Integration of Finfish in Shrimp (*Penaeus monodon*) Culture: An Effective Disease Prevention Strategy

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ABSTRACT

A farm trial on integration of finfish (*i.e.*, tilapia) in shrimp (*Penaeus monodon*) culture was conducted in Negros Occidental, Philippines to prevent luminous vibriosis in shrimp. The farm engaged in shrimp monoculture from 1987 to 1995. However, the prevailing luminous vibriosis outbreaks that started in 1994 prompted the farm operator to shift to tilapia culture in 1995-1996. The farm resumed shrimp operations in 1996 but by this time tilapia had already been integrated in the culture system. This paper reports on the results of the trial for 1999 using three ponds (ponds 7, 9, 29). These ponds had previously been used for tilapia culture for two years. During shrimp culture, they drew water from reservoirs stocked with tilapia and within the shrimp ponds tilapia are also stocked inside cages. This technology integrates crop rotation, biological pre-treatment and polyculture into one system. During the culture period the chemical and bacteriological quality of soil, water and shrimp were monitored. Water quality parameters were within normal ranges for shrimp culture. Luminous bacterial counts in water and shrimp were consistently below 10 colony forming units (cfu)/ml and 10^3 cfu/hepatopancreas (hp), respectively. These levels are below threshold levels associated with luminous vibriosis outbreaks. With a stocking density of 19.43 shrimp postlarvae (PL)/m^2, pond 7 yielded 2,605 kg shrimp/ha with an estimated survival of 35.65% after 109 days of culture (DOC). With a stocking density of 18.69 PL/m^2, pond 9 yielded 5,472 kg shrimp/ha with survival of 100% after 148 DOC. With a stocking density of 19.33 PL/m^2, Pond 29 yielded 5,702 kg shrimp/ha with survival of 82.66% after 151 DOC. The relatively low production in pond 7 can be attributed to the inferior quality of the batch of stocked shrimp PL that already had a low survival of 50% at DOC 30. Comparing the production performance from this present trial with that of this and other farms before the 1994 outbreaks, these good results cannot simply be attributed to chance despite of the lack of control in this farm trial. These results are consistent with the results of a previous trial of the same farm, the on-going verification trials in Negros Occidental, and the observations of many farmers in other parts of the country on the potential of shrimp-finfish integration in preventing luminous vibriosis in shrimp.

INTRODUCTION

Shrimp (*Penaeus monodon*) production in the Philippines peaked at 30,462 MT in 1991. Negros Occidental, the hub of intensive shrimp fanning in the Philippines, registered the highest production of 1,000 MT/month in the early 1990s. However, because of disease outbreaks (usually luminous vibriosis) production dropped to less than 100 MT/month in late 1994 and has not recovered since.

When the first outbreaks occurred in late 1994, the Bureau of Fisheries and Aquatic Resources (BFAR) immediately dispatched a technical team to investigate the problem. Throughout these past few years, numerous farm trials on the use of antibiotics and disinfectants (Baticados and Paclibare, 1992); bioaugmentation (Boyd and Gross, 1998); nutritional enrichment (Darachai *et al*., 1998); ozonation (Matsumura *et al*., 1998); immunostimulation (Horne *et al*., 1995); and ultraviolet (UV)-disinfection were either conducted or monitored by BFAR and the Negros Prawn Producers' Marketing Cooperative, Inc. (NPPMCI). However, none of these techniques proved consistently effective.

Luminous vibriosis in the Philippines is usually caused by *Vibrio harveyi* and high numbers of the bacterium in water has been associated with numerous disease outbreaks (Lavilla-Pitogo and dela Peña, 1998). In a 1994-1995 pond monitoring done by Lavilla-Pitogo (1998), they found that the onset of mortalities was always preceded by an exposure of the shrimp postlarvae (PL) to a high luminous bacterial population (10^2 to above 10^4 colony forming units (cfu)/ml) for three or more days.

In 1995-1996, laboratory analysts of NPPMCI monitored the water and soil quality of a semi-intensive shrimp farm in E.B. Magalona, Negros Occidental. In this set-up, polluted water (contaminated by domestic and agro-industrial pollutants) was pre-treated in a reservoir where tilapia was being cultured. The luminous bacterial count (LBC) of the water usually decreased from 10^2-10^3 to 10^1-10^2 cfu/ml (LBC in reservoir). A portion of this reservoir water was then transferred to the shrimp pond and the addition of this water caused the temporary decrease in LBC in the shrimp pond from the usual $l0^1$-$l0^2$ to $>10^1$-10^1. After a few days, the LBC in the shrimp pond would return to the usual 10^1-10^2 cfu/ml. This finding led us to try combining biological pre-treatment and polyculture. We hypothesized that tilapia culture can potentially decrease LBC but this potential can only be maintained if tilapia are also cultured simultaneously in the same pond containing the shrimp. Trials were therefore conducted in that farm and in the NPPMCI Demonstration Farm, Bago City, Negros Occidental. These early attempts on integrating tilapia in semi-intensive shrimp culture did not produce satisfactory results. However, the many insights learned from those trials were applied in two farms in San Enrique and Pulupandan, Negros Occidental.

Since 1996, these two farms have been integrating tilapia in their shrimp culture operations as an alternate species in crop rotation, as a biological pre-treatment agent in reservoir, and as an additional species in polyculture. Crop rotation is the culture of one organism after another in a same area. It is a type of "sanitation" practice which reduces initial inoculum of pathogen to a sufficiently low level so that the normal development of pathogen population will not reach a high level enough to cause appreciable yield loss, provided there is no unusual influx of pathogens (Berger, 1977). Biological pre-treatment is the use of an organism that causes the improvement of

quality of incoming water. Polyculture is the simultaneous cultivation of two or more organisms in an area and may improve soil and water quality during the culture period.

This paper presents the results of these trials on one of these two farms in Negros Occidental. The trials on the other farm are still in progress.

