrove habitats. Further, because significantly higher vibrio levels were found in mangrove habitats near the farms than in mangroves away from the farms (Fig. 3), the study has provided measurable evidence of impacts of the farms on their own local environments. The increase in the vibrio levels around these farms is probably due to the regular enrichment of river sediment with organic material in the discharges.

These findings have important implications for the industry. By using vibrio levels in sediments as biological indicators, it may be possible to estimate the sustainable carrying capacity of waterways which are used for shrimp farming. Other factors which would be required include the discharge volumes of the farms, the distance between neighboring farms, the flushing rates and circulation patterns of receiving waters as well as the size of the receiving waterbody. The sediment is a more appropriate media to sample than the water column because it is less influenced by perturbations caused by rainfall, tidal flushing and transient movements of waterborne substances.

Smith (1995) analyzed the composition of the effluent from the farms on the Clarence River and found that >90% of suspended sediments had a settlement velocity of (2.7 m s^{-1}) . This was used to determine that a settlement pond of 0.75 ha could be used to treat effluent from a farm with a maximum discharge of $1,000 \text{ l s}^{-1}$ (Smith 1995). Also, that study determined that the net load to the river from the discharge from a 1-ha pond was equivalent to a BOD of $6 \text{ kg O}_2 \text{ d}^{-1}$. Since that study found that 66% of the total carbon in the effluent was particulate carbon, a high proportion of the effluent has suspended solids which could settle near the farm and impact on the local environment. The finding in the present study that vibrio levels in river sediment were elevated near the farms, supports this hypothesis and demonstrates how the impact can be measured.

Conclusions

A comparative study of two commercial shrimp farms revealed that the practice of removing accumulated sediments between crops had no effect on the spatial and temporal distributions of vibrios in pond sediments. Maximum levels of vibrios occurred in the center of ponds during times of peak temperature and feed rate. Also, removing sediment had no effect on the spatial and temporal patterns for sulfate-reducing bacteria.

Vibrio levels in shrimp pond sediment were usually an order of magnitude higher than in mangrove sediment. Also, vibrio levels in mangrove sediment near effluent canals were significantly higher than those from mangrove sites 2 km from farms.

It was concluded that a) drying sediment between each crop reduced levels of vibrios and sulfate-reducing bacteria at the start of growout; b) removing accumulated sediment did not reduce levels of vibrios, sulfate-reducing bacteria or heterotrophic bacteria; and c) levels of vibrios and sulfate-reducing bacteria were better biological indicators of pond and environmental conditions than heterotrophic bacteria. It is hypothesized that the bacteriology of pond sediments in semi-intensive shrimp farms is largely driven by a combination of three dominant factors, namely, input of pelleted feed, ambient temperature and limiting levels of oxygen.

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References

- Apud, F., J.H. Primavera and P.L. Torres. 1983. Farming of prawns and shrimp. Extension Manual No. 5, AQD-SEAFDEC, Iloilo.
- Anon. 1990. Fish health management study of Asia-Pacific completed. NACA Newsletter, 7(4):1-7.
- Anon. 1994a. Antimicrobials for shrimp bacterial disease control. CP Shrimp News 2(6):2-3.
- Anon. 1994b. Pond bottom management. CP Shrimp News 2(1):2.
- Baumann, P. and R.H.W. Schubert. 1984. Family II. *Vibrionaceae*. In: Bergey's manual of systematic bacteriology, vol. 1 (eds. N.R. Krieg and J.G. Holt), pp. 516-550. Williams and Wilkins, Baltimore.
- Boyd, C.E., P. Munsiri and B.F. Hajek. 1994. Composition of sediments from intensive shrimp ponds in Thailand. Journal of the World Aquaculture Society 25(3):53-55.
- Cameron, D.E., C.D. Garland, T.E. Lewis and P.J. Machin. 1988. A survey of *Vibrionaceae* in Tasmanian coastal waters, with special reference to bacterial species pathogenic to fish or shellfish. Australian Journal of Marine and Freshwater Research 39:145-52.
- Cropp, C.M. and C.D. Garland. 1988. A scheme for the identification of marine bacteria. Australian Microbiology 9:27-34.
- Flegel, T.W., D.F. Fegan, S. Kongsom, S. Vuthikomudomkit, S. Sriurairatana, S. Boonyaratpalin, C. Chantanachookhin, J. Vickers and O.D. Macdonald. 1992. Occurrence, diagnosis and treatment of shrimp diseases in Thailand. In: Diseases of cultured Penaeid shrimp in Asia and the United States (eds. W. Fulks and K.L. Main), pp. 3-33. The Oceanic Institute, Hawaii.