MATERIALS AND METHODS

Farm

The farm is located in San Enrique, Negros Occidental, four kilometers from the sea. It has 32 ponds with a total area of 43 hectares (ha). The farm started shrimp culture operation in 1982 and experienced luminous vibriosis outbreaks in 1994. The farm started to culture tilapia in 1996 because of the drop in market price of shrimp and the unavailability of technology to address the luminous vibriosis problem.

Water source

The farm draws brackish water (8-20 ppt) from the adjacent Bagonawan River. The luminous bacterial count of the river water ranges from 10^1 to 10^2 cfu/ml. The water goes to a sedimentation pond (around 4 ha) before it is transferred to the reservoirs stocked with tilapia. Although the use of sedimentation pond is not essential, the fanner decided to use one of his unutilized ponds for this purpose to receive the relatively turbid water from the river before transferring it to the reservoirs.

Reservoirs (Tilapia culture ponds)

The farm maintains more than one reservoir for every culture pond to help ensure a ready supply of good quality water at all times. (Ponds 2, 8, 21, 28 and 31, having areas of 1.24, 1.00, 0.66, 0.52, and 0.43 ha, respectively, served as reservoirs).

Table 1 shows a brief history of these ponds.

Table 1. Culture operations of ponds 2, 8, 21, 28 and 31 from 1992 to 1999

Year	Pond 2	Pond 8	Pond 21	Pond 28	Pond 31
1992	shrimp culture	shrimp culture	shrimp culture	shrimp culture	shrimp culture
1993	shrimp culture	shrimp culture	shrimp culture	shrimp culture	shrimp culture
1994	shrimp culture	shrimp culture	shrimp culture	shrimp culture	shrimp culture
1995	shrimp culture	shrimp culture	shrimp culture	shrimp culture	shrimp culture
1996	shrimp culture	tilapia culture	shrimp culture	tilapia culture	shrimp culture
1997	tilapia culture	-	tilapia culture	-	tilapia culture
1998	tilapia culture	-	tilapia culture	-	tilapia culture
1999	tilapia culture	tilapia culture	tilapia culture	tilapia culture	tilapia culture

The reservoirs were prepared to favor the growth of natural food. They were stocked with 3-4 saline tolerant tilapia/m^2. The farm produces its so-called "Jewel Tilapia" which is a hybrid of *Oreochromis mossambicus* and *T. hornorum* imported from the USA. Supplemental feeding with commercial feed started after 30 days of culture (DOC). Aeration started at DOC 90 when the biomass reached around 3 tons/ha. During nighttime, the aerators were operated for 12 h, from 6 PM to 6 AM. During daytime, aerators were occasionally operated (*e.g.*, before water was transferred to shrimp ponds and depending on weather conditions).

A tilapia biomass of 3,000-3,500 kg/ha was maintained in reservoirs. It is at this biomass that the luminous bacterial count becomes undetectable and the water quality is stable.

Shrimp-tilapia culture ponds

For the trial being described, three ponds were used - ponds 7, 9 and 29 having areas of 1.0007 ha, 0.931 ha, 0.5276 ha, respectively. The pond preparation followed the industry practice.

For the design and placement of tilapia cages, four cages with dimensions of 10 m x 10 m x 10 m were placed around and 0.5 m away from the pond center. Each cage had two layers of nets: a fine-meshed net and a large-meshed net. To enhance water circulation within and outside the cage, the fine-meshed net was removed when the shrimp were big enough (average body weight, ABW, 5 g) not to enter the tilapia cages. The bottom of the cages had a distance of 0.3 m from the pond bottom.

Tilapia weighing 100 g each was stocked in the cages as soon as the ponds were filled with water from the reservoir. A total tilapia biomass of 400 kg/ha was stocked in four cages in each of the three ponds.

Shrimp PLs (PL 16-18; 16-18 days after molting to postlarval stage) were stocked in the pond at around 18-19 PL/m^2. Since the shrimp pond water came from the reservoirs (stocked with tilapia) and the chemical and microbial quality of the water was determined to be acceptable (*e.g.*, Secchi disc visibility, 30-60 cm, pH, 7.5-8.5, luminous bacterial count, less < than 10^1 cfu/ml), shrimp PLs were stocked 5-7 days after water filling.

Table 2 shows a brief history of these ponds. Ponds 7 and 9 were devoted to shrimp-milkfish (*Chanos chanos*) polyculture from 1982 to 1987. Pond 29 started shrimp culture operation in 1988.

Table 2. Culture operations of ponds 7, 9 and 29 from 1987 to 1999

Year	Pond 7	Pond 9	Pond 29
1987	shrimp culture	shrimp culture	-
1988	shrimp culture	shrimp culture	shrimp culture
1989	shrimp culture	shrimp culture	shrimp culture
1990	shrimp culture	shrimp culture	shrimp culture
1991	shrimp culture	shrimp culture	shrimp culture
1992	shrimp culture	shrimp culture	shrimp culture
1993	shrimp culture	shrimp culture	shrimp culture
1994	shrimp culture	shrimp culture	shrimp culture
1995	shrimp culture	shrimp culture	shrimp culture
1996	tilapia culture	tilapia culture	shrimp culture
1997	tilapia culture	tilapia culture	tilapia culture
1998	-	integrated shrimp-tilapia culture	tilapia culture
1999	integrated shrimp-tilapia culture	integrated shrimp-tilapia culture	integrated shrimp-tilapia culture

Soil and water quality monitoring

Before pond preparation and after harvest, pond soil was analyzed for organic matter (OM) content and pH using standard methods of soil analyses (Boyd and Tucker, 1992). Pond water quality was monitored closely. Dissolved oxygen (DO) was measured using DO meter four times a day at 4:00 P.M., 10:00 P.M., 2:00 A.M., and 4:00 A.M.. Temperature, salinity, pH, secchi disc visibility, depth, color and weather were recorded twice a day at 6:00 A.M. and 3:00 P.M.

Unionized ammonia, nitrite, alkalinity, and phytoplankton count were determined occasionally using standard methods (Strickland and Parsons 1972; Boyd and Tucker, 1992). Although water quality parameters were determined daily throughout the culture periods for both ponds 9 and 29, for pond 7 DO and temperature were determined only on DOC 17-57 and pH, salinity, transparency and depth were determined only on DOC 1-77.

Quantitative bacteriology

Quantitative bacterial counts on pond water and shrimp were made twice a week using standard methods (Lavilla-Pitogo *et al*., 1998; Leaño *et al*., 1998). For water samples, ten-fold serial dilutions were made and 0.1 ml aliquots of the dilutions were plated in duplicate on two culture media: nutrient agar (NA) and thiosulphate citrate bile-salt sucrose (TCBS) agar. For shrimp samples, five random samples were dissected aseptically and the hepatopancreas tissues (hp) removed, homogenized and suspended in sterile seawater (SSW) at one hp/ml SSW. Aliquots (0.1 ml) of the serially diluted homogenates were also spread on NA and TCBS plates. Total plate count (TPC), LBC, and presumptive *Vibrio* count (PVC) were determined after 18-24 h incubation at room temperature. Quantitative bacteriology of reservoir, source water and pond soil was also conducted occasionally.

Water management

Replenishment water was drawn from the reservoirs. The decision to replenish water was based on laboratory analysis and pond observation. Starting on DOC 17, 10-15% of the water in the shrimp-tilapia culture ponds was replaced with water from the reservoirs during water exchange.

Feeding management

The feeding of shrimp followed industry practice using commercial pellets and supplemental food (*e.g*., golden snail *Pomacea canaliculata*). The tilapia was also fed with commercial pellets.

Shrimp growth and health monitoring

Shrimp samples (n=5) were taken randomly and weighed every five days starting at DOC 30 until harvest time. Gross inspection of shrimp for signs of disease was done during this sampling period.

RESULTS

Production

Ponds 9 and 29 had good shrimp production of more than 5,000 kg/ha and more than 80% survival (Table 3). Pond 7 had a lower production which is explained below in the Discussion.

Table 3. Shrimp production in ponds 7, 9 and 29

Pond number	Stocking density (PL/m^2)	DOC at harvest (days)	Total production (kg/ha)	ABW (g)	Survival (%)	FCR
7	19.43	109	2,605	37.61	35.7	1.17
9	18.69	148	5,472	29.15	100	1.70
29	19.33	151	5,702	35.69	82.66	1.69

PL – postlarvae
DOC – days of culture
ABW – average body weight
FCR – feed conversion ratio

In 1998, pond 9 was also used for integrated shrimp-tilapia culture. With a stocking density of 18 PL/m^2, shrimp production was 4,385 kg/ha after DOC 108 with survival of 90%.

The tilapia production from ponds 7,9 and 29 are shown in Table 4. The tilapia production data from the reservoirs are not available because the intention is not to remove them from the reservoirs.

Table 4. Tilapia production performance of ponds 7, 9 and 29

Pond number	Stocking density (fish/m^2)	DOC at harvest (days)	Total production (kg)	ABW (g)	Survival (%)	FCR
7	10	101	1,250	313	100	1.77
9	10	148	1,500	375	100	1.70
29	10	151	1,200	300	100	1.69

DOC - days of culture
ABW - average body weight
FCR - feed conversion ratio

Soil and water quality

The OM and pH of the pond soil ranged from 1.75% to 2.5% and 6.9 and 7.4, respectively. Water quality parameters were within ideal ranges for shrimp culture (Table 5.) Dissolved oxygen, temperature and pH changed significantly during the day while salinity and transparency did not.

Table 6 shows the differences of water quality among ponds 7, 9 and 29. Dissolved oxygen and temperature were compared for DOC 17-57 because it is only during that period that data from the three ponds can be compared. For the other parameters, ponds were compared for the first 77 days.

From DOC 17-57, the DO and temperature of ponds 7,9 and 29 did not differ from each other. Likewise, pH was also not significantly different for the three ponds for the first 77 days of their culture.

Table 7 shows the correlation of the various water parameters with each other. Dissolved oxygen was directly related to salinity ($P < 0.05$) while depth was likewise directly related to temperature ($P < 0.05$) and salinity ($P < 0.001$).

Chlorella spp. was the most numerous species of phytoplankton present throughout the culture period (89.2%±9.5%, 86.6%±8.5%, 85.8%±7.4% for ponds 7, 9, and 29, respectively).

Table 5. Overall means and standard deviation (SD) of water quality parameters at different sampling times for ponds 7, 9 and 29

Pond Number	Dissolved oxygen (ppm)				Temperature (°C)		pH		Salinity (‰)		Transparency (cm)		Depth (cm)	
	4PM	10PM	2AM	4AM	6AM	3 PM	6AM	3 PM	6AM	3 PM	6AM	3 PM	6AM	3 PM
7	10.2 ± 1.9[a]	6.0 ± 1.1[b]	5.0 ± 1.0[c]	4.0 ±0.8[d]	29.3 ± 1.6[a]	31.5 ± 1.9[b]	7.6 ± 0.2[a]	8.1 ± 0.1[b]	11.3 ± 1.7[a]	11.2 ± 1.7[a]	29.1 ± 2.4[a]	28.8 ± 2.3[a]	91.2 ± 3.5[a]	91.4 ± 3.6[a]
9	10.9 ± 2.1[a]	5.9 ± 1.4[b]	4.6 ± 1.1[c]	3.8 ± 1.0[d]	29.2 ± 1.5[a]	31.4 ± 1.7[b]	7.7 ± 0.1[a]	8.1 ± 0.1[b]	11.8 ± 2.6[a]	11.8 ± 2.6[a]	25.1 ± 2.9[a]	24.9 ± 2.9[a]	94.9 ± 9.0[a]	98.2 ± 3.7[b]
29	10.0 ± 3.3[a]	5.8 ± 1.4[b]	4.7 ± 1.2[c]	4.0 ± 1.1[d]	29.2 ± 1.6[a]	31.2 ± 1.9[b]	7.7 ± 0.2[a]	8.1 ± 0.2[b]	11.7 ± 2.4[a]	11.7 ± 2.3[a]	25.6 ± 4.3[a]	25.2 ± 4.1[a]	95.4 ± 9.0[a]	99.0 ± 2.5[b]