- Fulks, W. and K.L. Main. 1992. Part 1. Introduction. In: Diseases of cultured Penaeid shrimp in Asia and the United States (eds. W. Fulks and K.L. Main), pp. 3-33. The Oceanic Institute, Hawaii.
- Hart, B.T., J.L. Ross and D.A. Veal. 1996. Microbial indicators of river health. Occasional Paper 07/96. pp. 27-45. Land and Water Resources Research and Development Corporation, Canberra.
- Lee, K.K., S.R. Yu F.R. Chen, T.I. Yang and P.C. Liu. 1996. Virulence of *Vibrio alginolyticus* isolated from diseased tiger prawn, *Penaeus monodon*. *Current Microbiology* 32:229-231.
- Lightner, D.V. 1978. Possible toxic effects of the marine blue-green algae, *Spirulina subsalsa*, on the blue shrimp *Penaeus stylirostris*. *Journal of Applied Phycol.* 4, 79-86.
- Lightner, D.V. 1983. Diseases of cultured penaeid shrimp. In: CRC handbook of mariculture, vol. 1: Crustacean aquaculture (ed. J.P. McVey), pp. 289-320. CRC Press, Florida.
- Lightner, D.V. 1996. A handbook of shrimp pathology and diagnostic procedures for disease of cultured Penaeid shrimp. World Aquaculture Society, Baton Rouge, Louisiana, USA.
- Masters, S.J. and P.T. Smith. 1995. Origin of reduced sulfur compounds in pond sediment. In: Sustainable aquaculture '95 (eds. J. Bardach et al.), pp. 255-260. Pacific Congress on Marine Science and Technology, Honolulu, Hawaii.
- Masters, S.J. and P.T. Smith. 1996. Management of oxidizable sulfur in earthen aquaculture. In: Proceedings of East Meets West, pp.248, World Aquaculture Conference, Bangkok, Thailand.
- Mohney, L.L., D.V. Lightner and T.A. Bell. 1994. An epizootic of vibriosis in Ecuadorian pond-reared *Penaeus vannamei* Boone (Crustacea: Decapoda). *Journal of the World Aquaculture Society*, 25 (1): 116-125.
- Muir, P.R. 1990. Factors affecting the survival of penaeid prawns in culture with particular reference to the larval stages. Ph. D. Thesis, James Cook University of North Queensland.
- Nash, G., C. Nithimathachoke, A. Tungmandi, P. Arkarjamorn, P. Prathanpipat and P. Ruamthaveesub. 1992. Vibriosis and its control in pond-reared *Penaeus monodon* in Thailand. In: Diseases in Asian Aquaculture I. M. Shariff, R.P. Subasinghe and J.R. Arthur (eds), pp. 143-155. Asian Fisheries Society, Manila.
- New, M.B. and H.R. Rabanal. 1985. A review of the status of penaeid aquaculture in South East Asia. In: Second Australian Prawn Seminar, (eds P.C. Rothlisberg and D.J. Staples), pp. 307-326, Cleveland, Queensland.
- Owens, L. 1996. Research on the effect of sulfur-cycle bacteria on the production in prawn ponds. *Austasia Aquaculture*, 10(3):64-5.
- Pankhurst, E.S. 1971. The isolation and enumeration of sulfate-reducing bacteria. In: Isolation of anaerobes (eds. D.A. Shapton and R.G. Board), pp. 223-240. Academic Press, London.
- Postgate, J.R. 1984. The sulphate-reducing bacteria. Cambridge University Press, Cambridge. 208 pp.
- Revord, T. and D. Weidner. 1992. Shrimp farming in Ecuador. *Fish Farming International* 19(6):38-41.
- Rosenberry, 1993. Production drops 16 % in 1993. In: World Shrimp Farming 1993. *Aquaculture Digest* 1-29.
- Smith, P.T. 1993. Prawn farming in Australia - sediment is a major issue. *Australian Fisheries*, 52(12):29-32.
- Smith, P.T. 1995. Characterisation of effluent from prawn farms. In: Sustainable aquaculture '95 (eds. J. Bardach et al.), pp. 327-338. Pacific Congress on Marine Science and Technology, Honolulu, Hawaii.
- Smith, P.T. 1996a. Physical and chemical characteristics of sediments from prawn farms and mangrove habitats on the Clarence River, Australia. *Aquaculture* 146:47-83.
- Smith, P.T. 1996b. Toxic effects of blooms of marine species of Oscillatoriales on farmed prawns (*Penaeus monodon*, *Penaeus japonicus*) and brine shrimp (*Artemia salina*). *Toxicon*, 34: 857-869.
- Suplee, M.W. and J.B. Cotner. 1996. Temporal changes in oxygen demand and bacterial sulfate reduction in inland shrimp ponds. *Aquaculture* 145:141-158.