Means of each water quality parameter within a row with different superscripts are significantly different ($P < 0.05$)

Table 6. Means and standard deviation (SD) of water quality parameters at days of culture (DOC) 17-57 for dissolved oxygen and temperature; and DOC 1-77 for pH, salinity, transparency and depth for ponds 7, 9, and 29

Pond number	Dissolved oxygen (ppm)	Temperature (C)	pH	Salinity (‰)	Transparency	Depth
7	6.3±2.7[a]	30.4±2.0[a]	7.9±0.3[a]	11.2 ± 1.7[a]	29.0±2.8[a]	91.3±3.5[a]
9	7.0±2.4[a]	30.9±1.5[a]	7.9±0.2[a]	12.9±2.9[b]	26.3±2.9[c]	96.6±5.5[b]
29	7.0±2.9[a]	31±1.5[a]	7.9±0.2[a]	13.0±2.4[b]	27.5±4.5[b]	97.0±5.3[b]

Means within a column with different superscripts are significantly different (P<0.05)

Table 7. Pearson coefficients of correlation* between means of water quality parameters in ponds 7,9 and 29. (DOC 17-57 for dissolved oxygen and temperature; DOC 1-77 for pH, salinity, transparency and depth)

	Dissolved oxygen (ppm)	Temperature (°C)	pH	Salinity (‰)	Transparency	Depth
Dissolved oxygen	x					
Temperature	0.987	x				
pH	0.950	0.988	x			
Salinity	0.998	0.995	0.968	x		
Transparency	-0.897	-0.814	-.716	-0.868	x	
Depth	0.995	0.997	0.976	0.999	0.999	x

* r>0.997, P<0.05; r>0.999, P<0.001

Quantitative bacteriology

Luminous bacterial counts were consistently lower than 10^1 cfu/ml and 10^3 cfu/hp in pond water and shrimp, respectively (Table 8). Table 9 compares the three ponds in terms of bacterial counts in water and shrimp during the first 109 DOC.

Although the three ponds varied greatly in terms of TPC and PVC, all of them consistently had LBC lower than 10^1 cfu/ml and 10^3 cfu/hp in pond water and shrimp, respectively, for the first 109 DOC. The Pearson correlation analysis of the above data revealed no relationship between the TPC of water and shrimp (r=-0.438) and PVC of water and shrimp (r =-0.807). The LBC of water and shrimp could not be compared because of their inexact values.

Table 8. Overall means for bacterial counts for water and shrimp in ponds 7, 9 and 29

Pond number	TPC, water (cfu/ml)	PVC, water (cfu/ml)	LBC, water (cfu/ml)	TPC, shrimp (cfu/hp)	PVC, shrimp (cfu/hp)	LBC, shrimp (cfu/hp)
7	2.9×10^4	2.2×10^3	$<10^1$	1.9×10^7	4.0×10^5	$<10^3$
9	1.4×10^4	1.4×10^3	$<10^1$	1.8×10^7	2.4×10^5	$<10^3$
29	1.4×10^4	1.4×10^3	$<10^1$	1.8×10^7	2.4×10^5	$<10^3$

Means within a column with different superscripts are significantly different ($P<0.05$)
TPC - total plate count
PVC - presumptive *Vibrio* count
LBC - luminous bacterial count
cfu - colony forming unit
hp - hepatopancreas

Table 9. Means of bacterial counts for water and shrimp in ponds 7, 9 and 29 for the first 109 days of culture (DOC)

Pond number	TPC, water (cfu/ml)	PVC, water (cfu/ml)	LBC, water (cfu/ml)	TPC, shrimp (cfu/hp)	PVC, shrimp (cfu/hp)	LBC, shrimp (cfu/hp)
7	2.9×10^{4b}	2.2×10^{3b}	$<10^1$	1.9×10^{7b}	4.0×10^{5c}	$<10^3$
9	1.5×10^{4c}	1×10^{3a}	$<10^1$	1.7×10^{7a}	3.2×10^{5b}	$<10^3$
29	1.0×10^{6a}	1.5×10^{7c}	$<10^1$	1.7×10^{7a}	2.4×10^{5a}	$<10^3$

Means within a column with different superscripts are significantly different ($P <0.05$)
TPC - total plate count
PVC - presumptive *Vibrio* count
LBC - luminous bacterial count
cfu - colony forming unit
hp - hepatopancreas

Figures 1 to 6 show the changes of bacterial counts in water and shrimp in the three ponds. The LBC was consistently lower than PVC and TPC. Except for one time, the LBCs of water and shrimp never exceeded 10 cfu/ml and 1,000 cfu/hp, respectively.

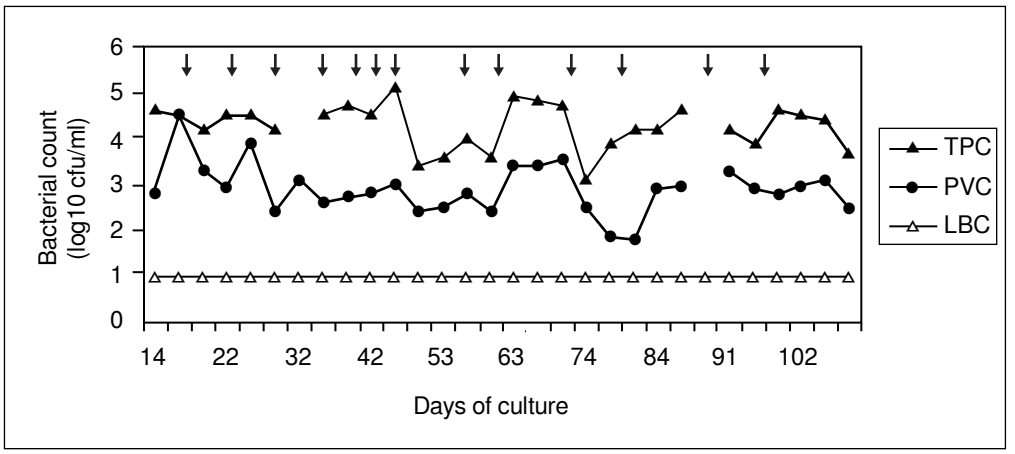

Figure 1. Bacterial count in water of pond 7. The dates when water from tilapia reservoir was added are indicated by an arrow. TPC - total plate count; PVC presumptive - *Vibrio* count; LBC - luminous bacterial count; cfu - colony forming unit

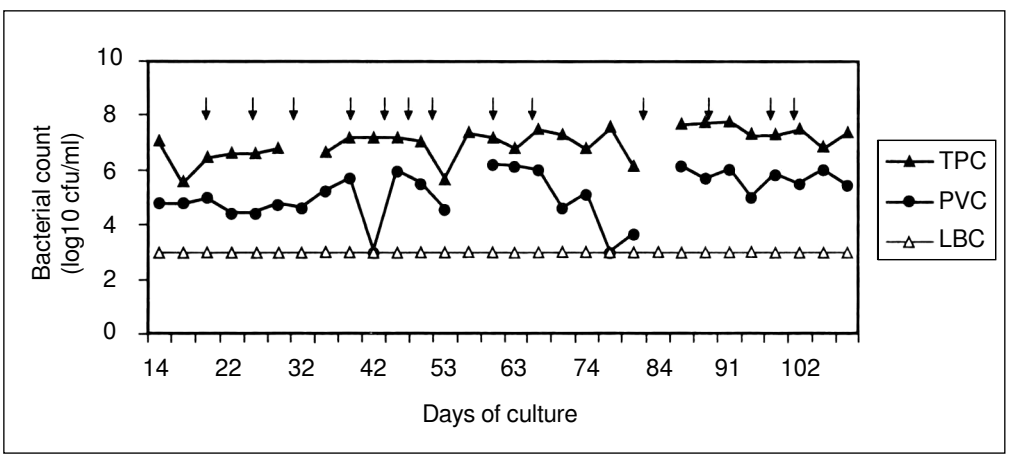

Figure 2. Bacterial count in shrimp of pond 7. The dates when water from tilapia reservoir was added are indicated by an arrow. TPC - total plate count; PVC - presumptive *Vibrio* count; LBC - luminous bacterial count; cfu - colony forming unit; hp - hepatopancreas

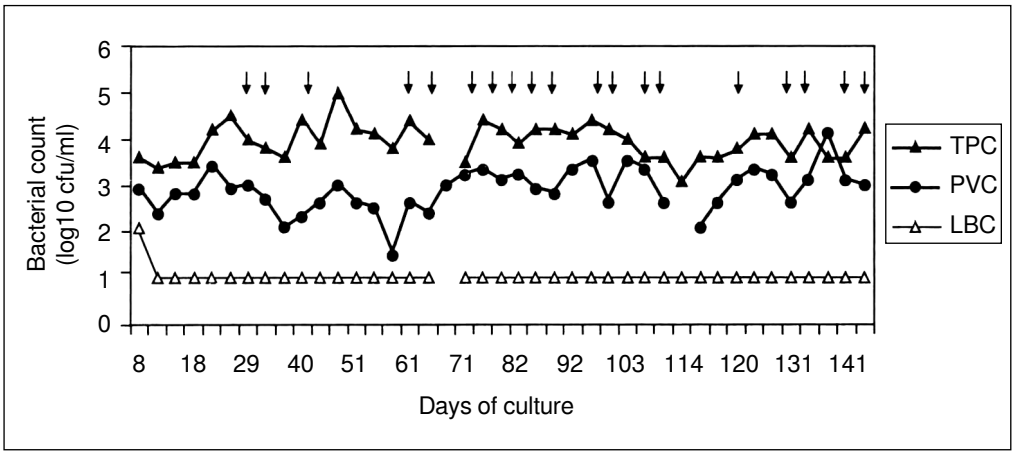

Figure 3. Bacterial count in water of pond 9. The dates when water from tilapia reservoir was added are indicated by an arrow. TPC - total plate count; PVC - presumptive *Vibrio* count; LBC - luminous bacterial count; cfu - colony forming unit

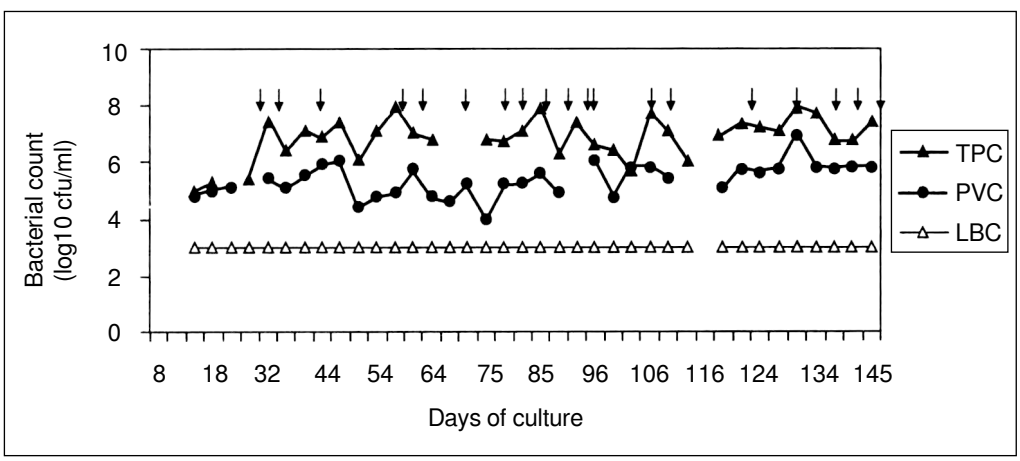

Figure 4. Bacterial count in shrimp of pond 9. The dates when water from tilapia reservoir was added are indicated by an arrow. TPC - total plate count; PVC - presumptive *Vibrio* count; LBC - luminous bacterial count; cfu - colony forming unit; hp - hepatopancreas

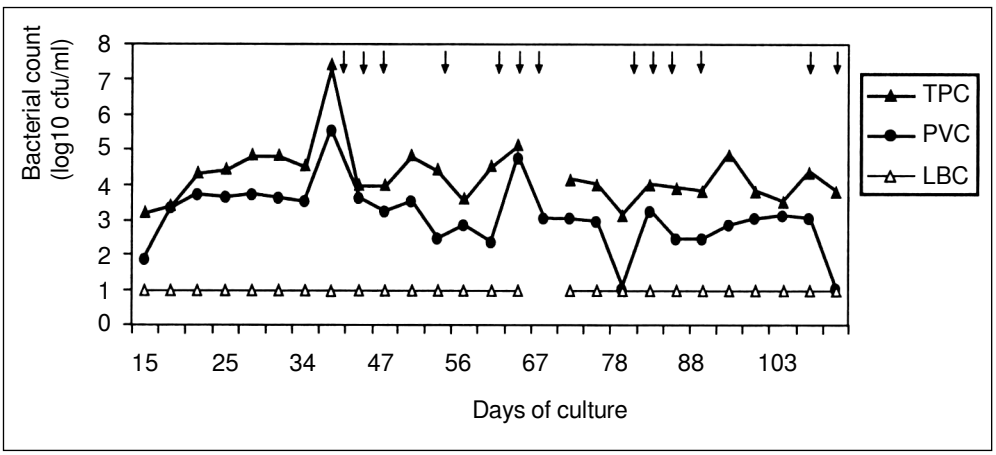

Figure 5. Bacterial count in water of pond 29. The dates when water from tilapia reservoir was added are indicated by an arrow. TPC - total plate count; PVC - presumptive *Vibrio* count; LBC - luminous bacterial count; cfu - colony forming unit

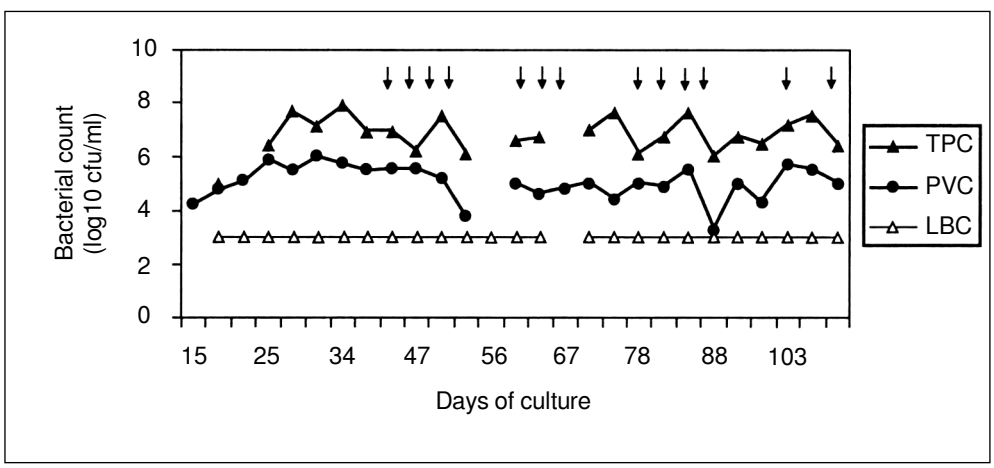

Figure 6. Bacterial count in shrimp of pond 29. The dates when water from tilapia reservoir was added are indicated by an arrow. TPC - total plate count; PVC - presumptive vibrio count; LBC - luminous bacterial count; cfu - colony forming unit; hp - hepatopancreas

DISCUSSION

Shrimp production

Although there was no control pond for this farm trial, the good production performance of Ponds 9 and 29 cannot simply be attributed to chance.

Table 10 shows progressive decline in shrimp survival of various ponds in FSD Farm from 1992 to 1996.

Table 10. Survival in shrimp monoculture of FSD Farm from 1992 to 1996

Year	Pond 7	Pond 9	Pond 29	Pond 2	Pond 8	Pond 21	Pond 28	Pond 31
1992	89.7	73.6	55.3	23.2	35.4	86.5	79.4	98.3
1993	75.3	75.6	85.3	78.8	74.6	55.7	59.4	86.4
1994	?	42	?	?	26	24.5	?	35
1995	40.48	28	21.2	16	14	11	27.6	11
1996	*	-	*	*	-	40	-	15

* Aborted operation
− No shrimp culture operation
? Missing data

Luminous vibriosis has been prevalent not only in Negros Occidental but also in other areas (*e.g.* the nearby province of Iloilo). A certain farm in Iloilo, for instance, had declining yearly survival rates of 86.6%, 63% and 29.3% from 1992 to 1994 (Lavilla-Pitogo *et al.*, 1998). Table 11 shows the decline in the production of export quality shrimp in the Philippines starting in 1995. The sharp decline in 1995 apparently reflects the first outbreaks of luminous vibriosis throughout the country in 1994.

Table 11. Philippine shrimp export from 1987 to 1997

Year	Quantity (MT)
1987	14,935
1988	24,288
1989	26,052
1990	24,146
1991	31,156
1992	23,003
1993	22,206
1994	21,518
1995	12,095
1996	13,514
1997	10,532

In view of the many unsuccessful attempts to control luminous vibriosis in other farms for the past few years, the declining shrimp survival in these farms suggests that only an effective control strategy can reverse the trend. The survival in ponds 9 and 29 in 1995 was 28 and 21%, respectively. With this present intervention, the survival in both ponds increased tremendously to 100% and 82.66%.

The relatively low shrimp production in pond 7 can be attributed to the inferior quality of stocked postlarvae. At DOC 30, the survival was already estimated at a low 50% and appeared to be unrelated to *V. harveyi.* In all the three ponds, for the first 30 DOC, the LBC for the shrimp was < 10^3 cfu/hp and <10^1 cfu/ml for water. Likewise, water quality was similar for all three ponds.

The on-going verification trial in another farm (Pulupandan, Negros Occidental) is also showing promising results. The results of this present trial and other on-going trials are consistent with the observation of many shrimp farmers throughout the country that crop rotation and shrimp-finfish polyculture is a promising technique for avoiding shrimp losses.

Finfish-based biological control

The first outbreaks of luminous vibriosis in grow-out ponds in the Philippines occurred in 1994. Many of these luminous vibriosis outbreaks have been associated with phytoplankton die-off (Paclibare, 1998). Another major risk factor is the high number of luminous bacteria (10^2 to above 10^4 cfu/ml) in pond water especially during the first 45 days of culture (Lavilla-Pitogo *et al*., 1998).

In a shrimp pond system, the most likely sources of luminous bacteria are the soil and incoming water (Karunasagar *et al*., 1996a; Lavilla-Pitogo *et al*., 1998). As soon as the pond is filled with water, the luminous bacterial population tends to increase, probably because of the organic matter remaining after pond preparation (Lavilla-Pitogo *et al*., 1998; Smith, 1998). During the culture period, organic matter (uneaten feed, shrimp faeces, exuviae, and plankton debris) accumulated. It is expected therefore that the luminous bacteria population would also increase.

The luminous bacterial population in soil and water can be reduced by either chemical or biological approaches. However, it is difficult to reduce the luminous bacterial population by disinfectants and antibiotics because the bacteria are protected in biofilms. Bacterial biofilms are composed of populations or communities of microorganisms adhering to environmental surfaces. These microorganisms are usually encased in an extracellular polysaccharide that they themselves synthesize. Biofilms may be found on essentially any environmental surface in which sufficient moisture is present. Biofilms are notably resistant to drying and disinfection. The role of biofilms in the development of bacterial diseases in shrimp ponds has not been investigated yet. However, the findings of Karunasagar *et al.* (1994; 1996b) may have some relevance to shrimp ponds. Karunasagar *et al.* (1994) reported mass mortality of *P. monodon* larvae due to an antibiotic-resistant *V. harveyi* infection and suggested that antibiotic-resistant, virulent strains of *V. harveyi* were colonizing larval tanks. In their follow-up study (Karunasagar *et al*., 1996b), they observed that *V. harveyi* can form biofilms on all three substrates they tested: cement slabs, high density polyethylene plastic and steel surfaces. Furthermore, the bacteria in biofilm were found to be more resistant to chlorine disinfection than their planktonic counterparts. In shrimp ponds, Karunasagar *et al.* (1996a) found that *V. harveyi* can survive even in sediments that are treated

with high doses of disinfectants. The addition of unspecified type of lime at 100 ppm to microcosms containing pond soil and water affected only *V. harveyi* counts in the soil marginally. The addition of chlorine at 10 ppm also led to a slight reduction of *V. harveyi* counts followed by an increase. When experiments were conducted on bacterial populations suspended in seawater, *V. harveyi* was found to be completely eliminated after 30 min exposure to 10 ppm chlorine.

Because of the difficulty in reducing the concentration of pathogenic bacteria in shrimp ponds by conventional chemical disinfection, other effective means such as biological control have been explored. Biological control may be divided into two approaches: a) the addition into the environment of beneficial microorganisms that serve as antagonists of the target pathogens and b) the manipulation of the environment in such a way that the proliferation of beneficial microorganisms is favored. Examples of these approaches are bioaugmentation (Boyd and Gross, 1998) and crop rotation, respectively. Experiences on bioaugmentation in shrimp culture indicate conflicting results. For instance, many of the commercial bioaugmentation products that were claimed by their producers to be effective did not perform well in Negros Occidental. Assuming bioaugmentation can reduce pathogenic bacteria in shrimp ponds, the method may still not be cost-effective because high amounts of the costly bioaugmentation products must be added to the ponds frequently. The second approach, *i.e.*, crop rotation is increasingly being recognized as an effective disease control strategy in shrimp culture similar to agriculture.

In the field of agriculture in Peru, for instance, a mandatory seven-year rotation for potatoes was established before the arrival of the Spaniards. It is now known that this practice was used to control potato cyst-nematodes (Sieczka, 1989). Kommendahl and Todd (1991) list approximately 64 fungal, 19 nematodes, 1 viral and 16 bacterial disease in plants in which crop rotation was found to be an effective control practice.

Crop rotation as applied in shrimp culture addresses problems due to luminous bacteria (and also possibly other pathogens) in the pond soil previously used for shrimp culture. The continuous monoculture of shrimp over the past few years may have caused the increase in luminous bacteria in the culture environments. Although many of the farms employ thorough pond preparation techniques, these bacteria may be carried over into succeeding cultures as they may be protected by bacterial biofilms. When a different kind of aquatic organism is reared in the pond previously used for shrimp culture, a different microenvironment is created. This change in the microenvironment may cause a change in the microflora of the pond soil. It has been observed that the greater the phylogenetic differences between the culture organisms used in crop rotation, the better the sanitary effect (Francis and Clegg, 1990). Since shrimp and finfish (*e.g.*, tilapia) belong to different orders within the animal kingdom, they are considered good candidates for crop rotation.

The potential of crop rotation in shrimp culture is also now being recognized in other countries. In Indonesia, Akiyama and Anggawati (1998) mentioned about an interesting case of a problematic shrimp pond which may lend support to the value of crop rotation. In the first semester of 1994, this pond had emergency harvest. During the succeeding three cycles, the operation had to be aborted within 40 days. In 1996, this pond was used instead as a tilapia broodstock pond. In the first semester of 1997, this pond was used for intensive monoculture of shrimp. During this cycle the production level was 5.2 MT/ha of 19g shrimp at 59% survival with FCR of 1.9. Apparently, the culture of tilapia may have “rejuvenated the pond.” Other countries like Bangladesh (Hossain, 1998) and Thailand (P. Chanratchakool, pers. com.) are also having

good experiences on shrimp-finfish crop rotation.

Since crop rotation can only be effective if there is no influx of luminous bacteria in the pond system, other sources of the bacteria should also be addressed. Biological pre-treatment using finfish and polyculture with finfish can be used to reduce luminous bacteria from incoming water and rearing water, respectively. In this farm trial, the LBC in all three ponds (ponds 7,9 and 29) were maintained below 10 cfu/ml. This level is much lower than the levels previously associated with luminous vibriosis outbreaks (10^2 to above 10^4 cfu/ml; Lavilla-Pitogo *et al.*, 1998). During the previous trial of the farm (using pond 8 as a reservoir stocked with tilapia and pond 9 as shrimp-tilapia pond), LBC would reach non-detectable level once the biomass of tilapia at the reservoirs reached 3,500 kg/ha. That biomass of tilapia was therefore maintained at the reservoirs during this present trial. Hence, maintaining low LBC at the shrimp-tilapia ponds which drew water from those reservoirs. The presence of tilapia in cages within the shrimp pond (at around 1,300 kg/ha biomass) may have also helped maintain that low LBC.

Aside from lowering LBC, tilapia culture may also have helped improve soil and water quality during this trial. In a trial on polyculture of shrimp and tilapia in Ecuador, Akiyama and Anggawati (1998) observed that water pH was maintained in the optimum range of 7.7 to 8.3 in polyculture ponds in contrast to the control ponds pH which ranged from pH 7.5 to 8.5. In addition, phytoplankton bloom in the polyculture ponds was more stable than that in the control ponds. It was also observed that upon harvest, the polyculture ponds did not smell as bad as the control ponds. The ecological role of tilapia in an intensive shrimp (*P. chinensis*)-tilapia culture was discussed by Ji-Qiao *et al.* (1998). Selective feeding of tilapia on large plankton, particularly zooplankton, results in a decrease in predatory pressure on small phytoplankton. These small phytoplankton have high productivity and fully utilize nutrients in the water due to their large absorptive surface area and low precipitating index. This may help explain the dominance of *Chlorella* spp. in the present trial. Furthermore, the disturbance by tilapia while swimming around enhances water movement and nutrient cycle. Nitrogen and phosphorus excreted by tilapia function as slow and even fertilization which helps maintain an optimal and constant biomass of phytoplankton. Tilapia has also a positive effect on dissolved oxygen. Dissolved oxygen level reached its peak and was significantly higher in enclosures with tilapia than in control enclosures (without tilapia) when the tilapia biomass was 300 kg/ha.

The term “finfish-based biological control” is hereby coined to describe the above approach. This approach uses finfish in crop rotation, biological pre-treatment and polyculture to manipulate the pond environment in such a way that beneficial microorganisms are favored to proliferate.

Research Needs

The present trial is among the first reported attempts to integrate finfish in shrimp culture as a disease prevention strategy. Because of the promising results obtained in this trial, refinements of the technology should be further investigated. Technology verification trials are now being conducted under various farm conditions in Negros Occidental. Controlled experiments to determine the effects of biomass and species of finfish on physico-chemical and microbiological quality of soil and water in shrimp culture are also worth pursuing. The information from these experiments will be useful in refining the technology.

As observed in many trials in Negros Occidental, there is a dominance of *Chlorella* spp. in brackishwater tilapia culture. Some hypothesize that *Chlorella* may be inhibiting luminous bacteria in nature. Direkbusarakom *et al.* (1997) found that extracellular products of *Chlorella* have antibiotic activity against shrimp pathogens *V. harveyi, V. parahaemolyticus* and *V. penaeicida.* However, one should bear in mind that the ability to produce an inhibitory material does not necessarily mean that it plays an ecological role (Sieburth, 1968). An excellent example is the autoinhibitor chlorellin from *Chlorella vulgaris.* Scutts (1964), cited by Sieburth, 1968, stated that "inhibitor production is not a general phenomenon with *Chlorella* and only occurs under certain conditions." The demonstration of ecologically sufficient concentrations of specific inhibitors under natural conditions is essential to the proof of their importance in ecology (Sieburth, 1968). As discussed above, *Chlorella* (and other phytoplankton) may be beneficial in improving water quality and may help reduce luminous bacteria indirectly but not directly as in the case of antibiosis. This hypothesis needs to be tested.

Tilapia culture may change the microenvironment in such a way that it favors the proliferation of microorganisms other than the luminous vibrios. Among these microorganisms may include the resident antagonists of luminous vibrios. Resident antagonists are biological agents present in the local environment which have the potential to interfere in the life processes of target pathogens. Other species of bacteria such as *V. alginolyticus* and *Bacillus* sp. have been shown to inhibit growth and reproduction of *V. harveyi* and its closely related species *V. parahaemolyticus* (Garriques and Arevalo, 1995; Rengpipat *et al*., 1998). The presence and activity of these resident antagonists in tilapia culture is therefore worth studying to further provide scientific basis on the finfish-based biological control.

ACKNOWLEDGMENTS

We thank the following organizations and persons for their technical and logistical support: BFAR especially former Director Guillermo L. Morales, Juan D. Albaladejo, Rolando C. Miranda, and Susan L. Mayo; and NPPMCI especially Chairman Roberto A. Gatuslao, Alex L. Montelibano, Alfredo O. Barcelona, and Francisco Y. Domingo.

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